

CRYSTALLINE FORM

The present specification claims benefit of priority to European Patent Application No. 23161547.7, filed 13 March 2023, the content of which is hereby incorporated by reference in its entirety for all purposes.

Technical Field

The present specification relates to a crystalline form of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione, and to compositions and uses thereof.

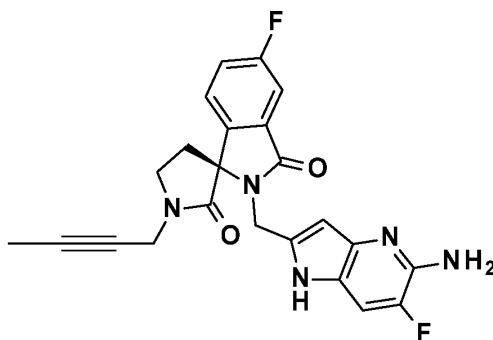
Background

Protein arginine methyltransferase 5 (PRMT5) is a member of the PRMT family of arginine methyltransferase enzymes that catalyse the addition of methyl groups to the guanidine motif of arginine residues, using S-adenosyl-L-methionine (SAM) as a methyl donor. PRMT5 is a type II arginine methyltransferase that symmetrically dimethylates the guanidine group of arginine residues thus converting a guanidine NH₂ group of arginine to a NMe₂ group. PRMT5 methylates a number of diverse substrates including histone and non-histone proteins, and in so doing regulates processes such as RNA splicing, cellular proliferation and DNA repair. Significantly, PRMT5 is overexpressed in a number of cancer types and has been identified as a candidate for therapeutic intervention through the development of small molecules that inhibit PRMT5 methyltransferase activity (see e.g. Kim et al., (2020) Cell Stress 4(8) 199-2151).

Cyclin dependent kinase inhibitor 2A (CDKN2A) is a tumour suppressor gene that is homozygously deleted in approximately 15% of cancers. Loss of the 9p21 chromosome locus results in the co-deletion of a number of additional genes including the gene encoding methylthioadenosine phosphorylase (MTAP). MTAP is a metabolic enzyme involved in methionine salvage and loss of MTAP results in increased concentrations of the MTAP substrate methylthioadenosine (MTA) in CDKN2A/MTAP deleted cancer cells. MTA itself acts as a weak PRMT5 inhibitor and MTA accumulation in CDKN2A/MTAP deleted cancer cell lines accordingly leads to a partial inhibition of PRMT5 activity. Compromised PRMT5 activity renders CDKN2A/MTAP deleted cancer cells susceptible to further targeting of PRMT5, for example using short hairpin RNA (shRNA). A "collateral vulnerability" in cancer, where CDKN2A/MTAP deleted tumours may be selectively targeted through PRMT5 inhibition, has been identified (see Marjon et al., (2016) Cell Reports 15, 574-587; Mavrakis et al., (2016) Science 11;351(6278):1208-13; Kryukov et al., (2016) Science 11;351(6278):1214-8).

“MTA-synergistic” PRMT5 inhibitors (i.e. inhibitors which bind to PRMT5 preferentially in the presence of MTA) exert a greater inhibitory effect on PRMT5 in environments where relatively high concentrations of MTA are present such as CDKN2A/MTAP deleted tumour cells and not in healthy tissues. Consequently, “MTA-synergistic” PRMT5 inhibitors should possess a high therapeutic index (and low off target toxicity) as their anti-proliferative activity will selectively manifest in the targeted, CDKN2A/MTAP deleted, tumour cells. Several “MTA-synergistic” PRMT5 inhibitors have entered clinical trials, such as MRTX-1719 (NCT05245500); TNG-908 (NCT05275478); TNG-462 (NCT05732831); AMG-193 (NCT05094336, NCT05094336); and AZD3470 (NCT06130553; NCT06137144). However, to date no inhibitors of PRMT5, let alone “MTA-synergistic” PRMT5 inhibitors, have been approved for therapeutic use.

PCT/EP2022/075248 (published as International Publication Pamphlet WO2023/036974) discloses PRMT5 inhibitors, particularly MTA-synergistic PRMT5 inhibitors, including the compound (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[indoline-3,3'-pyrrolidine]-1,2'-dione (herein referred to as Compound (I)):



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Compound (I),

alongside its activity as an inhibitor of PRMT5 enzyme in MTA present (IC_{50} 0.006 μ M) and MTA absent (IC_{50} 0.027 μ M) assays, its activity in HCT116 wild type cells (IC_{50} 0.2 μ M) and HCT116 MTAP knock-out cells (IC_{50} 0.0059 μ M), and its activity against cell proliferation in HCT116 wild type cells (IC_{50} 6.1 μ M) and HCT116 MTAP knock-out cells (IC_{50} 0.24 μ M). Therefore,

Compound (I) is a PRMT5 inhibitor and is in particular an MTA-synergistic PRMT5 inhibitor. To further study the therapeutic potential of Compound (I) it is desirable to have solid forms of the compound with suitable properties for pharmaceutical development.

In the formulation of drug substances, it is important for the drug substance (active compound) to be in a form in which it can be conveniently handled and processed. This is of importance, not only from the point of view of obtaining a commercially-viable manufacturing process for the drug substance itself, but also from the point of view of subsequent manufacture of pharmaceutical formulations comprising the active compound and suitable excipients. The chemical stability and the physical stability of the active compound are important factors in determining the suitability of a solid form for use in the development of pharmaceutical formulations. The active

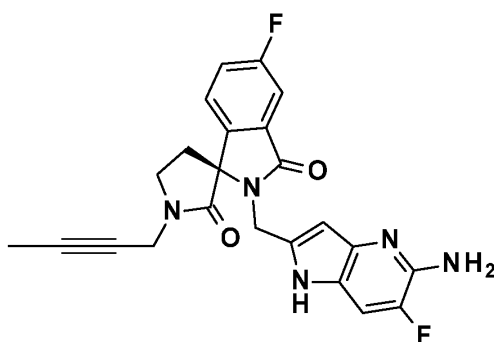
compound, and formulations containing it, should be capable of being effectively stored over appreciable periods of time, without exhibiting any significant change in the physico-chemical characteristics (e.g. chemical composition, density, hygroscopicity and solubility) of the active compound.

