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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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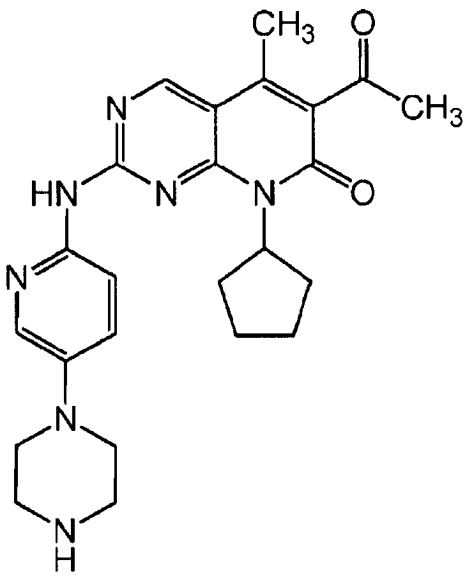
[Continued on next page]

(54) Title: SOLID FORMS OF A SELECTIVE CDK4/6 INHIBITOR



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(57) Abstract: This invention relates to the crystalline free base of acetyl-8- cyclopentyl-5-methyl-2-(5-piperazin-l-yl-pyridin-2-ylamino)-8H- pyrido[2,3-d]pyrimidin-7-one, formula (1) having improved properties, to pharmaceutical compositions and dosage forms comprising the free base, and to methods for making and using such compounds, compositions and dosage forms in the treatment of cell proliferative diseases, such as cancer.

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SOLID FORMS OF A SELECTIVE CDK4/6 INHIBITOR

Cross-Reference to Related Applications

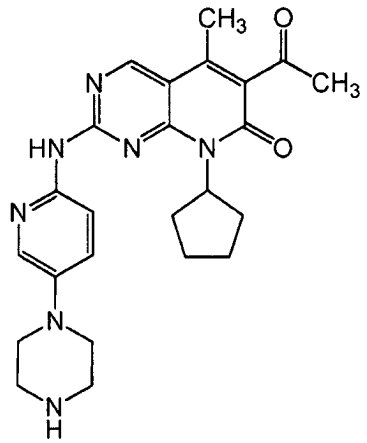
This application claims the benefit of priority to U.S. Provisional Application No. 61/767,761, filed on February 21, 2013, which is incorporated by reference herein in its entirety.

Field of the Invention

This invention relates to the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one having improved physicochemical properties. The invention also relates to pharmaceutical compositions and dosage forms comprising the free base, and to methods for making and using such compounds, compositions and dosage forms in the treatment of cell proliferative diseases, such as cancer.

Background of the Invention

The compound 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (also referred to herein as "compound **1**"), may be represented by the structure:



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and is also known as palbociclib or PD-0332991. Compound **1** is a potent and selective inhibitor of CDK4 and CDK6.

Compound **1** and pharmaceutically acceptable salts thereof are disclosed in International Publication No. WO 2003/062236 and U.S. Patent Nos. 6,936,612, 7,208,489 and 7,456,168, which describe the preparation of compound **1** as its hydrochloride salt. International Publication No. WO 2005/005426 and U.S. Patent Nos. 7,345,171 and 7,863,278 describe preparation of the free base and various mono- and di-acid addition salts of compound **1**, including polymorphic forms of the isethionate salt. A process for the preparation of compound **1** as a mono-isethionate salt is described in International Publication No. WO 2008/032157 and U.S.

Patent No. 7,781,583. The contents of each of the foregoing references are incorporated herein by reference in their entirety.

While compound 1 is a potent and selective CDK4/CDK6 inhibitor, its use as a free base presented challenges for pharmaceutical development. The free base provided by traditional salt break procedures, e.g., as in Example 4 of WO 2005/005426, was highly static prone and formed small primary particles, which agglomerated into large, hard agglomerates that were difficult to disperse by sieving and were unsuitable for further development. The present invention provides compound 1 free base having larger primary particle size that demonstrates improved physicochemical and manufacturability properties.

Summary of the Invention

The free base of compound 1, 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, can exist in one or more polymorphic forms, including Form A and Form B, wherein Form A is the more stable crystalline form. The free base may be anhydrous, or may contain varying amounts of water or one or more solvents.

The present invention provides the crystalline free base of compound 1 having larger primary particle size, greatly reduced specific surface area, and lower surface energy measurements than the free base provided by traditional salt break methods described in the art. The large particle size compound 1 free base disclosed herein is distinguishable by a variety of methods.

The polymorphic and solid forms of the invention can be distinguished by powder X-ray diffractometry (PXRD), solid state NMR (ssNMR), differential scanning calorimetry (DSC), vibrational spectroscopy (e.g., IR and Raman spectroscopy), polarized light microscopy (PLM), scanning electron microscopy (SEM), hot stage optical microscopy, electron crystallography, single crystal X-ray diffractometry, quantitative analysis, particle size analysis (PSA) (e.g., particle size, particle size distribution (PSD), and particle shape), specific surface area (SSA) analysis, surface energy analysis (e.g., inverse gas chromatography or IGC), by solubility studies and dissolution studies, or a combination of these techniques.

In one aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one having a specific surface area of ≤ 2 m^2/g. In some embodiments, the free base has a specific surface area of ≤ 1 m^2/g.

In preferred embodiments, the crystalline free base of compound 1 is a polymorph Form A of the free base. In some such embodiments, the crystalline free base has a PXRD pattern comprising a peak at diffraction angle (2θ) of 10.1± 0.2. In other such embodiments, the crystalline free base has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2 and 10.1 ± 0.2. In still other embodiments, the crystalline free base has a PXRD pattern

comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, and 11.5 ± 0.2. In further embodiments, the crystalline free base has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, 10.3 ± 0.2, and 11.5 ± 0.2. In further embodiments, the crystalline free base has a PXRD pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 1.

In some embodiments, the crystalline free base of compound 1 (Form A) has a ^{13}C solid state NMR (ssNMR) spectrum comprising the following resonance (ppm) values: 12.5 ppm ± 0.2 ppm. In other embodiments, the crystalline free base has a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: 12.5 ppm and 112.4 ppm ± 0.2 ppm. In further embodiments, the crystalline free base has a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: or 12.5 ppm, 112.4 ppm and 143.2 ppm ± 0.2 ppm.

In some embodiments described herein, the compound 1 free base of the invention is distinguished by particle size analysis. In some such embodiments, the crystalline free base has a primary particle size of from about 5 μm to about 150 μm, preferably from about 10 μm to about 100 μm, or more preferably from about 15 μm to about 80 μm. In other such embodiments, the crystalline free base has a primary particle size distribution characterized by: (i) a D10 value of from about 5 μm to about 10 μm; (ii) a D50 value of from about 10 μm to about 45 μm; or (iii) a D90 value of from about 30 μm to about 125 μm; or a combination of (i), (ii) and (iii). In additional embodiments, the crystalline free base has a primary particle size distribution ratio of (D90-D10)/D50 of from about 2 to about 3. In further embodiments, the crystalline free base has a volume mean diameter (D[4,3]) of from about 15 μm to about 125 μm.

In some embodiments, the crystalline free base of compound 1 is anhydrous. In other embodiments, the crystalline free base of compound 1 is a solvate, in particular a hydrate.

In another aspect, the invention provides a pharmaceutical composition comprising a crystalline free base of compound 1, having the large primary particle size according to the invention, and a pharmaceutically acceptable carrier, diluent or excipient. Frequently, the pharmaceutical composition comprises polymorph Form A of the free base.

The invention further provides a capsule comprising such a pharmaceutical composition of the invention. In some such embodiments, the capsule comprises from 0.1 to 200 mg, and preferably from 25 to 150 mg, of compound 1 free base (preferably as polymorph Form A), having the large primary particle size as described herein.

In another aspect, the invention provides a method of treating cancer in a mammal, preferably a human, comprising administering to the mammal a therapeutically effective amount of a pharmaceutical composition of the invention. The method of treatment may further comprise administration of compound 1 in combination with one or more additional therapeutic agents.

In further aspects, the invention provides methods of making the free base of compound 1 having a large primary particle size, as described herein. One method involves dissolving the small particle size free base of compound 1 in mixture of a first solvent and a second solvent and heating to achieve dissolution, cooling to appropriate temperature, providing seed crystals of compound 1 free base (Form A), followed by crystallization to provide the large particle size free base of compound 1. The small particle size free base used in this process may be isolated from a traditional salt break procedure, e.g., by acidic hydrolysis of the intermediate vinyl ether to provide an acid addition salt, followed by basification, as described in Example 5. Another method involves acidic hydrolysis of the intermediate vinyl ether in a mixture of water and a first solvent, which may require heating to obtain dissolution, addition of a second solvent and basification to provide a second mixture comprising the free base generated *in situ*, heating if required to obtain dissolution and to distill off water, and providing seed crystals of compound 1 free base (Form A) at an appropriate temperature, followed by crystallization to provide the free base of compound 1 having a large primary particle size. The invention further provides the free base of compound 1 prepared by these methods, having the properties described herein.

In each of the above methods, the first solvent is an alcohol and the second solvent is an aromatic solvent. Suitable alcohols include, but are not limited to, relatively high boiling alcohols such as n-butanol, t-butanol, n-propanol, pentanol, 1,4-butanediol or propylene glycol, and the like. Suitable aromatic solvents include, but are not limited to, anisole, mesitylene, m-xylene, chlorobenzene, pyridine, and the like. To improve yields, the methods may include heating or cooling to temperatures above or below room temperature. Frequently, the reaction mixtures may be heated to temperatures ranging from about 30°C to about 150°C, and more frequently from about 50°C to about 120°C to achieve dissolution. During crystallization, it may be desirable to cool the reaction mixture to a temperature that is at or below room temperature, for example between about 0°C and about 30°C, preferably to about 5°C, about 10°C, about 15°C, or about 20°C.

These and other aspects and embodiments are further described by the detailed description provided herein. Each of the embodiments described herein can be combined with any other embodiment described herein not inconsistent with the embodiment with which it is combined.

Brief Description of the Drawings

Figure 1 shows a PXRD pattern of compound 1 free base, polymorph Form A.

Figure 2 shows the Carbon CPMAS spectrum of compound 1 free base, polymorph Form A. Peaks marked by asterisks are spinning sidebands.

Figure 3 shows a PXRD pattern of compound 1 free base, polymorph Form B.

Figure 4 shows the Carbon CPMAS spectrum of compound 1 free base, polymorph Form B. Peaks marked by asterisks are spinning sidebands.

Figure 5 shows a scanning electron microscopy (200x magnification) image of compound 1 free base API, polymorph Form A, recrystallized from 40% n-BuOH/anisole.

Figure 6 shows a scanning electron microscopy (1500x magnification) image of compound 1 free base API, polymorph Form A, isolated from a standard free basing process.

Figure 7 shows the particle size distribution of compound 1 free base API, polymorph Form A, recrystallized from 40% n-BuOH/anisole.

Figure 8 shows the particle size distribution of compound 1 free base API, polymorph Form A, isolated from a standard free basing process.

Figure 9 shows a polarized light microscopy (PLM) image (200x) of compound 1 free base API, polymorph Form A, recrystallized from 40% n-BuOH/anisole.

Detailed Description of the Invention

The present invention may be understood more readily by reference to the following detailed description and the Examples included herein. It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting. It is further to be understood that unless specifically defined herein, the terminology used herein is to be given its traditional meaning as known in the relevant art.

As used herein, the singular form "a", "an", and "the" include plural references unless indicated otherwise. For example, "a" substituent includes one or more substituents.

As used herein, the term "about" means within a statistically meaningful range of a value, such as a stated concentration range, time frame, molecular weight, particle size, temperature or pH. Such a range can be within an order of magnitude, typically within 20%, more typically within 10%, and even more typically within 5% of the indicated value or range. Sometimes, such a range can be within the experimental error typical of standard methods used for the measurement and/or determination of a given value or range. The allowable variation encompassed by the term "about" will depend upon the particular system under study, and can be readily appreciated by one of ordinary skill in the art. Whenever a range is recited within this application, every whole number integer within the range is also contemplated as an embodiment of the invention.

As used herein, unless otherwise indicated, the term "abnormal cell growth" refers to cell growth that is independent of normal regulatory mechanisms (e.g., loss of contact inhibition). "Abnormal cell proliferative diseases" are diseases characterized by abnormal cell growth, such as cancer.

The term "cancer" includes both solid tumors and hematological malignancies. Cancers include, but are not limited to, breast cancer, ovarian cancer, cervical cancer, endometrial

cancer, prostate cancer, testicular cancer, pancreatic cancer, esophageal cancer, head and neck cancer, gastric cancer, bladder cancer, lung cancer (e.g., adenocarcinoma, NSCLC and SCLC), bone cancer (e.g., osteosarcoma), colon cancer, rectal cancer, thyroid cancer, brain and central nervous system cancers, glioblastoma, neuroblastoma, neuroendocrine cancer, rhabdoid cancer, keratoacanthoma, epidermoid carcinoma, seminoma, melanoma, sarcoma (e.g., liposarcoma), bladder cancer, liver cancer (e.g., hepatocellular carcinoma), kidney cancer (e.g., renal cell carcinoma), myeloid disorders (e.g., AML, CML, myelodysplastic syndrome and promyelocytic leukemia), and lymphoid disorders (e.g., leukemia, multiple myeloma, mantle cell lymphoma, ALL, CLL, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma).

The phrase "pharmaceutically acceptable" refers to substances, which are within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use.

The term "mammal", as used herein, may be a human or non-human mammal (e.g., dog, cat, rabbit, rat, mouse, horse, monkey, other lower-order primate, etc.). Preferably the mammal is a human.

As used herein, unless otherwise indicated, the term "treating" means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" as defined immediately above.

As used herein, an "effective" amount refers to an amount of a compound, agent, substance, formulation or composition that is of sufficient quantity to result in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The amount may be as a single dose or according to a multiple dose regimen, alone or in combination with other compounds, agents or substances. One of ordinary skill in the art would be able to determine such amounts based on such factors as a subject's size, the severity of a subject's symptoms, and the particular composition or route of administration selected.

"Unit dosage form", as used herein, refers to a physically discrete unit of inventive formulation appropriate for the subject to be treated. It will be understood, however, that the total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, duration of the treatment; drugs and/or

additional therapies used in combination or coincidental with the inventive compositions, and like factors well known in the medical arts.

As used herein, the term "essentially the same" with reference to X-ray diffraction peak positions means that typical peak position and intensity variability are taken into account. For example, one skilled in the art will appreciate that the peak positions (2θ) will show some inter-apparatus variability, typically as much as 0.2° or 0.1°. Further, one skilled in the art will appreciate that relative peak intensities will show inter-apparatus variability as well as variability due to degree of crystallinity, preferred orientation, prepared sample surface, and other factors known to those skilled in the art, and should be taken as qualitative measures only.

The term, "solvate," as used herein, refers to a crystal form of a substance which contains solvent. The term "hydrate" refers to a solvate wherein the solvent is water.

The term "seeding," as used herein, means the addition of crystals to a crystallization system, for the purpose of initiating or enhancing nucleation or acting as substrate for further crystallization.

As used herein, the terms "API" or "active pharmaceutical ingredient" refer to the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-d]pyrimidin-7-one.

As used herein, the term "primary particles" refers to individual API crystals.

As used herein, the term "agglomerates" refers to tightly bound API crystals that are difficult to disperse into primary particles during processing and particle size analysis.

The present invention provides compound **1** free base having larger primary particle size, greatly reduced specific surface area, and lower surface energy measurements than the free base provided by traditional salt break methods. For convenience, the compound **1** free base provided by the invention may sometimes be referred to herein as the "large (primary) particle size" free base. This is in contrast to the free base of compound **1** prepared through traditional salt break methods, which is sometimes referred to as the "small (primary) particle size" free base. It will be understood by those of skill in the art that the reference to "small particle size" in this case refers to the particle size of individual API crystals, and does not take into account the propensity of the "small" particles to form large agglomerates.

In some embodiments of the invention described herein, the crystalline free base of compound **1** is distinguished by specific surface area (SSA). Thus, in one aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one having a specific surface area (SSA) of ≤ 2 m^2/g. In some embodiments, the free base has a specific surface area (SSA) of ≤ 1 m^2/g. In other embodiments, the free base of compound **1** has an SSA of ≤ 0.9 m^2/g, ≤ 0.8 m^2/g or ≤ 0.7 m^2/g. In further embodiments, the free base of compound **1** has an SSA of between 0.2 m^2/g and 2 m^2/g, between 0.5 m^2/g and 1.5 m^2/g, or between 0.5 m^2/g and 1 m^2/g.

In some embodiments described herein, the crystalline free base of compound **1** is distinguished by dispersive surface energy. Thus, in one aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one having a dispersive surface energy of ≤ 60 mJ/m^2. In some embodiments, the free base has a dispersive surface energy of ≤ 55 mJ/m^2, ≤ 50 mJ/m^2, ≤ 45 mJ/m^2 or ≤ 40 mJ/m^2. In further embodiments, the free base of compound **1** has a dispersive surface energy of between 20 mJ/m^2 and 60 mJ/m^2, between 25 mJ/m^2 and 50 mJ/m^2, or between 30 mJ/m^2 and 50 mJ/m^2.

In preferred embodiments, the crystalline free base of compound **1** is a polymorph Form A of the free base. In some such embodiments, the crystalline form has a PXRD pattern comprising a peak at diffraction angle (2θ) of 10.1± 0.2. In other such embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2 and 10.1 ± 0.2. In still other embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, and 11.5 ± 0.2. In further embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, 10.3 ± 0.2, and 11.5 ± 0.2. In other embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) of 5.1 ± 0.2, 8.0 ± 0.2, 10.1 ± 0.2, and 11.5 ± 0.2. In further embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, 11.5 ± 0.2, and 19.7 ± 0.2. In still further embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, 11.5 ± 0.2, and 22.5 ± 0.2. In further embodiments, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 1.

In some embodiments, the crystalline free base of compound **1** (Form A) has a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: 12.5 ppm ± 0.2 ppm. In other embodiments, the crystalline form has a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: 12.5 ppm and 112.4 ppm ± 0.2 ppm. In further embodiments, the crystalline form has a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: or 12.5 ppm, 112.4 ppm and 143.2 ppm ± 0.2 ppm.

In some embodiments described herein, the crystalline free base of compound **1** is distinguished by particle size analysis. In some such embodiments, the free base has a primary particle size of from about 5 μm to about 150 μm, preferably from about 10 μm to about 100 μm, and more preferably from about 15 μm to about 80 μm.

In other such embodiments, the free base has a primary particle size distribution characterized by: (i) a D10 value of from about 5 μm to about 10 μm; (ii) a D50 value of from about 10 μm to about 45 μm; or (iii) a D90 value of from about 30 μm to about 125 μm; or a combination of (i), (ii) and (iii). In additional embodiments, the free base has a primary particle

size distribution ratio of (D90-D10)/D50 of from about 2 to about 3. In further embodiments, the free base has a volume mean diameter (D[4,3]) of from about 15 μm to about 125 μm.

In one aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a primary particle size of greater than about 5 μm. In some embodiments, the free base has a primary particle size of greater than about 7.5 μm. In other embodiments, the free base has a primary particle size of greater than about 10 μm. In other such embodiments, the free base has a primary particle size of greater than about 12.5 μm. In other such embodiments, the free base has a primary particle size of greater than about 15 μm.

In another aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a primary particle size of from about 5 μm to about 200 μm. In some embodiments, the free base has a primary particle size of: from about 5 μm to about 175 μm; from about 5 μm to about 150 μm; from about 5 μm to about 125 μm; from about 5 μm to about 100 μm; from about 5 μm to about 75 μm; from about 10 μm to about 200 μm; from about 10 μm to about 175 μm; from about 10 μm to about 150 μm; from about 10 μm to about 125 μm; from about 10 μm to about 100 μm; from about 10 μm to about 75 μm; from about 15 μm to about 200 μm; from about 15 μm to about 175 μm; from about 15 μm to about 150 μm; from about 15 μm to about 125 μm; from about 15 μm to about 100 μm; or from about 15 μm to about 75 μm.

In another aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a primary particle size distribution having at least one of:

(a) a D10 value of from about 5 μm to about 10 μm;

(b) a D50 value of from about 10 μm to about 45 μm; and

(c) a D90 value of from about 30 μm to about 125 μm.

In some such embodiments, the free base has a D10 value of from about 5 μm to about 10 μm. In other such embodiments, the free base has a D90 value of from about 30 μm to about 125 μm. In other such embodiments, the free base has a D50 value of from about 10 μm to about 45 μm. In some such embodiments, the free base has a D10 value of from about 5 μm to about 10 μm and a D90 value of from about 30 μm to about 125 μm. In further embodiments, the free base has a D10 value of from about 5 μm to about 10 μm, a D90 value of from about 30 μm to about 125 μm, and a D50 value of from about 10 μm to about 45 μm.

In another aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a primary particle size distribution having at least one of:

(d) a D10 value of from about 5 μm to about 10 μm;

(e) a D50 value of from about 10 μm to about 25 μm; and

(f) a D90 value of from about 30 μm to about 75 μm.

In some such embodiments, the free base has a D10 value of from about 5 μm to about 10 μm. In other such embodiments, the free base has a D90 value of from about 30 μm to about 75 μm. In other such embodiments, the free base has a D50 value of from about 10 μm to about 25 μm. In some such embodiments, the free base has a D10 value of from about 5 μm to about 10 μm and a D90 value of from about 30 μm to about 75 μm. In further embodiments, the free base has a D10 value of from about 5 μm to about 10 μm, a D90 value of from about 30 μm to about 755 μm, and a D50 value of from about 10 μm to about 25 μm.

In other embodiments, the free base has a primary particle size distribution having a D10 value of: from about 5 μm to about 7.5 μm; from about 5 μm to about 10 μm; from about 5 μm to about 12.5 μm; or from about 5 μm to about 15 μm.

In other embodiments, the free base has a primary particle size distribution having a D50 value of: from about 10 μm to about 50 μm; from about 10 μm to about 45 μm; from about 10 μm to about 40 μm; from about 10 μm to about 35 μm; from about 10 μm to about 30 μm; from about 10 μm to about 25 μm; or from about 10 μm to about 20 μm..

In still other embodiments, the free base has a primary particle size distribution having a D90 value of: from about 30 μm to about 175 μm; from about 30 μm to about 160 μm; from about 30 μm to about 150 μm; from about 30 μm to about 140 μm; from about 30 μm to about 130 μm; from about 30 μm to about 125 μm; from about 30 μm to about 120 μm; from about 30 μm to about 115 μm; from about 30 μm to about 110 μm; from about 30 μm to about 100 μm; from about 30 μm to about 75 μm; from about 30 μm to about 70 μm; from about 30 μm to about 65 μm; from about 30 μm to about 60 μm; from about 30 μm to about 55 μm; from about 30 μm to about 50 μm; or from about 30 μm to about 45 μm.

Each of the foregoing values of embodiments for D10 can be combined with any value for D50 and/or D90 value not inconsistent with it. Each of the foregoing values of embodiments for D50 can be combined with any value for D10 and/or D90 value not inconsistent with it. Each of the foregoing values of embodiments for D90 can be combined with any value for D10 and/or D50 value not inconsistent with it.

In another aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a primary particle size distribution ratio of (D90-D10)/D50 of from about 2 to about 3. In some such embodiments, the free base has a primary particle size of from about 5 μm to about 150 μm.

In some embodiments of this aspect, the free base has a primary particle size distribution ratio of (D90-D10)/D50 of: from about 2 to about 2.75; from about 2 to about 2.5;

from about 2 to about 2.25. In other embodiments, the ratio is about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, or about 3.0.

In yet another aspect, the invention provides a crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a volume mean diameter (D[4,3]) of from about 15 μm to about 125 μm. In some embodiments, the free base has a D[4,3] of from about 50 μm to about 100 μm. In other embodiments, the free base has a D[4,3] of from about 15 μm to about 30 μm.

In still other embodiments, the free base has a D[4,3] of: from about 15 μm to about 100 μm; from about 15 μm to about 90 μm; from about 15 μm to about 80 μm; from about 15 μm to about 70 μm; from about 15 μm to about 60 μm; from about 15 μm to about 50 μm; from about 15 μm to about 40 μm; from about 25 μm to about 120 μm; from about 25 μm to about 100 μm; from about 25 μm to about 90 μm; from about 25 μm to about 80 μm; from about 25 μm to about 70 μm; from about 25 μm to about 60 μm; from about 25 μm to about 50 μm; from about 25 μm to about 40 μm; about 25 μm; about 30 μm; about 35 μm; about 40 μm; about 45 μm; about 50 μm; about 55 μm; about 60 μm; about 65 μm; about 70 μm; about 75 μm; to about 80 μm; about 90 μm; about 100 μm; about 105 μm; about 110 μm; about 115 μm; or about 120 μm.

In another aspect, the invention provides a pharmaceutical composition comprising the free base of the invention, and a pharmaceutically acceptable carrier, diluent or excipient. The invention further provides capsule comprising such a pharmaceutical composition of the invention.

In some embodiments, the capsule comprises from 0.1 to 200 mg of polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one. In other embodiments, the capsule comprises from 25 to 150 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one. In other embodiments, the capsule comprises from 50 to 150 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one. In other embodiments, the capsule comprises from 50 to 100 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one. In other embodiments, the capsule comprises from 75 to 150 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one

In another aspect, the invention provides a method of treating cancer in a mammal, including a human, comprising administering to the mammal a therapeutically effective amount of a pharmaceutical composition of the invention. In some such embodiments, the pharmaceutical composition is administered in a capsule. The capsule may comprise from 0.1 to 200 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-

2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base. In other embodiments, the capsule may comprise from 25 to 150 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base. In further embodiments, the capsule may comprise from 50 to 150 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base.

Techniques for characterizing the crystalline free base of compound 1 according to the invention include, but are not limited to, powder X-ray diffractometry (PXRD), solid state NMR (ssNMR), differential scanning calorimetry (DSC), vibrational spectroscopy (e.g., IR and Raman spectroscopy), polarized light microscopy (PLM), scanning electron microscopy (SEM), hot stage optical microscopy, electron crystallography, single crystal X-ray diffractometry, quantitative analysis, particle size analysis (PSA) (e.g., particle size, particle size distribution (PSD), and particle shape), specific surface area (SSA) analysis, surface energy analysis (e.g., inverse gas chromatography or IGC), by solubility studies and dissolution studies, or a combination of these techniques.

In further aspects, the invention provides methods of making the free base of compound 1 having a large primary particle size, as described herein. One method involves dissolving the small particle size free base of compound 1 in mixture of a first solvent and a second solvent and heating to achieve dissolution, cooling to appropriate temperature, providing seed crystals of compound 1 free base (Form A), followed by crystallization to provide the large particle size free base of compound 1. The small particle size free base used in this process may be isolated from a traditional salt break procedure, e.g., by acidic hydrolysis of the intermediate vinyl ether to provide an acid addition salt, followed by basification, as described in Example 5.

In one embodiment, the invention provides a method of making the large particle size free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A), comprising: (a) suspending 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base in mixture of a first solvent and a second solvent and heating to achieve dissolution; (b) cooling to an appropriate temperature and providing seed crystals of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base (Form A); (c) gradually cooling the mixture to achieve crystallization; and (d) isolating the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having large particle size.

In another embodiment, the invention provides a method of making the large particle size free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A), comprising: (a) suspending 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base in

mixture of n-butanol and anisole and heating to about 95-100°C to achieve dissolution; (b) cooling to about 80 °C and providing seed crystals of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base (Form A); (c) maintaining the mixture at about 80°C for about 3 hours and then gradually cooling to about 10°C to achieve crystallization; and (d) filtering to isolate the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having large particle size.

Another method involves acidic hydrolysis of the intermediate vinyl ether in a mixture of water and a first solvent, which may require heating to obtain dissolution, addition of a second solvent and basification to provide a second mixture comprising the free base generated *in situ*, heating if required to obtain dissolution and to distill off water, cooling to appropriate temperature, providing seed crystals of compound 1 free base (Form A), followed by crystallization to provide the free base of compound 1 having a large primary particle size

In one embodiment, the invention provides a method of making the large particle size free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A), comprising: (a) suspending 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester in a mixture of water and a first solvent and heating to achieve dissolution; (b) addition of acid and reaction to produce the acid addition salt of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]-pyrimidin-7-one *in situ*; (c) addition of a second solvent and aqueous base to a pH of ≥10; (d) separation of the organic layer and heating to distill off water; (e) cooling to an appropriate temperature and providing seed crystals of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base (Form A); (f) gradually cooling the mixture to achieve crystallization; and (g) isolating the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having large particle size.

In another embodiment, the invention provides a method of making the large particle size free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A), comprising: (a) suspending 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester in a mixture of water and n-butanol and heating to about 70°C to achieve dissolution; (b) addition of concentrated HCl and heating at about 70°C for 4-6 hrs; (c) addition of anisole and aqueous NaOH to achieve a biphasic mixture having a pH of >10; (d) separation of the layers and heating the organic layer to about 120°C to distill off water; (e) cooling to about 80 °C and providing seed crystals of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base (Form A); (g) maintaining the mixture at about 80°C for about 3 hours and then gradually cooling to about

10°C to achieve crystallization; and (g) filtering to isolate the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having large particle size.

In some embodiments of each of the foregoing methods, the method provides the free base of compound **1** having a specific surface area of ≤ 2 m^2/g. In other embodiments of each of the foregoing methods, the method provides the free base of compound **1** having a specific surface area of ≤ 1 m^2/g. In other embodiments of each of the foregoing methods, the method provides the free base of compound **1** having a primary particle size of from about 5 μm to about 150 μm, preferably from about 10 μm to about 100 μm, and more preferably from about 15 μm to about 80 μm. In other embodiments of each of the foregoing methods, the method provides the free base of compound **1** having a primary particle size distribution characterized by: (i) a D10 value of from about 5 μm to about 10 μm; (ii) a D90 value of from about 30 μm to about 125 μm; or (iii) a D50 value of from about 10 μm to about 45 μm; or a combination of (i), (ii) and (iii). In further embodiments of each of the foregoing methods, the method provides the free base of compound **1** having a primary particle size distribution ratio of (D90-D10)/D50 of from about 2 to about 3. In further embodiments of each of the foregoing methods, the method provides the free base of compound **1** having a volume mean diameter (D[4,3]) of from about 15 μm to about 125 μm.

In another aspect, the invention provides the free base of compound **1**, as described herein, prepared according to one of these methods. In some embodiments, the invention provides the crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A), prepared according to any of the methods described herein. In some such embodiments, the free base prepared by the methods described herein may be characterized by its SSA, PSA, or surface energy, or a combination of these methods, alone or in further combination with PXRD or ssNMR. In some such embodiments, the crystalline free base has a residual solvent content of between 0.05-0.25 wt% anisole and/or between 0.05-0.25 wt% n-butanol. In other such embodiments, the crystalline free base has a residual solvent content of ≤ 0.5 wt% anisole and ≤ 0.5 wt% n-butanol, and preferably ≤ 0.25 wt% anisole and ≤ 0.25 wt% n-butanol.

In each of the above methods, the first solvent is an alcohol and the second solvent is an aromatic solvent. Suitable alcohols include, but are not limited to, relatively high boiling alcohols such as n-butanol, t-butanol, n-propanol, pentanol, 1,4-butanediol or propylene glycol, and the like. Suitable aromatic solvents include, but are not limited to, anisole, mesitylene, m-xylene, chlorobenzene, pyridine, and the like.

In some such embodiments, the solvent mixture comprises 10% alcohol, 15% alcohol, 20% alcohol, 25% alcohol, 30% alcohol, 35% alcohol, 40% alcohol, 45% alcohol, 50% alcohol, 60% alcohol, 70% alcohol, or >70% alcohol, with the balance being the aromatic solvent. In

other such embodiments, the solvent mixture comprises 90% aromatic, 85% aromatic, 80% aromatic, 75% aromatic, 70% aromatic, 65% aromatic, 60% aromatic, 55% aromatic, 50% aromatic, 40% aromatic, 30% aromatic, or <30% aromatic, with the balance being the alcohol solvent.

In one preferred embodiment, the first solvent is n-butanol. In another preferred embodiment, the second solvent is anisole. In a particularly preferred embodiment, the first solvent is n-butanol and the second solvent is anisole. In some such embodiments, the solvent mixture comprises 10% n-butanol/anisole, 15% n-butanol/anisole, 20% n-butanol/anisole, 25% n-butanol/anisole, 30% n-butanol/anisole, 35% n-butanol/anisole, 40% n-butanol/anisole, 45% n-butanol/anisole, 50% n-butanol/anisole, 60% n-butanol/anisole, 70% n-butanol/anisole, or >70% n-butanol/anisole. In some preferred embodiments, the solvent mixture comprises from about 20 to about 50% n-butanol/anisole. In a particularly preferred embodiment, the solvent mixture comprises about 40% n-butanol/anisole.

To improve yields, the methods may include heating or cooling to temperatures above or below room temperature. Frequently, the reaction mixtures may be heated to temperatures ranging from about 30°C to about 150°C, and more frequently from about 50°C to about 120°C to achieve dissolution. During crystallization, it may be desirable to cool the reaction mixture to a temperature that is at or below room temperature, for example between about 0°C and about 30°C, preferably to about 5°C, about 10°C, about 15°C, or about 20°C.

In additional embodiments, the free base of compound **1** is polymorph Form A having a powder X-ray diffraction pattern comprising a peak at diffraction angle (2θ) of 10.1± 0.2. In other embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 10.1 ± 0.2 and 22.5 ± 0.2. In further embodiments of this aspect, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 5.1 ± 0.2, 10.1 ± 0.2, and 22.5 ± 0.2. In further embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 5.1 ± 0.2, 10.1 ± 0.2, 19.7 ± 0.2, and 22.5 ± 0.2. In still other embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 5.1 ± 0.2, 10.1 ± 0.2, 17.1 ± 0.2, 19.7 ± 0.2, and 22.5 ± 0.2. In additional embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 5.1 ± 0.2, 10.1 ± 0.2, 11.5 ± 0.2, 17.1 ± 0.2, 19.7 ± 0.2, and 22.5 ± 0.2. In yet other embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 5.1 ± 0.2, 10.1 ± 0.2, 11.5 ± 0.2, 17.1 ± 0.2, 18.7 ± 0.2, 19.7 ± 0.2, and 22.5 ± 0.2. In some embodiments of this aspect, the crystalline form has a powder X-ray diffraction (PXRD) pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 1.

The powder X-ray diffraction (PXRD) pattern of free base polymorph Form A is shown in Figure 1 and the corresponding data is tabulated in Table 1.

Table 1: PXRD data for polymorph Form A of compound 1.

2θ (°) ± 0.2	Peak Intensity (%)
5.1	63
8.0	18
10.1	100
10.3	70
11.5	42
14.0	20
15.1	14
16.0	16
17.1	47
18.7	33
19.7	51
20.2	30
21.2	22
22.5	87
23.0	31

The solid state nuclear magnetic resonance (ssNMR) for crystalline free base Form A of compound 1 is shown in Figure 2 and the corresponding data is tabulated in Table 2.

Table 2. ^{13}C chemical shifts in parts per million for polymorph Form A of compound 1.

^{13}C Chemical Shifts [ppm][a] ± 0.2
12.50
25.40
26.54
29.04
32.03
46.15
51.01
55.66
107.34
112.44
125.94
131.14

140.15
143.15
144.85
156.32
157.35
158.06
161.88
201.94

(a) Referenced to external sample of solid phase adamantane at 29.5 ppm.

In another aspect, the invention provides a crystalline free base of compound **1**, wherein the crystalline free base is a polymorph Form B of the free base of compound **1**. In some embodiments of this aspect, the crystalline form has a powder X-ray diffraction pattern comprising a peak at diffraction angle (2θ) of 6.0 ± 0.2. In other embodiments of this aspect, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 6.0 ± 0.2 and 19.8 ± 0.2. In further embodiments of this aspect, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 6.0 ± 0.2, 19.8 ± 0.2, and 26.7 ± 0.2. In further embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 6.0 ± 0.2, 16.4 ± 0.2, 19.8 ± 0.2, and 26.7 ± 0.2. In still other embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 6.0 ± 0.2, 12.8 ± 0.2, 16.4 ± 0.2, 19.8 ± 0.2, and 26.7 ± 0.2. In additional embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 6.0 ± 0.2, 12.8 ± 0.2, 16.4 ± 0.2, 19.8 ± 0.2, 22.6 ± 0.2, and 26.7 ± 0.2. In yet other embodiments, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 6.0 ± 0.2, 10.9 ± 0.2, 12.8 ± 0.2, 16.4 ± 0.2, 19.8 ± 0.2, 22.6 ± 0.2, and 26.7 ± 0.2. In some embodiments of this aspect, the crystalline form has a PXRD pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 3. The powder X-ray diffraction (PXRD) pattern of free base polymorph Form B is shown in Figure 3 and the corresponding data is tabulated in Table 3.

Table 3: PXRD data for polymorph Form B of compound **1**.