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There remains a need to provide a solid form of Compound (I) which has appropriate physical and chemical stability and other drug related properties suitable for pharmaceutical development.

10 Summary of the Specification

In one aspect, the specification provides a crystalline form which is a co-crystal of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione:



Compound (I),

15 and adipic acid in a 2:1 ratio (herein referred to as "Compound (I) Form A").

The skilled person will appreciate that although a specific tautomer of Compound (I) is shown above, Compound (I) may exist in multiple tautomeric forms. The specification encompasses all such tautomeric forms.

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The specification also provides a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient.

25 The specification also provides Compound (I) Form A for use as a medicament, for example in the prophylaxis or treatment of a disorder mediated by PRMT5.

The specification also provides the use of Compound (I) Form A in the manufacture of a medicament, for example for the prophylaxis or treatment of a disorder mediated by PRMT5.

The specification also provides a method of prevention or treatment, for example preventing or treating a disorder mediated by PRMT5, which comprises administering Compound (I) Form A.

PCT/EP2022/075248 (published as International Publication Pamphlet WO2023/036974)

5 describes the synthesis of a crystalline form of Compound (I) (herein referred to as "Reference Form 1"). However, as discussed in the Examples below, experimental investigation of its properties has revealed that Reference Form 1 displays characteristics which may be deleterious for pharmaceutical development. For example, at ambient conditions Reference Form 1 exists as a hemi-hydrate which can reversibly be converted to the anhydrous form or
10 mono-hydrate upon changes in temperature and/or relative humidity. The reversible (de)hydration behaviour of Reference Form 1 may result in unpredictable changes in physical properties due to interconversion between the discrete forms, consequently affecting storage and manufacture, and may also create variability in analytical assaying which could complicate its development and use as a pharmaceutical active.

15 Compound (I) Form A as described herein possesses a distinct crystal structure (as determined by XRPD) and surprisingly displays a combination of characteristics which are attractive in the context of pharmaceutical development. For example, Compound (I) Form A displays high thermal stability (melting point $\approx 217.6^\circ\text{C}$ as determined by DSC); is un-solvated (as determined
20 by DSC and TGA); displays low hygroscopicity (as determined by DVS); appears to be physically stable (as determined by XRPD analysis after a water slurry stability experiment); and appears to have improved solubility in a range of biorelevant media compared to Reference Form 1 (as determined by experiments in intestinal and gastric fluid models).

25 Compound (I) may exist in other solid forms with alternative counter-ions such as acetic acid, 1,5-naphthalenedisulfonic acid, glutaric acid, oxalic acid, propionic acid, camphoric acid, 5-nitroisophthalic acid, 5-chlorosalicylic acid, 3,5-dinitrobenzoic acid, benzoic acid, fumaric acid, sulphuric acid, 2-mesitylenesulfonic acid, gallic acid, 3,5-dihydroxybenzoic acid, or 2,4-dihydroxybenzoic acid. These may exist in crystalline forms which are i) co-crystals of
30 Compound (I) and the counter-ion; ii) salts of Compound (I); or mixtures thereof. However, Compound (I) Form A is a crystalline form which appears to be particularly suitable for pharmaceutical development.

Brief Description of the Figures

35 **Figure 1:** X-ray powder diffraction pattern of Compound (I) Form A, a physical form of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione and adipic acid in a 2:1 ratio.

Figure 2: Differential scanning calorimetry and thermogravimetric analysis overlay thermogram of Reference Form 1.

Figure 3: Differential scanning calorimetry and thermogravimetric analysis overlay thermogram of Compound (I) Form A.

5 **Figure 4:** Dynamic vapor sorption isotherm plot of Reference Form 1.

Figure 5: Dynamic vapor sorption isotherm plot of Compound (I) Form A.

Detailed Description

10 X-ray powder diffraction analysis may be performed according to standard methods, examples of which can be found in e.g. Kitaigorodsky, A.I. (1973), *Molecular Crystals and Molecules*, Academic Press, New York; Bunn, C.W. (1948), *Chemical Crystallography*, Clarendon Press, London; or Klug, H.P. & Alexander, L.E. (1974), *X-ray Diffraction Procedures*, John Wiley & Sons, New York.

15 It is known in the art that an X-ray powder diffraction pattern may be obtained which has one or more measurement errors depending on measurement conditions (such as equipment, sample preparation or machine used). In particular, it is generally known that intensities in an X-ray powder diffraction pattern may fluctuate depending on measurement conditions and sample preparation. For example, persons skilled in the art of X-ray powder diffraction will realise that the
20 relative intensities of peaks may vary according to the orientation of the sample under test and on the type and setting of the instrument used. The skilled person will also realise that the position of reflections can be affected by the precise height at which the sample sits in the diffractometer and the zero calibration of the diffractometer. The surface planarity of the sample may also have a small effect. The skilled person will also realize that the relative intensity of peaks can be
25 affected by, for example, grains above approximately 30 micrometer in size and non-unitary aspect ratios which may affect analysis of samples. Furthermore, it should be understood that intensities may fluctuate depending on experimental conditions and sample preparation such as preferred orientation of the particles in the sample. The use of automatic or fixed divergence slits will also influence the relative intensity calculations. A person skilled in the art can handle such
30 effects when comparing diffraction patterns. Hence a person skilled in the art will appreciate that the diffraction pattern data presented herein is not to be construed as absolute and any crystalline form that provides a power diffraction pattern substantially identical to those disclosed herein fall within the scope of the present specification (for further information see Jenkins, R & Snyder, R.L. 'Introduction to X-Ray Powder Diffractometry' John Wiley & Sons, 1996).