2θ (°) ± 0.2	**Peak Intensity (%)**
6.0	100
10.9	39
12.8	40
16.4	41
19.8	50
18.1	24
12.1	23
22.6	40
26.7	48
28.2	20

The solid state nuclear magnetic resonance (ssNMR) for crystalline free base Form B of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one is shown in Figure 4, with corresponding tabulated data shown in Table 4.

Table 4. ^{13}C chemical shifts in parts per million for polymorph Form B of compound **1**.

^{13}C Chemical Shifts [ppm][a] ± 0.2
13.06
27.10
28.04
30.23
46.90 [b]
52.32 [b]
54.63
107.28
113.35
125.67
127.04
140.40
145.21
146.37
147.34
155.57
157.59
159.18
161.29
201.35

(a) Referenced to external sample of solid phase adamantane at 29.5 ppm.
(b) Broad peak

For each powder X-ray diffraction measurement, a sample of a free base was placed into a cavity located on a planar surface of the holder, and a glass slide was used to level the surface of the sample. The holder, which contains the sample, was placed in the diffractometer, and the source of the X-ray beam irradiated the sample, initially at a small angle relative to the planar surface of the holder. The X-ray beam was subsequently moved through an arc in a step-wise manner, which successively increased the angle between the incident beam and the planar surface of the holder. At each step of the scan, the scintillation counter detected the amount of

diffracted radiation, which was recorded as a function of 2θ (°). The instrument software displays the diffracted radiation results of the scan as intensity versus 2θ (°).

Tables 1 and 3 list significant PXRD peaks (i.e., those exhibiting peak height to noise ratio greater than 3.5) for the free base of compound **1** having polymorph Form A or Form B, respectively. The list of characteristic peaks provided is not the only possible list of characteristic peaks. Persons of ordinary skill in the art of polymorph identification may choose other sets of characteristic peaks that will also distinguish one polymorph from another.

Differences in PXRD patterns among separate measurements of the same polymorph may arise for many reasons. Sources of error include variations in sample preparation (e.g. sample height), instrument errors, calibration errors, and operator errors (including errors in determining peak locations). Preferential orientation, i.e., a lack of random orientation of crystals in the PXRD sample, can result in significant differences in relative peak heights. Calibration errors and sample height errors often result in a shift of all of the peaks of the diffractogram in the same direction and by the same amount. Small differences in sample height on a flat holder may lead to large displacements in PXRD peak positions. For a systematic study showing that sample height differences of 1 mm may lead to peak shifts as high as 1° 2θ, see Chen et al., *J. Pharmaceutical and Biomedical Analysis* (2001) 26:63.

In many instances, peak shifts among diffraction patterns resulting from systematic error can be eliminated by compensating for the shift (e.g., applying a correction factor to all peak position values) or by recalibrating the diffractometer. Generally, the same techniques can be used to compensate for differences among diffractometers so that PXRD peak positions obtained from two different instruments can be brought into agreement. Furthermore, when these techniques are applied to PXRD measurements from the same or different diffractometers, the peak positions for a particular polymorph will usually agree to within about ±0.2° 2θ.

The disclosed compounds embrace all pharmaceutically acceptable isotopic variations. An isotopic variation is a compound in which at least one atom is replaced by an atom having the same atomic number, but an atomic mass different from the atomic mass usually found in nature. Useful isotopes include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, and chlorine. Exemplary isotopes thus include, without limitation, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl.

Substitution of the disclosed compounds with isotopes such as deuterium, i.e. ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements, and hence may be more useful in some circumstances. In addition, certain isotopic variations, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The

radioactive isotopes tritium, i.e. ^{3}H, and carbon-14, i.e. ^{14}C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Isotopic variations of the disclosed compounds can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using appropriate isotopic variations of suitable reagents. Pharmaceutically acceptable solvates of the disclosed compounds include those in which the solvent of crystallization may be isotopically substituted, e.g. D_2O, d_6-acetone, d_6-DMSO.

Solubility Experiments

U.S. Patent No. 7,345,171 reported that the free base of compound 1, prepared by a traditional salt break procedure, had poor water solubility (9 μg/mL) at pH 7.9 and exhibited low bioavailability in animal studies. The free base was reported to be in its most stable crystal phase according to slurry experiments (i.e.., Form A). Figure 17 of U.S. Patent No. 7,345,171 provided the water adsorption/desorption isotherms for the free base of Form A. As noted previously, this material corresponds to the small particle size free base of compound 1 described herein.

The free base of compound 1 (Form A) has a high propensity for punch sticking in the drug particle manufacturing process. As punch sticking is related to API surface area, API particle size control is critical for minimizing sticking during drug product manufacturing. In addition to issues with punch sticking, compound 1 free base isolated directly from a standard salt break process was found to be highly static prone and found to form large (approximately 500 microns) hard agglomerates that were not dispersed by sieving. Free base API with similarly poor physical properties was produced by free basing of the existing isethionate salt API or by neutralization of the *in situ* salt formed in the final step of the API synthesis. In either process, small API primary particles were produced due to the rapid crystallization caused by the dramatic change in solubility with adjustment of the pH. In all cases the free base was isolated as the more stable polymorph of Form A.

Figure 6 shows a scanning electron microscopy (SEM) image of typical small primary particles formed by the free basing and neutralization experiments described above. The particle size distribution measurement for a batch of compound 1 (Form A) produced by this free base isolation process is provided in Figure 8. The second mode in the particle size distribution was caused by the presence of large agglomerates, which are also seen in the SEM image in Figure 6. Attempts to modify the free basing process were not successful in improving the physical properties of the API produced. As the process for producing free base resulted in the isolation of API with poor physical properties, work was undertaken to identify a recrystallization process that could improve the API physical properties.

Early crystallization screening experiments for compound 1 free base were completed to identify a solvent system that allows for the isolation of particles with improved physical properties. A combination of solubility screening and small-scale recrystallization studies examined multiple potential solvent systems.

Small-Scale Crystallization Studies

A series of small-scale crystallization experiments was run to identify a potential recrystallization solvent system as well as to assess the impact of solvent on the shape of the free base primary particles isolated. An initial set of 14 screening studies were run on a 10 mg scale using sealed vials and an external heat source to warm the 50 mg/mL samples up to reflux temperature. Visual observation identified the samples that went into solution, and photomicroscopy was used to characterize the particles produced. The results of these initial crystallization screening experiments are summarized in Table 5.

Table 5: Summary of results from preliminary small scale crystallization studies

Solvent System	Results of recrystallization
Cyclopentylmethyl ether	did not dissolve
n-Butyl Acetate	did not dissolve
n-Butanol	did not dissolve
Trifluorotoluene	did not dissolve
Toluene	did not dissolve
Chlorobenzene	small irregular shaped particles
DMF	small needle shaped particles
NMP	small irregular shaped particles
Propylene glycol	small irregular shaped particles
Anisole	large particles (lathes or tomahawk shape)
Pyridine	small lathe shaped particles
Sulfolane	small irregular shaped particles
m-Xylene	small/medium tomahawk shaped particles
Mesitylene	small needle shaped particles

Based on these small-scale crystallization studies, anisole became the focus of additional crystallization and solubility studies as the particles produced were large and as anisole is an ICH Class III solvent. This screening study also identified pyridine, m-xylene, and mesitylene as potential solvent systems based on the particles produced, although none of these solvents also have the ICH class III listing similar to anisole.

The following solvents have also been used for recrystallization of the solid: isopropanol, isobutanol, ethanol, ethyl acetate, toluene, tetrahydrofuran, and dioxane. Each of the solvents

generated the polymorph Form A crystalline solid of compound 1 which was the same as the original crystalline form obtained from dichloromethane.

Solubility Studies:

In parallel with the initial small-scale crystallization studies, a series of solubility studies were conducted on the free base of compound 1 to identify a possible recrystallization system. In an initial room temperature solubility screening study, a total of 23 solvents were screened. This study indicated that the compound 1 free-base has low solubility in a range of organic solvents, with only methylene chloride displaying a solubility greater than 1 mg/mL (3.0 mg/mL). Subsequent targeted higher temperature solubility studies were conducted. In a follow-up study, a set of 16 solvent systems were examined at a fixed concentration of 25 mg/mL, and the dissolution temperature was measured using a kinetic solubility method up to a maximum temperature of 110 °C.

Synergistic solubility behavior predicted by a COSMOtherm solubility model of compound 1 was used to select the binary and ternary solvent systems included in this screening study. The results of these studies are listed in Table 6. For experiments listed as >110 °C in the table, compound 1 did not dissolve in the solvent upon heating to 110 °C, indicating that the solubility is less than 25 mg/mL at 110°C in this solvent.

Table 6: Kinetic solubility measurements for 25 mg/mL compound 1 free base solutions

Experiment #	Solvent	Dissolution Temp. (° C)
1	n-BuOH	>110 °C
2	DMF	>110 °C
3	NMP	97.9
4	DMSO	>110 °C
5	DMAc	>110 °C
6	n-Butyl acetate	>110 °C
7	Anisole	>110 °C
8	10 % n-BuOH/Anisole (v/v)	>110 °C
9	20 % n-BuOH/Anisole (v/v)	109.7
10	40 % n-BuOH/Anisole (v/v)	101.4
11	10 % n-BuOH/NMP (v/v)	103.7
12	25 % n-BuOH/NMP (v/v)	>110 °C
13	10 % 1,4-butanediol/anisole (v/v)	109.8
14	25 % 1,4-butanediol/anisole(v/v)	104.8
15	1:1:8 propylene glycol/n-BuOH/anisole (v/v)	91.2
16	2:1:7 propylene glycol/n-BuOH/anisole (v/v)	84.1

Subsequent UPLC/MS testing of the saturated solution from experiments #3 and #11 in Table 6 indicated the presence of a previously unseen impurity peak, indicating that degradation occurred in these experiments.

Although the propylene glycol/n-BuOH/anisole mixtures showed improved solubility as compared to the n-BuOH/anisole mixtures, the former solvent system was not pursued because of the potential challenges of working with propylene glycol due to its high viscosity and boiling point which may cause issues on-scale.

Based on these screening studies, a mixture of 40% n-butanol and anisole was selected as the crystallization solvent system for further work, in view of the relatively high solubility, chemical stability of the API, and particle properties of the recrystallized compound 1 API. This solvent system was used in subsequent production to provide larger primary particle size API that had reduced sticking, was not static prone, and was free of agglomerates.

Using this solvent mixture, compound 1 was dissolved with 40 mL/g of solvent (concentration of 25 mg/mL) by heating to 95 – 100 °C, before being crystallized using a controlled cooling profile and seeding to induce nucleation. Figure 9 is a PLM image of a lab-scale lot of compound 1 recrystallized using this recrystallization procedure, while Figure 7 displays a particle size distribution for three lots of recrystallized API. This recrystallization process results in the isolation of compound 1 API particles with a larger primary particle size, which leads to a decrease in the sticking tendency in the drug product manufacturing process. This recrystallized compound 1 API does not form agglomerates and also has the positive attribute of not being static prone.

The combination of solubility screening and small-scale recrystallization studies examined multiple potential solvent systems for the recrystallization of compound 1 free base. Based on the results from these screening studies, a mixture of 40% n-butanol/anisole was selected as the preferred crystallization solvent system based on the relatively high solubility, chemical stability of the API, and particle properties of the recrystallized compound 1. The larger particle size and improved particle properties of the API isolated from this recrystallization process facilitated the development of a drug product manufacturing process for compound 1 free base.

Particle size assessment

Particle sizes for the recrystallized materials were assessed using laser diffraction methods. Laser diffraction is recognized by standards and guidance agencies including ISO and ASTM and is widely used to determine particle size distributions. In conducting the assessment, the sample is passed through a laser beam which results in laser light scattered at a range of angles. Detectors placed at fixed angles measure the intensity of light scattered at

that position. A mathematical model (Mie or Fraunhoffer Theory) is then applied to generate a particle size distribution.

The particle size was analyzed using the laser diffraction (or small angle light scattering) technique by dispersing the dry sample powder with compressed air. Specifically, the particle size distribution was analyzed using the Sympatec HELOS RODOS system equipped with a Vibri dry powder feeder. The powder sample was dispersed with a dispersion pressure of 0.5bar. In some instances, an Aspiros micro-dosing device was used, and the powder sample was dispersed with a dispersion pressure of 0.2bar. A suitable lens was selected to cover the particle size range of each sample.

In particle size determinations, the median value is defined as the value where half of the population resides above this point, and half resides below this point. For particle size distributions the median is called the D50. The D50 is the size in microns that splits the distribution with half above and half below this diameter. The expression Dv50 or D[v,0.5] is sometimes used for the median of a volume distribution.

The mode is the peak of a frequency distribution. A particle distribution may include more than one mode, e.g., where the particles exist as primary particles and agglomerations.

The span is sometimes used as a measurement of distribution width, and is defined as the ratio of (D[v,0.9]– D[v,0.1]) / D[v,0.5] or (D90-D10)/D50.

The distribution width may also be characterized by citing one, two or preferably three values on the x-axis, typically some combination of the D10, D50, and D90. The D50, the median, has been defined above as the diameter where half of the population lies below this value. Similarly, 90 percent of the distribution lies below the D90, and 10 percent of the population lies below the D10.

The term D[4,3] refers to the volume mean or mass moment mean. Laser diffraction results are reported on a volume basis and the volume mean can be used to define the central point of the distribution. The D[4,3] value is sensitive to the presence of large particles in the distribution.

Formulation

The present invention also relates to pharmaceutical compositions comprising the free base polymorph Form A of compound 1 described herein. Pharmaceutical compositions of the present invention may, for example, be in a form suitable for oral administration as a tablet, capsule, pill, powder, sustained release formulations, solution, suspension, for parenteral injection as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository. The pharmaceutical composition may be in unit dosage forms suitable for single administration of precise dosages. The pharmaceutical composition will include a conventional pharmaceutical carrier or excipient and a compound according to the

invention as an active ingredient. In addition, it may include other medicinal or pharmaceutical agents, carriers, adjuvants, etc.

Suitable pharmaceutical carriers include inert diluents or fillers, water and various organic solvents. The pharmaceutical compositions may, if desired, contain additional ingredients such as flavorings, binders, excipients and the like. Thus for oral administration, tablets containing various excipients, such as citric acid may be employed together with various disintegrants such as starch, alginic acid and certain complex silicates and with binding agents such as sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tableting purposes. Solid compositions of a similar type may also be employed in soft and hard filled gelatin capsules. Preferred materials include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration the active compound therein may be combined with various sweetening or flavoring agents, coloring matters or dyes and, if desired, emulsifying agents or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin, or combinations thereof.

Methods of preparing various pharmaceutical compositions with a specific amount of active compound are known, or will be apparent, to those skilled in this art. For examples, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easter, Pa., 15th Edition (1975).

The disclosed compound may be administered alone or in combination with other drugs and will generally be administered as a formulation in association with one or more pharmaceutically acceptable excipients. The term "excipient" describes any ingredient other than compound **1** and its salts. The choice of excipient will to a large extent depend on the particular mode of administration.

The disclosed compounds may be administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, or buccal or sublingual administration may be employed by which the compound enters the blood stream directly from the mouth.

Formulations suitable for oral administration include solid formulations such as tablets, capsules containing particulates, liquids, or powders, lozenges (including liquid-filled), chews, multi- and nano-particulates, gels, solid solution, liposome, films (including muco-adhesive), ovules, sprays and liquid formulations. Liquid formulations include suspensions, solutions, syrups and elixirs. Such formulations may be employed as fillers in soft or hard capsules and typically comprise a carrier, for example, water, EtOH, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

The disclosed compounds may also be used in fast-dissolving, fast-disintegrating dosage forms such as those described in Liang and Chen, *Expert Opinion in Therapeutic Patents* (2001) 11(6):981-986.

For tablet dosage forms, depending on dose, the drug may make up from 1 wt % to 80 wt % of the dosage form, more typically from 5 wt % to 60 wt % of the dosage form. In addition to the drug, tablets generally contain a disintegrant. Examples of disintegrants include sodium starch glycolate, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, croscarmellose sodium, crospovidone, polyvinylpyrrolidone, methylcellulose, microcrystalline cellulose, lower alkyl-substituted hydroxypropyl cellulose, starch, pregelatinized starch, and sodium alginate. Generally, the disintegrant will comprise from 1 wt % to 25 wt %, preferably from 5 wt % to 20 wt % of the dosage form.

Binders are generally used to impart cohesive qualities to a tablet formulation. Suitable binders include microcrystalline cellulose, gelatin, sugars, polyethylene glycol, natural and synthetic gums, polyvinylpyrrolidone, pregelatinized starch, hydroxypropyl cellulose, and hydroxypropyl methylcellulose. Tablets may also contain diluents, such as lactose (monohydrate, spray-dried monohydrate, anhydrous and the like), mannitol, xylitol, dextrose, sucrose, sorbitol, microcrystalline cellulose, starch, and dibasic calcium phosphate dihydrate.

Tablets may also optionally include surface-active agents, such as sodium lauryl sulfate and polysorbate 80, and glidants such as silicon dioxide and talc. When present, surface-active agents may comprise from 0.2 wt % to 5 wt % of the tablet, and glidants may comprise from 0.2 wt % to 1 wt % of the tablet.

Tablets also generally contain lubricants such as magnesium stearate, calcium stearate, zinc stearate, sodium stearyl fumarate, and mixtures of magnesium stearate with sodium lauryl sulfate. Lubricants generally comprise from 0.25 wt % to 10 wt %, preferably from 0.5 wt % to 3 wt % of the tablet. Other ingredients may include preservatives, anti-oxidants, flavors, and colorants.

Tablet blends may be directly compressed to form tablets. Tablet blends or portions of blends may alternatively be wet-, dry-, or melt-granulated, melt congealed, or extruded before tabletting. The final formulation may comprise one or more layers and may be coated or uncoated. Exemplary tablets contain up to about 80 % drug, from about 10 wt % to about 90 wt % binder, from about 0 wt % to about 85 wt % diluent, from about 2 wt % to about 10 wt % disintegrant, and from about 0.25 wt % to about 10 wt % lubricant. For additional details concerning the formulation of tablets, see H. Lieberman and L. Lachman, *Pharmaceutical Dosage Forms: Tablets, Vol. 1* (1980).

Solid formulations for oral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted-, and programmed-release. For a general description of suitable modified

release formulations, see US Patent No. 6,106,864. For details of other useful release technologies, such as high energy dispersions and osmotic and coated particles, see Verma et al, *Pharmaceutical Technology On-line* (2001) 25(2):1-14. For a discussion of the use of chewing gum to achieve controlled release, see WO 00/35298.

The disclosed compounds may also be administered directly into the blood stream, into muscle, or into an internal organ. Suitable means for parenteral administration include intravenous, intra-arterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, and subcutaneous. Suitable devices for parenteral administration include needle (including micro-needle) injectors, needle-free injectors and infusion techniques.

Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates, and buffering agents (preferably to a pH of from 3 to 9), but for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. The preparation of parenteral formulations under sterile conditions, for example, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art. Exemplary parenteral administration forms include solutions or suspensions of active compounds in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired.

The solubility of the disclosed compounds used in the preparation of parenteral solutions may be increased by the use of appropriate formulation techniques, such as the incorporation of solubility-enhancing agents. Formulations for parenteral administration may be formulated to be immediate and/or modified release as described above. Thus the disclosed compounds may be formulated in a more solid form for administration as an implanted depot providing long-term release of the active compound.

The compounds of the invention may also be administered topically to the skin or mucosa, either dermally or transdermally. Typical formulations for this purpose include gels, hydrogels, lotions, solutions, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges, fibers, bandages, and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol and propylene glycol. Topical formulations may also include penetration enhancers. See, for example, Finnin and Morgan, *J Pharm Sci* (1999) 88(10):955-958.

Other means of topical administration include delivery by iontophoresis, electroporation, phonophoresis, sonophoresis and needle-free (e.g. POWDERJECT) or micro-needle injection. Formulations for topical administration may be formulated to be immediate and/or modified release as described above.

The disclosed compounds can also be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, for example, in a dry blend with lactose, or as a mixed component particle, for example, mixed with phospholipids) from a dry powder inhaler or as an aerosol spray from a pressurized container, pump, spray, atomizer (preferably an atomizer using electrohydrodynamics to produce a fine mist), or nebulizer, with or without the use of a suitable propellant, such as dichlorofluoromethane. The pressurized container, pump, spray, atomizer, or nebulizer contains a solution or suspension, which comprises the active compound, an agent for dispersing, solubilizing, or extending release of the active compound (e.g., EtOH or aqueous EtOH), one or more solvents, which serve as a propellant, and an optional surfactant, such as sorbitan trioleate or an oligolactic acid.

Prior to use in a dry powder or suspension formulation, the drug product is micronized to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying.

Capsules, blisters and cartridges (made, for example, from gelatin or hydroxypropylmethyl cellulose) for use in an inhaler or insufflator may be formulated to contain a powder mix of the active compound, a suitable powder base such as lactose or starch, and a performance modifier such as L-leucine, mannitol, or magnesium stearate. The lactose may be anhydrous or, preferably, monohydrated. Other suitable excipients include dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose and trehalose.

A suitable solution formulation for use in an atomizer using electrohydrodynamics to produce a fine mist may contain from 1 μg to 20 mg of the compound of the invention per actuation and the actuation volume may vary from 1 μl to 100 μl. A typical formulation may comprise compound **1**, propylene glycol, sterile water, EtOH, and NaCl. Alternative solvents, which may be used instead of propylene glycol, include glycerol and polyethylene glycol.

Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release using, for example, poly(DL-lactic-coglycolic acid (PGLA). Suitable flavors, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to formulations intended for inhaled/intranasal administration.

In the case of dry powder inhalers and aerosols, the dosage unit is determined by means of a valve that delivers a metered amount. Units in accordance with the invention are typically arranged to administer a metered dose or "puff" containing from 100 to 1000 μg of the active pharmaceutical ingredient. The overall daily dose will typically be in the range 100 μg to 10 mg which may be administered in a single dose or, more usually, as divided doses throughout the day.

The active compounds may be administered rectally or vaginally, for example, in the form of a suppository, pessary, or enema. Cocoa butter is a traditional suppository base, but various alternatives may be used as appropriate. Formulations for rectal/vaginal administration may be formulated to be immediate and/or modified release as described above.

The disclosed compounds may also be administered directly to the eye or ear, typically in the form of drops of a micronized suspension or solution in isotonic, pH-adjusted, sterile saline. Other formulations suitable for ocular and aural administration include ointments, biodegradable (e.g. absorbable gel sponges, collagen) and non-biodegradable (e.g. silicone) implants, wafers, lenses and particulate or vesicular systems, such as niosomes or liposomes. A polymer such as crossed-linked polyacrylic acid, polyvinylalcohol, hyaluronic acid, a cellulosic polymer (e.g., hydroxypropylmethylcellulose, hydroxyethylcellulose, or methyl cellulose), or a heteropolysaccharide polymer (e.g., gelan gum), may be incorporated together with a preservative, such as benzalkonium chloride. Such formulations may also be delivered by iontophoresis. Formulations for ocular/andial administration may be formulated to be immediate and/or modified release as described above.

The disclosed compounds may be combined with soluble macromolecular entities such as cyclodextrin or polyethylene glycol-containing polymers to improve their solubility, dissolution rate, taste masking, bioavailability and/or stability. Drug-cyclodextrin complexes, for example, are found to be generally useful for most dosage forms and administration routes. Both inclusion and non-inclusion-complexes may be used. As an alternative to direct complexation with the drug, the cyclodextrin may be used as an auxiliary additive, i.e. as a carrier, diluent, or solubilizer. Alpha-, beta- and gamma-cyclodextrins are commonly used for these purposes. See, for example, International Patent Applications WO 91/11172, WO 94/02518, and WO 98/55148.

The therapeutically effective dose of compound **1** will vary from approximately 0.01 mg/kg to approximately 100 mg/kg of body weight per day. Typical adult doses will be approximately 0.1 mg to approximately 3000 mg per day. The quantity of active component in a unit dose preparation may be varied or adjusted from approximately 0.1 mg to approximately 500 mg, preferably from about 0.6 mg to 100 mg according to the particular application and the potency of the active component. The composition can, if desired, also contain other compatible therapeutic agents. A subject in need of treatment is administered a dosage of about 0.6 to about 500 mg per day, either singly or in multiple doses over a 24-hour period. Such treatment may be repeated at successive intervals for as long as necessary.

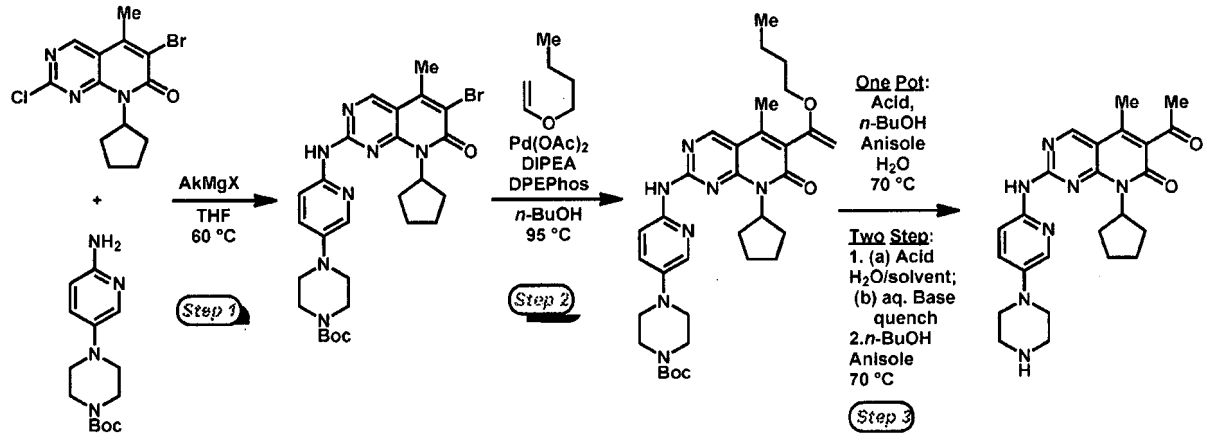
Disorders or conditions caused by abnormal cell proliferation include cancer and vascular smooth muscle proliferation associated with atherosclerosis, post-surgical vascular stenosis and restenosis, and endometriosis. Autoimmune diseases include psoriasis,

inflammation-like rheumatoid arthritis, lupus, type 1 diabetes, diabetic nephropathy, multiple sclerosis, glomerulonephritis, and organ transplant rejection, including host versus graft disease.

In one embodiment, the present invention provides a method of treating abnormal cell growth in a mammal, including a human, in need of such treatment comprising, administering to said mammal a therapeutically effective amount of a crystalline free base of compound 1 according to the invention described herein. In frequent embodiments, the free base is a polymorph of Form A.

In another embodiment, the abnormal cell growth is cancer, including both solid tumors and hematological malignancies. In some such embodiments, the cancer is selected from breast cancer, ovarian cancer, cervical cancer, endometrial cancer, prostate cancer, testicular cancer, pancreatic cancer, esophageal cancer, head and neck cancer, gastric cancer, bladder cancer, lung cancer (e.g., adenocarcinoma, NSCLC and SCLC), bone cancer (e.g., osteosarcoma), colon cancer, rectal cancer, thyroid cancer, brain and central nervous system cancers, glioblastoma, neuroblastoma, neuroendocrine cancer, rhabdoid cancer, keratoacanthoma, epidermoid carcinoma, seminoma, melanoma, sarcoma (e.g., liposarcoma), bladder cancer, liver cancer (e.g., hepatocellular carcinoma), kidney cancer (e.g., renal cell carcinoma), myeloid disorders (e.g., AML, CML, myelodysplastic syndrome and promyelocytic leukemia), and lymphoid disorders (e.g., leukemia, multiple myeloma, mantle cell lymphoma, ALL, CLL, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma).

General Synthetic Scheme



The examples and preparations provided below further illustrate and exemplify particular aspects of embodiments of the invention. It is to be understood that the scope of the present invention is not limited in any way by the scope of the following examples.

Examples

General Methods and Materials

Powder X-ray Diffraction (PXRD)

PXRD data were collected according to the following protocol. A sample (2 mg) was placed on a microscopic slide with zero background. The sample was then placed in a Discover D8 (Bruker AXS Instruments) equipped with a GADDS detector. The system used a copper X-ray source maintained at 40kV and 40 mA to provide CUα1 emission at 1.5406 angstroms. Data were collected from 4 to 40 °2θ using a step scan of 0.02° with a step time of 60.1 seconds. Diffraction peaks are typically measured with an error of ± 0.2 degrees (2θ).

SSNMR Instrumentation and Method

SSNMR data were collected according to the following protocol. Spectra were collected on Bruker-Biospin 4 mm and 7 mm BL CPMAS probe positioned into a wide-bore Bruker-Biospin Avance III 500 MHz NMR spectrometer. The 4 mm rotors were oriented at the magic angle and spun at 15.0 kHz. The 7 mm rotors were oriented at the magic angle and spun at 7.0 kHz. All spectra were acquired at ambient conditions (temperature uncontrolled).

The ^{13}C solid state spectra were collected using a proton decoupled cross-polarization magic angle spinning (CPMAS) experiment. Peak resonances are reported in parts-per-million (ppm) ± 0.2 ppm.

Differential Scanning Calorimetery (DSC):

DSC measurements, are carried out using a Q1000, Thermal Analysis Instruments. A sample is placed in a hermetically sealed aluminum pan with a pinhole. A typical sample weight is 1.6 mg. The sample is equilibrated to 25°C and then ramped to 250°C at a scan rate of 10°C/min. Dry nitrogen is used as the purge gas.

Brunauer, Emmet and Teller (BET) Specific Surface area (SSA) Measurement:

SSA measurements were collected according to the following protocol. Monolayer formation of gas molecules on the crystal surface was used to determine the specific surface area of a dry powder of active pharmaceutical ingredient. The sample was made free of moisture and atmospheric vapours by applying heat and purging with nitrogen gas. The sample temperature was then reduced to that of liquid nitrogen for the adsorbate gas (nitrogen) to be adsorbed. The quantity of adsorbed gas and pressure data were used to generate an adsorption isotherm plot. The data were then converted into specific surface area value using a mathematical algorithm based on the so-called Brunauer, Emmett, and Teller (BET) theory (*see, e.g., J. Am. Chem. Soc.*, 1938, 60:309). Specific surface area was measured using a static

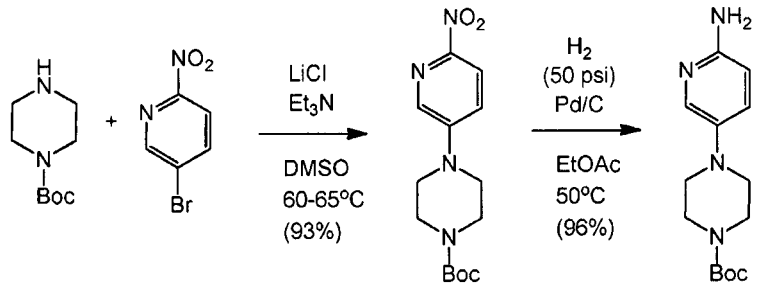
multi-point or single-point gas adsorption method, as fully described in ISO 9277:2010 and in the experimental below.

Inverse-phase Gas Chromatography (IGC) Surface Energy Measurement:

Surface energy measurements were collected using IGC according to the following protocol. A sufficient quantity of sample was packed into a silanised glass column with the powder mass secured within the column by glass wool plugs inserted at both ends. The column was conditioned by flowing a stream of dry nitrogen through the powder mass for sufficient time for any surface adsorbates to be removed. Measurements were made by injecting a series of alkane vapour probes (Nonane, Octane, Heptane and Hexane) into the carrier gas stream at concentrations low enough to assume infinite dilution of the alkane vapour in the nitrogen stream and recording the time taken for each vapour to elute through the column. A plot of the retention time (corrected for the 'dead volume' of interstitial space within the packed column) versus a function of the cross sectional area and surface tension of the alkane vapour probe molecules used yielded a line with a slope indicative of the surface energy of the solid powder under examination.

Synthetic Examples

Example 1. Preparation of 4-(6-amino-pyridin-3-yl)piperazine-1-carboxylic acid *tert*-butyl ester



Step A Step B

Step A. Preparation of 4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

To a vessel was added 5-bromo-2-nitropyridine (10.0 g, 1.0 equiv.) along with DMSO (25 mL, 2.5 vol). N-Boc piperazine (13.8 g, 1.5 equiv.) was added, followed by triethylamine (7.5 g, 1.5 equiv.) and LiCl (2.1 g, 1.0 equiv.). The mixture was warmed to 60-65°C for a minimum of 12 hours.

Water (5 mL, 0.5 vol) was added slowly to the vessel at 60-65°C. The mixture was kept at 60-65 °C for one hour, then cooled to room temperature. The slurry was kept at 20-25 °C for 1 hour and then filtered onto a #2 Whatman™ paper filter. The cake was rinsed with water (50 mL, 5 vol.). The crude solids were collected and transferred back to a clean vessel.

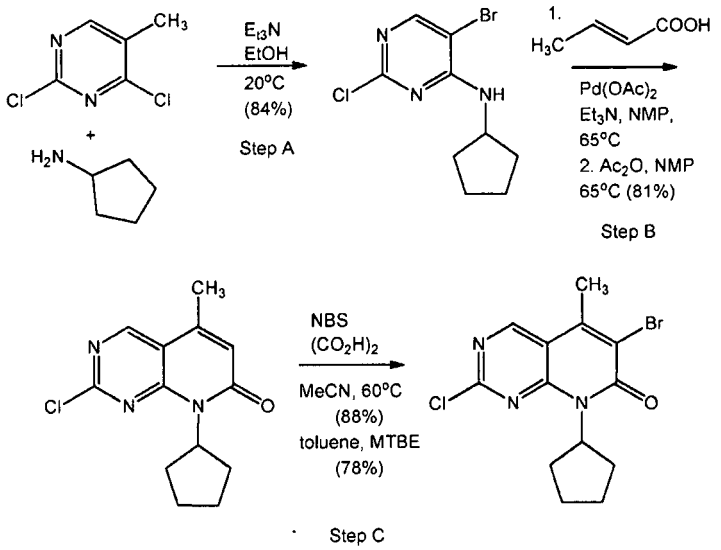
Water (100 mL, 10 vol.) was added to the vessel containing the solids and the mixture was warmed to 35-40°C for 2 hours, then filtered while warm onto a #2 Whatman paper™ filter. The solids were rinsed with water (40 mL, 4 vol.) and allowed to dry overnight in the vacuum oven at 50-55°C. The 4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid tert-butyl ester was isolated as a yellow solid (14.1 g collected; ~93% yield).

Step B. Preparation of 4-(6-amino-pyridin-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

To a vessel was added 4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (12.0 g, 1.0 equiv.) along with ethyl acetate (48 mL, 4.0 vol.). To the slurry was added 50% water wet 5% Pd/C (480 mg, 4% w/w) and the vessel was purged three times with nitrogen. The vessel was purged three times with hydrogen and then pressurized to 50 psi hydrogen. The mixture was heated to 42-47°C and allowed to stir until hydrogen uptake ceased (at least 8 hours).

The product mixture was filtered and washed with ethyl acetate (2 x 1.5 mL). The combined filtrate was concentrated under reduced pressure to a volume of 6 mL (2 vol.). To the solution was added *n*-heptane (54 mL, 4.5 vol.) and the mixture was distilled under reduced pressure to a volume of 6 mL (2 vol.). To the solution was added *n*-heptane (54 mL, 4.5 vol.). The resulting thick slurry was cooled to 20-25°C and allowed to stir for 2 hours. The slurry was filtered and the filter cake washed with *n*-heptane (36 mL, 3 vol.). The solids were allowed to dry overnight in a vacuum oven at 50-55°C. The 4-(6-amino-pyridin-3-yl)-piperazine-1-carboxylic acid tert-butyl ester was isolated as a pale orange solid (10.4 g collected; ~96% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.62 (dd, J = 2.99, 0.60 Hz, 1H), 7.17 (dd, J = 8.85, 2.99 Hz, 1H), 6.40 (dd, J = 8.85, 0.60 Hz, 1H), 5.45 (bs, 2H), 3.43 (m, 2H), 2.85 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.8, 153.8, 138.7, 136.8, 125.9, 108.3, 78.9, 50.5, 43.8, 43.0, 28.0; HRMS: Calcd for $C_{14}H_{23}N_4O_2$ $(M+H)^+$: 279.18155, Found: 279.18173.

Example 2. Preparation of 6-bromo-2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one



Step A. Preparation of 5-bromo-2-chloro-6-cyclopentylamino-pyrimidine

To a vessel was added absolute ethanol (3000 mL, 3.0 vol) followed by 5-bromo-2,4-dichloropyrimidine (mw 227.87; 1000 g, 1.0 equiv.). Triethylamine (612 mL, 1.0 equiv.) was added, and then cyclopentylamine (mw 85.15; 520 mL, 1.2 equiv.) was added slowly over 2 hours to control the mild exotherm. After completion of cyclopentylamine addition, the reaction was seeded with 5-bromo-2-chloro-6-cyclopentylamino-pyrimidine (5 g, 0.5 wt%) to induce crystallization, if needed. The reaction was stirred at 25°C for 2 hours.

Water (2500 mL, 2.5 vol) was added to the vessel at 20-25 °C at a rate of 30 mL/min. The mixture was cooled to 8-12°C at 2°C/min. The slurry was kept at 8-12°C for 1 hour and then filtered onto a #2 Whatman™ paper filter. The cake was rinsed with n-heptane (2000 mL). The cake was reslurried with n-heptane on the filter drier (2000 mL). The material was dried overnight in the vacuum oven at 50-55°C to give 5-bromo-2-chloro-6-cyclopentylamino-pyrimidine (1020 g; 84%) as a white solid.

Step B. Preparation of 2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one

To a vessel was added 5-bromo-2-chloro-6-cyclopentylamino-pyridimidine (10.0 g, 1.0 equiv.) along with N-methylpyrrolidone (NMP) (50 mL, 5.0 vol.) at ambient temperature. To the reaction mixture was added crotonic acid (4.7 g, 1.5 equiv.) and triethylamine (20.2 mL, 4.0 equiv.). The vessel was degassed and purged three times with nitrogen. To the degassed reaction mixture was added $Pd(OAc)_2$ (0.25 g, 0.03 equiv.). The vessel was degassed and purged three times with nitrogen using the same method as step 3. The mixture was heated to 65°C and allowed to stir until starting material was consumed (at least 6 hours).

Acetic anhydride (6.8 mL, 2.0 equiv) was added to the reaction mixture. The reaction was allowed to react at 65 °C until starting material was consumed (usually 1-2 hours).

The reaction mixture was cooled to 20°C and H_2O (100 mL, 10 vol) was added to dissolve triethylamine·HBr salts and precipitate out 2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one. The material was granulated at 20 °C for 1 hour. The solids were filtered and washed with H_2O (20 mL, 2.0 vol), and a 4:1 mixture of isopropanol/H_2O (50 mL, 5.0 vol). The crude product was dried under vacuum at 55-70°C to give 2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, (7.8 g; 81%) as a tan to gray solid.

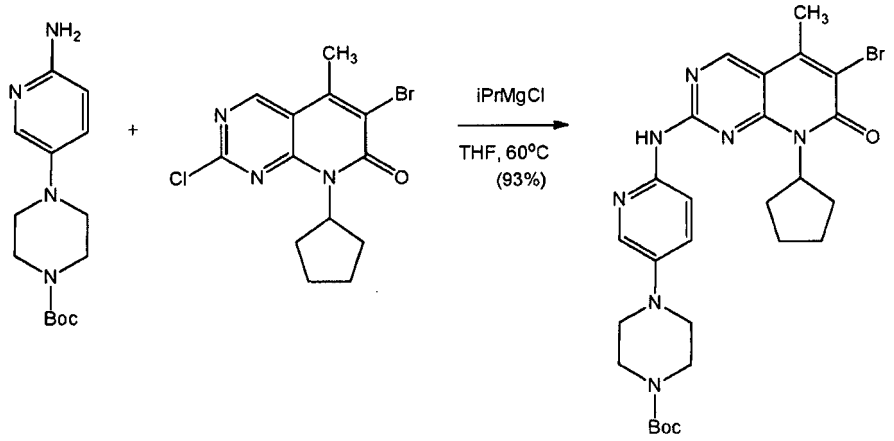
Step C. Preparation of 6-bromo-2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one

To a glass lined vessel was added 2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (9.35 g, 1.0 equiv.) along with acetonitrile (65 mL, 7.0 vol). N-Bromosuccinimide (9.67 g, 1.5 equiv.) and oxalic acid (0.65 g, 0.2 equiv.) were added. The reaction mixture was heated to 60±5 °C. The reaction was stirred at 60°C until starting material was consumed (at least 6 hours). The slurry was cooled to 20°C and H_2O (9 mL, 1 vol) was added. To the slurry was added a solution of sodium bisulfite (3.88 g, 1.0 equiv) in H_2O (38 mL, 4 vol). The slurry was granulated for 1 hour, then filtered directly onto a #2 Whatman paper filter. The reaction vessel was washed with water (19 mL, 2 vol) followed by a 7:3 mix of methanol/acetonitrile (28 mL, 3 vol), and the washes were transferred onto the filter cake. The product was dried in the vacuum oven at 50-55°C. 6-Bromo-2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (10.52 g; 87%) was isolated as a pale yellow solid.

The product was further purified by recrystallization from toluene and n-heptanes. Toluene (60 mL, 6 vol) and 6-bromo-2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (10.00 g, 1 equiv) were added to a reaction vessel and heated to 80°C. The warm reaction mixture was filtered through an appropriate cartridge to ensure the removal of insoluble Pd and other insoluble contaminants. The filter cartridge was washed with 80°C toluene (5 mL, 0.5 vol). The slurry was cooled to 25°C at 1 °C/min. n-Heptane (70 mL, 7 vol) was added to the reaction slurry at 1 mL/min. The slurry was further cooled to 0°C at 1°C/min. The slurry was granulated at 0°C for at least 1 hour.

The slurry was filtered directly onto a #2 Whatman paper filter. n-Heptane (30 mL, 3 vol) was charged to the reaction vessel and the wash was transferred onto the filter cake and the product was dried in the vacuum oven at 50-55°C. 6-Bromo-2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (8.73 g, 87%) was isolated as a cream colored solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.20 (s, 1H), 5.82 (m, 1H), 2.65 (s, 3H), 2.11 (m, 2H), 2.04 (m, 2H), 1.86 (m, 2H), 1.64 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.2, 158.2, 157.6, 154.1, 144.0, 120.9, 113.0, 54.4, 28.3, 25.7, 18.3; HRMS: Calcd for $C_{13}H_{14}N_3O_1Br_1Cl_1$ $(M+H)^+$: 342.00033, Found: 342.00037.

Example 3. Preparation of 4-{6-[6-bromo-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidin-2-ylamino]pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester

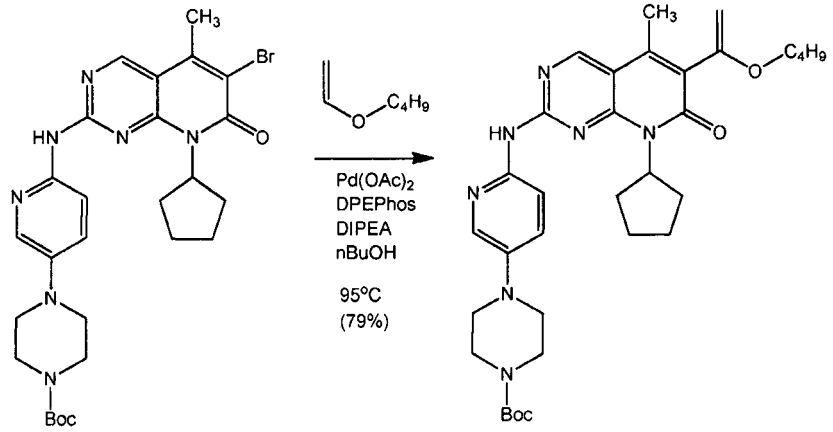


A dry, nitrogen purged reactor was charged with tetrahydrofuran (900 mL, 15 mL/g). The batch temperature was set at 20°C and agitation at 250 RPM was started. The reactor was charged with 4-(6-amino-pyridin-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (63.4g, 0.2278 moles, 1.3 equiv.) and the mixture held at 20°C for 30 min to dissolve the starting material. The reactor was charged with isopropylmagnesium chloride (93.9 g, 0.193 moles, 1st charge 1.1 eq) (2.0M in THF, 1.1 equiv.) by pump over 30 min. The batch was maintained at 20°C for 40 min. The reactor was charged with 6-bromo-2-chloro-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (60.1g, 0.1755 moles, 1 eq.) all at once and rinsed with THF (50 mL rinse). An additional charge of isopropylmagnesium chloride (93.9g, 0.193 moles, 1.1 eq - 2nd charge (2.0M in THF, 1.1 equiv.) was added by pump over 30 min. The batch was held at 20°C for 90 min. and then heated from 20°C to 60°C.

After reaction, a mixture of THF (2.86 vol) and HOAc (1 equiv.) was used to quench the reaction. The batch was then seeded with 0.5 wt/wt% of 4-{6-[6-bromo-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester and a mixture of THF (1.14 vol) and HOAc (0.4 equiv.) was charged to complete the precipitation. After cooling to 20°C, the batch was filtered, washed with acetone (4 vol), water (6 vol) and acetone (4 vol).

The wet cake was dried under vacuum at 65°C to a constant weight to give 4-{6-[6-bromo-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester in 93% yield. ^{1}H NMR (600 MHz, THF-d_8): δ 9.36 (s, 1H), 8.87 (s, 1H), 8.22 (d, J = 8.8 Hz, 1H), 8.04 (d, J = 2.9 Hz, 1H), 7.39 (dd, J = 8.8, 2.9 Hz, 1H), 6.10 (m, 1H), 3.55 (broad, 4H), 3.09 (broad, 4H), 2.60 (s, 3H), 2.30 (m, 2H), 2.09 (m, 2H), 1.85 (m, 2H), 1.66 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (150 MHz, THF-d_8): δ 159.5, 158.9, 157.7, 156.0, 155.0, 147.2, 144.62, 144.56, 138.0, 126.7, 117.6, 114.2, 108.4, 79.9, 55.5, 50.6, 44.7, 29.0, 28.7, 26.9, 18.1; HRMS: Calcd for $C_{27}H_{35}N_7O_3Br_1$ $(M+H)^+$: 584.19797, Found: 584.19811.

Example 4. Preparation of 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester



A dry, nitrogen-purged reactor was charged with 1-butanol (60 mL, 6 mL/g) and 4-{6-[6-bromo-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester (10 g, 0.017 moles) and butyl vinyl ether (5.1g, 0.051 moles, 3.0 eq) were added. Diisopropylethylamine (5.3g, 0.041 moles, 2.4 eq) was added and the mixture was sparged with nitrogen through a sparge tube for 30 minutes. Palladium acetate (0.16g, 0.00068 moles, 0.0400 eq) and bis(2-diphenyphosphinophenyl)ether (0.45g, 0.00082 moles, 0.04800 eq) were added. The mixture was heated to 95°C over 30 minutes and the batch was stirred at 95°C for 2 hours. The mixture was cooled to 80°C and sampled to monitor reaction completion. Following completion, water (15 mL, 1.5 mL/g) and 1-butanol (30 mL, 3 mL/g) were added.

The solution was filtered through a 0.45 micron filter to remove precipitated palladium. Water (35 mL, 3.5 mL/g) was added, followed by 1,2 diaminopropane (6.3g, 0.085 moles, 5.0 eq). The mixture was stirred at 70°C for at least 30 minutes. The agitation was stopped and the mixture was allowed to settle for 15 minutes. The bottom aqueous phase was separated off and the mixture was cooled to 60°C over 30 minutes. The mixture was seeded with 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester (Form C) (50 mg, 0.005 g/g) and held at 60°C for 90 minutes.

Once crystallization was observed, the mixture was cooled to 50°C over one hour and held at 50°C for three hours. The mixture was cooled to 30°C over three hours and held at 30°C for two hours, then cooled to 20°C over four hours and held at 20°C for four hours. The slurry was filtered and washed with 1-butanol (10 mL, 1 mL/g). The filter cake was blown down and the mixture was charged with 1-butanol (10 mL, 1 mL/g) and the slurry was stirred at 20°C for 1 hour. The filter cake was blown down. The mixture was washed with methyl t-butyl ether (20 mL, 2 mL/g) and the cake was fully deliquored using extended blow through times (2 hours or

more). The cake was dried at 70°C. Yield is 75-80%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.0 (s, 1H), 8.87 (s, 1H), 8.07 (d, J = 2.9 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.48 (dd, J = 9.0, 2.9 Hz, 1H), 5.83 (m, 1H), 4.47 (d, J = 1.6 Hz, 1H), 4.05 (d, J = 1.6 Hz, 1H), 3.77 (t, J = 6.4 Hz, 2H), 3.48 (broad, 4H), 3.11 (broad, 4H), 2.37 (s, 3H), 2.22 (m, 2H), 1.89 (m, 2H), 1.75 (m, 2H), 1.61 (m, 2H), 1.58 (m, 2H), 1.43 (s, 9H), 1.38 (m, 2H), 0.90 (t, J = 7.39 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.9, 158.2, 157.3, 155.2, 154.6, 153.7, 145.0, 143.0, 142.6, 136.0, 125.8, 125.5, 114.6, 106.6, 87.8, 78.9, 66.8, 52.8, 48.5, 43.4, 42.5, 30.3, 28.0, 27.4, 25.1, 18.8, 14.4, 13.6; HRMS: Calcd for $C_{33}H_{46}N_7O_4$ $(M+H)^+$: 604.36058, Found: 604.36049.

The intermediate butoxyl-vinyl ether may be isolated in one of several polymorphic forms. Form A was isolated as the kinetic product in the absence of seeding, while Form B was isolated in a few cases but is rarely observed. The most stable crystalline form of the butoxyl-vinyl ether, Form C, was obtained by seeding the reaction mixture with Form C crystals. Any of these polymorphic forms may be utilized in the preparation of Compound **1** free base, but polymorph Form C of the butoxyl-vinyl ether is preferred for ease of filterability.

PXRD data for polymorph Forms A, B and C of the intermediate butoxyl-vinyl ether are tabulated in Tables 7, 8 and 9, respectively.

Table 7: PXRD data for polymorph Form A of intermediate butoxyl-vinyl ether

2θ (°) ± 0.2	Peak Intensity (%)
4.3	100
4.8	85
6.2	39

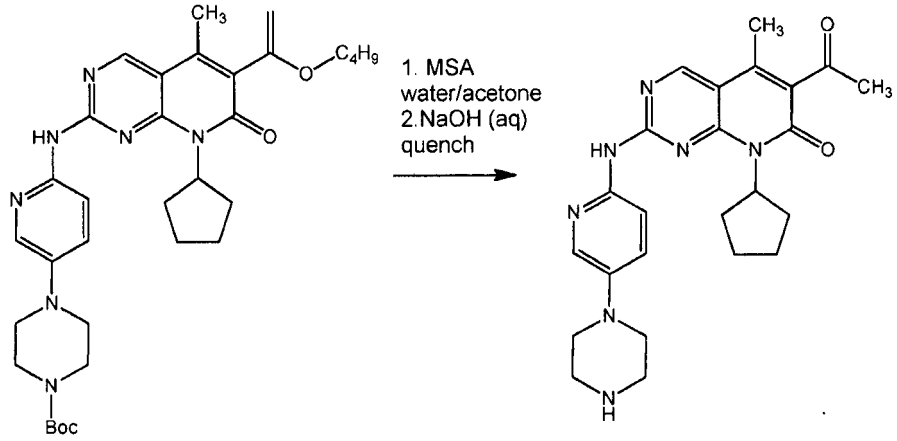
Table 8: PXRD data for polymorph Form B of intermediate butoxyl-vinyl ether

2θ (°) ± 0.2	Peak Intensity (%)
5.5	100
7.5	3
9.7	3
11.1	4
14.8	3
16.7	4
17.5	5
20.1	4

Table 9: PXRD data for polymorph Form C of intermediate butoxyl-vinyl ether

2θ (°) ± 0.2	Peak Intensity (%)
5.4	100
9.7	11
10.8	58
12.7	10
13.3	24
13.5	27
16.1	12
16.6	8
17.0	14
17.5	22
18.1	8
18.8	8
19.6	16
20.6	16
21.7	17
22.9	8
23.8	8
24.4	8
25.0	8

Example 5. Preparation of Small Particle Size Free Base of Compound 1 by Salt Break Method



To a reactor was added 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid tert-butyl ester (2.70 kg, 4.47 mol, 1.0 equiv.) followed by a mixture of water (27.00 L, 10 L/kg) and

acetone (13.50 L, 5 L/kg). The yellow slurry was warmed to between 50°C and 55°C. A solution of methanesulfonic acid (2.15 kg, 22.36 mol, 5.0 eq.) diluted with water (5.40 L, 2 L/kg of starting material) and acetone (5.40 L, 2 L/kg of starting material) was added to the reactor over approximately 10 minutes. The reaction mixture was kept between 45°C and 55°C for at least 12 hours. A clear yellow solution was achieved during the reaction.

The reaction mixture was cooled to 35°C, and a mixture of 5 wt% sodium hydroxide solution was added in portions to the reactor to raise the reaction mixture to a pH > 9. The reactor was cooled to between 20°C and 25°C, granulated, and filtered. The cake was washed with water followed by acetone and dried under vacuum.

This method generated the small primary particle size free base of compound 1, which was equivalent to the material prepared from treatment of the compound 1 hydrochloride salt with aqueous NaOH in Example 4 of WO 2005/005426.

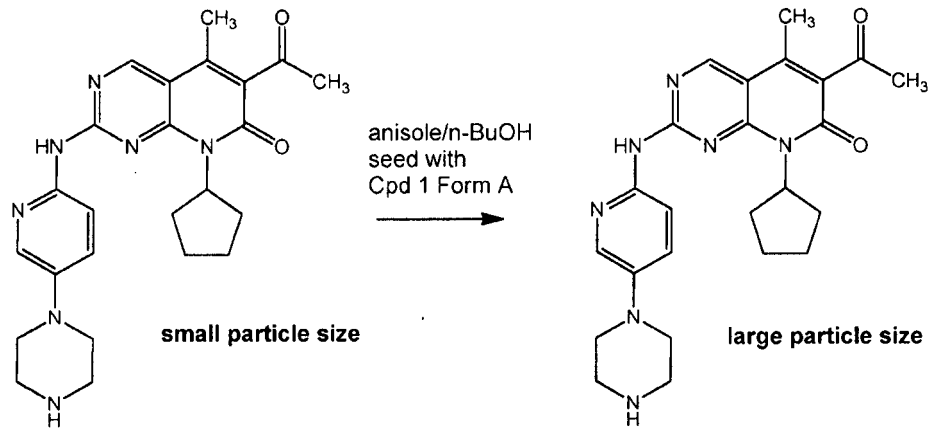
In addition to the representative procedure provided above (corresponding to Experiment S in Table 10), a range of acids and aqueous solvent systems were screened to determine the impact on the reaction and subsequent quench and isolation of free base of compound 1. Lab-scale screening experiments were run to identify reaction conditions for converting the intermediate vinyl ether to the free base compound 1. The results of these reaction screening experiments are summarized in Table 10, indicating the generality of the method.

Table 10: Summary of results from reaction screening experiments

Experiment	Acid	Solvent system	Yield	Purity
A	Isethionic acid	water	99	99.93
B	Isethionic acid	16% THF/water	>100	98.77
C	Isethionic acid	28% THF/water	95	97.95
D	HCl	water	>100	99.59
E	H_2SO_4	water	98	98.6
F	MSA	water	98	99.42
G	MSA	16% THF/water	>100	97.86
H	Isethionic acid	15% NMP/water	88	97.7
I	Isethionic acid	15% DMF/water	90	98.94
J	TFA (8 eq.)	water	100	99.14
K	Isethionic acid	15% CH_3CN/water	>100	99.56
L	Isethionic acid	15% acetone/water	92	99.54
M	Isethionic acid	15% DMAC/water	>100	98.91
N	Isethionic acid	15% sulfolane/water	92	98.67
O	MSA	15% CH_3CN/water	100	99.52

P	MSA	15% acetone/water	97	99.54
Q	CF_3SO_3H (incomplete)	water	N/A	N/A
R	MSA	33% CH_3CN/water	99	99.7
S	MSA	33% acetone/water	98	99.74
T	MSA	33% MeOH/water	98	99.74
U	MSA	33% THF/water	96	99.76

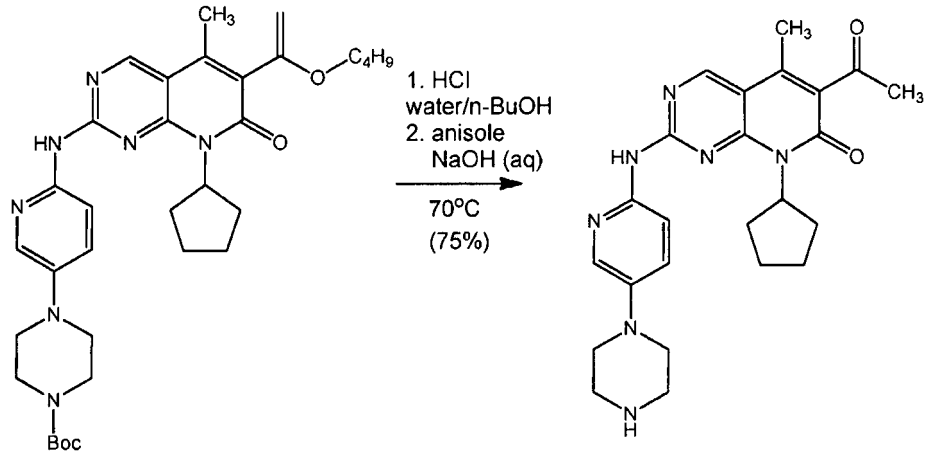
Example 6. Conversion of Small Particle Size Free Base to Large Particle Size Free Base of Compound 1



To a reactor was added compound **1** free base (20g, 44.69 mmol, 1.0 eq.), prepared according to Example 5, followed by 1-butanol (320 ml, 16 ml/g) and anisole (480 ml, 24 ml/g). The yellow slurry was warmed to between 95°C and 100°C to achieve dissolution. The reactor was cooled to 80°C. To the solution in the reactor, a seed slurry containing compound **1** free base (Form A) seed crystals (0.1 g, 0.2 mmol, 0.005 eq.) suspended in 1-butanol (5 mL, 0.25 mL/g of starting material) was charged to induce crystallization. The resulting slurry was stirred at 80°C for 3 hours. The slurry was cooled to 10°C at 0.2 °C/min over 350 minutes, granulated, and filtered. The cake was washed with anisole followed by heptane, and dried under vacuum.

This method generated the large primary particle size crystals of the free base of compound **1**, which were equivalent to the free base prepared using the one-pot method described in Example 7 below.

Example 7. One-Pot Method for Preparation of the Large Particle Size Free Base of Compound 1



To a reactor was added water (200 mL, 10 mL/g) and 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester (20 g, 33.1 mmol, 1.0 equiv.) followed by 1-BuOH (232 mL, 11.6 mL/g) to rinse any solids down into reactor. The yellow slurry was warmed to 70°C. A two-liquid phase mixture formed. Concentrated HCl solution (16.3 g, 165.5 mmol, 5.0 eq.) was added to the reactor over approximately 10 minutes. The reaction mixture was kept at 70°C for 4 to 6 hours. A clear yellow biphasic solution was achieved after 3 hours.

To the reaction mixture was added anisole (356 mL, 17.8 mL/g). While maintaining the mixture at 70°C, a solution of aq. NaOH (17.2 g, 172.1 mmol, 5.2 eq.) (40 wt% solution) was added to the reactor over 20 minutes to raise the reaction mixture to a pH > 10. The two-phase mixture was stirred for 30 minutes after the NaOH addition was complete.

The phases were separated and the organic phase was washed with water twice. The batch was then heated to 80°C and speck-free filtered into the crystallizing vessel, rinsing the filter with butanol. The batch was then distilled to remove water and achieve a temperature of 120°C. The batch was then cooled to 80 °C and seeded with a seed slurry of compound **1** free base (Form A) seed crystals (0.015 g, 0.033 mmol, 0.1 wt.% wrt compound **1**) and 1-BuOH (10 mL, 0.5 mL/g). The batch was then cooled to 30°C at 0.2°C /min and then ripened with three cycles where the temperature was stepped down by 10°C each time. On the final cycle, the batch was cooled to 10°C, granulated and filtered. The cake was washed with twice with heptane and dried under vacuum. After drying, the sample was confirmed to be a single crystalline polymorph Form A.

^{1}H NMR (600 MHz, DMSO-d_6/TFA): δ 10.41 (s, 0.75H), 9.03 (s, 0.25H), 8.98 (s, 2H), 8.12 (d, J = 3.0 Hz, 1H), 7.90 (d, J = 9.1 Hz, 1H), 7.63 (dd, J = 9.1, 3.0 Hz, 1H), 5.84 (m, 1H), 3.40 (broad, 4H), 3.29 (broad, 4H), 2.43 (s, 3H), 2.33 (s, 3H), 2.21 (m, 2H), 1.91 (m, 2H), 1.79 (m, 2H), 1.59 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6/TFA): δ 202.4, 160.7, 154.8, 158.3, 158.0,

144.9, 142.3, 142.0, 134.6, 129.7, 126.7, 115.3, 107.0, 53.0, 45.6, 42.6, 31.3, 27.6, 25.2, 13.7; HRMS: Calcd for $C_{24}H_{30}N_7O_2$ $(M+H)^+$: 448.24555, Found: 448.24540.

Comparative PSA, SSA and surface energy data for the small primary particle size and large primary particle size formulations of the free base of compound **1** are provided below. In all cases, the free base was isolated as polymorph Form A.

Powder X-ray Diffraction (PXRD)

Experimental:

Powder Diffraction analysis was conducted using a Bruker D8 diffractometer equipped with a Cu radiation source, fixed slits (divergence=1.0 mm, anti-scatter=0.6 mm, and receiving=0.6 mm) and a scintillation counter detector. Data were collected in the Theta-Theta goniometer at the Cu wavelength $K\alpha_1$ =1.54056 Å from 3.0 to 40.0 degrees 2-Theta using a step size of 0.040 degrees and a step time of 2.0 second. X-ray tube voltage and amperage were set at 40 kV and 40 mA respectively. Samples were prepared by placement in a Nickel Disk (Gasser & Sons, Inc. Commack, NY) and rotated during data collection. Data were collected and analyzed using Bruker DIFFRAC Plus software (Version 2.6). PXRD data files (.raw) were not processed prior to peak searching. Generally, a Threshold value of 1 and a Width value of 0.3 were used to make preliminary peak assignments. The output of automated assignments was visually checked to ensure validity and adjustments manually made if necessary. Additionally, peaks were manually assigned within spectra if appropriate.

SSNMR Experimental:

Carbon spectra on Form A were acquired on a 4 mm rotor for 2048 scans with recycle delay of 25 seconds and 2 ms of cross polarization. 100 kHz of proton decoupling was applied during acquisition. Carbon spectra on Form B were acquired on a 4 mm rotor for 2048 scans for 128 scans were collected with recycle delay of 4.5 seconds with 2 ms of cross polarization. 70 kHz of proton decoupling and total suppress of spinning sideband (TOSS) was applied during acquisition.

Instrument Method:

Approximately 80 mg of sample were packed into a 4 mm ZrO_2 rotor. Spectra were collected at ambient temperature and pressure on a Bruker-Biospin 4 mm CPMAS probe positioned into a wide-bore Bruker-Biospin Avance III 500 MHz (^{1}H frequency) NMR spectrometer. The packed rotor was oriented at the magic angle and spun at 15.0 kHz. The ^{13}C solid state spectra were collected using a proton phase modulated decoupled cross-polarization magic angle spinning (CPMAS) experiment. The cross-polarization contact time was set to 2.0 ms. A proton decoupling field of approximately 100 kHz was applied during acquisition. The

carbon spectrum of compound 1 Form A was acquired for 512 scans with a 25 second recycle delay. The spectrum is shown Figure 2 and the data is tabulated in Table 2. The carbon spectrum of compound 1 Form B was acquired for 2048 scans with a 4.5 second recycle delay. The carbon spectra were referenced using an external standard of crystalline adamantane, setting its upfield resonance to 29.5 ppm. The spectrum is shown Figure 4 and the data is tabulated in Table 4.

Particle size analysis

The particle size was analyzed using the laser diffraction (or small angle light scattering) technique by dispersing the dry sample powder with compressed air. Specifically, the particle size distribution was analyzed using the Sympatec HELOS RODOS system equipped with a Vibri dry powder feeder. The powder sample was dispersed with a dispersion pressure of 0.5bar. In some instances, an Aspiros micro-dosing device was used, and the powder sample was dispersed with a dispersion pressure of 0.2bar. A suitable lens was selected to cover the particle size range of each sample.

Results

Comparative data for four batches of API are provided in Table 11 below, using either the Vibri or Aspiros devices to disperse the sample. Batch No. 4 had a D90 of around 75µm, whereas Batch Nos. 1 and 2 both had a D90 of approximately 45 µm. The laser diffraction particle size data confirms the SEM observations for these batches.

Table 11. Comparative Size Distribution Data

Summary of PSD data		Particle size (µm)			
Batch No.	Disp. Method.	D[v,0.1]	D[v,0.5]	D[v,0.9]	D[4,3]
1	0.2 Bar ASPIROS	5.21	17.00	43.59	21.33
2	0.2 Bar ASPIROS	6.20	20.83	46.15	23.87
3	0.2 Bar ASPIROS	11.64	46.08	130.26	59.07
	0.5 bar VIBRI	9.96	41.23	116.43	53.02
4	0.2 Bar ASPIROS	7.41	24.97	76.56	35.06
	0.5 Bar VIBRI	6.33	23.19	69.20	32.16

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy was performed under standard conditions. Figure 5 provides a SEM (200x magnification) image of compound 1 free base Form A recrystallized from

40% BuOH/anisole. Figure 6 provides a SEM (1500x magnification) image of compound 1 free base Form A isolated from a standard free basing process

Sticking Analysis

The MASS (Material Adhesion Screen for Sticking) Punch was developed in-house to quantitatively assess the sticking propensity of tablet formulations by weighing the amount of adhered powder on removable punch tip after a series of compressions. This test enables formulators to objectively and quickly evaluate the risk of punch sticking during drug product development and troubleshoot sticking observed during clinical tablet manufacturing.

To prepare the sample for MASS Punch testing, 10 g of API was diluted in a lightly lubricated standard blend (10% API, 89.75% Avicel PH102 and 0.25% magnesium stearate) and bottle blended (500mL amber glass bottle) for 500 rotations. The weight of powder adhered to the removable punch tip (1/2" round flat faced) was assessed using a microbalance periodically up to 100 compressions of ~250 mgW tablets at a target solid fraction of 0.85.

The MASS Punch profile for compound 1 free base mixed in the standard blend showed a positive response. Photos of the punch tips at the end of the compression runs confirmed that powder adhered to the tips (data not shown). For reference, a control sample of the standard blend is not sticky and would have less than 10 μg powdered adhered. The test method was found to rank the sticking propensity of new API lots relative to those of known materials.

Specific Surface Area (SSA) Measurement (BET Nitrogen)

Apparatus

Specific Surface Area (SSA) measurement (BET Nitrogen) were determined using a Micromeritics TriStar II 3020 specific surface area analyser together with Micromeritics SmartPrep station (Micromeritics U.K. Ltd., Ste 2, The Stables Hexton Manor, Hexton, Hertfordshire SG5 3JH, England). Samples were subjected to the BET-nitrogen adsorption analysis to determine the specific surface area of the samples.

Setup

Software version: TriStar II Confirm (1.03 or equivalent)
Adsorbate: Nitrogen
Sample tube: 3/8" mm flat bottom cell with glass filler rods
Sample masses*: Approximately ¾ full cell
Sample preparation: SmartPrep (Flow degassing using nitrogen)
Out gassing conditions: 16 hrs at 25 °C under gas flow (ramping at 10°C/min)
Isothermal jacket: Used
Isothermal collection points: 11 point BET in the range 0.05-0.30 P/Po
Isothermal data analysis range: 7 point BET in the range 0.05-0.20 P/Po
Leak test: 120s

Free space: Measured

Evacuation time: 1hr

Outgas test duration: 180s

Equilibration interval: 10s

Equilibration timeout: 600s

*The mass of sample varies according to the particle size of the test sample. For samples where the particle size is relatively small, approximately 0.50g of material was required to ¾ fill the cell bulb, and where the particle size of the sample is relatively large, 0.75g of material was required to ¾ fill the cell bulb.

Calculations and Reporting

The specific surface area was reported in the range 0.05-0.20 P/Po using 7 point BET from a triplicate determination. The sample mass, specific surface area, BET constant ('C' value) and correlation coefficient for each replicate were determined.

Results

Table 12 provides BET-N2 SSA for four batches of compound 1 free base API, one comprising the small primary particle size API prepared by the traditional salt break method (batch 5), and three batches comprising the large particle size API prepared according to the present invention. Batch 5 contained compound 1 free base having small primary particles and large agglomerates, which was very static-prone and sticky. Batch 6 was prepared using temperature cycling and had a typical particle size distribution (PSD) for the large particle size free base of compound 1, with a VMD of approximately 17 μm. Batch 7 demonstrated a similar PSD to batch 6. Batch 8 is a representative ICH batch of the large particle size free base of compound 1, also prepared by temperature cycling. The same batches were used in the surface energy determinations below.

Table 12: BET SSA by N_2

Batch No.	BET SSA by N_2
5	6.6
6	0.62
7	0.69
8	0.67

Inverse-phase Gas Chromatography (IGC) Surface Energy measurement:

A sufficient quantity of sample was packed into a silanised glass column with the powder mass secured within the column by glass wool plugs inserted at both ends. The column was conditioned by flowing a stream of dry nitrogen through the powder mass for sufficient time for any surface adsorbates to be removed. Measurements were made by injecting a series of

alkane vapour probes (Nonane, Octane, Heptane and Hexane) into the carrier gas stream at concentrations low enough to assume infinite dilution of the alkane vapour in the nitrogen stream and recording the time taken for each vapour to elute through the column. A plot of the retention time (corrected for the 'dead volume' of interstitial space within the packed column) versus a function of the cross sectional area and surface tension of the alkane vapour probe molecules used yields a line with a slope indicative of the surface energy of the solid powder under examination.

Results

Table 13 provides Dispersive Surface Energy (mJ/m^2) data generated for the four batches of compound **1** free base, i.e., batches 5-8, described above with respect to the SSA data. Batch 5 is the small particle size free base, and batches 6-8 include the large particle size of the free base API.

Table 13: Dispersive Surface Energy (mJ/m^2)

Batch No.	Dispersive Surface Energy (mJ/m^2)
5	61.63
6	49.42
7	35.75
8	42.27

All publications and patent applications cited in this specification and all references cited therein are herein incorporated by reference as if each individual publication or patent application or reference were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Claims

We Claim:

1. A crystalline free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, having a specific surface area of ≤ 2 m^2/g.

2. The free base of claim 1, having a specific surface area of ≤ 1 m^2/g.

3. The free base of claim 1 or 2, wherein the crystalline free base is a polymorph Form A of the free base.

4. The free base of claim 3, having a powder X-ray diffraction pattern comprising a peak at diffraction angle (2θ) of 10.1 ± 0.2.

5. The free base of claim 3, having a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2 and 10.1 ± 0.2.

6. The free base of claim 3, having a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, and 11.5 ± 0.2.

7. The free base of claim 3, having a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 8.0 ± 0.2, 10.1 ± 0.2, 10.3 ± 0.2, and 11.5 ± 0.2.

8. The free base of claim 3, having a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 1.

9. The free base of any one of claims 1 to 8, having a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: 12.5 ppm ± 0.2 ppm.

10. The free base of claim 9, having a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: 12.5 ppm and 112.4 ppm ± 0.2 ppm.

11. The free base of claim 9 or 10, having a ^{13}C solid state NMR spectrum comprising the following resonance (ppm) values: 12.5 ppm, 112.4 ppm and 143.2 ppm ± 0.2 ppm.

12. The free base of any one of claims 1 to 8, having a primary particle size of from about 5 μm to about 150 μm.

13. The free base of any one of claims 1 to 12, having a primary particle size distribution characterized by: (i) a D10 value of from about 5 μm to about 10 μm; (ii) a D90 value of from about 30 μm to about 125 μm; or (iii) a D50 value of from about 10 μm to about 45 μm; or a combination of (i), (ii) and (iii).

14. The free base of any one of claims 1 to 13, having a primary particle size distribution ratio of (D90-D10)/D50 of from about 2 to about 3.

15. A pharmaceutical composition comprising the free base of any one of claims 1 to 14, and at least one pharmaceutically acceptable carrier, diluent or excipient.

16. A capsule comprising the pharmaceutical composition of claim 15, containing from 0.1 to 200 mg of the polymorph Form A of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base.

17. A method of treating cancer, comprising administering to a human in need of such treatment a therapeutically effective amount of the pharmaceutical composition of claim 15.

18. A method of making the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having a specific surface area of ≤ 2 m^2/g, comprising:

(a) suspending 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base in mixture of n-butanol and anisole and heating to about 95-100°C to achieve dissolution;

(b) cooling to about 80 °C and providing seed crystals of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base (Form A);

(c) maintaining the mixture at about 80°C for about 3 hours and then gradually cooling to about 10°C to achieve crystallization; and

(d) filtering to isolate the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having a specific surface area of ≤ 2 m^2/g.