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Generally, a measurement error of a diffraction angle in an X-ray powder diffractogram is about 5% or less, in particular plus or minus 0.2° 2-theta, and such degree of a measurement error should be taken into account when considering the X-ray powder diffraction pattern in Figure 1,

and when reading Tables 2 and Table 3. Furthermore, it should be understood that intensities may fluctuate depending on experimental conditions and sample preparation (preferred orientation). Definition of relative intensity is described in Table 1:

5 **Table 1. Definition of relative intensity.**

% Relative intensity	Definition
25-100	very strong
10-25	strong
3-10	medium
< 3	weak

An X-ray powder diffraction pattern was obtained directly from a sample of Compound (I) Form A, prepared as described herein. From visual analysis of the pattern (Figure 1) it is evident that Compound (I) Form A is highly crystalline. The ten most prominent peaks are shown in Table 2:

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Table 2. Ten most prominent peaks in X-ray powder diffraction pattern of Form A.

2 θ , °	Relative intensity
8.9	very strong
14.5	strong
16.9	strong
17.4	strong
19.1	very strong
19.7	very strong
21.2	strong
22.9	very strong
23.7	strong
25.8	strong

The term “about” as used herein when referring to any given numerical value means within $\pm 5\%$ of that value.

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In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least one specific peak at (± 0.2) 8.9 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least one specific peak at about 8.9 degrees 2-theta when measured using CuK α radiation.

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In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least one specific peak at (± 0.2) 19.1 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least one specific peak at
5 about 19.1 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least two specific peaks at (± 0.2) 8.9 and 19.1 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound
10 (I) Form A which has an X-ray powder diffraction pattern containing at least two specific peaks at about 8.9 and 19.1 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least one specific peak selected from (± 0.2) 8.9, 19.1, 19.7 and
15 22.9 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least one specific peak selected from about 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least two specific peaks selected from (± 0.2) 8.9, 19.1, 19.7 and
20 22.9 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least two specific peaks selected from about 8.9, 19.1, 19.7 and 22.9 degrees 2-
25 theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least three specific peaks selected from (± 0.2) 8.9, 19.1, 19.7
30 and 22.9 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least three specific peaks selected from about 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 19.1, 19.7 and 22.9 degrees 2-theta
35 when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at about 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation, and one, two, three, four, five or six specific peaks selected from (± 0.2) 14.5, 16.9, 17.4, 21.2, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at about 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation, and one, two, three, four, five or six specific peaks selected from about 14.5, 16.9, 17.4, 21.2, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least two peaks selected from (± 0.2) 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing at least two peaks selected from about 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at about 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation.

The complete list of XRPD pattern peaks for Compound (I) Form A is shown in Table 3. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing the specific peaks substantially as shown in Table 3 when measured using CuK α radiation.

In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation, and one, two or three specific peaks selected from (± 0.2) 27.0, 28.1 and 29.2 degrees 2-theta when measured using CuK α radiation. In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern containing specific peaks at about 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α

radiation, and one, two or three specific peaks selected from about 27.0, 28.1 and 29.2 degrees 2-theta when measured using CuK α radiation.

5 In one embodiment the specification provides Compound (I) Form A which has an X-ray powder diffraction pattern substantially as shown in Figure 1 when measured using CuK α radiation.

10 In the present specification in instances where the crystalline form is described as having “an X-ray powder diffraction pattern containing at least one specific peak at (± 0.2)...” or “an X-ray powder diffraction pattern containing at least one specific peak at about...” the XRPD of the crystalline form may contain one or more of the 2-theta values listed, for example one or more of the 2-theta values, 2 or more of the 2-theta values or 3 or more of the 2-theta values listed. Similar descriptions with reference to different numbers of peaks (such as “...containing at least two specific peaks...” etc) are to be interpreted in the same manner.

15 Compound (I) Form A has also been characterised by differential scanning calorimetry (DSC). A person skilled in the art will understand that the value or range of values observed in a particular compound’s DSC thermogram will show variation between batches of different purities. Therefore, whilst for one compound the range may be small, for others the range may be quite large. Generally, a measurement error of a diffraction angle in DSC thermal events is
20 approximately plus or minus 5°C, and such degree of a measurement error should be taken into account when considering the DSC data included herein, such as for Figure 3.

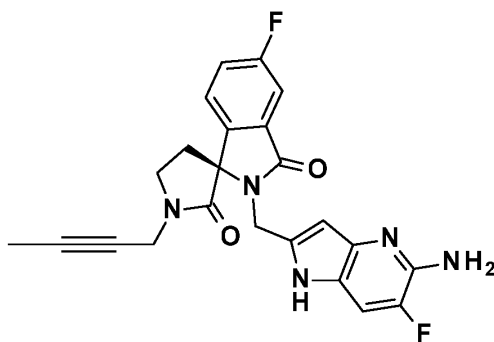
When heated in a differential scanning calorimeter (conditions as described in the Examples section), Compound (I) Form A exhibits a melting with an onset temperature at about 216.6°C,
25 and a peak temperature at about 217.7°C as illustrated in Figure 3.

In one embodiment the specification provides Compound (I) Form A which has a DSC thermogram with an onset melting at 216.6°C plus or minus 5°C and a peak at 217.7°C plus or minus 5°C. In one embodiment the specification provides Compound (I) Form A which has a
30 DSC thermogram with an onset melting at about 216.6°C and a peak at about 217.7°C. In one embodiment the specification provides Compound (I) Form A which has a DSC thermogram substantially as shown in Figure 3.

Thermogravimetric analysis (TGA) (in combination with DSC as described above) also
35 demonstrates that in contrast to Reference Form 1 (which as discussed above has variable and reversible (de)hydration), Compound (I) Form A is un-solvated, which may provide advantages during storage, manufacture and pharmaceutical development.