19. A method of making the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having a specific surface area of ≤ 2 m^2/g, comprising:

(a) suspending 4-{6-[6-(1-butoxyl-vinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido-[2,3-*d*]pyrimidin-2-ylamino]-pyridin-3-yl}-piperazine-1-carboxylic acid *tert*-butyl ester in a mixture of water and n-butanol and heating to about 70°C to achieve dissolution;

(b) addition of concentrated HCl and heating at about 70°C for 4-6 hrs;

(c) addition of anisole and aqueous NaOH to achieve a biphasic mixture having a pH of >10;

(d) separation of the layers and heating the organic layer to about 120°C to distill off water;

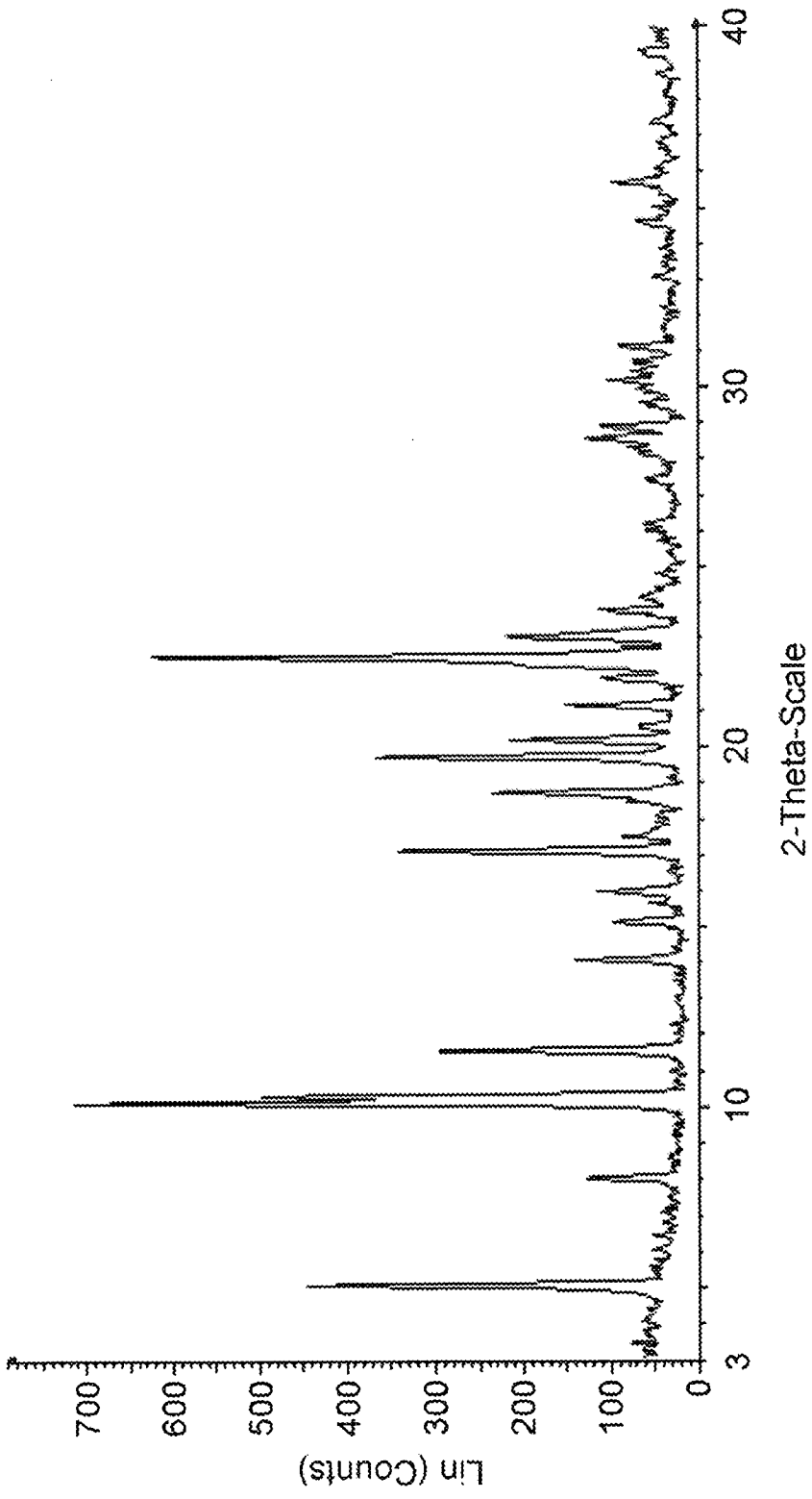
(e) cooling to about 80 °C and providing seed crystals of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one free base (Form A);

(g) maintaining the mixture at about 80°C for about 3 hours and then gradually cooling to about 10°C to achieve crystallization; and

(g) filtering to isolate the free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) having a specific surface area of ≤ 2 m^2/g.

20. The free base of 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (Form A) prepared according to the method of claim 18 or 19.

FIG. 1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600
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3
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20
30
40
2-Theta-Scale

FIG. 2

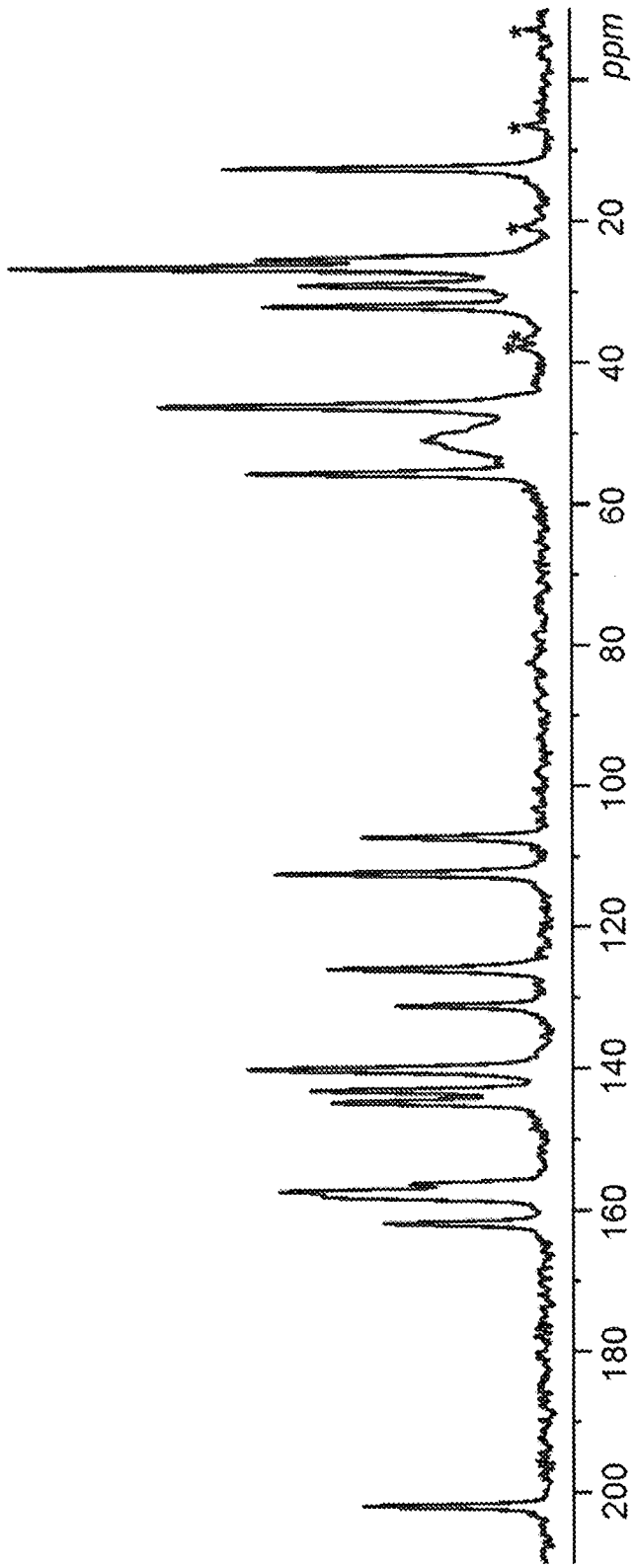
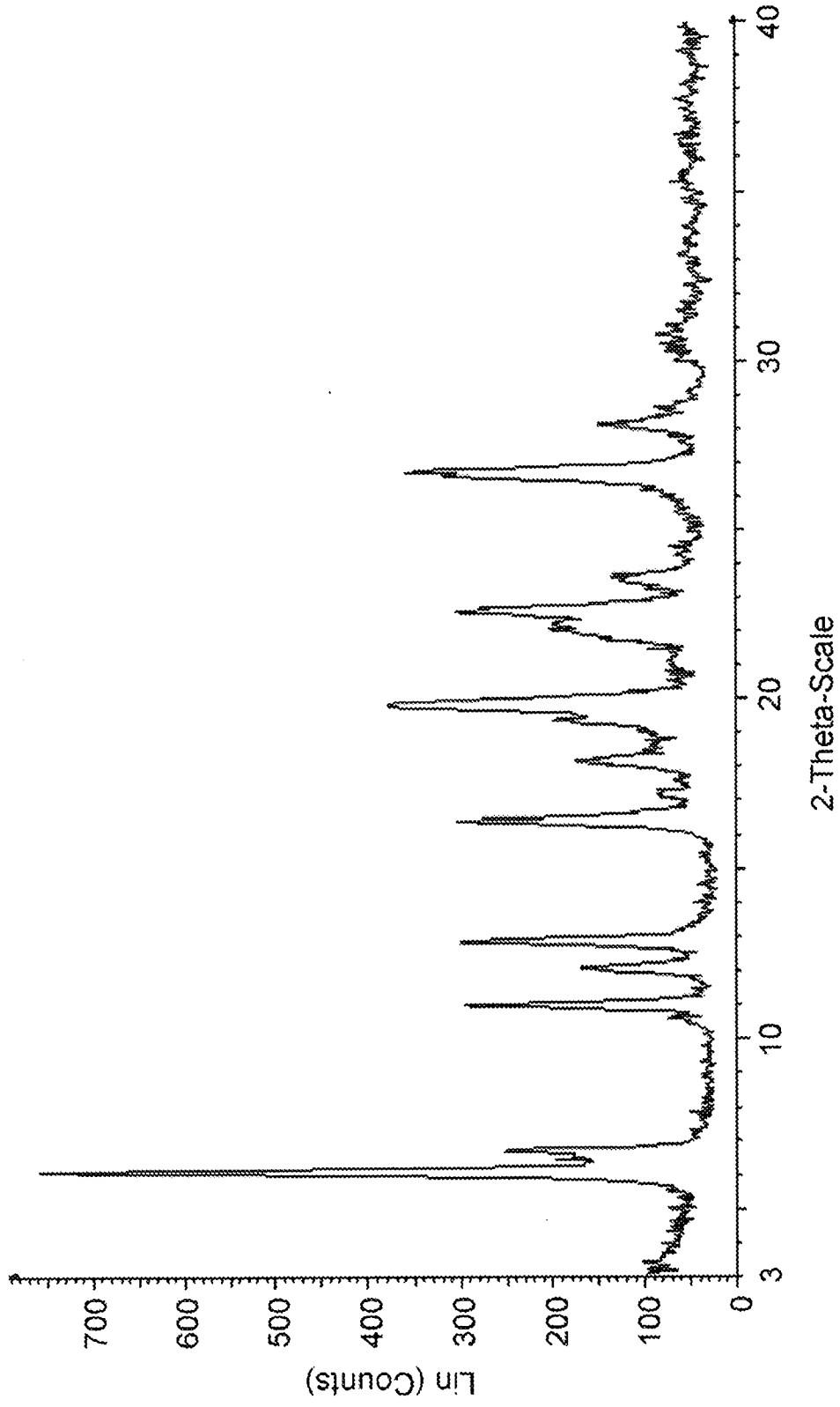


FIG. 3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2-Theta-Scale

FIG. 4

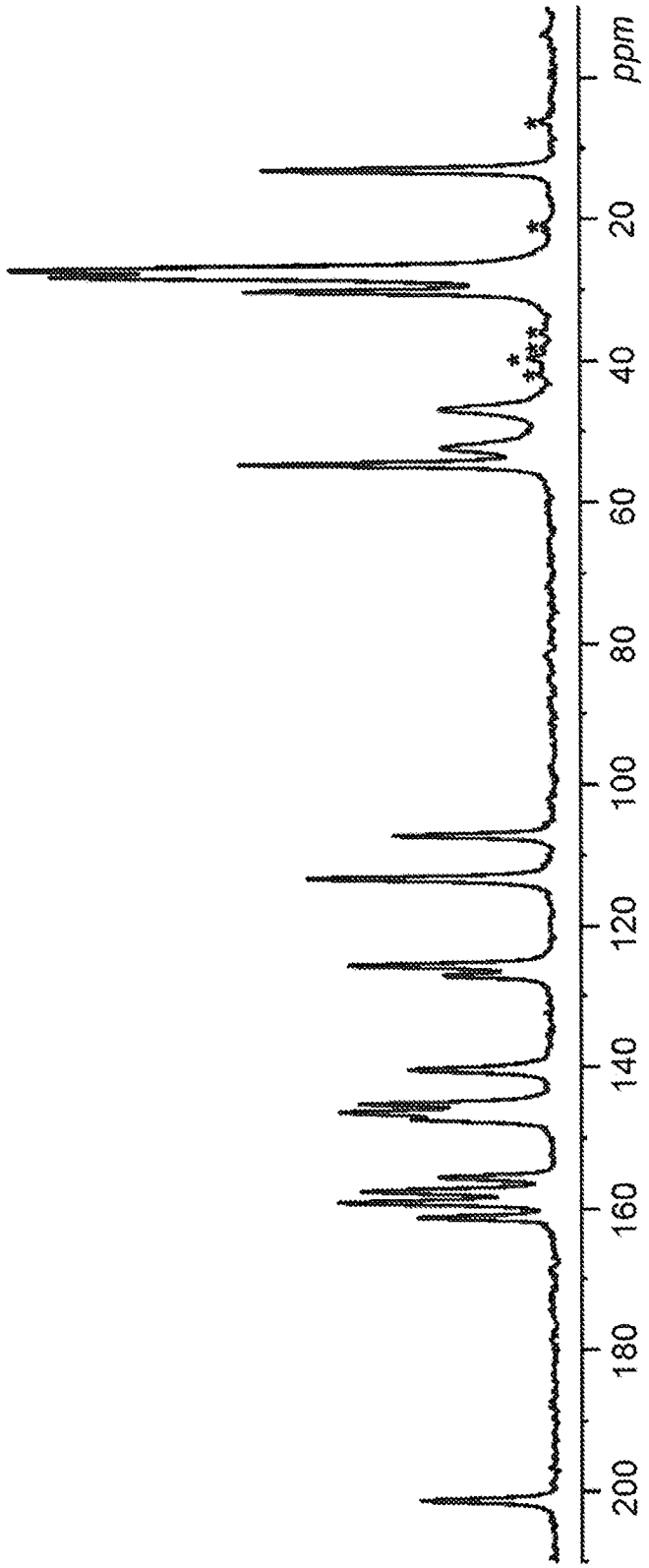


FIG. 5

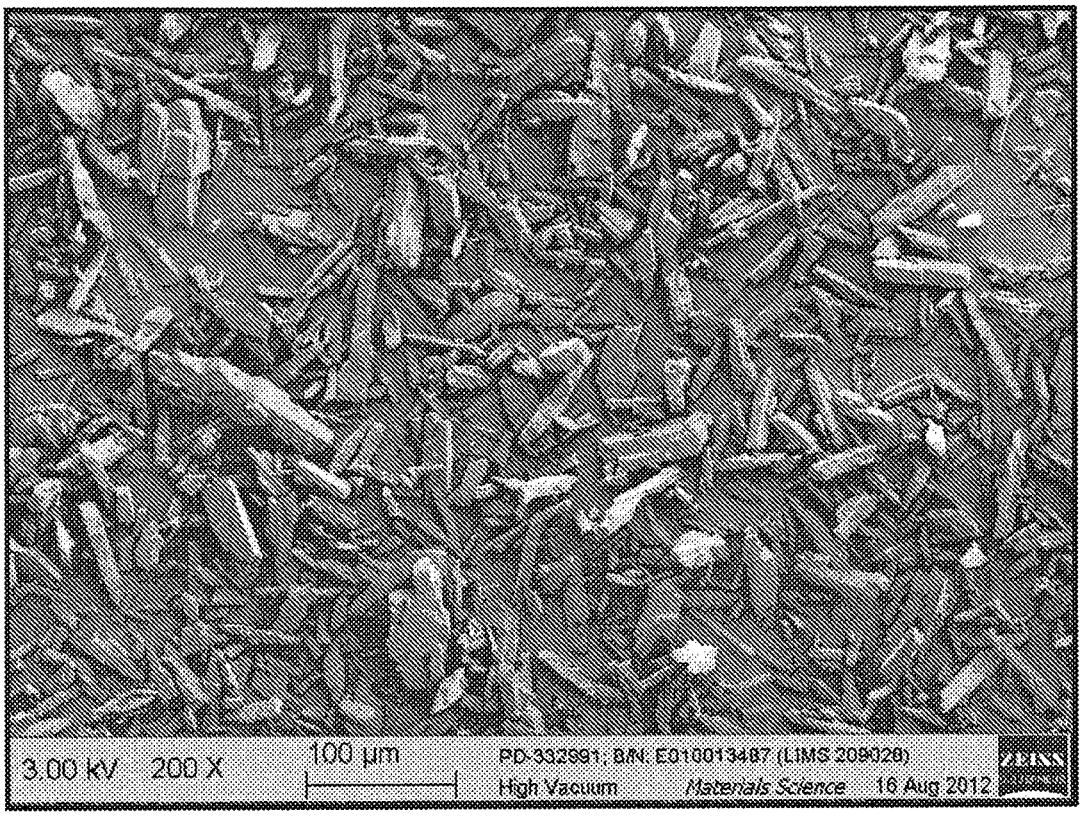

3.00 kV 200 X
100 µm
PD-332991; B/N: E0100134B7 (LIMS 209328)
High Vacuum
Materials Science
16 Aug 2012
ZEISS

FIG. 6

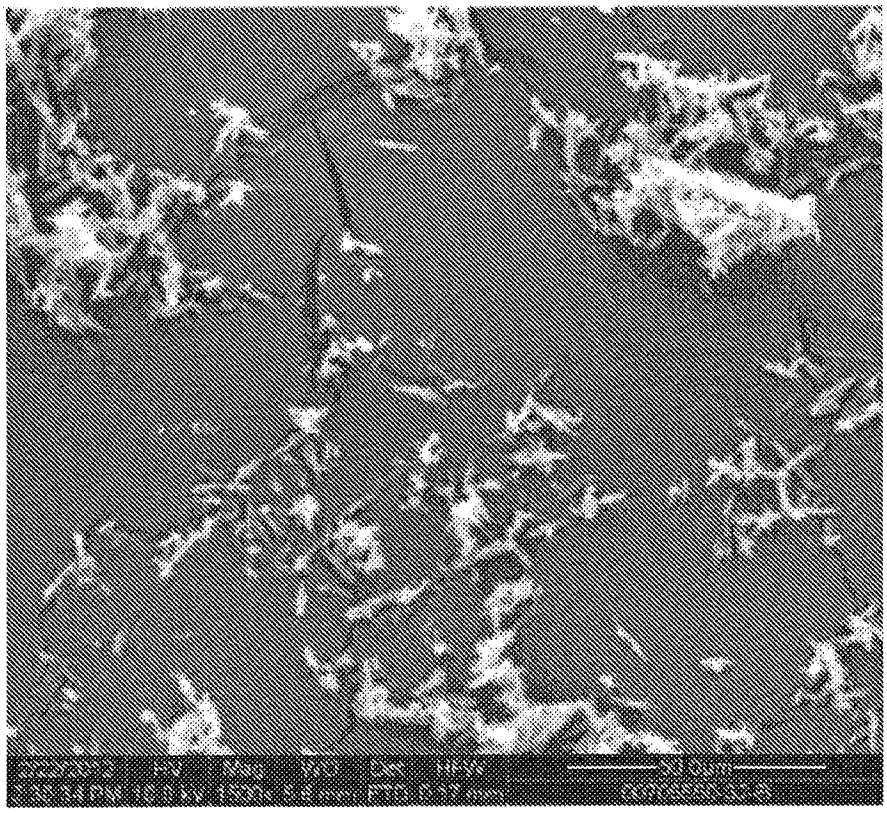


FIG. 7

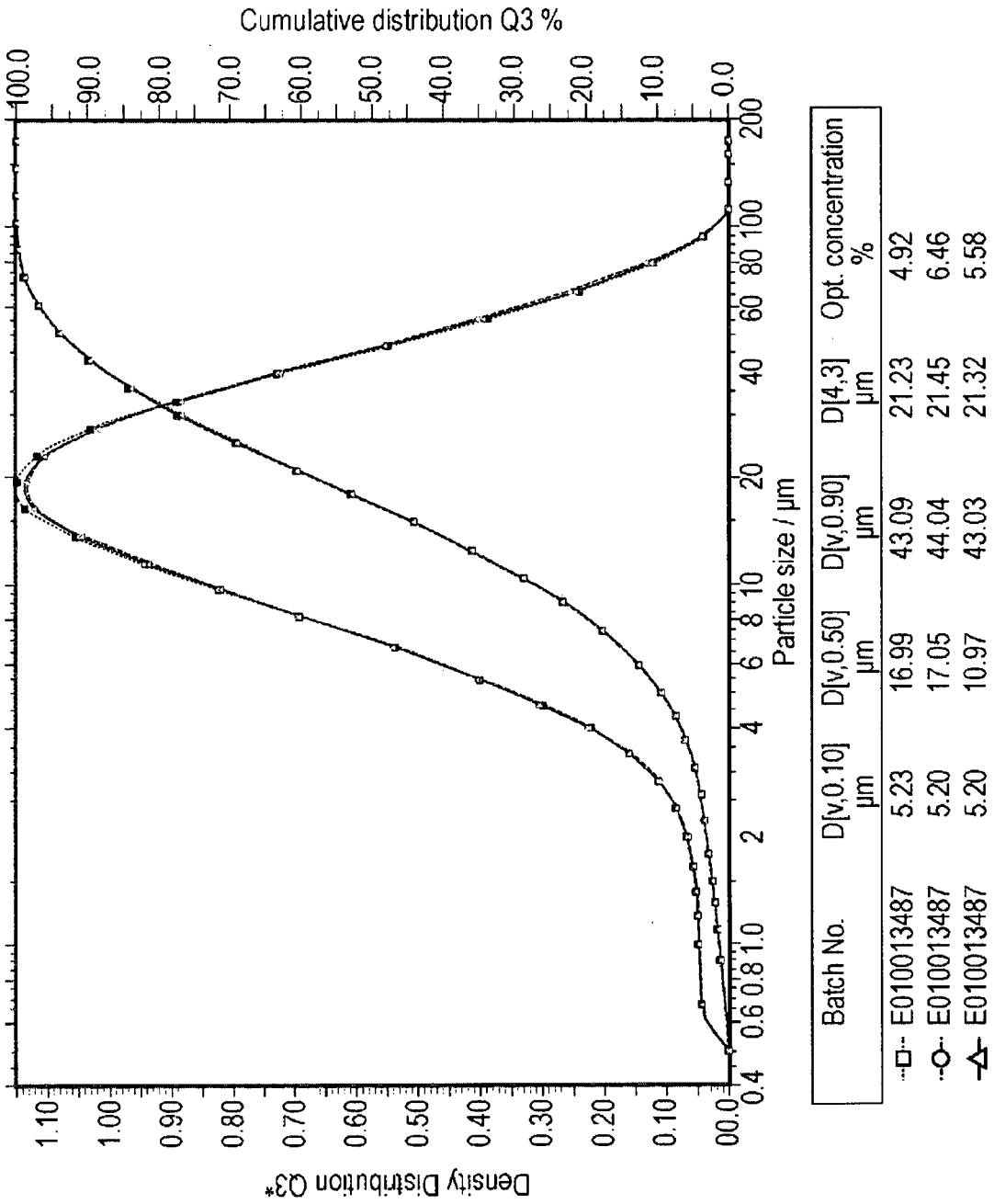
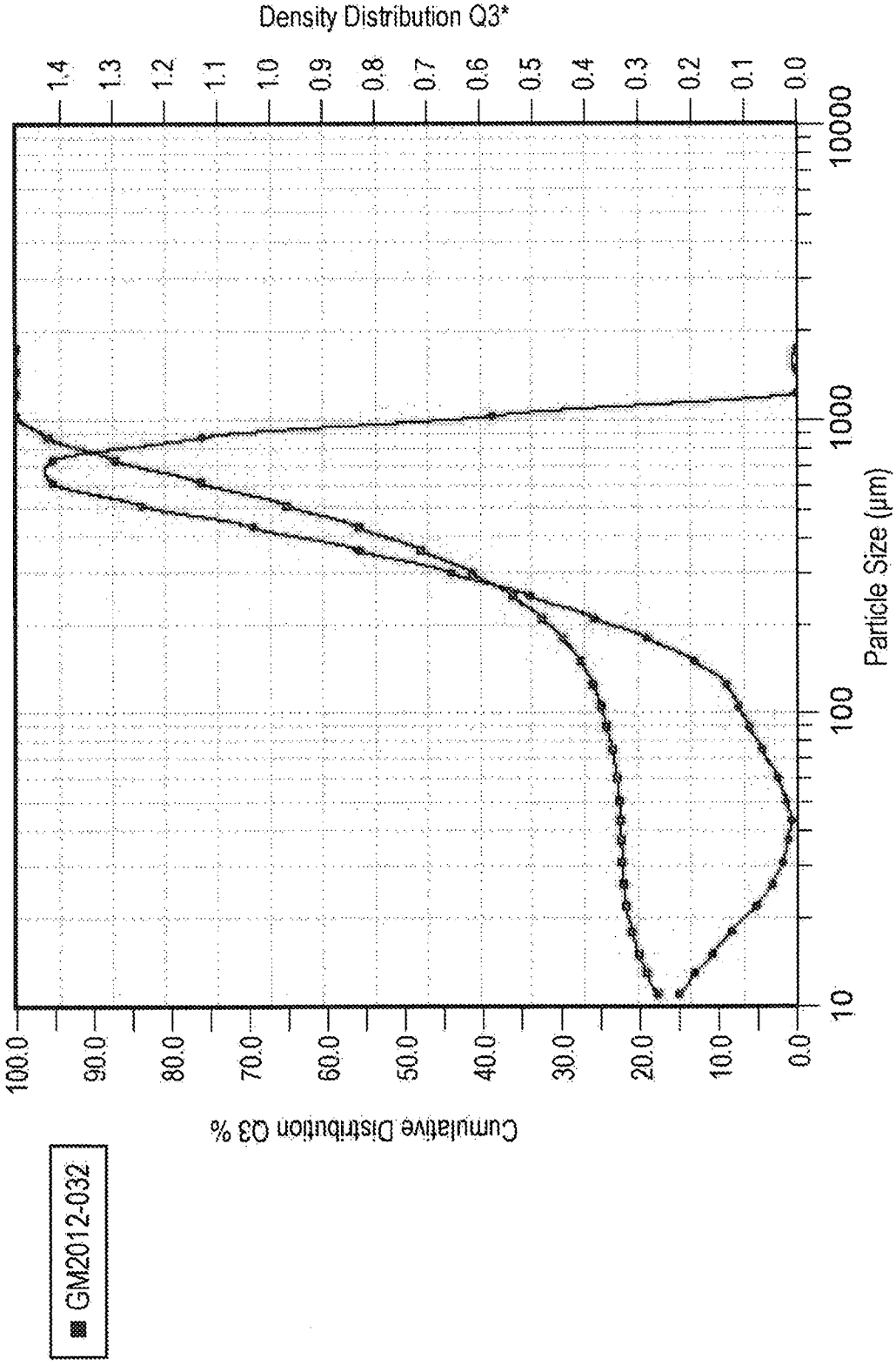

Cumulative distribution Q3 %
Density Distribution Q3*
Particle size / µm
Batch No. D[v,0.10] µm D[v,0.50] µm D[v,0.90] µm D[4,3] µm Opt. concentration %
E01013487 5.23 16.99 43.09 21.23 4.92
E01013487 5.20 17.05 44.04 21.45 6.46
E01013487 5.20 10.97 43.03 21.32 5.58

FIG. 8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0.6
0.5
0.4
0.3
0.2
0.1
0.0
10000
1000
100
10
Particle Size (µm)
100.0
90.0
80.0
70.0
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50.0
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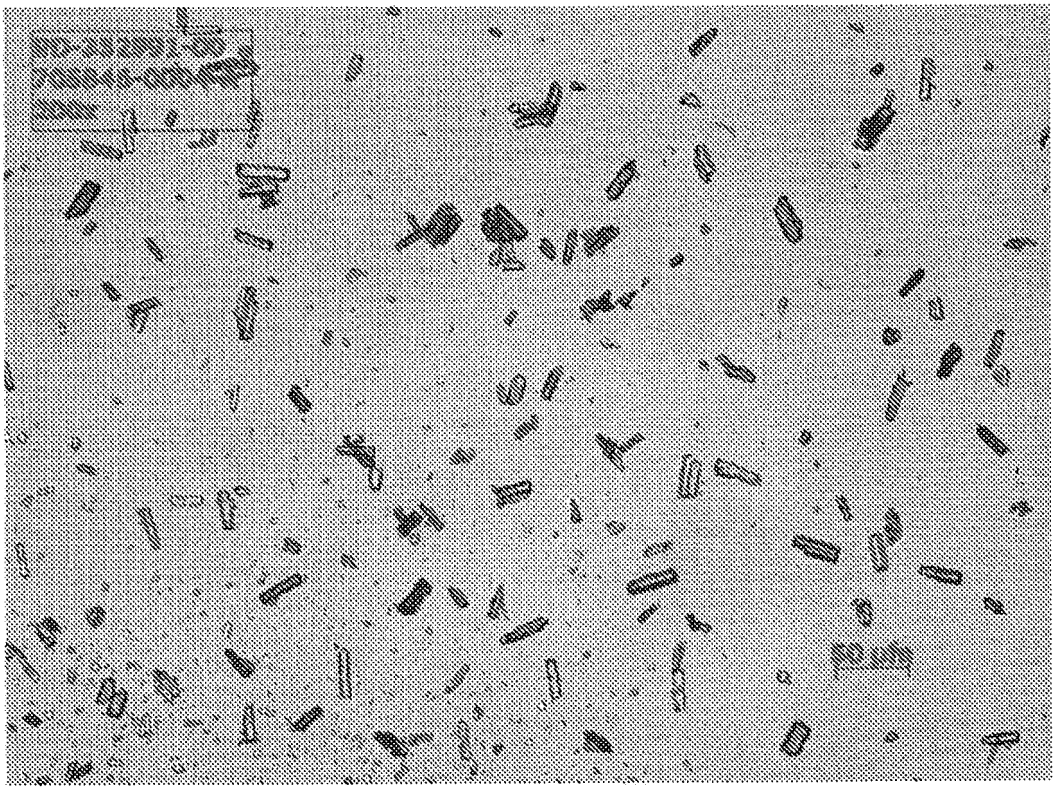
30.0
20.0
10.0
0.0
Cumulative Distribution Q3 %
GM2012-032

FIG. 9



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/058865

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D471/04 A61K31/519 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/005426 A1 (WARNER LAMBERT CO [US]; BEYLIN VLADIMIR GENUKH [US]; BLACKBURN ANTHONY) 20 January 2005 (2005-01-20) cited in the application page 22; example 4 -----	1-20

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 2014	22/04/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Fink, Dieter

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2014/058865

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005005426 A1	20-01-2005	AR 045898 A1	16-11-2005
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		UY 28404 A1	28-02-2005
		WO 2005005426 A1	20-01-2005
		ZA 200600230 A	25-04-2007

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(86)PCT国际申请的申请数据
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(71)申请人 辉瑞大药厂
地址 美国纽约州
(72)发明人 B·P·柴卡尔 N·D·伊德
(74)专利代理机构 中国国际贸易促进委员会专利商标事务所 11038
代理人 袁志明
(51)Int.Cl.
C07D 471/04(2006.01)
A61K 31/519(2006.01)
A61P 35/00(2006.01)

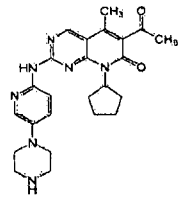
权利要求书2页 说明书38页 附图9页

(54)发明名称

选择性CDK4/6抑制剂的固态形式

(57)摘要

本发明涉及具有改善的性质的式(1)乙酰基-8-环戊基-5-甲基-2-(5-哌嗪-1-基-吡啶-2-基氨基)-8H-吡啶并[2,3-d]嘧啶-7-酮的结晶游离碱、包含该游离碱的药物组合物及剂型、以及制备及使用所述化合物、组合物及剂型用于治疗细胞增生疾病(诸如癌症)的方法。



(1)

CN 105008357 A

1. 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的结晶游离碱，其具有≤ 2 平方米 / 克的比表面积。

2. 权利要求 1 的游离碱，其具有≤ 1 平方米 / 克的比表面积。

3. 权利要求 1 或 2 的游离碱，其中该结晶游离碱为该游离碱的 A 型多晶型。

4. 权利要求 3 的游离碱，其具有包含在衍射角 (2θ)10. 1±0. 2 的峰的粉末 X 射线衍射图案。

5. 权利要求 3 的游离碱，其具有包含在衍射角 (2θ)8. 0±0. 2 及 10. 1±0. 2 的峰的粉末 X 射线衍射图案。

6. 权利要求 3 的游离碱，其具有包含在衍射角 (2θ)8. 0±0. 2、10. 1±0. 2 及 11. 5±0. 2 的峰的粉末 X 射线衍射图案。

7. 权利要求 3 的游离碱，其具有包含在衍射角 (2θ)8. 0±0. 2、10. 1±0. 2、10. 3±0. 2 及 11. 5±0. 2 的峰的粉末 X 射线衍射图案。

8. 权利要求 3 的游离碱，其具有包含在实质上与图 1 中所示相同的衍射角 (2θ) 的峰的粉末 X 射线衍射图案。

9. 权利要求 1 ～ 8 任一项的游离碱，其具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :12. 5ppm±0. 2ppm。

10. 权利要求 9 的游离碱，其具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :12. 5ppm 及 112. 4ppm±0. 2ppm。

11. 权利要求 9 或 10 的游离碱，其具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 : 12. 5ppm、112. 4ppm 及 143. 2ppm±0. 2ppm。

12. 权利要求 1 ～ 8 任一项的游离碱，其具有约 5 微米至约 150 微米的原生粒径。

13. 权利要求 1 ～ 12 任一项的游离碱，其具有特征如下所示的原生粒径分布 :(i) 约 5 微米至约 10 微米的 D10 值 ;(ii) 约 30 微米至约 125 微米的 D90 值 ;或 (iii) 约 10 微米至约 45 微米的 D50 值 ;或 (i)、(ii) 及 (iii) 的组合。

14. 权利要求 1 ～ 13 任一项的游离碱，其具有 (D90-D10)/D50 为约 2 至约 3 的原生粒径分布比。

15. 一种药物组合物，其包含权利要求 1 ～ 14 任一项的游离碱及至少一种药学上可接受的载体、稀释剂或赋形剂。

16. 一种胶囊，其包含权利要求 15 的药物组合物，其含有 0. 1 至 200 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮游离碱的 A 型多晶型。

17. 一种治疗癌症的方法，其包括给予需要该治疗的人治疗有效量的权利要求 15 的药物组合物。

18. 一种制备具有≤ 2 平方米 / 克的比表面积的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的游离碱 (A 型) 的方法，其包括 :

(a) 将 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮游离碱悬浮于正丁醇及茴香醚的混合物中，并加热至约 95 ～ 100℃以获得溶解 ;

(b) 冷却至约 80℃并提供 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱 (A 型) 的晶种 ;

(c) 将所述混合物维持在约 80℃约 3 小时,然后逐渐冷却至约 10℃以获得结晶 ;以及

(d) 过滤以分离具有≤ 2 平方米 / 克的比表面积的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型)。

19. 一种制备具有≤ 2 平方米 / 克的比表面积的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型) 的方法,其包括 :

(a) 将 4-{6-[6-(1- 丁氧基 - 乙烯基)-8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基]- 吡啶 -3- 基 } 哌嗪 -1- 甲酸叔丁酯悬浮于水及正丁醇的混合物中,并加热至约 70℃以获得溶解 ;

(b) 添加浓 HCl 并在约 70℃加热达 4 ～ 6 小时 ;

(c) 添加茴香醚及 NaOH 水溶液以获得 pH 为 >10 的双相混合物 ;

(d) 分离层并加热该有机层至约 120℃以馏出水 ;

(e) 冷却至约 80℃并提供 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱 (A 型) 的晶种 ;

(g) 在约 80℃将所述混合物维持约 3 小时,然后逐渐冷却至约 10℃以获得结晶 ;以及

(g) 过滤以分离具有≤ 2 平方米 / 克的比表面积的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型)。

20. 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型),其是根据权利要求 18 或 19 的方法制备的。

选择性 CDK4/6 抑制剂的固态形式

[0001] 相关申请的交叉引用

[0002] 本申请主张 2013 年 2 月 21 日提交的美国临时申请第 61/767,761 号的优先权利益，以引用的方式将该临时申请的全文并入本申请中。

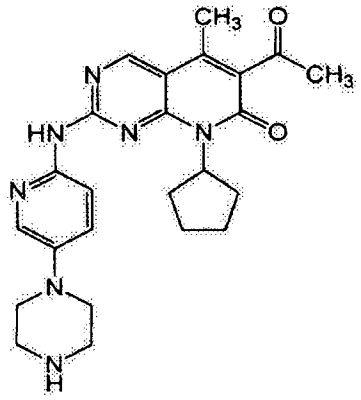
[0003] 本发明的领域

[0004] 本发明涉及具有改良的理化性质的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱。本发明还涉及包含该游离碱的药物组合物及剂型、以及制备所述化合物、组合物及剂型的方和使用所述化合物、组合物及剂型用于治疗细胞增生疾病（诸如癌症）的方法。