In one embodiment the specification provides Compound (I) Form A which has a DSC and TGA overlay thermogram substantially as shown in Figure 3.

In one embodiment, the specification provides a crystalline form which is a co-crystal of (3S)-2-
 5 [(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-
 3,3'-pyrrolidine]-1,2'-dione:



Compound (I),

and adipic acid in a 2:1 ratio (Compound (I) Form A), having at least one of the following:

- a) an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 19.1, 19.7
 10 and 22.9 degrees 2-theta when measured using CuK α radiation;
- b) an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 14.5, 16.9,
 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α
 radiation;
- c) an X-ray powder diffraction pattern substantially as shown in Figure 1;
- d) a DSC thermogram with an onset melting at 216.6°C plus or minus 5°C and a peak at
 15 217.7°C plus or minus 5°C;
- e) a DSC thermogram and optionally a TGA thermogram substantially as shown in Figure
 3.

20 When it is stated that the present specification relates to a crystalline form, the degree of
 crystallinity is greater than about 60%. In one embodiment, the degree of crystallinity is greater
 than about 80%. In one embodiment, the degree of crystallinity is greater than about 90%. In one
 embodiment, the degree of crystallinity is greater than about 95%. In one embodiment, the degree
 of crystallinity is greater than about 98%.

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It is desirable that the crystalline form is substantially free from other crystalline forms of
 Compound (I) (i.e. a crystalline form or forms other than Compound (I) Form A). Therefore, in one
 embodiment, the crystalline form contains less than 20%, 15%, 10%, 5%, 3% or 1% by weight of
 other crystalline forms of Compound (I). In one embodiment, the crystalline form contains more
 30 than 80%, 85%, 90%, 95%, 97% or 99% by weight of Compound (I) Form A.

Based on present experimental data, Compound (I) Form A is believed to be a co-crystal of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione and adipic acid in a 2:1 ratio i.e. having a 2:1 stoichiometric ratio of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione:adipic acid. Compound (I) Form A may also be referred to as Compound (I):hemi-adipic acid co-crystal.

Without wishing to be bound by theory, co-crystal formation results from situations whereby an acid or base "co-former" is a solid at room temperature and there is no or only partial proton transfer between the free compound and such an acid or base co-former. Consequently, a co-crystal of the co-former and free compound results rather than a salt. The definition of the co-former acid or base being a solid at room temperature is intended to distinguish co-crystals from solvates. It is accepted that the proton transfer is in fact a continuum, and can change with temperature, and therefore the point at which a co-crystal is better described as a salt can be somewhat subjective. However, as noted above, based on all present experimental evidence Compound (I) Form A is believed to be a co-crystal.

In one embodiment, there is provided Compound (I) Form A obtainable by any of the methods disclosed herein. In one embodiment, there is provided Compound (I) Form A obtainable by the method of Example 1, Method A. In one embodiment, there is provided Compound (I) Form A obtainable by the method of Example 1, Method B. In one embodiment, there is provided Compound (I) Form A obtainable by the method of Example 1, Method C.

Compound (I) Form A may be further characterised by additional techniques well known to the skilled person, such as single crystal X-ray diffraction (for example to evaluate proton position, bond lengths or bond angles), solid state ¹H-NMR, (to evaluate for example, C or N chemical shifts) or spectroscopic techniques (to measure for example, O-H or N-H signals and IR peak shifts resulting from hydrogen bonding).

Compound (I) Form A may be prepared as described in the Examples herein. Crystallisation of the desired Compound (I) Form A may be aided by seeding with crystals of the desired form. The seed crystals may be obtained using one of the methods described in the Examples, such as Method B. The use of seeding is particularly advantageous in larger-scale manufacture.

Medical Uses

As described above, Compound (I) is an inhibitor of PRMT5, and in particular Compound (I) is a MTA-synergistic PRMT5 inhibitor. Therefore, Compound (I) Form A is expected to be useful as

a medicament, such as in the prophylaxis or treatment of a disorder mediated by PRMT5 i.e. a disorder wherein inhibition of PRMT5 provides a prophylactic or therapeutic effect.

5 As used herein, "prophylaxis" is intended to have its normal meaning and includes primary prophylaxis to prevent the development of the disease or condition and secondary prophylaxis whereby the disease or condition has already developed and the subject is temporarily or permanently protected against exacerbation or worsening of the disease or condition, or the development of new symptoms associated with the disease or condition. The terms "prophylactic", "preventing" and "prevention" are used synonymously with "prophylaxis".

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As used herein, "treatment" is intended to have its normal meaning of dealing with a disease or condition in order to entirely or partially relieve one, some or all of its symptoms in a subject, or to correct or compensate for the underlying pathology. The terms "treatment" and "treating" are used synonymously with "therapy".

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A subject will typically be a subject in need of prophylaxis or treatment according to the specification. In one embodiment, the subject is a human.

20 In one embodiment, the specification provides Compound (I) Form A for use as a medicament.

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In one embodiment, the specification provides Compound (I) Form A for use in the prophylaxis or treatment of a disorder mediated by PRMT5 (e.g. cancer). In one embodiment, the specification provides Compound (I) Form A for use in the prophylaxis of a disorder mediated by PRMT5 (e.g. cancer). In one embodiment, the specification provides Compound (I) Form A for use in the treatment of a disorder mediated by PRMT5 (e.g. cancer).

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In one embodiment, the specification provides the use of Compound (I) Form A in the manufacture of a medicament.

30 In one embodiment, the specification provides the use of Compound (I) Form A in the manufacture of a medicament for the prophylaxis or treatment of a disorder mediated by PRMT5 (e.g. cancer). In one embodiment, the specification provides the use of Compound (I) Form A in the manufacture of a medicament for the prophylaxis of a disorder mediated by PRMT5 (e.g. cancer). In one embodiment, the specification provides the use of Compound (I) Form A in the manufacture of a medicament for the treatment of a disorder mediated by PRMT5 (e.g. cancer).

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In one embodiment, the specification provides a method of preventing or treating a disorder mediated by PRMT5 (e.g. cancer), which comprises administering Compound (I) Form A. In one embodiment, the specification provides a method of preventing a disorder mediated by PRMT5 (e.g. cancer), which comprises administering Compound (I) Form A. In one embodiment, the
5 specification provides a method of treating a disorder mediated by PRMT5 (e.g. cancer).

In one embodiment, the disorder mediated by PRMT5 is cancer. In one embodiment, the cancer is a MTAP deleted cancer i.e. a cancer in which the MTAP gene has been deleted. In one embodiment the cancer is a CDKN2A deleted and MTAP deleted cancer i.e. a cancer in which
10 the CDKN2A and MTAP genes are deleted.

In one embodiment, the cancer is selected from gastric, pancreatic, colorectal, uterine, bile duct, stomach, bladder, cervical, testicular germ cell, lung (e.g. non-small cell lung cancer), multiple myeloma, lymphoma (e.g. diffuse large B cell lymphoma or Hodgkin's lymphoma),
15 rhabdomyosarcoma and cutaneous squamous cell carcinoma.

In one embodiment, the cancer is selected from gastric, lung (e.g. non-small cell lung cancer) and lymphoma (e.g. diffuse large B cell lymphoma or Hodgkin's lymphoma).

20 In one embodiment, the cancer is non-small cell lung cancer. In one embodiment, the cancer is diffuse large B cell lymphoma. In one embodiment, the cancer is Hodgkin's lymphoma.

Combination therapy

Compound (I) Form A may be administered in conjunction with other compounds used for the
25 treatment of the above conditions. In one embodiment, there is provided a combination therapy comprising Compound (I) Form A and a second active ingredient.

Compound (I) Form A and a second active ingredient may be administered concurrently, sequentially or in admixture, for the treatment of one or more of the conditions listed above. Such
30 a combination may be used in combination with one or more further active ingredients.

Pharmaceutical Compositions

For use in prophylaxis or therapy, Compound (I) Form A will typically be administered as a pharmaceutical composition. Therefore, in one embodiment the specification provides a
35 pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient.

In one embodiment, the specification provides a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient for use as a medicament.

- 5 In one embodiment, the specification provides a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient for use in the prophylaxis or treatment of a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer). In one embodiment, the specification provides a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable
- 10 excipient for use in the prophylaxis of a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer). In one embodiment, the specification provides a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient for use in the treatment of a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer).
- 15 In one embodiment, the specification provides the use of a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient, in the manufacture of a medicament.
- 20 In one embodiment, the specification provides the use of a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient, in the manufacture of a medicament for the prophylaxis or treatment of a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer). In one embodiment, the specification provides the use of a pharmaceutical composition comprising Compound (I) Form
- 25 A and at least one pharmaceutically acceptable excipient, in the manufacture of a medicament for the prophylaxis of a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer). In one embodiment, the specification provides the use of a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient, in the manufacture of a medicament for the treatment of a disorder mediated by
- 30 PRMT5, such as the disorders disclosed herein (e.g. cancer).

- In one embodiment, the specification provides a method of preventing or treating a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer), which comprises administering a pharmaceutical composition comprising Compound (I) Form A and at least one
- 35 pharmaceutically acceptable excipient. In one embodiment, the specification provides a method of preventing a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer), which comprises administering a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient. In one embodiment, the

specification provides a method of treating a disorder mediated by PRMT5, such as the disorders disclosed herein (e.g. cancer), which comprises administering a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient.

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Administration

Compound (I) Form A will normally be administered in the form of a pharmaceutical composition comprising Compound (I) Form A in a pharmaceutically acceptable dosage form via the oral, parenteral, intravenous, intramuscular, subcutaneous or in other injectable ways, buccal, rectal, vaginal, transdermal and/or nasal route and/or via inhalation. Depending upon the disorder and patient to be treated and the route of administration, the compositions may be administered at varying doses. In one embodiment, Compound (I) Form A or a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient are administered orally.

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Dosage forms suitable for oral use form one aspect of the specification. In one embodiment the specification provides a solid oral dosage form comprising Compound (I) Form A or a pharmaceutical composition comprising Compound (I) Form A and at least one pharmaceutically acceptable excipient. In one embodiment, the solid oral dosage form is a tablet.

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The compositions of the specification may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents.

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Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents; granulating and disintegrating agents; binding agents; and lubricating agents. Tablet formulations may be uncoated or coated using conventional coating agents and procedures well known in the art.

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For further information on formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

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The amount of active ingredient (i.e. Compound (I) Form A) that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration.

Suitable daily doses of Compound (I) Form A in prophylactic or therapeutic treatment of humans are about 0.0001-100 mg/kg body weight.

- 5 For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The specification may be further illustrated by the following, non-limiting examples.

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EXAMPLES

In general:

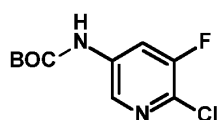
- (i) operations were carried out at ambient temperature, i.e. in the range 17 to 25 °C and
15 under an atmosphere of an inert gas such as nitrogen unless otherwise stated;
- (ii) evaporations were carried out by rotary evaporation or utilising Genevac equipment or Biotage v10 evaporator in vacuo and work up procedures were carried out after removal of residual solids by filtration;
- (iii) flash chromatography purifications were performed on an automated Teledyne Isco
20 CombiFlash® Rf or Teledyne Isco CombiFlash® Companion® using prepacked RediSep Rf Gold™ Silica Columns (20-40 µm, spherical particles), GraceResolv™ Cartridges (Davisil® silica) or Silicycle cartridges (40 - 63 µm);
- (iv) preparative reverse phase HPLC was performed on an Agilent 1290 Infinity II Preparative system equipped with a SQ MS detector (Multimode ESI/APCI source), with a
25 Waters CSH C18 OBD column (5 microns silica, 30 mm diameter, 100 mm length, flow rate of 50 mL/min) using decreasingly polar mixtures of water (containing 0.1—0.3% aqueous ammonium) or water (containing 0.1% formic acid) and acetonitrile as eluents. Preparative SFC purification was performed on either a Sepiatec P100 SFC system or Waters Prep 100 SFC system equipped with QDa MS detector, using the chromatographic conditions as detailed in
30 corresponding experimental data;
- (v) yields, where present, are not necessarily the maximum attainable;
- (vi) in general, NMR chemical shift values were measured on the delta scale [proton magnetic resonance spectra were determined using a Bruker Avance 400 (400 MHz) instrument]; measurements were taken at ambient temperature unless otherwise specified; the
35 following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; ddd, doublet of doublet of doublet; dt, doublet of triplets; bs, broad signal;