[0005] 本发明的背景

[0006] 化合物 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮（本申请中亦被称为“化合物 1”）可以下示结构表示：

[0007]



1

[0008] 且也被称为帕泊西力 (palbociclib) 或 PD-0332991。化合物 1 为 CDK4 及 CDK6 的有效且选择性抑制剂。

[0009] 化合物 1 及其药学上可接受的盐公开于国际专利公开文本第 WO 2003/062236 号及美国专利第 6,936,612 号、7,208,489 号及 7,456,168 号中，它们描述了化合物 1 的盐酸盐的制备。国际专利公开文本第 WO 2005/005426 号及美国专利第 7,345,171 号及 7,863,278 号描述了化合物 1 的游离碱及不同一酸及二酸加成盐的制备，包括羟乙磺酸盐的多晶型形式。制备化合物 1 的一羟乙磺酸盐的方法描述于国际专利公开文本第 WO 2008/032157 号及美国专利 7,781,583 号中。以全文引用方式将前述各参考文献的全部内容并入本申请中。

[0010] 虽然化合物 1 为有效且具选择性的 CDK4/CDK6 抑制剂，但其以游离碱形式的使用对药物开发显示出挑战。由传统盐断裂程序所提供的游离碱（例如在 WO 2005/005426 的实施例 4 中）非常容易产生静电且形成小的初级粒子，该初级粒子聚结成大的、硬的聚结物，该聚结物难以通过筛分分散且不适于进一步开发。本发明提供具有较大原生粒径的化合物

1 游离碱，其证实有改良的理化性质及制备性质。

[0011] 本发明概述

[0012] 化合物 1，即 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱可以一种或多种多晶型形式存在，包括 A 型和 B 型，其中 A 型为更稳定的晶型。该游离碱可为无水的或可含有不同量的水或一种或多种溶剂。

[0013] 本发明提供化合物 1 的结晶游离碱，其具有比通过本领域中所述的传统盐断裂方法所提供的游离碱更大原生粒径、极大减少的比表面积及更低的表面能测量值。本申请中公开的大粒径化合物 1 游离碱可通过多种方法区分。

[0014] 本发明的多晶型及固体形式可通过粉末 X 射线衍射测定法 (PXRD)、固态 NMR(ssNMR)、差示扫描量热法 (DSC)、振动光谱法 (例如 IR 及拉曼光谱法)、偏振光显微术 (PLM)、扫描电子显微术 (SEM)、热载台光学显微术、电子晶体学、单晶 X 射线衍射法、定量分析、粒径分析 (PSA)(例如，粒径、粒径分布 (PSD) 及粒子形状)、比表面积 (SSA) 分析、表面能分析 (例如反气相色谱法或 IGC)、溶解度研究及溶出度研究或这些技术的组合来辨别。

[0015] 一方面，本发明提供具有≤ 2 平方米 / 克的比表面积的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱。在一些实施方案中，所述游离碱具有≤ 1 平方米 / 克的比表面积。

[0016] 在优选的实施方案中，化合物 1 的结晶游离碱为该游离碱的 A 型多晶型。在一些这样的实施方案中，所述结晶游离碱具有包含在衍射角 (2θ)10.1±0.2 的峰的 PXRD 图案。在其它这样的实施方案中，所述结晶游离碱具有包含在衍射角 (2θ)8.0±0.2 及 10.1±0.2 的峰的 PXRD 图案。在再其它实施方案中，所述结晶游离碱具有包含在衍射角 (2θ)8.0±0.2、10.1±0.2 及 11.5±0.2 的峰的 PXRD 图案。在另外的实施方案中，所述结晶游离碱具有包含在衍射角 (2θ)8.0±0.2、10.1±0.2、10.3±0.2 及 11.5±0.2 的峰的 PXRD 图案。在另外的实施方案中，所述结晶游离碱具有包含在与图 1 中所示实质上相同的衍射角 (2θ) 的峰的 PXRD 图案。

[0017] 在一些实施方案中，化合物 1 的结晶游离碱 (A 型) 具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR(ssNMR) 光谱 :12.5ppm±0.2ppm。在其它实施方案中，所述结晶游离碱具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :12.5ppm 及 112.4ppm±0.2ppm。在另外的实施方案中，所述结晶游离碱具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :或 12.5ppm、112.4ppm 及 143.2ppm±0.2ppm。

[0018] 在本申请中所述的一些实施方案中，通过粒径分析辨别本发明的化合物 1 游离碱。在一些这样的实施方案中，所述结晶游离碱具有约 5 微米至约 150 微米，优选约 10 微米至约 100 微米，或更优选约 15 微米至约 80 微米的原生粒径。在其它这样的实施方案中，所述结晶游离碱具有特征如下所示的原生粒径分布 :(i) 约 5 微米至约 10 微米的 D10 值 ;(ii) 约 10 微米至约 45 微米的 D50 值 ;或 (iii) 约 30 微米至约 125 微米的 D90 值 ;或 (i)、(ii) 及 (iii) 的组合。在另外的实施方案中，所述结晶游离碱具有 (D90-D10)/D50 为约 2 至约 3 的原生粒径分布比。在另外的实施方案中，所述结晶游离碱具有约 15 微米至约 125 微米的体积平均直径 (D[4,3])。

[0019] 在一些实施方案中，化合物 1 的结晶游离碱为无水的。在其它实施方案中，化合物

1 的结晶游离碱为溶剂合物，特别是水合物。

[0020] 在其它方面，本发明提供一种包含根据本发明的具有大原生粒径的化合物 1 的结晶游离碱，以及药学上可接受的载体、稀释剂或赋形剂的药物组合物。经常地，该药物组合物包含所述游离碱的 A 型多晶型。

[0021] 本发明另外提供包含本发明的所述药物组合物的胶囊。在一些这样的实施方案中，所述胶囊包含 0.1 至 200 毫克及优选 25 至 150 毫克的化合物 1 游离碱（优选 A 型多晶型），其具有如本申请中所述的大原生粒径。

[0022] 在其它方面，本发明提供一种治疗哺乳动物（优选人类）的癌症的方法，其包括对所述哺乳动物给予治疗有效量的本发明药物组合物。所述治疗方法可以另外包括与一种或多种额外治疗剂联合给予化合物 1。

[0023] 在其它方面，本发明提供用于制备如本申请中所述的具有大原生粒径的化合物 1 游离碱的方法。一种方法包括将小粒径的化合物 1 游离碱溶解于第一溶剂与第二溶剂的混合物中并加热以获得溶解，冷却至适当温度，提供化合物 1 游离碱（A 型）的晶种，接着结晶以提供大粒径的化合物 1 游离碱。该方法中所使用的小粒径游离碱可从传统盐断裂程序，例如通过中间体乙烯基醚的酸水解分离，以提供酸加成盐，接着碱化，如实施例 5 中所述。另一种方法包括中间体乙烯基醚于水与第一溶剂的混合物中的酸水解，其可能需要加热以获得溶解，添加第二溶剂及碱化以提供包含在原位产生的游离碱的第二混合物，视需要加热以获得溶解并馏出水，并在适当温度下提供化合物 1 游离碱（A 型）的晶种，然后结晶以提供具有大原生粒径的化合物 1 游离碱。本发明另外提供通过这些方法制备的化合物 1 游离碱，其具有本申请中所述的性质。

[0024] 在上述各方法中，所述第一溶剂为醇且所述第二溶剂为芳族溶剂。适用的醇包括但不局限于相对高沸点的醇，诸如正丁醇、叔丁醇、正丙醇、戊醇、1,4- 丁二醇或丙二醇等。适用的芳族溶剂包括但不局限于茴香醚、1,3,5- 三甲苯、间二甲苯、氯苯、吡啶等。为了提高收率，所述方法可包括加热或冷却至高于或低于室温的温度。通常，所述反应混合物可被加热至约 30℃至约 150℃的温度，更通常为约 50℃至约 120℃的温度以获得溶解。在结晶期间，可能需要将所述反应混合物冷却至室温或低于室温的温度，例如介于约 0℃与约 30℃之间，优选约 5℃、约 10℃、约 15℃或约 20℃。

[0025] 这些及其它方面和实施方案通过本申请中所提供的详细描述进一步说明。本申请中所述的各实施方案可与本申请中所述的未与其所结合的实施方案抵触的任何其它实施方案结合。

[0026] 附图简要说明

[0027] 图 1 显示化合物 1 游离碱（A 型多晶型）的 PXRD 图案。

[0028] 图 2 显示化合物 1 游离碱（A 型多晶型）的碳 CPMAS 光谱。星号所标记的峰为旋转边带。

[0029] 图 3 显示化合物 1 游离碱（B 型多晶型）的 PXRD 图案。

[0030] 图 4 显示化合物 1 游离碱（B 型多晶型）的碳 CPMAS 光谱。星号所标记的峰为旋转边带。

[0031] 图 5 显示化合物 1 游离碱 API（A 型多晶型，从 40% n-BuOH/ 茴香醚再结晶）的扫描电子显微术（200 倍放大倍率）图像。

[0032] 图 6 显示化合物 1 游离碱 API(A 型多晶型，从标准游离碱化程序分离）的扫描电子显微术（1500 倍放大倍率）图像。

[0033] 图 7 显示化合物 1 游离碱 API(A 型多晶型，从 40% n-BuOH/ 茴香醚再结晶）的粒径分布。

[0034] 图 8 显示化合物 1 游离碱 API(A 型多晶型，从标准游离碱化程序分离）的粒径分布。

[0035] 图 9 显示化合物 1 游离碱 API(A 型多晶型，从 40% n-BuOH/ 茴香醚再结晶）的偏振光显微术 (PLM)(200 倍）图像。

[0036] 本发明的详细说明

[0037] 参考以下详细说明及本申请中所包括的实施例可更容易理解本发明。应理解此处所使用的术语仅为了说明特定实施方案，并非想限制。另外应理解，除非本申请中特别界定，否则本申请中所使用的术语应被给予其在相关技术领域中已知的传统意义。

[0038] 如本申请中所使用，除非另外指示，否则单数形“a”、“an”及“the”包括复数指代。例如，“a”取代基包括一个或多个取代基。

[0039] 如本申请中所使用的，术语“约”意指在某一值（诸如所述的浓度范围、时段、分子量、粒径、温度或 pH) 的统计学意义范围内。此范围可在一数量级内，通常在所指示值或范围的 20%内，更常在 10%内，及更常在 5%内。有时候，这类范围可在用于测量和 / 或测定给定值或范围的标准方法的一般实验误差内。通过术语“约”包含的容许偏差将视所研究的特定系统而定，而且本领域普通技术人员可轻易地理解。无论何时在本申请中引用某一范围时，在该范围内的每个整数全部亦被视为本发明的实施方案。

[0040] 如本申请中所使用，除非另外指示，否则术语“异常细胞生长”是指不依赖于正常调节机制（例如失去接触抑制）的细胞生长。“异常细胞增生疾病”是特征为异常细胞生长的疾病，诸如癌症。

[0041] 术语“癌症”包括实体肿瘤及血液学恶性病二者。癌症包括但不局限于乳腺癌、卵巢癌、宫颈癌、子宫内膜癌、前列腺癌、睾丸癌、胰腺癌、食道癌、头颈癌、胃癌、膀胱癌、肺癌（例如，腺癌、NSCLC 及 SCLC)、骨癌（例如，骨肉瘤）、结肠癌、直肠癌、甲状腺癌、脑及中枢神经系统癌症、神经胶质母细胞瘤、神经母细胞瘤、神经内分泌癌、杆状癌、角质棘皮瘤、表皮样癌、精细胞瘤、黑色素瘤、肉瘤（例如，脂质肉瘤）、膀胱癌、肝癌（例如，肝细胞癌）、肾癌（例如，肾细胞癌）、骨髓样障碍（例如，AML、CML、骨髓发育不良综合征及前髓细胞性白血病）及淋巴样障碍（例如，白血病、多发性骨髓瘤、外膜细胞淋巴瘤、ALL、CLL、B 细胞淋巴瘤、T 细胞淋巴瘤、何杰金淋巴瘤、非何杰金淋巴瘤、毛细胞淋巴瘤）。

[0042] 短语“药学上可接受的”是指在合理医学判断范围内适于与患者的组织接触而无过度毒性、刺激、过敏反应等、相称地具有合理益处 / 风险比且对其所计划的用途有效的物质。

[0043] 如本申请中所使用，术语“哺乳动物”可为人类或非人类哺乳动物（例如，狗、猫、兔、大鼠、小鼠、马、猴、其它低等灵长类等）。优选地，所述哺乳动物是人类。

[0044] 如本申请中所使用，除非另外指示，否则术语“治疗”意指逆转、减轻、预防应用此术语的障碍或病况或该障碍或病况的一种或多种症状、或抑制所述障碍或病况的进程。如本申请中所使用，除非另外指示，否则术语“治疗”是指前文所定义的作为“治疗”的治疗动

作。

[0045] 如本申请中所使用的，“有效”量是指化合物、药剂、物质、制剂或组合物的量，该量具有足以造成疾病症状严重性降低、无疾病症状期间的频率及持续期间增加或防止因疾病侵扰所致的损伤或失能的量。该量可为单一剂量或根据多剂量方案，单独或与其它化合物、药剂或物质组合。本领域普通技术人员能根据诸如受治疗者的身材、受治疗者的症状的严重性及所选择的特定组合物或给药途径来确定所述量。

[0046] 如本申请中所使用的，“单位剂型”是指适于被治疗的对象的发明制剂的物理离散单元。然而，将理解本发明的组合物的总每日使用将由主治医生在合理医学判断范围内决定。对于任何特定受治疗者的特定有效剂量水平将取决于许多因素，包括被治疗的障碍及该障碍的严重性；所使用的特定组合物；受治疗者的年龄、体重、一般健康状况、性别及饮食；给药时间、治疗持续期间；与本发明组合物组合或同时使用的药物和/或另外疗法、以及医疗领域中为人熟知的类似因素。

[0047] 如本申请中所使用，关于X射线衍射峰位置的术语“实质上相同”意指考虑典型峰位置及强度变异性。例如，本领域技术人员将理解，峰位置（2θ）会显示一些仪器间的变异性，通常多达0.2°或0.1°。此外，本领域技术人员将理解，相对峰强度将显示仪器间的变异性以及因结晶性的程度，优选定向、所制备的样品表面及本领域技术人员已知的其它因素所致的变异性，且应被仅视为定性度量。

[0048] 本申请中所使用的，术语“溶剂合物”是指含有溶剂的物质的晶体形式。术语“水合物”指所述溶剂为水的溶剂合物。

[0049] 如本申请中所使用的，术语“加晶种”意指为引发或加强成核或用作进一步结晶的基材的目的将晶体添加至结晶系统。

[0050] 如本申请中所使用的，术语“API”或“活性药物成分”是指6-乙酰基-8-环戊基-5-甲基-2-(5-哌嗪-1-基-吡啶-2-基氨基)-8H-吡啶并[2,3-d]嘧啶-7-酮的游离碱。

[0051] 如本申请中所使用的，术语“初级粒子”是指个体API晶体。

[0052] 如本申请中所使用的，术语“聚结物”是指难以在加工及粒径分析期间难以分散成初级粒子的紧密结合的API晶体。

[0053] 本发明提供具有比通过传统盐断裂方法所提供的游离碱更大的原生粒径、极大减少的比表面积及更低的表面能测量值的化合物1的游离碱。为方便起见，本发明所提供的化合物1游离碱在本申请中有时可被称为“大（原生）粒径”游离碱。这与经由传统盐断裂方法所制备的化合物1游离碱相反，该由传统盐断裂方法所制备的化合物1游离碱有时被称为“小（原生）粒径”游离碱。本领域技术人员将理解，在这种情况中提及“小粒径”是指个体API晶体的粒径，不考虑该“小”粒子形成大聚结物的倾向。

[0054] 在本申请中所述的一些实施方案中，化合物1的结晶游离碱通过比表面积(SSA)辨别。因此，一方面，本发明提供一种具有≤2平方米/克的比表面积(SSA)的6-乙酰基-8-环戊基-5-甲基-2-(5-哌嗪-1-基-吡啶-2-基氨基)-8H-吡啶并[2,3-d]嘧啶-7-酮的结晶游离碱。在一些实施方案中，所述游离碱具有≤1平方米/克的比表面积(SSA)。在其它实施方案中，所述化合物1游离碱具有≤0.9平方米/克、≤0.8平方米/克或≤0.7平方米/克的SSA。在另外的实施方案中，所述化合物1游离碱具有介于0.2平

方米 / 克与 2 平方米 / 克之间、介于 0.5 平方米 / 克与 1.5 平方米 / 克之间、或介于 0.5 平方米 / 克与 1 平方米 / 克之间的 SSA。

[0055] 在本申请中所述的一些实施方案中，化合物 1 的结晶游离碱通过分散表面能辨别。因此，一方面，本发明提供一种具有≤ 60mJ/m^2的分散表面能的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱。在一些实施方案中，所述游离碱具有≤ 55mJ/m^2、≤ 50mJ/m^2、≤ 45mJ/m^2或≤ 40mJ/m^2的分散表面能。在另外的实施方案中，化合物 1 游离碱具有介于 20mJ/m^2与 60mJ/m^2之间、介于 25mJ/m^2与 50mJ/m^2之间、或介于 30mJ/m^2与 50mJ/m^2之间的分散表面能。

[0056] 在优选的实施方案中，化合物 1 的结晶游离碱为该游离碱的 A 型多晶型。在一些这样的实施方案中，所述晶型具有包含在衍射角 (2θ)10.1±0.2 的峰的 PXRD 图案。在其它这样的实施方案中，所述晶型具有包含在衍射角 (2θ)8.0±0.2 及 10.1±0.2 的峰的 PXRD 图案。在再其它实施方案中，所述晶型具有包含在衍射角 (2θ)8.0±0.2、10.1±0.2 及 11.5±0.2 的峰的 PXRD 图案。在另外的实施方案中，所述晶型具有包含在衍射角 (2θ)8.0±0.2、10.1±0.2、10.3±0.2 及 11.5±0.2 的峰的 PXRD 图案。在其它实施方案中，所述晶型具有包含在衍射角 (2θ)5.1±0.2、8.0±0.2、10.1±0.2 及 11.5±0.2 的峰的 PXRD 图案。在另外的实施方案中，所述晶型具有包含在衍射角 (2θ)8.0±0.2、10.1±0.2、11.5±0.2 及 19.7±0.2 的峰的 PXRD 图案。在再另外的实施方案中，所述晶型具有包含在衍射角 (2θ)8.0±0.2、10.1±0.2、11.5±0.2 及 22.5±0.2 的峰的 PXRD 图案。在另外的实施方案中，所述晶型具有包含在与图 1 中所示实质上相同的衍射角 (2θ) 的峰的 PXRD 图案。

[0057] 在一些实施方案中，化合物 1 的结晶游离碱 (A 型) 具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :12.5ppm±0.2ppm。在其它实施方案中，所述晶型具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :12.5ppm 及 112.4ppm±0.2ppm。在另外的实施方案中，所述晶型具有包含以下共振 (ppm) 值的 ^{13}C 固态 NMR 光谱 :或 12.5ppm、112.4ppm 及 143.2ppm±0.2ppm。

[0058] 在本申请中所述的一些实施方案中，化合物 1 的结晶游离碱通过粒径分析来辨别。在一些这样的实施方案中，所述游离碱具有约 5 微米至约 150 微米，优选约 10 微米至约 100 微米，及更优选约 15 微米至约 80 微米的原生粒径。

[0059] 在其它这样的实施方案中，所述游离碱具有特征如下所示的原生粒径分布 :(i) 约 5 微米至约 10 微米的 D10 值 ;(ii) 约 10 微米至约 45 微米的 D50 值 ;或 (iii) 约 30 微米至约 125 微米的 D90 值 ;或 (i)、(ii) 及 (iii) 的组合。在另外的实施方案中，所述游离碱具有 (D90-D10)/D50 为约 2 至约 3 的原生粒径分布比。在另外的实施方案中，所述游离碱具有约 15 微米至约 125 微米的体积平均直径 (D[4,3])。

[0060] 一方面，本发明提供一种具有大于约 5 微米的原生粒径的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱。在一些实施方案中，所述游离碱具有大于约 7.5 微米的原生粒径。在其它实施方案中，所述游离碱具有大于约 10 微米的原生粒径。在其它这样的实施方案中，所述游离碱具有大于约 12.5 微米的原生粒径。在其它这样的实施方案中，所述游离碱具有大于约 15 微米的原生粒径。

[0061] 在其它方面，本发明提供一种具有约 5 微米至约 200 微米的原生粒径的 6- 乙酰

基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱。在一些实施方案中,所述游离碱具有以下原生粒径 :约 5 微米至约 175 微米 ;约 5 微米至约 150 微米 ;约 5 微米至约 125 微米 ;约 5 微米至约 100 微米 ;约 5 微米至约 75 微米 ;约 10 微米至约 200 微米 ;约 10 微米至约 175 微米 ;约 10 微米至约 150 微米 ;约 10 微米至约 125 微米 ;约 10 微米至约 100 微米 ;约 10 微米至约 75 微米 ;约 15 微米至约 200 微米 ;约 15 微米至约 175 微米 ;约 15 微米至约 150 微米 ;约 15 微米至约 125 微米 ;约 15 微米至约 100 微米 ;或约 15 微米至约 75 微米。

[0062] 在其它方面,本发明提供一种具有原生粒径分布的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱,所述原生粒径分布具有如下至少一种 :

[0063] (a) 约 5 微米至约 10 微米的 D10 值 ;

[0064] (b) 约 10 微米至约 45 微米的 D50 值 ;以及

[0065] (c) 约 30 微米至约 125 微米的 D90 值。

[0066] 在一些这样的实施方案中,所述游离碱具有约 5 微米至约 10 微米的 D10 值。在其它这样的实施方案中,所述游离碱具有约 30 微米至约 125 微米的 D90 值。在其它这样的实施方案中,所述游离碱具有约 10 微米至约 45 微米的 D50 值。在一些这样的实施方案中,所述游离碱具有约 5 微米至约 10 微米的 D10 值及约 30 微米至约 125 微米的 D90 值。在另外的实施方案中,所述游离碱具有约 5 微米至约 10 微米的 D10 值、约 30 微米至约 125 微米的 D90 值,及约 10 微米至约 45 微米的 D50 值。

[0067] 在其它方面,本发明提供一种具有原生粒径分布的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱,所述原生粒径分布具有如下至少一种 :

[0068] (d) 约 5 微米至约 10 微米的 D10 值 ;

[0069] (e) 约 10 微米至约 25 微米的 D50 值 ;以及

[0070] (f) 约 30 微米至约 75 微米的 D90 值。

[0071] 在一些这样的实施方案中,所述游离碱具有约 5 微米至约 10 微米的 D10 值。在其它这样的实施方案中,所述游离碱具有约 30 微米至约 75 微米的 D90 值。在其它这样的实施方案中,所述游离碱具有约 10 微米至约 25 微米的 D50 值。在一些这样的实施方案中,所述游离碱具有约 5 微米至约 10 微米的 D10 值及约 30 微米至约 75 微米的 D90 值。在另外的实施方案中,所述游离碱具有约 5 微米至约 10 微米的 D10 值、约 30 微米至约 755 微米的 D90 值,及约 10 微米至约 25 微米的 D50 值。

[0072] 在其它实施方案中,所述游离碱具有原生粒径分布,该原生粒径分布具有以下 D10 值 :约 5 微米至约 7.5 微米 ;约 5 微米至约 10 微米 ;约 5 微米至约 12.5 微米 ;或约 5 微米至约 15 微米。

[0073] 在其它实施方案中,所述游离碱具有原生粒径分布,该原生粒径分布具有以下 D50 值 :约 10 微米至约 50 微米 ;约 10 微米至约 45 微米 ;约 10 微米至约 40 微米 ;约 10 微米至约 35 微米 ;约 10 微米至约 30 微米 ;约 10 微米至约 25 微米 ;或约 10 微米至约 20 微米。

[0074] 在再其它实施方案中,所述游离碱具有原生粒径分布,该原生粒径分布具有以下 D90 值 :约 30 微米至约 175 微米 ;约 30 微米至约 160 微米 ;约 30 微米至约 150 微米 ;约 30

微米至约 140 微米；约 30 微米至约 130 微米；约 30 微米至约 125 微米；约 30 微米至约 120 微米；约 30 微米至约 115 微米；约 30 微米至约 110 微米；约 30 微米至约 100 微米；约 30 微米至约 75 微米；约 30 微米至约 70 微米；约 30 微米至约 65 微米；约 30 微米至约 60 微米；约 30 微米至约 55 微米；约 30 微米至约 50 微米；或约 30 微米至约 45 微米。

[0075] 关于 D10 的实施方案的上述值各自可与任何不与其抵触的 D50 和 / 或 D90 值组合。关于 D50 的实施方案的上述值各自可与任何不与其抵触的 D10 和 / 或 D90 值组合。关于 D90 的实施方案的上述值各自可与任何不与其抵触的 D10 和 / 或 D50 值组合。

[0076] 在其它方面，本发明提供一种具有约 2 至约 3 的 (D90-D10)/D50 的原生粒径分布比的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的结晶游离碱。在一些这样的实施方案中，所述游离碱具有约 5 微米至约 150 微米的原生粒径。

[0077] 在此方面的一些实施方案中，所述游离碱具有以下的 (D90-D10)/D50 原生粒径分布比：约 2 至约 2. 75；约 2 至约 2. 5；约 2 至约 2. 25。在其它实施方案中，所述比为约 2. 0、约 2. 1、约 2. 2、约 2. 3、约 2. 4、约 2. 5、约 2. 6、约 2. 7、约 2. 8、约 2. 9、或约 3. 0。

[0078] 在再另一方面，本发明提供具有约 15 微米至约 125 微米的体积平均直径 (D[4, 3]) 的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的结晶游离碱。在一些实施方案中，所述游离碱具有约 50 微米至约 100 微米的 D[4, 3]。在其它实施方案中，所述游离碱具有约 15 微米至约 30 微米的 D[4, 3]。

[0079] 在再其它实施方案中，所述游离碱具有以下的 D[4, 3]：约 15 微米至约 100 微米；约 15 微米至约 90 微米；约 15 微米至约 80 微米；约 15 微米至约 70 微米；约 15 微米至约 60 微米；约 15 微米至约 50 微米；约 15 微米至约 40 微米；约 25 微米至约 120 微米；约 25 微米至约 100 微米；约 25 微米至约 90 微米；约 25 微米至约 80 微米；约 25 微米至约 70 微米；约 25 微米至约 60 微米；约 25 微米至约 50 微米；约 25 微米至约 40 微米；约 25 微米；约 30 微米；约 35 微米；约 40 微米；约 45 微米；约 50 微米；约 55 微米；约 60 微米；约 65 微米；约 70 微米；约 75 微米；至约 80 微米；约 90 微米；约 100 微米；约 105 微米；约 110 微米；约 115 微米；或约 120 微米。

[0080] 在另一方面，本发明提供包含本发明游离碱及药学上可接受的载体、稀释剂或赋形剂的药物组合物。本发明另外提供包含本发明的所述药物组合物的胶囊。

[0081] 在一些实施方案中，所述胶囊包含 0. 1 至 200 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的 A 型多晶型。在其它实施方案中，所述胶囊包含 25 至 150 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的 A 型多晶型。在其它实施方案中，所述胶囊包含 50 至 150 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的 A 型多晶型。在其它实施方案中，所述胶囊包含 50 至 100 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的 A 型多晶型。在其它实施方案中，所述胶囊包含 75 至 150 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2, 3-d] 嘧啶 -7- 酮的 A 型多晶型。

[0082] 在另一方面，本发明提供一种治疗哺乳动物 (包括人类) 的癌症的方法，其包括对

所述哺乳动物给予治疗有效量的本发明药物组合物。在一些这样的实施方案中，所述药物组合物以胶囊形式给予。所述胶囊可包含 0.1 至 200 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱的 A 型多晶型。在其它实施方案中，所述胶囊可包含 25 至 150 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱的 A 型多晶型。在另外的实施方案中，所述胶囊可包含 50 至 150 毫克的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱的 A 型多晶型。

[0083] 用于表征根据本发明的化合物 1 的结晶游离碱的技术包括但不局限于粉末 X 射线衍射测定法 (PXRD)、固态 NMR(ssNMR)、差示扫描量热法 (DSC)、振动光谱法 (例如 IR 及拉曼光谱法)、偏振光显微术 (PLM)、扫描电子显微术 (SEM)、热载台光学显微术、电子晶体学、单晶 X 射线衍射法、定量分析、粒径分析 (PSA)(例如，粒径、粒径分布 (PSD) 及粒子形状)、比表面积 (SSA) 分析、表面能分析 (例如反相气相色谱法或 IGC)、通过溶解度研究及溶出度研究、或这些技术的组合。

[0084] 在其它方面，本发明提供用于制备如本申请中所述的具有大原生粒径的化合物 1 游离碱的方法。一种方法涉及将小粒径的化合物 1 游离碱溶解于第一溶剂与第二溶剂的混合物中并加热以获得溶解，冷却至适当温度，提供化合物 1 游离碱 (A 型) 的晶种，接着结晶以提供大粒径的化合物 1 游离碱。该方法中所使用的小粒径游离碱可从传统盐断裂程序，例如通过中间体乙烯基醚的酸水解分离，以提供酸加成盐，接着碱化，如实施例 5 中所述。

[0085] 在一个实施方案中，本发明提供一种制备大粒径 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型) 的方法，其包括 :(a) 将 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱悬浮于第一溶剂与第二溶剂的混合物中，并加热以获得溶解 ;(b) 冷却至适当温度并提供 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱 (A 型) 的晶种 ;(c) 将该混合物逐渐冷却以获得结晶 ;以及 (d) 分离该具有大粒径的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型)。

[0086] 在另一个实施方案中，本发明提供一种制备大粒径 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型) 的方法，其包括 :(a) 将 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱悬浮于正丁醇及茴香醚的混合物中，并加热至约 95 至 100℃以获得溶解 ;(b) 冷却至约 80℃并提供 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱 (A 型) 的晶种 ;(c) 将该混合物在约 80℃维持约 3 小时，然后逐渐冷却至约 10℃以获得结晶 ;以及 (d) 过滤以分离该具有大粒径的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱 (A 型)。

[0087] 另一种方法涉及中间体乙烯基醚于水与第一溶剂的混合物中的酸水解，其可能需要加热以获得溶解，添加第二溶剂及碱化以提供包含在原位产生的游离碱的第二混合物，

视需要加热以获得溶解及馏出水，冷却至适当温度，提供化合物 1 游离碱（A 型）的晶种，然后结晶以提供具有大原生粒径的化合物 1 游离碱。

[0088] 在一个实施方案中，本发明提供一种制备大粒径 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱（A 型）的方法，其包括：(a) 将 4-{6-[6-(1- 丁氧基 - 乙烯基)-8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基]- 吡啶 -3- 基 } 哌嗪 -1- 甲酸叔丁酯悬浮于水及第一溶剂的混合物中，并加热以获得溶解；(b) 添加酸及反应以在原位产生 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的酸加成盐；(c) 添加第二溶剂及碱水溶液至 pH 值大于或等于 10；(d) 将有机层分离并加热以馏出水；(e) 冷却至适当温度并提供 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱（A 型）的晶种；(f) 将该混合物逐渐冷却以获得结晶；以及 (g) 分离该具有大粒径的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱（A 型）。

[0089] 在另一个实施方案中，本发明提供一种制备大粒径 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱（A 型）的方法，其包括：(a) 将 4-{6-[6-(1- 丁氧基 - 乙烯基)-8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基]- 吡啶 -3- 基 } 哌嗪 -1- 甲酸叔丁酯悬浮于水及正丁醇的混合物中，并加热至约 70℃以获得溶解；(b) 添加浓 HCl 并在约 70℃加热 4 至 6 小时；(c) 添加茴香醚及 NaOH 水溶液以获得 pH 为 >10 的双相混合物；(d) 分离层并加热有机层至约 120℃以馏出水；(e) 冷却至约 80℃并提供 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮游离碱（A 型）的晶种；(g) 将该混合物在约 80℃维持约 3 小时，然后逐渐冷却至约 10℃以获得结晶；以及 (g) 过滤以分离该具有大粒径的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的游离碱（A 型）。

[0090] 在上述各方法的一些实施方案中，所述方法提供具有≤ 2 平方米 / 克的比表面积的化合物 1 游离碱。在上述各方法的其它实施方案中，所述方法提供具有≤ 1 平方米 / 克的比表面积的化合物 1 游离碱。在上述各方法的其它实施方案中，所述方法提供具有约 5 微米至约 150 微米，优选约 10 微米至约 100 微米，及更优选约 15 微米至约 80 微米的原生粒径的化合物 1 游离碱。在上述各方法的其它实施方案中，所述方法提供具有特征如下所示的原生粒径分布的化合物 1 游离碱：(i) 约 5 微米至约 10 微米的 D10 值；(ii) 约 30 微米至约 125 微米的 D90 值；或 (iii) 约 10 微米至约 45 微米的 D50 值；或 (i)、(ii) 及 (iii) 的组合。在上述各方法的另外的实施方案中，所述方法提供具有约 2 至约 3 的 (D90-D10)/D50 原生粒径分布比的化合物 1 游离碱。在上述各方法的另外的实施方案中，所述方法提供具有约 15 微米至约 125 微米的体积平均直径 (D[4,3]) 的化合物 1 游离碱。

[0091] 在另一方面，本发明提供如本申请中所述根据这些方法之一制备的化合物 1 游离碱。在一些实施方案中，本发明提供根据本申请中所述方法中的任一方法制备的 6- 乙酰基 -8- 环戊基 -5- 甲基 -2-(5- 哌嗪 -1- 基 - 吡啶 -2- 基氨基)-8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的结晶游离碱（A 型）。在一些这样的实施方案中，由本申请中所述方法制备的游离

碱可以其SSA、PSA或表面能、或这些方法的组合，单独或另外与PXRD或ssNMR组合来表征。在一些这样的实施方案中，所述结晶游离碱具有介于0.05至0.25重量百分比（wt%）之间的茴香醚和/或介于0.05至0.25wt%之间的正丁醇的残留溶剂含量。在其它这样的实施方案中，所述结晶游离碱具有≤0.5wt%的茴香醚及≤0.5wt%的正丁醇，优选≤0.25wt%的茴香醚及≤0.25wt%的正丁醇的残留溶剂含量。

[0092] 在上述各方法中，所述第一溶剂为醇且所述第二溶剂为芳族溶剂。适用的醇包括但不局限于相对高沸点的醇，诸如正丁醇、叔丁醇、正丙醇、戊醇、1,4-丁二醇或丙二醇等。适用的芳族溶剂包括但不局限于茴香醚、1,3,5-三甲苯、间二甲苯、氯苯、吡啶等。

[0093] 在一些这样的实施方案中，所述溶剂混合物包含10%的醇、15%的醇、20%的醇、25%的醇、30%的醇、35%的醇、40%的醇、45%的醇、50%的醇、60%的醇、70%的醇、或>70%的醇，其余部分为芳族溶剂。在其它这样的实施方案中，所述溶剂混合物包含90%的芳族溶剂、85%的芳族溶剂、80%的芳族溶剂、75%的芳族溶剂、70%的芳族溶剂、65%的芳族溶剂、60%的芳族溶剂、55%的芳族溶剂、50%的芳族溶剂、40%的芳族溶剂、30%的芳族溶剂、或<30%的芳族溶剂，其余部分为醇溶剂。

[0094] 在一个优选的实施方案中，所述第一溶剂为正丁醇。在另一个优选的实施方案中，所述第二溶剂为茴香醚。在特别优选的实施方案中，所述第一溶剂为正丁醇且所述第二溶剂为茴香醚。在一些这样的实施方案中，所述溶剂混合物包含10%的正丁醇/茴香醚、15%的正丁醇/茴香醚、20%的正丁醇/茴香醚、25%的正丁醇/茴香醚、30%的正丁醇/茴香醚、35%的正丁醇/茴香醚、40%的正丁醇/茴香醚、45%的正丁醇/茴香醚、50%的正丁醇/茴香醚、60%的正丁醇/茴香醚、70%的正丁醇/茴香醚、或>70%的正丁醇/茴香醚。在一些优选的实施方案中，所述溶剂混合物包含约20至约50%的正丁醇/茴香醚。在特别优选的实施方案中，所述溶剂混合物包含约40%的正丁醇/茴香醚。

[0095] 为了提高收率，所述方法可以包括加热或冷却至高于或低于室温的温度。经常地，所述反应混合物可加热至约30℃至约150℃的温度，更常为约50℃至约120℃的温度以获得溶解。在结晶期间，可能需要将所述反应混合物冷却至室温或低于室温的温度，例如介于约0℃与约30℃之间，优选约5℃、约10℃、约15℃或约20℃。

[0096] 在另外的实施方案中，所述化合物1的游离碱为具有包含在衍射角(2θ)10.1±0.2的峰的粉末X射线衍射图案的A型多晶型。在其它实施方案中，所述晶型具有包含在衍射角(2θ)10.1±0.2及22.5±0.2的峰的粉末X射线衍射图案。在该方面的其它实施方案中，所述晶型具有包含在衍射角(2θ)5.1±0.2、10.1±0.2及22.5±0.2的峰的粉末X射线衍射图案。在另外的实施方案中，所述晶型具有包含在衍射角(2θ)5.1±0.2、10.1±0.2、19.7±0.2及22.5±0.2的峰的粉末X射线衍射图案。在再其它实施方案中，所述晶型具有包含在衍射角(2θ)5.1±0.2、10.1±0.2、17.1±0.2、19.7±0.2及22.5±0.2的峰的粉末X射线衍射图案。在另外的实施方案中，所述晶型具有包含在衍射角(2θ)5.1±0.2、10.1±0.2、11.5±0.2、17.1±0.2、19.7±0.2及22.5±0.2的峰的粉末X射线衍射图案。在再其它实施方案中，所述晶型具有包含在衍射角(2θ)5.1±0.2、10.1±0.2、11.5±0.2、17.1±0.2、18.7±0.2、19.7±0.2及22.5±0.2的峰的粉末X射线衍射图案。在该方面的一些实施方案中，所述晶型具有包含在实质上与图1中所示相同的衍射角(2θ)的峰的粉末X射线衍射(PXRD)图案。

[0097] 游离碱 A 型多晶型的粉末 X 射线衍射 (PXRD) 图案显示于图 1，且对应数据列表于表 1 中。

[0098] 化合物 1 的 A 型多晶型的 PXRD 数据

[0099]

2θ (°) ±0.2	峰强度 (%)
5.1	63
8.0	18
10.1	100
10.3	70
11.5	42
14.0	20
15.1	14
16.0	16
17.1	47
18.7	33
19.7	51
20.2	30
21.2	22
22.5	87
23.0	31

[0100] 化合物 1 的结晶游离碱 A 型的固态核磁共振 (ssNMR) 显示于图 2 中，且对应数据列表于表 2 中。

[0101] 表 2. 化合物 1 的 A 型多晶型的以每百万之份数表示的 ^{13}C 化学位移

[0102]

^{13}C 化学位移 [ppm][a] ±0.2
12.50
25.40

26.54
29.04
32.03
46.15
51.01
55.66
107.34
112.44
125.94
131.14
140.15
143.15
144.85
156.32
157.35
158.06
161.88
201.94

[0103] (a) 参考 29.5ppm 处的固相金刚烷的外部样品。

[0104] 在另一方面，本发明提供化合物 1 的结晶游离碱，其中该结晶游离碱为化合物 1 游离碱的 B 型多晶型。在该方面的一些实施方案中，所述晶型具有包含在衍射角 (2θ)6.0±0.2 的峰的粉末 X 射线衍射图案。在该方面的其它实施方案中，所述晶型具有包含在衍射角 (2θ)6.0±0.2 及 19.8±0.2 的峰的粉末 X 射线衍射图案。在该方面的其它实施方案中，所述晶型具有包含在衍射角 (2θ)6.0±0.2、19.8±0.2 及 26.7±0.2 的峰的粉末 X 射线衍射图案。在其它实施方案中，所述晶型具有包含在衍射角 (2θ)6.0±0.2、16.4±0.2、19.8±0.2 及 26.7±0.2 的峰的粉末 X 射线衍射图案。在再其它实施方案中，所述晶型具有包含在衍射角 (2θ)6.0±0.2、12.8±0.2、16.4±0.2、19.8±0.2 及 26.7±0.2 的峰的粉末 X 射线衍射图案。在另外的实施方案中，所述晶型具有包含在衍射

角（2θ）6.0±0.2、12.8±0.2、16.4±0.2、19.8±0.2、22.6±0.2 及 26.7±0.2 的峰的粉末 X 射线衍射图案。在再其它实施方案中，所述晶型具有包含在衍射角（2θ）6.0±0.2、10.9±0.2、12.8±0.2、16.4±0.2、19.8±0.2、22.6±0.2 及 26.7±0.2 的峰的粉末 X 射线衍射图案。在该方面的一些实施方案中，所述晶型具有包含在实质上与图 3 所示相同的衍射角（2θ）的峰的 PXRD 图案。游离碱 B 型多晶型的粉末 X 射线衍射（PXRD）图案显示于图 3 中，且对应数据列表于表 3 中。

[0105] 表 3：化合物 1 的 B 型多晶型的 PXRD 数据

[0106]

2θ（°）±0.2	峰强度（%）
6.0	100
10.9	39
12.8	40
16.4	41
19.8	50
18.1	24
12.1	23
22.6	40
26.7	48
28.2	20

[0107] 6-乙酰基-8-环戊基-5-甲基-2-(5-哌嗪-1-基-吡啶-2-基氨基)-8H-吡啶并[2,3-d]嘧啶-7-酮的结晶游离碱 B 型的固态核磁共振（ssNMR）显示于图 4 中，对应数据列表于表 4 中。

[0108] 表 4. 化合物 1 的 B 型多晶型的以每百万之份数表示的 ^{13}C 化学位移

[0109]

^{13}C 化学位移 [ppm][a]±0.2
13.06
27.10
28.04
30.23

46.90[b]
52.32[b]
54.63
107.28
113.35
125.67
127.04
140.40
145.21
146.37
147.34
155.57
157.59
159.18
161.29
201.35

[0110] (a) 参考 29.5ppm 处的固相金刚烷的外部样品。

[0111] (b) 宽峰

[0112] 就各粉末 X 射线衍射测量而言，将游离碱的样品置入位于支架的平坦表面上的孔穴，并使用载玻片整平该样品表面。将含有该等样品的支架置入衍射仪中，且 X 射线光束源照射所述样品，最初以相对于所述支架的平坦表面的小角度照射。然后以逐步方式将所述 X 射线光束移动通过弧，所述方式连续增加在入射光束与所述支架的平坦表面之间的角度。在扫描的每一步，闪烁计数器检测被衍射的照射的量，其作为 2θ（°）的函数记录。仪器软件显示所述扫描的被衍射的照射结果，其表示为强度对 2θ（°）。

[0113] 表 1 及表 3 分别列出了具有 A 型或 B 型多晶型的化合物 1 游离碱的显著 PXRD 峰（即，展现峰高对噪声比大于 3.5 峰）。所提供的特征峰列表不是唯一可能的特征峰列表。多晶型鉴别领域中的技术人员可选择亦可将一种多晶型与另一种多晶型相区分的其它组特征峰。

[0114] 在相同多晶型的单独测量中 PXRD 图案的差异可因许多原因引起。误差来源包括

样品制备中的变异（例如样品高度）、仪器误差、校准误差、以及操作者误差（包括确定峰位置的误差）。优先定向（即，PXRD 样品中缺乏晶体的随机定向）可造成相对峰高的显著差异。校准误差及样品高度误差经常造成衍射图的全部峰向相同方向和以相同量偏移。在平坦支架上的样品高度的小差异可能导致PXRD峰位置的大幅位移。系统研究显示1毫米的样品高度差异可导致峰偏移高达1° 2θ，详见Chen等人，J. Pharmaceutical and Biomedical Analysis(2001)26:63。

[0115] 在许多情况中，由系统误差造成的衍射图案中的峰偏移可通过补偿该偏移（例如对所有峰位置值应用校准因子）或通过再校准衍射仪来消除。通常，相同技术可用以补偿衍射仪之间的差异，使得从两个不同仪器获得的PXRD峰位置能够一致。此外，当将这些技术应用于来自相同或不同衍射仪的PXRD测量时，特定多晶型的峰位置经常一致在约±0.2° 2θ 内。

[0116] 所公开的化合物包括所有药学上可接受的同位素变化形式。同位素变化形式是至少一个原子被具有相同原子序数但原子质量与自然界中所发现的原子质量不同的原子置换的化合物。可用的同位素包括氢、碳、氮、氧、磷、硫、氟及氯的同位素。因此典型的同位素包括但不局限于 ^{2}H、^{3}H、^{13}C、^{14}C、^{15}N、^{17}O、^{18}O、^{32}P、^{35}S、^{18}F 及 ^{36}Cl。

[0117] 所公开的化合物经诸如氘（即 ^{2}H）取代可提供某些由较大代谢稳定性（例如活体内半衰期增加或剂量需要减少）的治疗优点，因此在许多状况下可能更有用。此外，特定同位素变化形式（例如结合放射性同位素）可用于药物和/或底物组织分布研究。从容易结合及检测工具便利性的角度看，放射性同位素氘（即 ^{3}H）及碳14（即 ^{14}C）特别有用。

[0118] 所公开的化合物的同位素变化形式通常可通过本领域技术人员已知的惯用技术或通过与随后的实施例中所述方法类似的方法，使用适用试剂的适当同位素变化形式来制备。所公开的化合物的药学上可接受的溶剂合物包括其中结晶的溶剂可被同位素取代（例如 D_2O、d_6-丙酮、d_6-DMSO）的溶剂合物。

[0119] 溶解度实验

[0120] 美国专利第7,345,171号报道，通过传统盐断裂程序所制备的化合物1游离碱于pH 7.9下具有不良水溶解度（9μg/mL），且在动物研究中展现低生物利用度。报道指出所述游离碱为其根据淤浆实验的最稳定晶体相（即，A型）。美国专利第7,345,171号的图17提供了A型的游离碱的水吸附/脱附等温线。如先前所述，该材料对应于本申请中所述的小粒径化合物1游离碱。

[0121] 化合物1游离碱（A型）在药物粒子制备程序中具有高度冲压黏附倾向。由于冲压黏附与API表面积相关，故API粒径控制是最小化药物产品制备期间的黏附的关键。除了冲压黏附问题外，还发现从标准盐断裂程序直接分离的化合物1游离碱极容易产生静电，且发现形成不能通过筛分进行分散的大的（大约500微米）硬聚结物。通过现有羟乙磺酸盐API的游离碱化或通过中和在API合成最终步骤中所形成的原位盐产生具有相似不良物理性质的游离碱API。在任一方法中，因由调整pH产生溶解性剧烈改变导致的快速结晶而产生小API初级粒子。在所有情况下，所述游离碱作为更稳定A型多晶型分离。

[0122] 图6显示通过上述游离碱化及中和实验所形成的典型小初级粒子的扫描电子显微术（SEM）图像。通过此游离碱分离方法所产生的一批化合物1（A型）的粒径分布测量提供于图8中。该粒径分布中的第二模式由大聚结物的存在导致，在图6中的SEM图像亦可

看到它。改变所述游离碱化方法的尝试在改善所产生的 API 的物理性质方面未能成功。因为所述产生游离碱的方法造成具有不良物理性质的 API 的分离，进行了研究以鉴别能改善 API 物理性质的再结晶方法。

[0123] 完成了化合物 1 游离碱的早期结晶筛选实验以鉴别容许分离具有改良的物理性质的粒子的溶剂系统。溶解度筛选与小规模再结晶研究的组合检验了多种可能的溶剂系统。

[0124] 小规模结晶研究

[0125] 进行了一系列小规模结晶实验以鉴别可能的再结晶溶剂系统以及评估溶剂对于所分离的游离碱初级粒子的形状的影响。以 10mg 模规进行了最初一组 14 个筛选研究，其使用密封管及外部热源以将 50mg/mL 样品加温高至回流温度。目视观察鉴别成为溶液的样品，且使用照相显微术来表征所产生的粒子。这些初始结晶筛选实验的结果汇总于表 5 中。

[0126] 表 5 ：来自初步小规模结晶研究的结果概要

[0127]

溶剂系统	再结晶的结果
环戊基甲基醚	不溶解
乙酸正丁酯	不溶解
正丁醇	不溶解
三氟甲苯	不溶解
甲苯	不溶解
氯苯	小的不规则形状粒子
DMF	小的针状粒子
NMP	小的不规则形状粒子
丙二醇	小的不规则形状粒子
茴香醚	大粒子（车床或斧形）
吡啶	小车床形粒子
环丁砜	小的不规则形状粒子
间二甲苯	小 / 中等斧形粒子
1, 3, 5- 三甲苯	小的针状粒子

[0128]

[0129] 基于这些小规模结晶研究，由于所产生的粒子大且茴香醚为 ICH 第 III 类溶剂，茴

香醚成为额外结晶及溶解度研究的焦点。基于所产生的粒子，此筛选研究亦将吡啶、间二甲苯及 1,3,5- 三甲苯鉴别为可能的溶剂系统，但这些溶剂中无一亦具有类似于茴香醚的 ICH 第 III 类列表。

[0130] 以下溶剂亦一直用于固体的再结晶：异丙醇、异丁醇、乙醇、乙酸乙酯、甲苯、四氢呋喃及二噁烷。所述溶剂各产生化合物 1 的 A 型多晶型结晶固体，其与从二氯甲烷获得的原始晶型相同。

[0131] 溶解度研究：

[0132] 与初始小规模结晶研究平行，对化合物 1 游离碱进行了一系列溶解度研究以鉴别可能的再结晶系统。在初始室温溶解度筛选研究中，筛选了总计 23 种溶剂。此研究显示出化合物 1 游离碱在一系列有机溶剂中具有低溶解度，只有二氯甲烷显示出大于 1mg/mL 的溶解度 (3.0mg/mL)。进行了随后的靶向较高温度溶解度研究。在追踪研究中，在 25mg/mL 的固定浓度下检验了一组 16 种溶剂系统，且使用直到 110℃的最大温度的动力学溶解度方法测量了溶解温度。

[0133] 将通过化合物 1 的 COSMOtherm 溶解度模型预测的协同溶解度行为用于选择此筛选研究中包括的二元及三元溶剂系统。这些研究的结果列于表 6 中。就该表中所列为 >110℃的实验而言，当加热至 110℃时，化合物 1 不溶解于所述溶剂中，这表明在此溶剂中于 110℃下溶解度低于 25mg/mL。

[0134] 表 6. 25mg/mL 化合物 1 游离碱溶液的动力学溶解度测量

[0135]

实验 #	溶剂	溶解温度（℃）
1	n-BuOH	>110℃
2	DMF	>110℃
3	NMP	97.9
4	DMSO	>110℃
5	DMAc	>110℃
6	乙酸正丁酯	>110℃
7	茴香醚	>110℃
8	10% n-BuOH/ 茴香醚 (v/v)	>110℃
9	20% n-BuOH/ 茴香醚 (v/v)	109.7
10	40% n-BuOH/ 茴香醚 (v/v)	101.4
11	10% n-BuOH/NMP(v/v)	103.7

12	25% n-BuOH/NMP(v/v)	>110℃
13	10% 1,4- 丁二醇 / 茴香醚 (v/v)	109.8
14	25% 1,4- 丁二醇 / 茴香醚 (v/v)	104.8
15	1:1:8 丙二醇 /n-BuOH/ 茴香醚 (v/v)	91.2
16	2:1:7 丙二醇 /n-BuOH/ 茴香醚 (v/v)	84.1

[0136] 来自表 6 中的实验编号 3 及编号 11 的饱和溶液的随后 UPLC/MS 测试显示存在先前未看到的杂质峰，此表明这些实验中发生了降解。

[0137] 虽然丙二醇 /n-BuOH/ 茴香醚混合物显示出相比于 n-BuOH/ 茴香醚混合物而言改良的溶解度，但前者溶剂系统因其可能造成规模化问题的高粘度及沸点导致与丙二醇一起操作的潜在挑战，故不推行。

[0138] 基于这些筛选研究，鉴于 API 的相对高溶解度、化学稳定性及再结晶化合物 1API 的粒子性质，选择 40% 的正丁醇与茴香醚的混合物作为用以进一步处理的结晶溶剂系统。此溶剂系统用于随后生产以提供较大原生粒径 API，该较大原生粒径 API 的黏附减少、不易产生静电及无聚结物。

[0139] 使用此溶剂混合物，在使用受控冷却曲线及晶种引发成核而结晶之前，通过加热至 95 至 100℃，以 40mL/g 溶剂溶解化合物 1（浓度为 25mg/mL）。图 9 为使用此再结晶程序再结晶的化合物 1 的实验室规模批次的 PLM 图像，而图 7 显示三批再结晶的 API 的粒径分布。此再结晶过程导致具有较大原生粒径的化合物 1API 粒子的分离，较大原生粒径导致在药物产品生产过程中的黏附倾向性降低。此再结晶的化合物 1API 不形成聚结物，且亦具有不易产生静电的正面贡献。

[0140] 溶解度筛选与小规模再结晶研究的组合检验了多种用于再结晶化合物 1 游离碱的可能溶剂系统。基于这些筛选研究的结果，基于所述 API 的相对高溶解度、化学稳定性及再结晶化合物 1 的粒子性质，选择 40% 的正丁醇 / 茴香醚的混合物作为优选的结晶溶剂系统。从该再结晶过程分离的 API 的较大粒径及改良的粒子性质促进了化合物 1 游离碱的药物产品生产过程的发展。

[0141] 粒径评估

[0142] 使用激光衍射法评估了再结晶材料的粒径。激光衍射由包括 ISO 及 ASTM 的标准及指导机构认可，且广泛用以测定粒径分布。在进行评估中，使样品通过导致在一定范围角度散射的激光的激光束。以固定角度放置的检测器测量该位置所散射的光强度。然后应用数学模型（Mie 或 Fraunhoffer 理论）产生粒径分布。

[0143] 使用激光衍射（或小角度光散射）技术，通过用压缩空气分散干燥样品粉末来分析粒径。特别的是，使用配备有 Vibri 干粉进料器的 Sympatec HELOS RODOS 系统来分析粒径分布。该粉末样品以 0.5 巴的分散压力来分散。在一些情况中，使用 Aspiros 微定量给料装置，且以 0.2 巴的分散压力分散粉末样品。选择适用的透镜以涵盖每个样品的粒径范围。

[0144] 在粒径测定中，将中位值定义为一半群体停留在此点上方及一半停留在此点下方

的值。就粒径分布而言，该中位数称为 D50。该 D50 为将分布分成一半在此直径上方且一半在此直径下方的以微米计的尺寸。Dv50 或 D[v,0.5] 的表达方式有时用于体积分布的中位数。

[0145] 所述模式是频率分布的峰。粒子分布可包括超过一个模式，例如粒子作为初级粒子及聚结物存在的情况。

[0146] 跨距有时用作分布宽度的测量，且定义为 (D[v,0.9]-D[v,0.1])/D[v,0.5] 或 (D90-D10)/D50 的比。

[0147] 所述分布宽度亦可通过引用在 x 轴上的一、二或优选三个值，通常为 D10、D50 及 D90 的一些组合，来表征。中位数 D50 已于上文定义为一半群体位于此值下方的直径。类似地，分布的 90％位于 D90 下方，及群体的 10％位于 D10 下方。

[0148] 术语 D[4,3] 是指体积平均或质量矩平均值。激光衍射结果以体积为基础报告，且体积平均值可用以定义分布的中心点。D[4,3] 值对于分布中大粒子的存在是敏感的。

[0149] 制剂

[0150] 本发明还涉及包含本申请中所述化合物 1 的游离碱 A 型多晶型的药物组合物。本发明的药物组合物可例如呈适于口服的形式，诸如片剂、胶囊、药丸、粉末、缓释制剂、溶液、悬浮液；用于肠胃外注射，如灭菌溶液、悬浮液或乳液；用于局部给予，诸如油膏或乳霜；或用于直肠给予，如栓剂。所述药物组合物可为适于单次给予精确剂量的单位剂型。该药物组合物将包括常规药物载体或赋形剂及根据本发明的化合物作为活性成分。此外，可包括其它药物或制药用物质、载体、助剂等。

[0151] 适用的药物载体包括惰性稀释剂或填料、水及各种有机溶剂。视需要，所述药物组合物可含有额外成分，诸如调味剂、粘合剂、赋形剂等。因此，就口服而言，含有各种赋形剂（诸如柠檬酸）的片剂可与各种崩解剂（诸如淀粉、藻酸及特定复合硅酸盐）以及粘合剂（诸如蔗糖、明胶及阿拉伯树胶）一起使用。另外，润滑剂（诸如硬脂酸镁、硫酸月桂酯钠及滑石）经常可用于制片目的。相似类型的固态组合物亦可用于软质及硬质填充明胶胶囊。优选的材料包括乳糖及高分子量聚乙二醇。当需要水性悬浮液或酏剂以供口服时，其中的活性化合物可与各种甜味剂或调味剂、着色物质或染料，以及视需要与乳化剂或悬浮剂，连同稀释剂（诸如水、乙醇、丙二醇、甘油或其组合）并用。

[0152] 制备具有特定量的活性化合物的各种药物组合物的方法为本领域技术人员已知或对本领域技术人员而言显而易见。例如，详见 Remington's Pharmaceutical Sciences, Mack Publishing Company, Easter, Pa.，第 15 版 (1975)。

[0153] 所公开的化合物可单独给予或与其它药物合并给予，且通常将作为与一种或多种药学上可接受的赋形剂相关联的制剂形式给予。术语“赋形剂”描述任何化合物 1 及其盐以外的成分。赋形剂的选择在很多程度上取决于特定给药模式。

[0154] 所公开的化合物可口服。口服给予可包括吞咽，因此该化合物进入胃肠道，或可使用颊或舌下给予，藉此该化合物从口腔直接进入血流。

[0155] 适于口服给予的制剂包括固态制剂，诸如片剂、含有颗粒、液体或粉末的胶囊、锭剂（包括填充液体者）、咀嚼剂、多颗粒剂及纳米粒、凝胶、固溶体、脂质体、薄膜剂（包括粘膜黏附剂）、阴道栓、喷雾剂及液态制剂。液态制剂包括悬浮液、溶液、糖浆及酏剂。所述制剂亦可作为填料用于软胶囊或硬胶囊中，且通常包含载体，例如水、EtOH、聚乙二醇、丙二

醇、甲基纤维素或适用的油，及一种或多种乳化剂和/或悬浮剂。液态制剂亦可通过将固体（例如来自小药囊）复原而制备。

[0156] 所公开的化合物亦可用于快速溶解、快速崩解剂型，诸如 Liang 及 Chen 于 Expert Opinion in Therapeutic Patents(2001)11(6):981-986 中所描述。

[0157] 至于片剂剂型，视剂量而定，该药物可占剂型的 1wt%至 80wt%，更常剂型的占 5wt%至 60wt%。除了药物的外，片剂通常含有崩解剂。崩解剂的实例包括淀粉羟乙酸钠、羧甲基纤维素钠盐、羧甲基纤维素钙、交联羧甲基纤维素钠、交聚维酮、聚乙烯吡咯烷酮、甲基纤维素、微晶纤维素、低级烷基取代的羟丙基纤维素、淀粉、预胶化淀粉及褐藻酸钠。通常，崩解剂占剂型的 1wt%至 25wt%，更优选为 5wt%至 20wt%。

[0158] 通常使用粘合剂以使片剂制剂具有粘结性质。适用的粘合剂包括微晶纤维素、明胶、糖、聚乙二醇、天然及合成胶、聚乙烯吡咯烷酮、预胶化淀粉、羟丙基纤维素及羟丙基甲基纤维素。片剂亦可含有稀释剂，诸如乳糖（一水合物、喷雾干燥的一水合物、无水物等）、甘露醇、木糖醇、右旋糖、蔗糖、山梨醇、微晶纤维素、淀粉及磷酸氢钙二水合物。

[0159] 片剂亦可任选地包括表面活性剂，诸如硫酸月桂酯钠及聚山梨酯 80，以及助流剂，诸如二氧化硅及滑石。当存在上述物质时，表面活性剂可占片剂的 0.2wt%至 5wt%，及助流剂可占片剂的 0.2wt%至 1wt%。

[0160] 片剂亦通常含有润滑剂，诸如硬脂酸镁、硬脂酸钙、硬脂酸锌、反丁烯二酸硬脂酰钠及硬脂酸镁与硫酸月桂酯钠的混合物。润滑剂通常占片剂的 0.25wt%至 10wt%，更优选为 0.5wt%至 3wt%。其它成分可包括防腐剂、抗氧化剂、调味剂及着色剂。

[0161] 片剂掺合物可直接压制形成片剂。片剂掺合物或掺合物的部分或者可在制片前经湿式、干式或熔融粒化、熔融凝结或挤出。最终制剂可包含一层或多层，且可具有包衣或无包衣。典型片剂含有至多约 80%的药物、约 10wt%至约 90wt%的粘合剂、约 0wt%至约 85wt%的稀释剂、约 2wt%至约 10wt%的崩解剂及约 0.25wt%至约 10wt%的润滑剂。有关片剂配制的额外细节详见 H. Lieberman 及 L. Lachman, Pharmaceutical Dosage Forms:Tablets, Vol. 1(1980)。

[0162] 用于口服的固态制剂可配制成立即释放型和/或改良释放型。改良释出制剂包括延迟释放型、持续释放型、脉冲释放型、控制释放型、靶向释放型及程序释放型。适用的改良释放型制剂的一般描述详见美国专利第 6,106,864 号。就其它可用释放技术（诸如高能分散体及渗透且经涂覆的粒子）的细节详见 Verma 等人，Pharmaceutical Technology On-line(2001)25(2):1-14。用以获得受控制释放的口香糖用途的讨论详见 WO 00/35298。

[0163] 所公开的化合物亦可直接给予至血流、至肌肉或至内脏。肠胃外给予的适用方式包括静脉内、动脉内、腹膜内、椎管内、室内、尿道内、胸骨内、颅内、肌内及皮下给予。肠胃外给予的适用装置包括针式（包括微针）注射器、无针式注射器及输注技术。

[0164] 肠胃外制剂通常为水溶液，其可含有赋形剂，诸如盐类、碳水化合物及缓冲剂（优选的至 pH 为 3 至 9），但就一些应用而言，更适宜配制为灭菌非水溶液或待与适用载体（诸如灭菌无热原水）联合使用的干燥形式。在灭菌条件下例如通过冷冻干燥制备肠胃外制剂可容易地使用本领域技术人员详知的标准医药技术完成。典型肠胃外给予形式包括活性化合物在灭菌水溶液中的溶液或悬浮液形式，例如丙二醇水溶液或右旋糖溶液。若需要，所述剂型适合经缓冲。

[0165] 用于制备肠胃外溶液的所公开化合物的溶解度可通过使用适当配制技术（诸如结合溶解度增强剂）来提高。肠胃外给予的制剂可配制成如上述立即释放型和/或改良释放型。如此，这些公开的化合物可配制成更实体形式以供作为提供该活性化合物的长期释放的植入型贮库给予。

[0166] 本发明化合物亦可局部给予至皮肤或粘膜，表皮或经皮给予。用于此目的的典型制剂包括凝胶、水凝胶、洗剂、溶液、霜剂、软膏、敷粉、敷料、泡体、膜剂、皮肤贴片、糯米纸馕剂、植入物、海绵、纤维、绷带及微乳液。亦可使用脂质体。典型载体包括醇、水、矿油、液态石蜡脂、白石蜡脂、甘油、聚乙二醇及丙二醇。局部制剂亦可包括渗透增强剂。详见例如Finnin及Morgan，J Pharm Sci(1999)88(10):955-958。

[0167] 局部给予的其它方式包括通过离子电泳法、电穿孔、音波声波导入法(phonophoresis)、超音波导入(sonophoresis)及无针式（例如POWDERJECT）注射来递送。局部给予的制剂可配制成如上述立即释放型和/或改良释放型。

[0168] 所公开化合物亦可鼻内或通过吸入从干粉吸入器通常呈干粉形式（单独或为混合物，例如与乳糖的干燥掺合物，或为混合组分粒子，例如与诸如磷脂酰胆碱的卵磷脂混合）或从加压容器、泵、喷雾器、雾化器（通常为使用电流体力学以产生细微水雾的雾化器）或喷雾器呈气溶胶喷雾投药，其使用或不使用适用推进剂（诸如二氯氟甲烷）。该加压容器、泵、喷雾器、雾化器或喷雾器可含有溶液或悬浮液，该溶液或分散液包含该活性化合物、用于分散、溶解或延长该活性化合物的释放的用剂（例如EtOH或含水EtOH）、一种或多种用作推进剂的溶剂、以及随意的界面活性剂，诸如山梨醇酐三油酸酯或寡聚乳酸。

[0169] 在用于干粉或悬浮液制剂之前，该药物产物经微粉化至适于通过吸入递送的大小（通常小于5微米）。此可通过任何适当的粉碎方法获致，诸如螺旋射流粉碎、流体化床射流粉碎、超临界流体处理以形成纳米粒子、高压均质化或喷雾干燥。

[0170] 用于吸入器或吹入器的胶囊、泡罩及筒（例如从明胶或羟丙基甲基纤维素制得）可配制成含有该活性化合物、适用的粉末状基质（诸如诸如乳糖或淀粉）及性能改良剂（诸如诸如L-亮氨酸、甘露醇或硬脂酸镁）的粉末混合物。该乳糖可为无水或优选的呈一水合物形式。其它适用的赋形剂包括聚葡糖、葡萄糖、麦芽糖、山梨醇、木糖醇、果糖、蔗糖及海藻糖。

[0171] 适用于使用电流体力学以产生细微水雾的雾化器的溶液制剂可在每次启动含有1微克至20毫克的本发明化合物，且该启动体积可从1微升至100微升。典型制剂可包含化合物1、丙二醇、灭菌水、EtOH及NaCl。可用以代替丙二醇的替代溶剂包括甘油及聚乙二醇。

[0172] 吸入式/鼻内给予的制剂可例如使用聚(DL-乳酸-共聚羟乙酸(PGLA)配制成立即释出型和/或改良释出型。适用的调味剂（诸如薄荷醇及左薄荷醇或甜味剂（诸如糖精或糖精钠）可添加至欲用于吸入式/鼻内给予的制剂。

[0173] 在干粉吸入器及气溶胶的例中，单位剂量利用递送经计量的量的阀来决定。根据本发明的单元通常配置成给予经计量的剂量或含有100至1000微克该活性药学成分的“按压一次量(puff)”。每日总剂量通常在100微克至10毫克的范围，其可以单剂给予，或更常见的是分成一天数剂。

[0174] 该活性化合物可经直肠/阴道给予，例如呈栓剂、子宫托或灌肠剂形式。可可脂传统栓剂基质，但若情况适当的话，可使用各种替代品。肠胃外给予的制剂可配制成如上述经

直肠 / 阴道给予。

[0175] 所公开的化合物亦可直接给予至眼睛或耳朵，通常呈于等渗压 pH 经调整的灭菌盐水中的微粉化悬浮液或溶液的滴剂形式。适用于眼部及耳部给予的其它制剂包括软膏、生物可降解（例如可吸收凝胶海绵、胶原）及生物不可降解（例如聚硅氧）植入物、糯米纸馓剂、透镜及微粒，或多孔系统，诸如囊泡 (niosome) 或脂质体。聚合物（诸如交联聚丙烯酸、聚乙烯醇、玻尿酸）；纤维素聚合物（例如羟丙基甲基纤维素、羟乙基纤维素或甲基纤维素）；或异元多醣聚合物（例如吉兰胶）可结合防腐剂，诸如氯化烷基二甲基苄基铵。所述制剂亦可通过离子电泳法递送。肠胃外给予的制剂可配制成如上述眼部 / 耳部给予。

[0176] 所公开的化合物可与可溶性大分子实体（诸如环糊精或含聚乙二醇聚合物）结合，以改善其溶解度、溶解速率、掩味性、生物利用度和 / 或稳定性。例如，已发现药物 - 环糊精复合物通常可用于大部分剂型及给予途径。可使用包含型或非包含型复合物。与药物直接复合的替代方式可使用环糊精作为辅助添加剂，即，作为载体、稀释剂或助溶剂。α -、β - 及 γ - 环糊精常用于所述目的。详见例如国际专利申请 WO 91/11172、WO 94/02518 及 WO 98/55148。

[0177] 化合物 1 的治疗有效剂量可从每天大约 0.01mg/kg 体重至大约 100mg/kg 体重。通常成人剂量将为大约每天 0.1mg 至大约 3000mg。在单位剂量制剂中的活性组分的量可根据特定应用及该活性组分的效力在 0.1mg 至大约 500mg，优选约 0.6mg 至 100mg 变化或调整。视需要，该组合物亦可含有其它兼容治疗剂。对需要治疗的对象给予每天约 0.6 至约 500mg，其为单剂或在 24 小时期间的多剂。此种治疗可以连续间隔重复必要的时间长度。

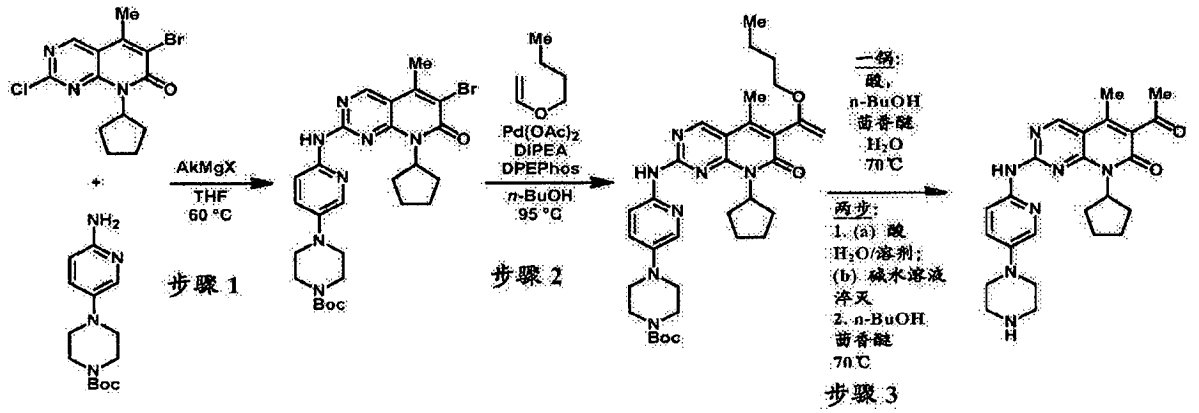
[0178] 由异常细胞增生造成的障碍或病况包括癌症及与动脉粥样硬化、手术后血管狭窄和再狭窄相关联的血管平滑肌增生、及子宫内膜异位。自体免疫疾病包括银屑病、炎症样类风湿性关节炎、狼疮、1 型糖尿病、糖尿病肾病变、多发性硬化症、肾小球肾炎及器官移植排斥，包括宿主抗移植物病。

[0179] 在一个实施方案中，本发明提供一种治疗需要此种治疗的哺乳动物（包括人类）异常细胞生长的方法，其包括对该哺乳动物给予治疗有效量的本申请中所述的本发明化合物 1 结晶游离碱。在常见实施方案中，所述游离碱为 A 型多晶型。

[0180] 在另一个实施方案中，所述异常细胞生长为癌症，包括实体肿瘤及恶性血液疾病二者。在一些这样的实施方案中，所述癌症选自乳腺癌、卵巢癌、宫颈癌、子宫内膜癌、前列腺癌、睾丸癌、胰腺癌、食道癌、头颈癌、胃癌、膀胱癌、肺癌（例如腺癌、NSCLC 及 SCLC）、骨癌（例如骨肉瘤）、结肠癌、直肠癌、甲状腺癌、脑及中枢神经系统癌症、神经胶质母细胞瘤、神经母细胞瘤、神经内分泌癌、杆状癌、角质棘皮瘤、表皮样癌、精细胞瘤、黑色素瘤、肉瘤（例如，脂质肉瘤）、膀胱癌、肝癌、（例如，肝细胞癌）、肾癌（例如，肾细胞癌）、骨髓障碍（例如，AML、CML、骨髓发育不良综合征及前髓细胞性白血病）及淋巴障碍（例如，白血病、多发性骨髓瘤、外膜细胞淋巴瘤、ALL、CLL、B 细胞淋巴瘤、T 细胞淋巴瘤、何杰金淋巴瘤、非何杰金淋巴瘤、毛细胞淋巴瘤）。

[0181] 一般合成方案

[0182]



[0183] 以下所提供的实施例及制备例进一步阐明及举例说明本发明实施方案的特定方面。应理解，本发明范围绝不受以下实施例的范围限制。

实施例

[0184] 一般方法及材料

[0185] 粉末 X 射线衍射 (PXRD)

[0186] 根据以下方案收集 PXRD 数据。将样品 (2mg) 置于零背景的显微镜载玻片上。然后将该样品置入配备有 GADDS 检测器的 Discover D8(Bruker AXS Instruments)。该系统使用维持在 40kV 与 40mA 的铜 X 射线源以提供 1.5406 埃的 CU α 1 发射。使用 0.02° 的步进扫描、60.1 秒的步进时间收集 4 至 40° 2θ 的数据。通常测量衍射峰具有 ±0.2 度 (2θ) 的误差。

[0187] SSNMR 仪器及方法

[0188] 根据以下方案收集 SSNMR 数据。在定位于大口径 Bruker-Biospin Avance III 500MHz NMR 光谱仪内的 Bruker-Biospin 4mm 及 7mm BL CPMAS 探针上收集光谱。使 4mm 转子以魔角定向且在 15.0kHz 下旋转。使 7mm 转子以魔角定向且在 7.0kHz 下旋转。所有光谱在环境条件（温度未受控制）下获取。