- (vii) in general, compounds were also characterized by mass spectrometry following liquid chromatography (LCMS or UPLC); reverse-phase C18 silica was used with a flow rate of 1 mL/min and detection was by Electrospray Mass Spectrometry and by UV absorbance recording a wavelength range of 220-320 nm. Analytical UPLC was performed on CSH C18 reverse-phase silica, using a Waters Acquity UPLC CSH C18 column with dimensions 2.1 x 50 mm and particle size 1.7 micron) Gradient analysis was employed using decreasingly polar mixtures as eluent, for example decreasingly polar mixtures of water (containing 0.1% formic acid or 0.1% ammonia) as solvent A and acetonitrile as solvent B. A typical 2 minute analytical UPLC method would employ a solvent gradient over 1.3 min, at approximately 1 mL/min, from a 97:3 mixture of solvents A and B respectively to a 3:97 mixture of solvents A and B. The reported molecular ion corresponds to the $[M+H]^+$ unless otherwise specified; for molecules with multiple isotopic patterns (Br, Cl etc.) the reported value is the one obtained for the lowest isotope mass unless otherwise specified;
- (viii) intermediate purity was assessed by thin layer chromatographic, mass spectral, HPLC (high performance liquid chromatography) and/or NMR analysis.

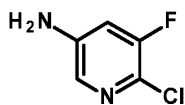
Preparation of Reference Form 1

- A synthesis of Compound (I) is described in PCT/EP2022/075248 (published as International Publication Pamphlet WO2023/036974), the contents of which are incorporated by reference for the purpose of describing Compound (I) and its synthesis. Compounds described below were named using Chemdraw version 20.0.2.51 and the synthetic methods described herein are given by way of illustration only and are non-limiting.

Intermediate AQ: *tert*-Butyl (6-chloro-5-fluoropyridin-3-yl)carbamate

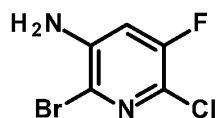


- To 5-bromo-2-chloro-3-fluoropyridine (100 g, 475.22 mmol) in dioxane (1 L) was added *tert*-butyl carbamate (61.20 g, 522.74 mmol) and caesium carbonate (310.00 g, 950.43 mmol). The solution was degassed under vacuum and purged with an inert atmosphere of nitrogen for 5 minutes followed by the addition of tris(dibenzylideneacetone)dipalladium(0) (13.06 g, 14.26 mmol) and (9,9-dimethyl-9H-xanthene-4,5-diyl)bis(diphenylphosphane) (Xantphos) (11.00 g, 19.01 mmol). The reaction mixture was heated to 85 °C under nitrogen for 16 hours and then cooled to rt. The solid was filtered off and washed with excess dioxane. The solvent was removed *in vacuo* to afford crude title compound (179 g, 153%) as a dark orange gum that solidified on standing. The crude gum was used directly in next step without further purification. ^1H NMR (400 MHz, DMSO- d_6 , 30°C) 1.49 (9H, s), 7.98 (1H, dd), 8.29 (1H, d), 9.98 (1H, s); m/z MH^+ 247.

Intermediate AR: 6-Chloro-5-fluoropyridin-3-amine

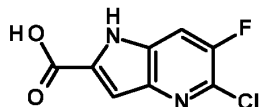
4M HCl in 1,4-dioxane (137 mL, 547.3 mmol) was added in one portion to a solution of *tert*-butyl (6-chloro-5-fluoropyridin-3-yl)carbamate (36 g, 109.5 mmol) in 1,4-dioxane (20 mL) at 20°C. The resulting suspension was stirred at 20 °C for 3 days. The reaction mixture was diluted with water (250 mL) and EtOAc (100 mL). The organic phase was separated and extracted with 2M HCl (3 x 100 mL) until no more product remained in the organic phase. The combined aqueous phases were stirred and cooled to 0°C in an ice bath. The reaction mixture was basified to pH 14 with 50% NaOH solution. The reaction mixture was then extracted with EtOAc (2 x 250 mL), the combined organics were washed with saturated brine (50 mL), dried over MgSO₄, filtered and the solvent was removed *in vacuo* to afford the title compound (12.4 g, 77%) as a brown solid. Used directly in next step with no further purification. ¹H NMR (400 MHz, CDCl₃, 27°C) 3.88 (s, 2H), 6.80 (dd, J = 9.6, 2.5 Hz, 1H), 7.68 (d, J = 2.5 Hz, 1H).

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Intermediate AS: 2-Bromo-6-chloro-5-fluoropyridin-3-amine

6-Chloro-5-fluoropyridin-3-amine (56.8 g, 379.3 mmol) in MeCN (250 mL) was cooled to 5 °C and a solution of NBS (67.50 g, 379.3 mmol) in MeCN (500 mL) was added over 15 minutes. The reaction mixture was warmed to rt and stirred for 45 minutes. Water (2 L) was added and the reaction mixture stirred for 30 minutes. The resulting solid was filtered off and washed with water (400 mL). The solid was dried under vacuum to afford the title compound (76 g, 89%) as a brown solid. ¹H NMR (400 MHz, DMSO-d₆, 30°C) 6.01 (2H, s), 7.12 (1H, d); *m/z* MH⁺ 225.