[0189] 使用质子去耦交叉极化魔角旋转 (CPMAS) 实验收集 ^{13}C 固态光谱。峰共振以每百万分之份数 (ppm) ±0.2ppm 报告。

[0190] 差示扫描量热法 (DSC)：

[0191] 使用 Q1000（热分析仪器）进行 DSC 测量。将样品置入具有针孔的密封铝盘。典型样品重量为 1.6 毫克。将样品平衡至 25℃，然后以 10℃ / 分钟的扫描速率升温至 250℃。使用干燥氮气作为冲洗气体。

[0192] 布鲁诺、埃梅特和特勒 (Brunauer, Emmet and Teller)(BET) 比表面积 (SSA) 测量：

[0193] 根据以下方案收集 SSA 测量值。使用气体分子在晶体表面上的单层形成测定活性药学成分的干燥粉末的比表面积。通过施加热及以氮气冲洗使该样品不含湿气及大气蒸气。然后将样品温度降至用作待吸附的被吸附气体（氮）的液态氮的温度。被吸附气体的量及压力数据用于产生吸附等温线图。然后使用数学算法基于所谓的布鲁诺、埃梅特和特勒 (BET) 理论，将数据转化成比表面积值（详见例如，J. Am. Chem. Soc., 1938, 60:309)。使

用静止多点或单点气体吸附法测量比表面积，该方法完整描述于 ISO 9277:2010 及以下实验中。

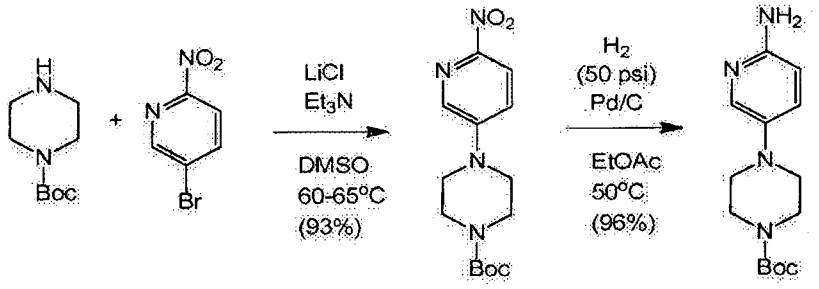
[0194] 反相气相色谱法（IGC）表面能测量：

[0195] 根据以下方案使用 IGC 收集表面能测量值。将充分量的样品装填于硅烷化玻璃柱中，通过插在两端的玻璃羊毛塞将该粉末物质固定在所述柱内。通过使干燥氮气流流动通过所述粉末物质充分时间以除去任何表面吸附物，调节所述柱。通过将一系列烷烃蒸气探针（壬烷、辛烷、庚烷及己烷）注入载体气流，其浓度低到足以假定所述烷烃蒸气在所述氮气流中无限稀释，并记录各蒸气通过所述柱洗出所花费的时间来进行测量。保留时间（针对填充柱内的填隙空间的“死体积”进行校准）对横断面积及所使用的烷烃蒸气探针分子的表面张力的函数作图产生直线，斜率具有表示被检验的固态粉末的表面能。

[0196] 合成实施例

[0197] 实施例 1. 4-(6- 氨基 - 吡啶 -3- 基）哌嗪 -1- 甲酸叔丁酯的制备

[0198]



[0199] 步骤 A. 4-(6- 硝基 - 吡啶 -3- 基）哌嗪 -1- 甲酸叔丁酯的制备

[0200] 于容器中添加 5- 溴 -2- 硝基吡啶（10.0g，1.0 当量）以及 DMSO(25mL，2.5vol)。添加 N-Boc 哌嗪（13.8g，1.5 当量），然后添加三乙胺（7.5g，1.5 当量）及 LiCl(2.1g，1.0 当量）。将该混合物加温至 60 至 65℃至少 12 小时。

[0201] 将水（5mL，0.5vol）缓慢添加至该于 60 至 65℃的容器内。使该混合物在 60 至 65℃维持 1 小时，然后冷却至室温。将该淤浆维持在 20 至 25℃为时 1 小时，然后在 #2Whatman™ 滤纸上过滤。以水清洗该滤饼（50ml，5vol）。收集粗制固体并将其移回清洁容器。

[0202] 将水（100mL，10vol）添加至该含有该固体的容器中，并将该混合物加温至 35 至 40℃为时 2 小时，然后于温热时在 #2Whatman™ 滤纸上过滤。以水（40mL，4vol）清洗这些固体，然后在真空烘箱中于 50 至 55℃下让其干燥过夜。分离 4-(6- 硝基 - 吡啶 -3- 基）- 哌嗪 -1- 甲酸叔丁酯，为黄色固体（收集 14.1g；收率～ 93%）。

[0203] 步骤 B. 4-(6- 氨基 - 吡啶 -3- 基）哌嗪 -1- 甲酸叔丁酯的制备

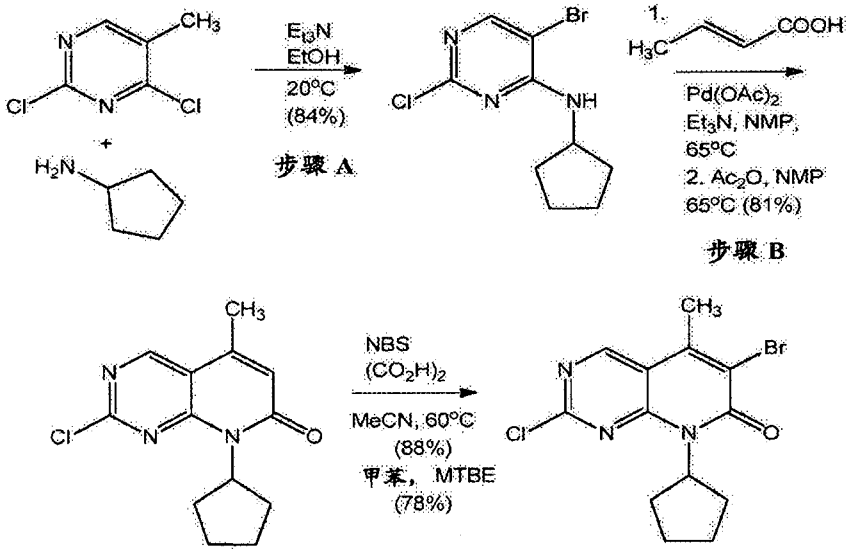
[0204] 于容器中添加 4-(6- 硝基 - 吡啶 -3- 基）- 哌嗪 -1- 甲酸叔丁酯（12.0g，1.0 当量）以及乙酸乙酯（48mL，4.0vol）。于淤浆中添加 50% 水、湿 5% Pd/C(480mg，4% w/w) 且以氮气冲洗该容器三次。该容器以氢气冲洗三次，然后加压 50psi 氢气。将该混合物加热至 42 至 47℃且允许搅拌直到氢气吸取停止为止（至少 8 小时）。

[0205] 过滤该产物混合物并以乙酸乙酯清洗（2×1.5mL）。在减压下将合并的滤液浓缩至体积为 6mL(2vol)。于该溶液中添加正庚烷（54mL，4.5vol），并在减压下蒸馏该混合物

至体积为 6mL(2vol)。于该溶液添加正庚烷 (54mL,4.5vol)。将所得的浓稠淤浆冷却至 20 至 25℃并允许搅拌 2 小时。过滤该淤浆并以正庚烷 (36mL,3vol) 清洗该滤饼。使该固体在 50 至 55℃的真空烘箱中干燥过夜。分离 4-(6- 氨基 - 吡啶 -3- 基)- 哌嗪 -1- 甲酸叔丁酯,为浅橘色固体 (收集 10.4g ;收率～ 96%)。^{1}H NMR(500MHz, DMSO-d_6) : δ 7.62(dd, J = 2.99, 0.60Hz, 1H), 7.17(dd, J = 8.85, 2.99Hz, 1H), 6.40(dd, J = 8.85, 0.60Hz, 1H), 5.45(bs, 2H), 3.43(m, 2H), 2.85(m, 2H), 1.41(s, 9H) ;^{13}C NMR(125MHz, DMSO-d_6) : δ 154.8, 153.8, 138.7, 136.8, 125.9, 108.3, 78.9, 50.5, 43.8, 43.0, 28.0 ;HRMS:$C_{14}H_{23}N_4O_2$(M+H)$^+$的计算值 : 279.18155,发现值 :279.18173。

[0206] 实施例 2. 6- 溴 -2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的制备

[0207]



[0208] 步骤 A. 5- 溴 -2- 氯 -6- 环戊基氨基 - 嘧啶的制备

[0209] 于容器中添加纯乙醇 (3000mL,3.0vol),然后添加 5- 溴 -2,4- 二氯嘧啶 (分子量 227.87 ;1000g,1.0 当量)。添加三乙胺 (612mL,1.0 当量),然后在 2 小时期间缓慢添加环戊胺 (分子量 85.15 ;520mL,1.2 当量) 以控制温和放热。在完成环戊胺添加之后,视需要,以 5- 溴 -2- 氯 -6- 环戊基氨基 - 嘧啶 (5g,0.5wt%) 对反应加晶种来引发结晶。该反应于 25℃下搅拌 2 小时。

[0210] 在 20 至 25℃下以 30mL/ 分钟的速率将水 (2500mL,2.5vol) 添加至该容器中。以 2℃ / 分钟将该混合物冷却至 8 至 12℃。将该淤浆维持在 8 至 12℃为时 1 小时,然后在 #2Whatman™滤纸上过滤。以正庚烷 (2000mL) 清洗该滤饼。以正庚烷 (2000mL) 在过滤干燥器上将该滤饼再浆化。该物质在真空烘箱中于 50 至 55℃下干燥过夜以产生呈白色固体形式的 5- 溴 -2- 氯 -6- 环戊基 - 氨基嘧啶 (1020g ;84%)。

[0211] 步骤 B. 2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的制备

[0212] 在周围温度下于容器中添加 5- 溴 -2- 氯 -6- 环戊基氨基 - 嘧啶 (10.0g,1.0 当量) 以及 N- 甲基吡咯烷酮 (NMP)(50mL,5.0vol)。于该反应混合物中添加巴豆酸 (4.7g,1.5 当

量）及三乙胺（20.2mL，4.0 当量）。将该容器脱气并使用氮气冲洗三次。于该经脱气的反应混合物中添加 $Pd(OAc)_2$（0.25g，0.03 当量）。使用与步骤 3 相同方法将该容器脱气并以氮气冲洗三次。将该混合物加热至 65℃，并允许搅拌直到原料消耗为止（至少 6 小时）。

[0213] 将乙酐（6.8mL，2.0 当量）添加至该反应混合物。使该反应在 65℃下反应，直到原料消耗为止（通常为 1 至 2 小时）。

[0214] 将该反应混合物冷却至 20℃并添加 H_2O（100mL，10vol）以溶解三乙胺·HBr 盐，并沉淀出 2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮。该物质在 20℃下粒化 1 小时。将该固体过滤并以 H_2O（20mL，2.0vol）以及 4:1 的异丙醇 /H_2O 混合物（50mL，5.0vol）清洗。该粗制产物在真空下于 55 至 70℃干燥以产生呈黄褐色至灰色固体形式的 2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮（7.8g；81%）。

[0215] 步骤 C. 6- 溴 -2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮的制备

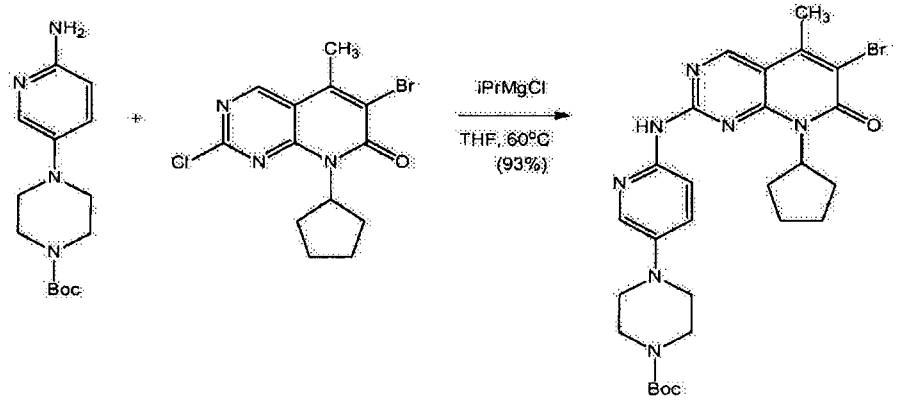
[0216] 于衬有玻璃的容器中添加 2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮（9.35g，1.0 当量）以及乙腈（65mL，7.0vol）。添加 N- 溴琥珀酰亚胺（9.67g，1.5 当量）及草酸（0.65g，0.2 当量）。然后将该反应混合物加热至 60±5℃。该反应在 60℃下搅拌直到原料消耗为止（至少 6 小时）。将该淤浆冷却至 20℃且添加 H_2O（9mL，1vol）。于该淤浆中添加于 H_2O（38mL，4vol）中的亚硫酸氢钠溶液（3.88g，1.0 当量）。使该淤浆粒化 1 小时，然后在 #2Whatman 滤纸上直接过滤。以水（19mL，2vol），然后以 7:3 的甲醇 / 乙腈混合物（28mL，3vol）清洗该反应容器，然后将该清洗液转移至该滤饼上。该产物在真空烘箱中于 50 至 55℃下干燥。分离出呈浅黄色固体形式的 6- 溴 -2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮（10.52g；87%）。

[0217] 通过从甲苯及正庚烷再结晶进一步纯化该产物。将甲苯（60mL，6vol）及 6- 溴 -2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮（10.00g，1 当量）添加至反应容器并加热至 80℃。将该温热反应混合物通过适当筒过滤以确保除去不可溶的 Pd 及其它不可溶的污染物。该过滤器筒以 80℃甲苯（5mL，0.5vol）清洗。以 1℃ / 分钟将该淤浆冷却至 25℃。以 1mL/ 分钟将正庚烷（70mL，7vol）添加至该反应淤浆。以 1℃ / 分钟将该淤浆进一步冷却至 0℃。该淤浆于 0℃粒化至少 1 小时。

[0218] 将该淤浆直接在 #2Whatman 滤纸上过滤。将正庚烷（30mL，3vol）装入该反应容器，并将清洗液转移至该滤饼上，且在真空烘箱中于 50 至 55℃下干燥该产物。分离出呈乳化色固体形式的 6- 溴 -2- 氯 -8- 环戊基 -5- 甲基 -8H- 吡啶并 [2,3-d] 嘧啶 -7- 酮（8.73g，87 %）。^1H NMR（500MHz，DMSO-d_6）：δ 9.20（s，1H），5.82（m，1H），2.65（s，3H），2.11（m，2H），2.04（m，2H），1.86（m，2H），1.64（m，2H）；^{13}C NMR（125MHz，DMSO-d_6）：δ 158.2，158.2，157.6，154.1，144.0，120.9，113.0，54.4，28.3，25.7，18.3；HRMS：$C_{13}H_{14}N_3O_1Br_1Cl_1(M+H)^+$ 的计算值：342.00033，发现值：342.00037。

[0219] 实施例 3. 4-{6-[6- 溴 -8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基] 吡啶 -3- 基 }- 哌嗪 -1- 甲酸叔丁酯的制备

[0220]



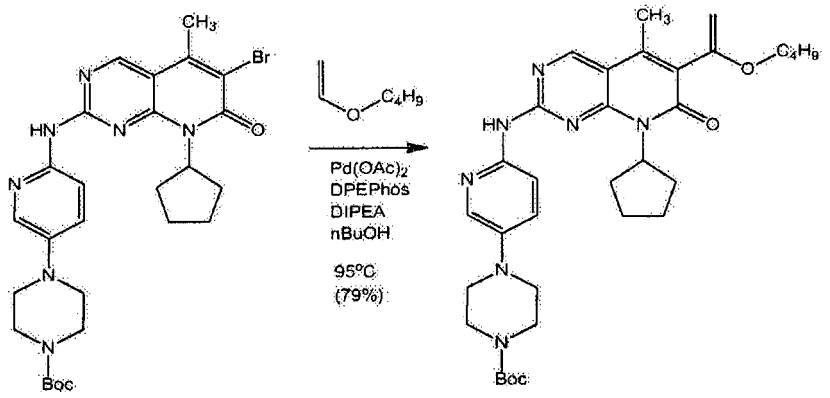
[0221] 以四氢呋喃(900mL,15mL/g)装填干燥、经氮气冲洗的反应器。批料温度设于20℃,且开始以250RPM搅动。以4-(6-氨基-吡啶-3-基)-哌嗪-1-甲酸叔丁酯(63.4g,0.2278摩尔,1.3当量)装填该反应器,并使该混合物保持在20℃下30分钟以使该原料溶解。通过泵在30分钟期间以氯化异丙基镁(93.9g,0.193摩尔,第1次装填1.1当量)(于THF中2.0M,1.1当量)装填反应器。该批料维持在20℃下40分钟。以6-溴-2-氯-8-环戊基-5-甲基-8H-吡啶并[2,3-d]嘧啶-7-酮(60.1g,0.1755摩尔,1当量)一次装填该反应器,并以THF冲洗(50mL冲洗)。通过泵在30分钟期间添加氯化异丙基镁的额外装填(93.9g,0.193摩尔,1.1当量,第2次装填(于THF中2.0M,1.1当量)。该批料保持在20℃下90分钟,然后从20℃加热至60℃。

[0222] 反应之后,THF(2.86vol)及HOAc(1当量)的混合物用于淬灭反应。然后以0.5wt/wt%的4-{6-[6-溴-8-环戊基-5-甲基-7-氧代-7,8-二氢-吡啶并[2,3-d]嘧啶-2-基氨基]-吡啶-3-基}-哌嗪-1-甲酸叔丁酯对该批料加晶种并装填THF(1.14vol)与HOAc(0.4当量)的混合物来完成沉淀。冷却至20℃之后,过滤该批料,以丙酮(4vol)、水(6vol)及丙酮(4vol)清洗。

[0223] 该湿滤饼在真空下于65℃干燥至恒定重量以产生4-{6-[6-溴-8-环戊基-5-甲基-7-氧代-7,8-二氢-吡啶并[2,3-d]嘧啶-2-基氨基]-吡啶-3-基}-哌嗪-1-甲酸叔丁酯,收率为93%。^{1}H NMR(600MHz,THF-d_8):δ 9.36(s,1H),8.87(s,1H),8.22(d,J = 8.8Hz,1H),8.04(d,J = 2.9Hz,1H),7.39(dd,J = 8.8,2.9Hz,1H),6.10(m,1H),3.55(宽,4H),3.09(宽,4H),2.60(s,3H),2.30(m,2H),2.09(m,2H),1.85(m,2H),1.66(m,2H),1.46(s,9H);^{13}C NMR(150MHz,THF-d_8):δ 159.5,158.9,157.7,156.0,155.0,147.2,144.62,144.56,138.0,126.7,117.6,114.2,108.4,79.9,55.5,50.6,44.7,29.0,28.7,26.9,18.1;HRMS:$C_{27}H_{35}N_7O_3Br_1$(M+H)$^+$的计算值:584.19797,发现值:584.19811。

[0224] 实施例4. 4-{6-[6-(1-丁氧基-乙烯基)-8-环戊基-5-甲基-7-氧代-7,8-二氢吡啶并[2,3-d]嘧啶-2-基氨基]-吡啶-3-基}-哌嗪-1-甲酸叔丁酯的制备

[0225]



[0226] 以 1- 丁醇 (60mL,6mL/g) 装填干燥、经氮气冲洗的反应器且添加 4-{6-[6- 溴 -8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢 - 吡啶并 [2,3-d] 嘧啶 -2- 基氨基]- 吡啶 -3- 基 }- 哌嗪 -1- 甲酸叔丁酯 (10g,0.017 摩尔) 及丁基乙烯基醚 (5.1g,0.051 摩尔,3.0 当量)。添加二异丙基乙胺 (5.3g,0.041 摩尔,2.4 当量) 且使氮气通过充气管为时 30 分钟来使该混合物充气。添加乙酸钯 (0.16g,0.00068 摩尔,0.0400 当量) 及双 (2- 二苯基膦基苯基) 醚 (0.45g,0.00082 摩尔,0.04800 当量)。在 30 分钟期间将该混合物加热至 95℃,且在 95℃下搅拌该批料 2 小时。将该混合物冷却至 80℃,并取样以监测反应完成。于完成之后,添加水 (15mL,1.5mL/g) 及 1- 丁醇 (30mL,3mL/g)。

[0227] 该溶液经由 0.45 微米的过滤器过滤以除去沉淀的钯。添加水 (35mL,3.5mL/g),然后添加 1,2- 二氨基丙烷 (6.3g,0.085 摩尔,5.0 当量)。该混合物在 70℃下搅拌至少 30 分钟。停止搅动,并使该混合物沉降 15 分钟。分离出底部水相,并在 30 分钟期间使该混合物冷却至 60℃。以 4-{6-[6-(1- 丁氧基 - 乙烯基)-8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基] 吡啶 -3- 基 }- 哌嗪 -1- 甲酸叔丁酯 (C 型) (50mg,0.005g/g) 对该混合物加晶种并保持于 60℃为时 90 分钟。

[0228] 一旦观察到结晶,在 1 小时期间将该混合物冷却至 50℃,并在 50℃保持 3 小时。在 3 小时期间将该混合物冷却至 30℃,并在 30℃保持 2 小时,然后在 4 小时期间冷却至 20℃,并在 20℃保持 4 小时。过滤该淤浆并以 1- 丁醇 (10mL,1mL/g) 清洗。排出该滤饼,并装填 1- 丁醇 (10mL,1mL/g),且在 20℃下搅拌该淤浆 1 小时。排出该滤饼。用甲基叔丁基醚 (20mL,2mL/g) 清洗该混合物,并使用延长吹过时间 (2 小时或更长) 使该滤饼完全脱液。在 70℃下干燥该滤饼。收率为 75 至 80%。^{1}H NMR(600MHz,DMSO-d_6) : δ 10.0(s,1H),8.87(s,1H),8.07(d,J = 2.9Hz,1H),7.91(d,J = 9.0Hz,1H),7.48(dd,J = 9.0,2.9Hz,1H),5.83(m,1H),4.47(d,J = 1.6Hz,1H),4.05(d,J = 1.6Hz,1H),3.77(t,J = 6.4Hz,2H),3.48(宽,4H),3.11(宽,4H),2.37(s,3H),2.22(m,2H),1.89(m,2H),1.75(m,2H),1.61(m,2H),1.58(m,2H),1.43(s,9H),1.38(m,2H),0.90(t,J = 7.39Hz,3H) ;^{13}C NMR(150MHz,DMSO-d_6) : δ 160.9,158.2,157.3,155.2,154.6,153.7,145.0,143.0,142.6,136.0,125.8,125.5,114.6,106.6,87.8,78.9,66.8,52.8,48.5,43.4,42.5,30.3,28.0,27.4,25.1,18.8,14.4,13.6 ;HRMS:$C_{33}H_{46}N_7O_4$(M+H)$^+$的计算值 :604.36058,发现值 :604.36049。

[0229] 所述中间体丁氧基 - 乙烯基醚可被分离成数种多晶型之一。A 型在不加晶种的情况下作为动力学产物分离,而 B 型在少数情况下分离但极少观察到。通过以 C 型晶体对反

应混合物加晶种获得丁氧基－乙烯基醚的最稳定晶型，即 C 型。这些多晶型中的任一种可用于制备化合物 1 游离碱，但因为容易过滤，丁氧基－乙烯基醚的 C 型为优选。

[0230] 所述中间体丁氧基－乙烯基醚的 A、B 及 C 型多晶型的 PXRD 数据分别列表于表 7、8 及 9。

[0231] 表 7. 中间体丁氧基－乙烯基醚的 A 型多晶型的 PXRD 数据

[0232]

2θ(°)±0.2	峰强度(%)
4.3	100
4.8	85
6.2	39

[0233] 表 8. 中间体丁氧基－乙烯基醚的 B 型多晶型的 PXRD 数据

[0234]

2θ(°)±0.2	峰强度(%)
5.5	100
7.5	3
9.7	3
11.1	4
14.8	3
16.7	4
17.5	5
20.1	4

[0235] 表 9. 中间体丁氧基－乙烯基醚的 C 型多晶型的 PXRD 数据

[0236]

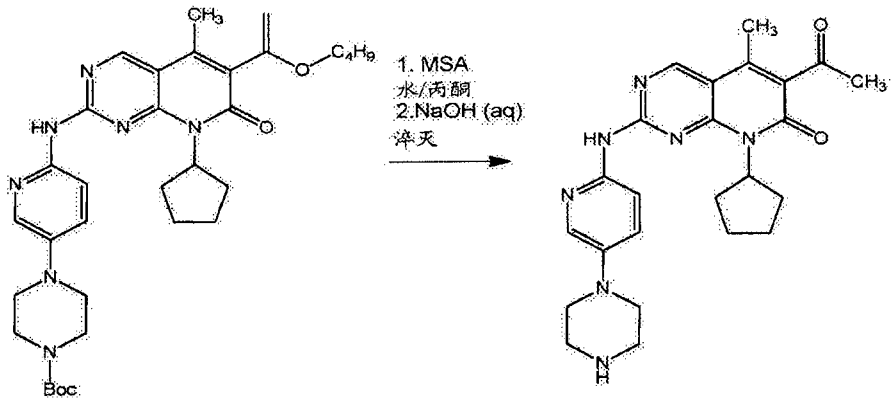
2θ(°)±0.2	峰强度(%)
5.4	100
9.7	11
10.8	58
12.7	10

13.3	24
13.5	27
16.1	12
16.6	8
17.0	14
17.5	22
18.1	8
18.8	8
19.6	16
20.6	16
21.7	17
22.9	8
23.8	8
24.4	8
25.0	8

[0237]

[0238] 实施例 5. 通过盐断裂法制备小粒径化合物 1 游离碱

[0239]



[0240] 于反应器中添加 4-{6-[6-(1- 丁氧基 - 乙烯基)-8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基]- 吡啶 -3- 基 }- 哌嗪 -1- 甲酸叔丁酯

(2.70kg,4.47 摩尔,1.0 当量),然后添加水 (27.00L,10L/kg) 及丙酮 (13.50L,5L/kg) 的混合物。将该黄色淤浆加温至介于 50℃与 55℃之间。在大约 10 分钟期间,将以水 (5.40L,2L/kg 的原料) 及丙酮 (5.40L,2L/kg 的原料) 稀释的甲磺酸溶液 (2.15kg,22.36 摩尔,5.0 当量) 添加至该反应器。使反应混合物保持在 45℃与 55℃之间至少 12 小时。在该反应期间获致透明黄色溶液。

[0241] 将该反应混合物冷却至 35℃,并将 5wt%的氢氧化钠溶液的混合物分数部分添加至该反应器以使该反应混合物升高至 pH>9。将该反应器冷却至介于 20℃与 25℃之间,粒化及过滤。以水然后以丙酮清洗该滤饼,并在真空下干燥。

[0242] 该方法产生小原生粒径化合物 1 游离碱,其与从 WO 2005/005426 的实施例 4 中使用 NaOH 水溶液处理化合物 1 盐酸盐所制备的物质等效。

[0243] 除了上文提供的代表性程序 (对应于表 10 中实验 S) 以外,还筛选了一定范围的酸及水性溶剂系统以测定对该反应及随后淬灭及分离化合物 1 游离碱的影响。进行了实验室规模筛选实验以鉴别用于将中间体乙烯基醚转化成游离碱化合物 1 的反应条件。这些反应筛选实验的结果汇总于表 10 中,其表明所述方法的一般性。

[0244] 表 10 :得自反应筛选实验的结果汇总

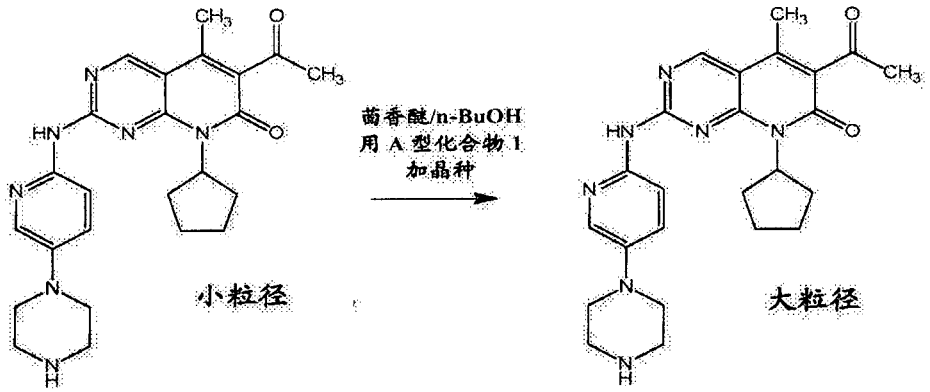
[0245]

实验	酸	溶剂系统	收率	纯度
A	羟乙磺酸	水	99	99.93
B	羟乙磺酸	16% THF/ 水	>100	98.77
C	羟乙磺酸	28% THF/ 水	95	97.95
D	HCl	水	>100	99.59
E	H_2SO_4	水	98	98.6
F	MSA	水	98	99.42
G	MSA	16% THF/ 水	>100	97.86
H	羟乙磺酸	15% NMP/ 水	88	97.7
I	羟乙磺酸	15% DMF/ 水	90	98.94
J	TFA(8 当量)	水	100	99.14
K	羟乙磺酸	15% CH_3CN/ 水	>100	99.56
L	羟乙磺酸	15%丙酮 / 水	92	99.54
M	羟乙磺酸	15% DMAC/ 水	>100	98.91

N	羟乙磺酸	15%环丁砜 / 水	92	98.67
O	MSA	15% CH_3CN/ 水	100	99.52
P	MSA	15%丙酮 / 水	97	99.54
Q	CF_3SO_3H（不完全）	水	N/A	N/A
R	MSA	33% CH_3CN/ 水	99	99.7
S	MSA	33%丙酮 / 水	98	99.74
T	MSA	33% MeOH/ 水	98	99.74
U	MSA	33% THF/ 水	96	99.76

[0246] 实施例 6. 化合物 1 的小粒径游离碱成为大粒径游离碱的转化

[0247]

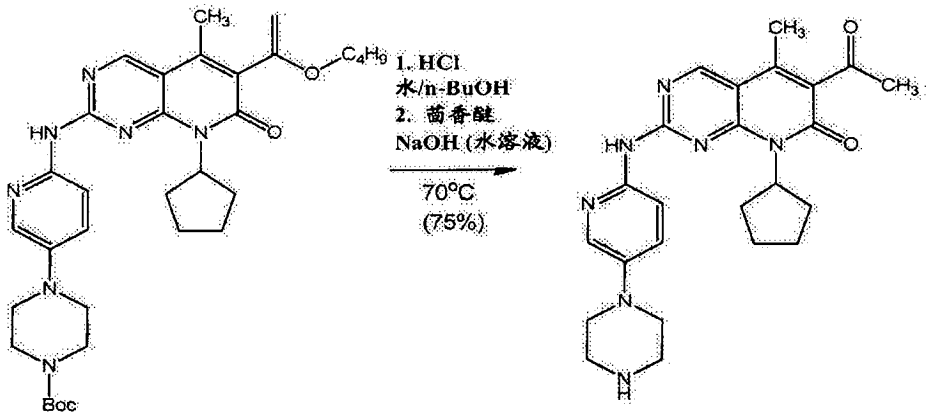


[0248] 于反应器中添加根据实施例 5 制备的化合物 1 游离碱（20g，44.69 毫摩尔，1.0 当量），然后添加 1- 丁醇（320mL，16ml/g）与茴香醚（480mL，24ml/g）。将该黄色淤浆加温至介于 95℃与 100℃之间以获得溶解。将该反应器冷却至 80℃。于该反应器中的溶液中，装填含有悬浮液于 1- 丁醇（5mL，0.25mL/g 的原料）中的化合物 1 游离碱（A 型）晶种（0.1g，0.2 毫摩尔，0.005 当量）的晶种淤浆以引发结晶。将所得到的淤浆于 80℃下搅拌 3 小时。将该淤浆以 0.2℃ / 分钟在 350 分钟期间冷却至 10℃、粒化及过滤。以茴香醚然后以庚烷清洗该滤饼，并在真空下干燥它。

[0249] 此方法产生了化合物 1 游离碱的大原生粒径晶体，其与使用下文实施例 7 中所述的一锅法所制备的游离碱等效。

[0250] 实施例 7. 用于制备大粒径化合物 1 游离碱的一锅法

[0251]



[0252] 于反应器中添加水 (200mL,10mL/g) 及 4-{6-[6-(1- 丁氧基 - 乙烯基)-8- 环戊基 -5- 甲基 -7- 氧代 -7,8- 二氢吡啶并 [2,3-d] 嘧啶 -2- 基氨基]- 吡啶 -3- 基 }- 哌嗪 -1- 甲酸叔丁酯 (20g,33.1 毫摩尔,1.0 当量),然后添加 1-BuOH(232mL,11.6mL/g) 以将任何固体冲入反应器。将该黄色淤浆加温至 70℃。形成两液相混合物。在大约 10 分钟期间将浓 HCl 溶液 (16.3g,165.5 毫摩尔,5.0 当量) 加入该反应器。使该反应混合物保持在 70℃为时 4 至 6 小时。在 3 小时后获致透明黄色双相溶液。

[0253] 于该反应混合物中添加茴香醚 (356mL,17.8mL/g)。在将该混合物维持在 70℃时,在 20 分钟期间将 NaOH(17.2g,172.1 毫摩尔,5.2 当量) 水溶液 (40wt% 溶液) 添加至该反应器以使该反应混合物提高至 pH>10。于 NaOH 添加完成之后,搅拌该两相混合物 30 分钟。

[0254] 将所述相分离并以水清洗有机相两次。然后将该批料加热至 80℃并无碎屑过滤至结晶容器,以丁醇冲洗该过滤器。然后蒸馏该批料以除去水并达到 120℃的温度。然后将该批料冷却至 80℃,以化合物 1 游离碱 (A 型) 晶种晶体 ((0.015g,0.033 毫摩尔,0.1wt% wrt 化合物 1) 及 1-BuOH(10mL,0.5mL/g) 的晶种淤浆加晶种。然后以 0.2℃ / 分钟将该批料冷却至 30℃,然后以每次使温度逐步下降 10℃的三个回合使其成熟。在最终回合时,将该批料冷却至 10℃、粒化及过滤。以庚烷清洗该滤饼两次,并在真空下干燥。干燥后,确认该样品为单晶 A 型多晶型。

[0255] ^{1}H NMR(600MHz, DMSO-d_6/TFA) : δ 10.41(s, 0.75H), 9.03(s, 0.25H), 8.98(s, 2H), 8.12(d, J = 3.0Hz, 1H), 7.90(d, J = 9.1Hz, 1H), 7.63(dd, J = 9.1, 3.0Hz, 1H), 5.84(m, 1H), 3.40(宽,4H), 3.29(宽,4H), 2.43(s, 3H), 2.33(s, 3H), 2.21(m, 2H), 1.91(m, 2H), 1.79(m, 2H), 1.59(m, 2H) ;^{13}C NMR(150MHz, DMSO-d_6/TFA) : δ 202.4, 160.7, 154.8, 158.3, 158.0, 144.9, 142.3, 142.0, 134.6, 129.7, 126.7, 115.3, 107.0, 53.0, 45.6, 42.6, 31.3, 27.6, 25.2, 13.7 ;HRMS:$C_{24}H_{30}N_7O_2$(M+H)$^+$的计算值 :448.24555, 发现值 : 448.24540。

[0256] 以下提供化合物 1 游离碱的小原生粒径及大原生粒径制剂的对比 PSA、SSA 及表面能数据。在所有情况下,所述游离碱作为 A 型多晶型分离。

[0257] 粉末 X 射线衍射 (PXRD)

[0258] 实验 :

[0259] 使用配备 Cu 辐射源、固定狭缝 (发散 = 1.0mm, 抗散射 = 0.6mm 及接收 = 0.6mm) 及闪烁计数器检测器的 Bruker D8 衍射仪进行粉末衍射分析。数据于 Cu 波长

$K\alpha_1$ =1.54056Å 从 3.0 至 40.0 度的 2θ 使用 0.040 度的步幅及 2.0 秒的步时收集在 θ-θ 测角仪中。X 射线管电压及安培值分别设于 40kV 及 40mA。样品通过放置在镍盘 (Gasser&Sons, Inc. Commack, NY) 中来制备并于数据收集期间使其旋转。收集数据并使用 Bruker DIFFRAC Plus 软件 (2.6 版) 分析。PXRD 数据文件 (.raw) 在峰搜寻之前未经处理。通常,使用临限值 1 及宽度值 0.3 来进行初步峰指定。目视检查自动化指定的输出以确保有效性及视需要进行人工调整。另外,若情况适当,在光谱内人工指定峰。

[0260] SSNMR 实验 :