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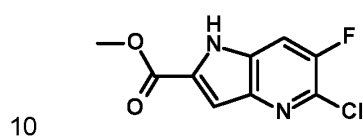
Intermediate AT: 5-Chloro-6-fluoro-1H-pyrrolo[3,2-*b*]pyridine-2-carboxylic acid

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Palladium acetate (3.35 g, 14.93 mmol), triphenylphosphine (3.92 g, 14.93 mmol), 2-bromo-6-chloro-5-fluoropyridin-3-amine (18.0 g, 74.65 mmol) and pyruvic acid (15.57 mL, 224 mmol) were placed in a flask with 1,4-dioxane (88 mL). Triethylamine (45.80 mL, 328.5 mmol) was added and the reaction was heated at 100 °C for 2.5 hours under nitrogen. The reaction mixture was cooled to rt and filtered to remove unwanted solids. The filtrate was diluted with 2M NaOH (200 mL) and

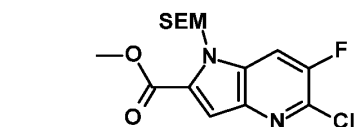
MTBE (200 mL) was added. The reaction mixture was then stirred vigorously and separated. The organic phase was washed with 2M NaOH (100 mL). The combined basic aqueous phases were carefully acidified with concentrated HCl (aqueous) and a brown solid precipitated which was collected by filtration and dried. The dark brown solid was suspended in MeOH (90 mL) and stirred for 2 hours at rt. The solid was filtered and dried under vacuum to afford the title compound (14.60 g, 91%) as a beige solid. ^1H NMR (400 MHz, DMSO- d_6 , 30°C) 7.16 (1H, dd), 7.84 (1H, dd), 12.36 (1H, s), 13.46 (1H, s); m/z MH^+ 214.

Intermediate AU: Methyl 5-chloro-6-fluoro-1H-pyrrolo[3,2-*b*]pyridine-2-carboxylate



Sulfuric acid (3.08 mL, 57.83 mmol) was added dropwise carefully to 5-chloro-6-fluoro-1H-pyrrolo[3,2-*b*]pyridine-2-carboxylic acid (14.60 g, 57.83 mmol) in MeOH (113 mL) at rt. The reaction mixture was stirred at reflux for 18 hours. The reaction mixture was allowed to cool and the solvent was removed *in vacuo*. Saturated NaHCO_3 (400 mL) was carefully added to the residue and the resulting precipitate was filtered off, washed with water and dried under vacuum to afford the title compound (14.20 g, 107%) as a brown solid. ^1H NMR (400 MHz, DMSO- d_6 , 30°C) 3.91 (3H, s), 7.24 (1H, dd), 7.88 (1H, dd), 12.56 (1H, s); m/z MH^+ 229.

Intermediate AV: Methyl 5-chloro-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-*b*]pyridine-2-carboxylate

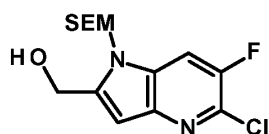


Potassium bis(trimethylsilyl)amide (1M in THF) (101 mL, 100.9 mmol) was added dropwise over 15 minutes to a solution of methyl 5-chloro-6-fluoro-1H-pyrrolo[3,2-*b*]pyridine-2-carboxylate (21.74 g, 77.60 mmol) and (2-(chloromethoxy)ethyl)trimethylsilane (18.83 mL, 100.9 mmol) in THF (419 mL) at 5 °C under nitrogen. The reaction mixture was stirred at 5 °C for 30 minutes. Potassium bis(trimethylsilyl)amide (1M in THF) (15.52 mL, 15.52 mmol) was added and the reaction mixture was stirred at 5 °C for a further 30 minutes. (2-(Chloromethoxy)ethyl)trimethylsilane (1.88 mL, 10.09 mmol) was added and stirred at 5 °C for a further 15 minutes. The reaction mixture was quenched with saturated NH_4Cl (400 mL) and diluted with EtOAc (400 mL). The aqueous phase was re-extracted with EtOAc (250 mL). The combined organic phases were dried over MgSO_4 , filtered and the solvent was removed *in vacuo*. The crude material was suspended in heptane (450 mL) and stirred for 5 minutes. The unrequired solid was filtered off and washed with heptane (50 mL). The solvent was removed *in vacuo* to afford the title

compound (32.50 g, 117%) as a brown gum which solidified on standing. The gum was used directly in next step without further purification. ^1H NMR (400 MHz, DMSO- d_6 , 30°C) -0.13 (9H, s), 0.70 – 0.80 (2H, m), 3.38 – 3.51 (2H, m), 3.89 (3H, s), 5.95 (2H, s), 7.41 (1H, d), 8.40 – 8.54 (1H, m); m/z MH^+ 359.

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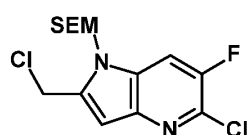
Intermediate AW: (5-Chloro-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-b]pyridin-2-yl)methanol



Diisobutylaluminum hydride (1M in toluene) (170 mL, 170.42 mmol) was added dropwise to a stirred solution of methyl 5-chloro-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-b]pyridine-2-carboxylate (27.80 g, 77.47 mmol) in DCM (333 mL) at 5 °C over 15 minutes. The reaction mixture was stirred at rt for 30 minutes. The reaction mixture was carefully poured into 2M NaOH (500 mL), diluted with DCM (500 mL) and stirred for 1 hour. The organic phase was separated and the aqueous phase was extracted with DCM (2 x 200 mL). The combined organics were dried with MgSO_4 , filtered and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (17.60 g, 68%) as a pale orange oil which solidified on standing. ^1H NMR (400 MHz, DMSO- d_6 , 30°C) -0.10 (9H, s), 0.75 – 0.83 (2H, m), 3.36 – 3.56 (2H, m), 4.72 (2H, d), 5.50 (1H, t), 5.60 (2H, s), 6.57 (1H, d), 8.24 (1H, dd); m/z MH^+ 331.