[0261] A 型的碳光谱在 4mm 转子上 2048 次扫描获取,其中再循环延迟为 25 秒及交叉极化为 2ms。于获取期间应用 100kHz 质子去耦。B 型的碳光谱在 4mm 转子上 2048 次扫描获取,针对再循环延迟为 4.5 秒及交叉极化为 2ms 的 128 次扫描收集。于获取期间应用 70kHz 质子去耦及旋转边带总抑制 (TOSS)。

[0262] 仪器方法 :

[0263] 将大约 80 毫克的样品装入 4mm ZrO_2转子。在周围温度及压力下,于定位于大口径 Bruker-Biospin Avance III 500MHz (^{1}H 频率) NMR 光谱仪内的 Bruker-Biospin 4mm CPMAS 探针上收集光谱。该经装填的转子以魔角定向且在 15.0kHz 下旋转。^{13}C 固态光谱使用质子去耦交叉极化魔角旋转 (CPMAS) 实验收集。交叉极化接触时间设为 2.0ms。在获取期间施加大约 100kHz 的质子去耦场。获取再循环延迟为 25 秒的 512 次扫描的化合物 1A 型的碳光谱。该光谱示于图 2,且数据列表于表 2。获取再循环延迟为 4.5 秒的 2048 次扫描的化合物 1B 型的碳光谱。这些碳光谱使用高场共振设于 29.5ppm 的结晶金刚烷的外部标准参考。该光谱示于图 4 中,且数据列表于表 4 中。

[0264] 粒径分析

[0265] 粒径使用激光衍射 (或小角度光散射) 技术,通过压缩空气分散干燥样品粉末来分析。特别是,该粒径分布使用配备 Vibri 干粉进料器的 Sympatec HELOS RODOS 系统来分析。该粉末样品以 0.5 巴的分散压力来分散。在一些实施例中,使用 Aspiros 微调剂装置,且以 0.2 巴的分散压力分散该粉末样品。选择适用的透镜以涵盖每一样品的粒径范围。

[0266] 结果

[0267] API 四个批料的对照数据提供于下表 11 中,使用 Vibri 或 Aspiros 装置来分散样品。4 号批料具有约 75 μm 的 D90,然而 1 号及 2 号批料二者均具有大约 45 微米的 D90。激光衍射粒径数据确认这些批料的 SEM 观察。

[0268] 表 11. 比较性尺寸分布数据

[0269]

PSD 数据的概述		粒径(μm)			
批次 No.	分散方法	D[v,0.1]	D[v,0.5]	D[v,0.9]	D[4,3]
1	0.2 Bar ASPIROS	5.21	17.00	43.59	21.33
2	0.2 Bar ASPIROS	6.20	20.83	46.15	23.87
3	0.2 Bar ASPIROS	11.64	46.08	130.26	59.07
	0.5 bar VIBRI	9.96	41.23	116.43	53.02
4	0.2 Bar ASPIROS	7.41	24.97	76.56	35.06
	0.5 Bar VIBRI	6.33	23.19	69.20	32.16

[0270] 扫描电子显微术 (SEM)

[0271] 扫描电子显微术在标准条件下进行。图 5 提供从 40% BuOH/ 茴香醚再结晶的化合物 1 游离碱 A 型的 SEM(200 倍放大倍率）图像。图 6 提供从标准游离碱化程序分离的化合物 1 游离碱 A 型的 SEM(1500 倍放大倍率）图像。

[0272] 黏附分析

[0273] 内部开发 MASS(用于黏附的物质粘着筛选）冲压以通过在一系列压制之后秤重可除去冲压尖端上黏附的粉末的量来定量评估片剂制剂的黏附倾向。此测试使配制者能客观且迅速评估药品发展期间的冲压黏附风险以及排除临床片剂制备期间所观察到的黏附问题。

[0274] 为了制备 MASS 冲压用的样品，将 10g 的 API 稀释于经稍微润滑的标准掺合物中（10% API，89.75% Avicel PH102 及 0.25%硬脂酸镁）且在瓶（500mL 琥珀色玻璃瓶）中旋转 500 次掺合的。使用微量天平，在目前固体摩擦力为 0.85 下定期评估至高达 100 次压制～ 250mgW 片剂时黏附于该可除去冲压尖端（1/2″ 圆形平面）的粉末重量。

[0275] 混合于该标准掺合物中的化合物 1 游离碱的 MASS 冲压曲线显示正响应。压制结束时的冲压尖端的相片确认粉末黏附于这些尖端（数据未显示）。为了供参考，该标准掺合物的对照样品不具黏附性，且具有少于 10 μg 的黏附粉末。已发现该测试方法将新 API 批料的黏附倾向相对于已知物质的黏附做排序。

[0276] 比表面积 (SSA) 测量 (BET 氮）

[0277] 装置

[0278] 比表面积 (SSA) 测量 (BET 氮）使用 Micromeritics TriStar II 3020 比表面积分析仪与 Micromeritics SmartPrep 站 (Micromeritics U.K.Ltd.,Ste 2,The Stables Hexton Manor,Hexton,Hertfordshire SG53JH, 英格兰）一起测定。对样品进行该 BET-氮吸附分析以测定这些样品的比表面积。

[0279] 设定

[0280] 软件版本 TriStar II Confirm(1.03 或等效物）

[0281] 被吸附物：氮

[0282] 样品管：具有玻璃填料棒的 3/8″mm 平底单元

[0283] 样品量*：单元的大约 3/4 满

[0284] 样品制备：SmartPrep（使用氮流动脱气）

[0285] 脱气条件：于 25℃在气体流下 16 小时（升温速率为 10℃ / 分钟）

[0286] 等温套：使用

[0287] 等温收集点：在 0.05-0.30P/Po 范围中的 11 点 BET

[0288] 等温数据分析范围：在 0.05-0.20P/Po 范围中的 7 点 BET

[0289] 渗漏测试：120s

[0290] 自由空间：测得

[0291] 抽空时间：1hr

[0292] 脱气测试持续期间：180s

[0293] 平衡化间隔：10s

[0294] 平衡化时限：600s

[0295] *样品的量根据测试样品的粒径而变化。就粒径相对小的样品而言，需要大约 0.50g 的物质以填满该单元圆头的 3/4，及而样品的粒径较大的情况下，需要 0.75g 的物质填满该单元圆头的 3/4。

[0296] 计算及报告

[0297] 使用来自一式三份测定的 7 点 BET 报告在 0.05-0.20P/Po 范围内的比表面积。测定各复制物的样品量、比表面积、BET 常数（“C”值）及相关数。

[0298] 结果

[0299] 表 12 提供 4 批化合物 1 游离碱 API 批料的 BET-N2SSA，其中一者包含通过传统盐断裂法所制备的小原生粒径 API（批料 5），及三种批料包含根据本发明所制备的大粒径 API。批料 5 含有具有小初级粒子及大型聚结物的化合物 1 游离碱，其非常容易产生静电及非常粘。批料 6 使用温度循环制备，且具有大粒径化合物 1 游离碱的典型粒径分布（PSD），VMD 为大约 17 微米。批料 7 显示与批料 6 相似的 PSD。批料 8 为显示大粒径化合物 1 游离碱的代表性 ICH 批料，其亦通过温度循环制备。在以下表面能测定中使用相同批料。

[0300] 表 12. 使用 N_2 的 BET SSA

[0301]

批次 No.	使用 N_2 的 BET SSA
5	6.6
6	0.62
7	0.69
8	0.67

[0302] 反相气相色谱法（IGC）表面能测量：

[0303] 将充分量的样品装填于硅烷化玻璃管中，这些粉末通过插在两端的玻璃羊毛塞固定在该管内。该管通过使干燥氮气流流动经过所述粉末充分时间以使任何表面被吸附物被除去来调理。通过将一系列烷烃蒸气探针（壬烷、辛烷、庚烷及己烷）注入该载体气体流使

其浓度低到足以假设所述烷烃蒸气在所述氮气流中无限稀释，并记录各蒸气经由该管洗出所花费的时间来进行测量。保留时间（针对该装填的管内的填隙空间的"死体积"校准）与横断面积及所使用的烷烃蒸气探针分子的表面张力的函数作图产生直线，斜率表示被检验的固态粉末的表面能。

[0304] 结果

[0305] 表 13 提供针对 SSA 数据所产生的四批化合物 1 游离碱批料（即，上述批料 5 至 8）的分散表面能（mJ/m^2）数据。批料 5 为小粒径游离碱，及批料 6 至 8 包括大粒径游离碱 API。

[0306] 表 13. 分散表面能（mJ/m^2）

[0307]

批次 No.	分散表面能（mJ/m^2）
5	61.63
6	49.42
7	35.75
8	42.27

[0308] 本说明书中所提及的所有公开案及专利申请及其中引用的所有参考数据以引用方式并入本申请中的程度如同个别公开案或专利申请或参考数据明确个别指示为以引用方式并入。虽然前述发明已经由为求清楚理解的说明及实施例而详细描述，但本技术的一般技术人士根据本发明教示将很容易理解可对其进行特定改变及修改而不违背附录申请专利范围的精神与范围。

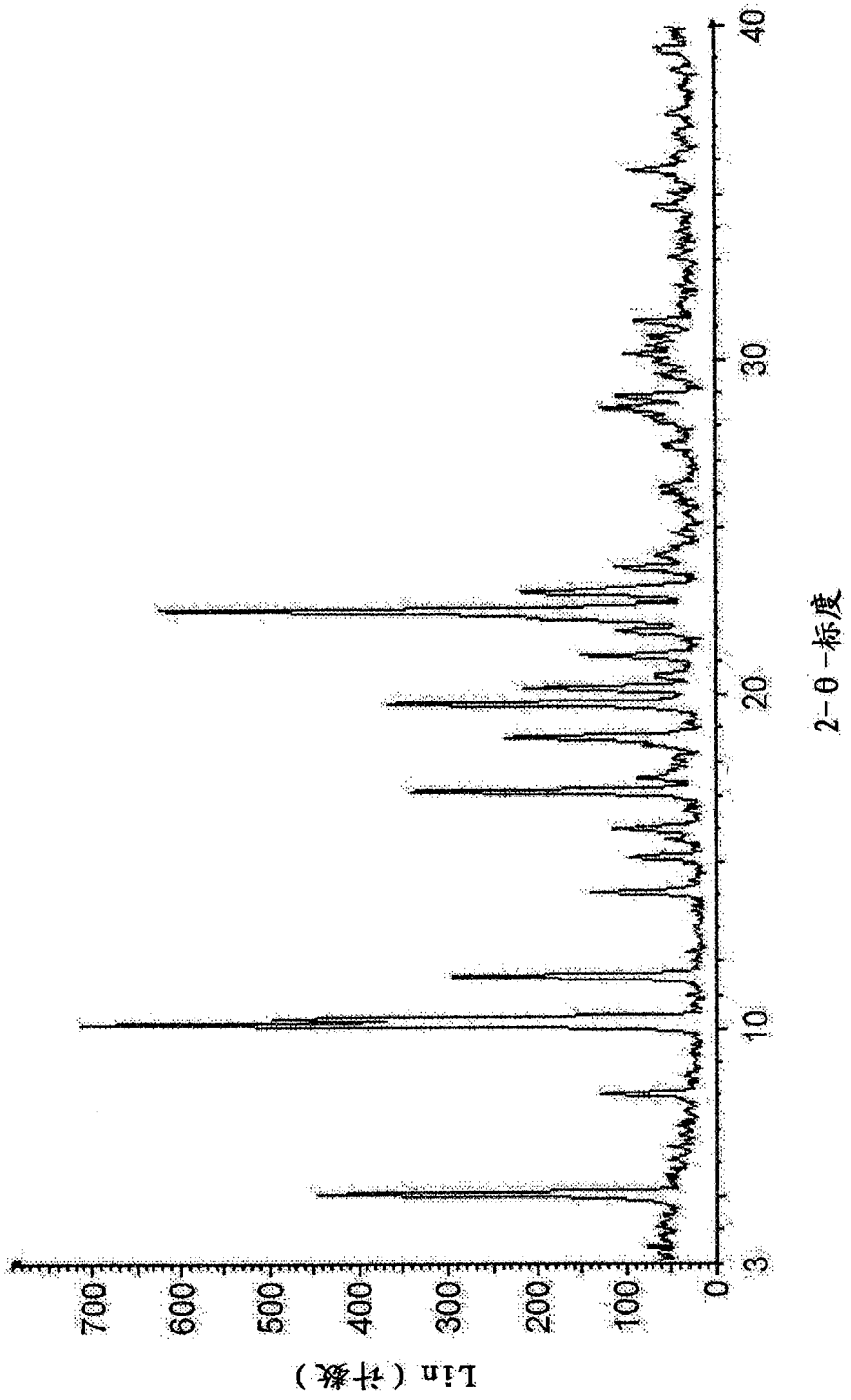


图 1

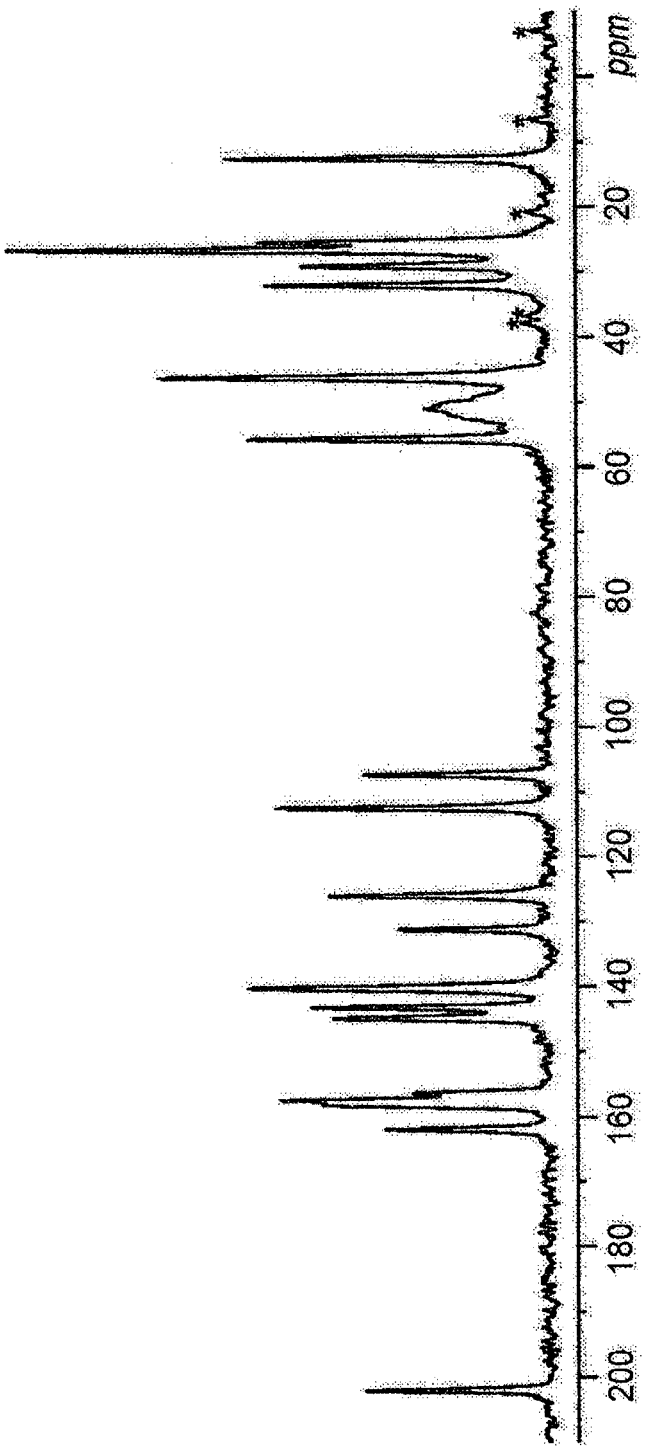


图 2

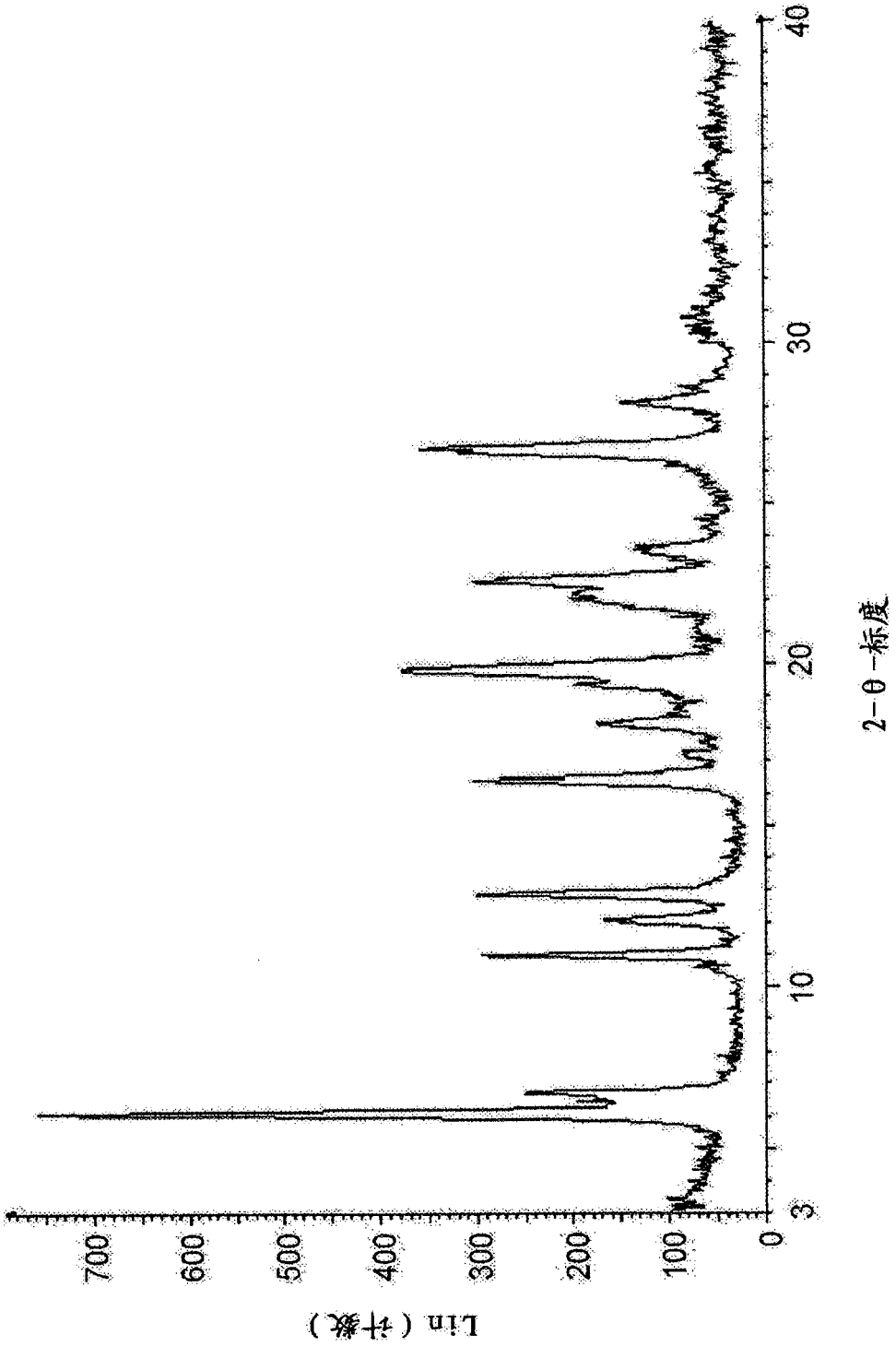


图 3

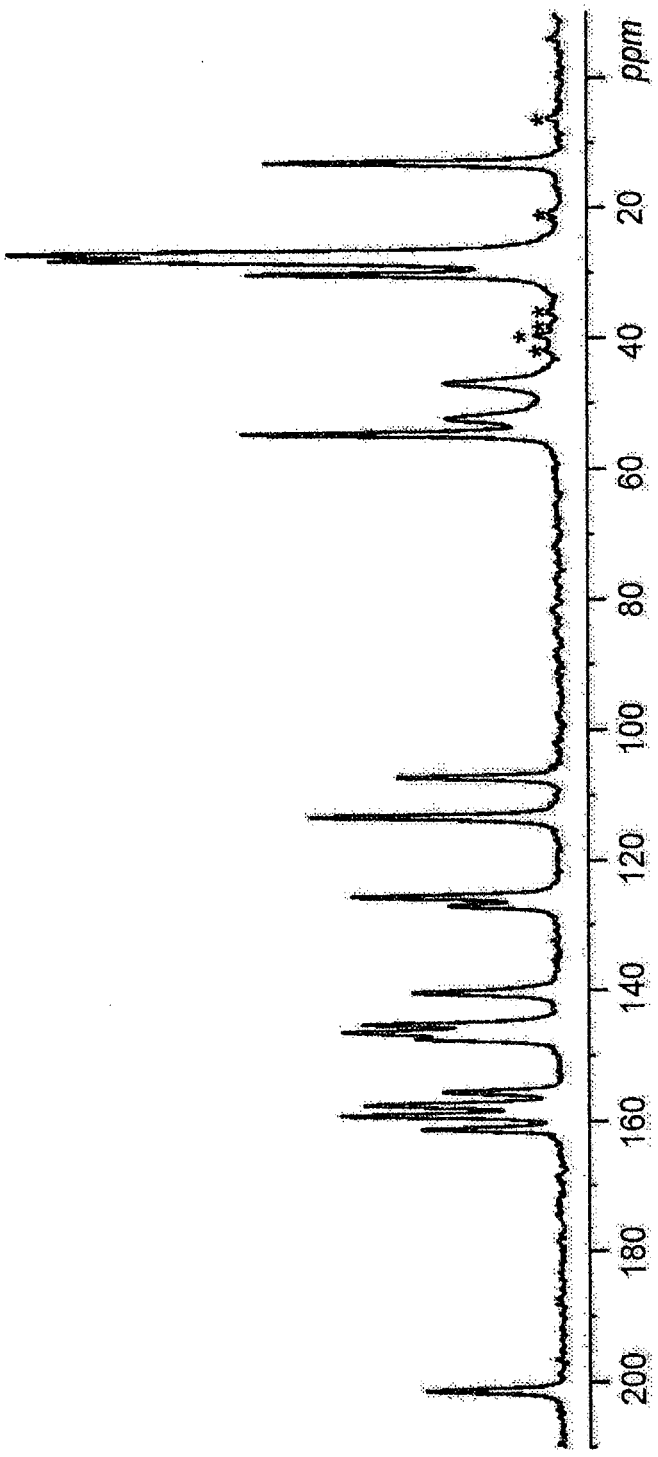


图 4

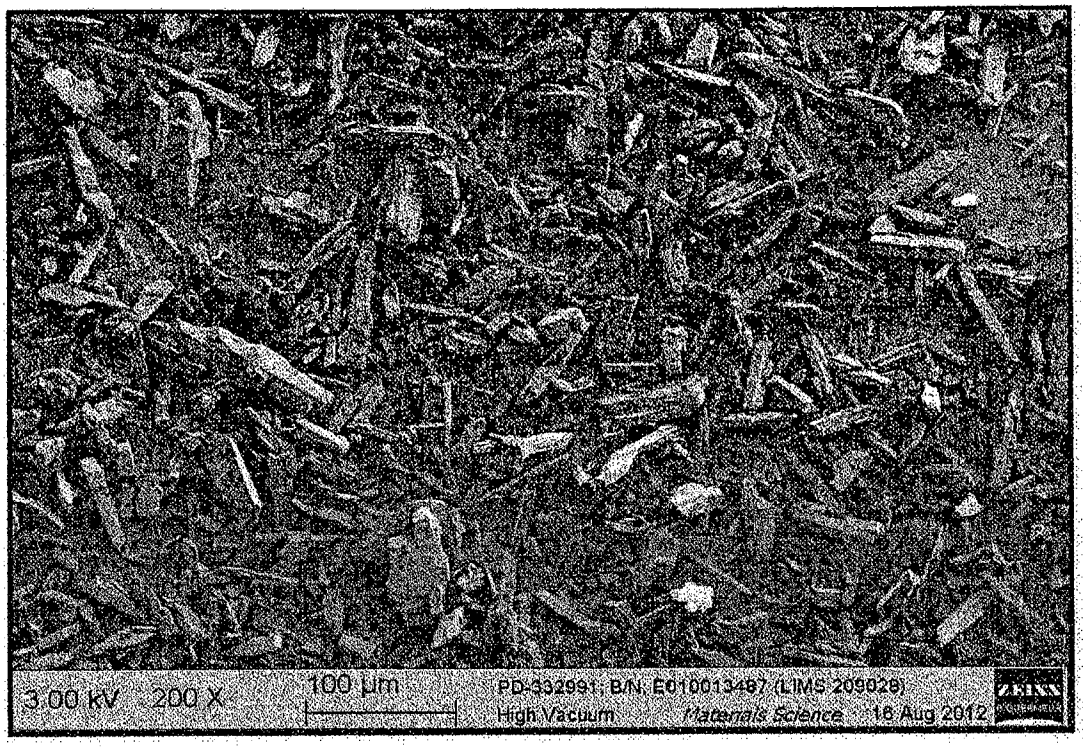


图 5

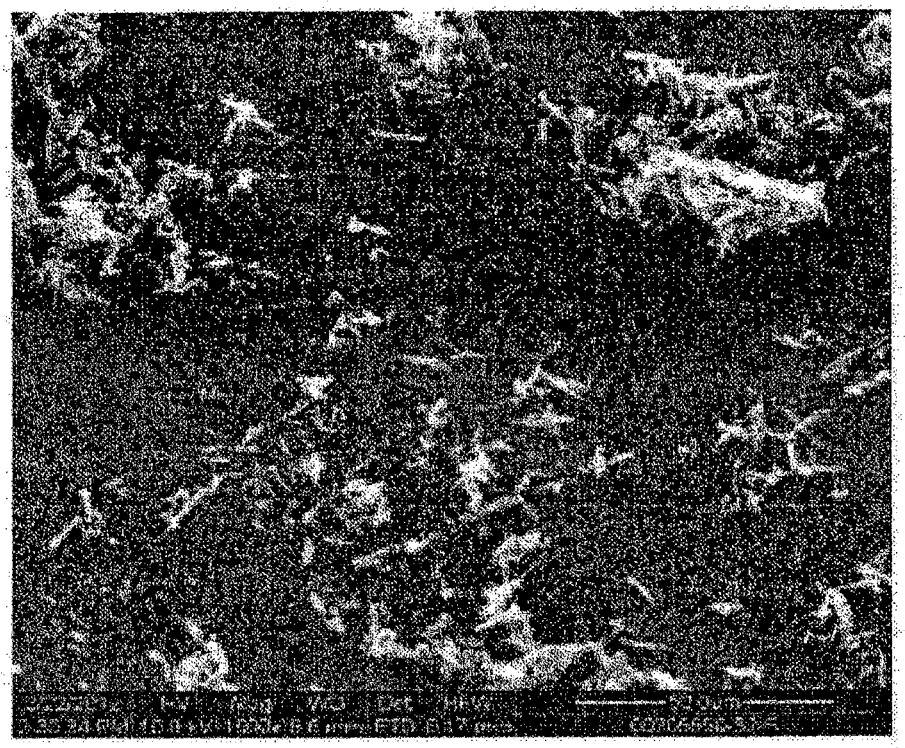


图 6

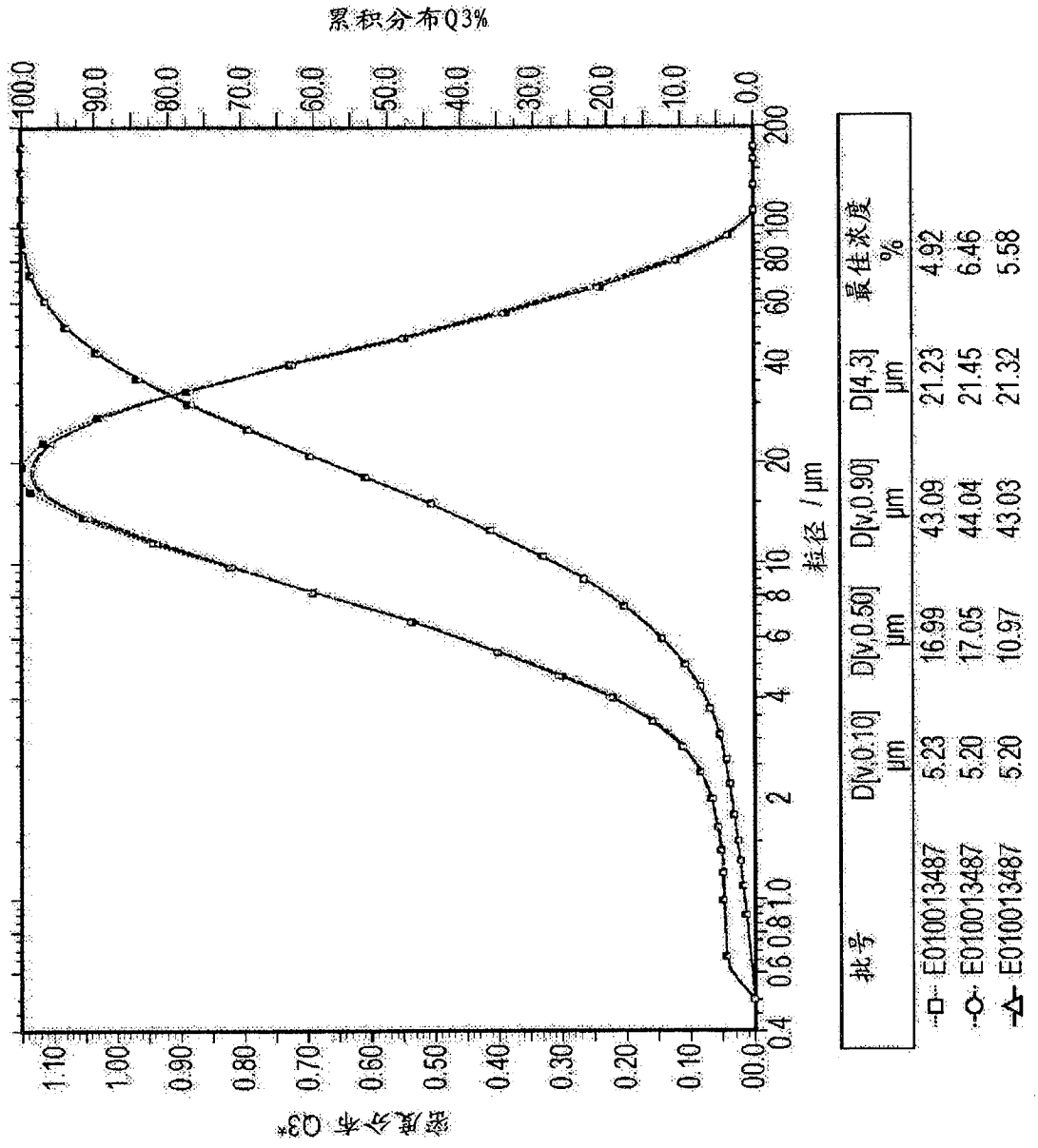


图 7

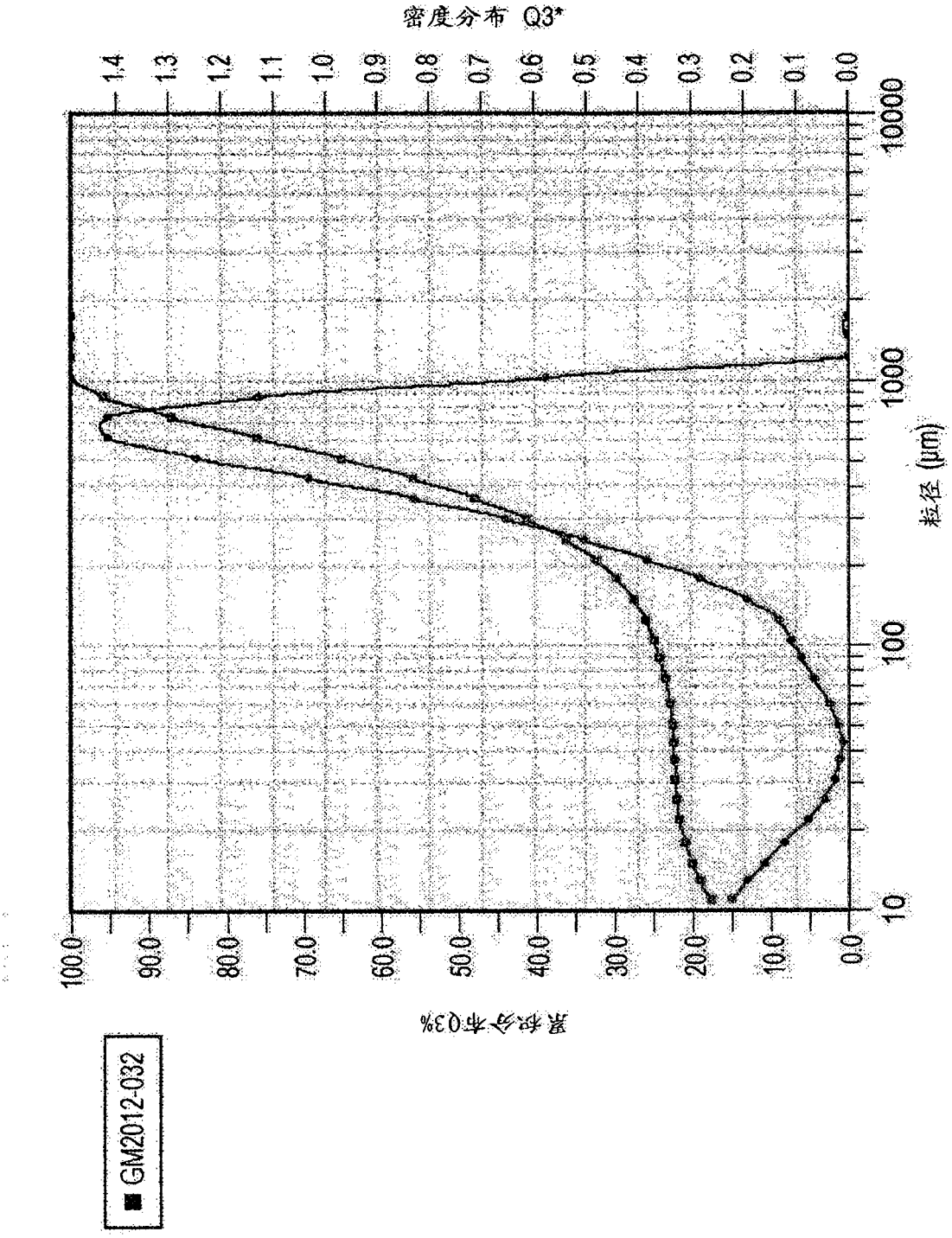

密度分布 Q3*
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10000
1000
100
10
粒径 (μm)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
累积分布Q3%
GM2012-032

图 8

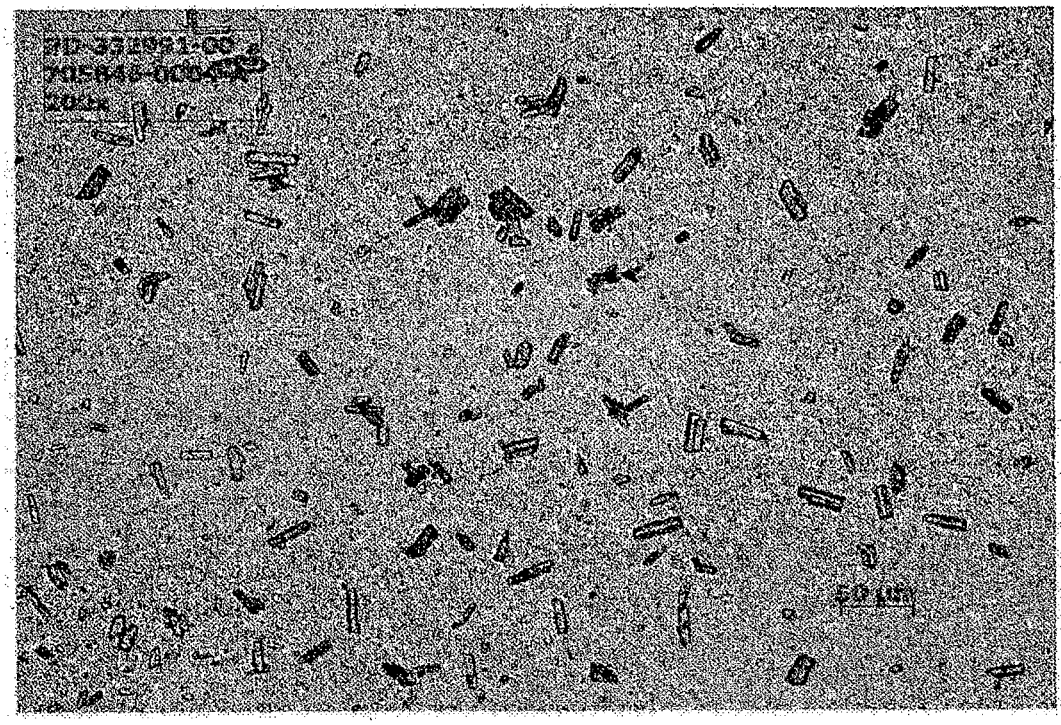


图 9