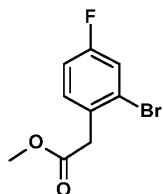
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Intermediate AX: 5-Chloro-2-(chloromethyl)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-b]pyridine

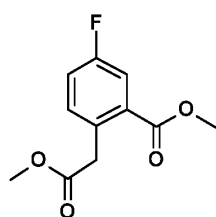


Thionyl chloride (13.2 mL, 181.4 mmol) was added dropwise carefully to (5-chloro-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-b]pyridin-2-yl)methanol (20.0 g, 60.45 mmol) in DCM (200 mL) at rt. The reaction mixture was stirred at rt for 1 hour. Saturated NaHCO_3 (500 mL) was then slowly added. Once gas evolution had ceased the phases were separated and the aqueous phase was re-extracted with DCM (300 mL). The organic phases were combined, washed with brine (200 mL), passed through a phase separating filter paper and the solvent was removed *in vacuo* to afford the title compound (19.2 g, 91%) as a brown crystalline solid. ^1H NMR (400 MHz, DMSO- d_6 , 27°C) -0.10 (9H, s), 0.75 – 0.9 (2H, m), 3.43 – 3.53 (2H, m), 5.07 (2H, s), 5.66 (2H, s), 6.7 – 6.9 (1H, m), 8.32 (1H, dd); m/z MH^+ 349.

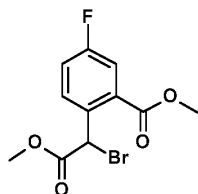
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Intermediate AA: Methyl 2-(2-bromo-4-fluorophenyl)acetate

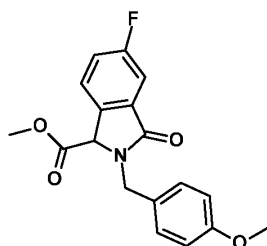
Thionyl chloride (31.3 mL, 429.1 mmol) was added dropwise carefully to 2-(2-bromo-4-fluorophenyl)acetic acid (CAS No. 61150-59-2) (100 g, 429.1 mmol) in MeOH (400 mL) at rt. The reaction mixture was stirred at 60°C for 4 hours, cooled and the solvent was removed *in vacuo*. The residue was partitioned between EtOAc (250 mL) and saturated NaHCO₃ (200 mL). The organic phase was washed with water (100 mL), brine (100 mL), passed through a phase separating filter paper and the solvent was removed *in vacuo* to afford the title compound (105 g, 99%) as a colourless oil. ¹H NMR (400 MHz, DMSO-d₆, 30°C) 3.64 (3H, s), 3.83 (2H, s), 7.25 (1H, td), 7.48 (1H, dd), 7.58 (1H, dd); *m/z* MH⁺ not observed.

Intermediate AB: Methyl 5-fluoro-2-(2-methoxy-2-oxoethyl)benzoate

Methyl 2-(2-bromo-4-fluorophenyl)acetate (45.0 g, 182.14 mmol) and triethylamine (27.90 mL, 200.35 mmol) were placed in a steel pressure vessel with MeOH (300 mL). [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (complex with dichloromethane) (4.46 g, 5.46 mmol) was added and the vessel was sealed. The vessel was purged with carbon monoxide and then charged to 7 bar with carbon monoxide. The pressure vessel was heated to 100 °C and stirred for 2 hours. The reaction mixture was allowed to cool, vented and filtered to remove catalyst. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc (250 mL), washed with water (2 x 200 mL) and brine (100 mL). The organic phase was passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (38.40 g, 93%) as a pale yellow oil. ¹H NMR (400 MHz, DMSO-d₆, 30°C) 3.60 (3H, s), 3.80 (3H, s), 3.99 (2H, s), 7.42 – 7.49 (2H, m), 7.66 (1H, ddd); *m/z* MH⁺ 227.

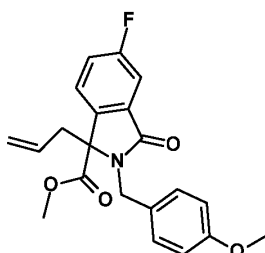
Intermediate AC: *rac*-Methyl-2-(1-bromo-2-methoxy-2-oxoethyl)-5-fluorobenzoate

Methyl 5-fluoro-2-(2-methoxy-2-oxoethyl)benzoate (47.0 g, 207.8 mmol) was dissolved in chloroform (450 mL). 1-Bromopyrrolidine-2,5-dione (55.5 g, 311 mmol) was added followed by 2,2'-azobis(2-methylpropionitrile) (3.41 g, 20.8 mmol) and the reaction mixture was stirred at reflux for 72 hours. The reaction mixture was cooled and washed with water (2 x 250 mL), brine (100 mL), passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (50.50 g, 80%) as a colourless oil. ¹H NMR (400 MHz, DMSO-d₆, 30°C) 3.71 (3H, s), 3.86 (3H, s), 6.51 (1H, s), 7.56 (1H, td), 7.66 (1H, dd), 7.81 (1H, dd); *m/z* MH⁺ not observed.

Intermediate AD: *rac*-Methyl 5-fluoro-2-(4-methoxybenzyl)-3-oxoisindoline-1-carboxylate

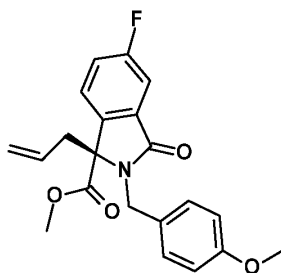
4-Methoxybenzylamine (23.5 g, 171 mmol) was placed in a flask with MeCN (300 mL) and sodium bicarbonate (23.9 g, 285 mmol) was added. *rac*-Methyl 2-(1-bromo-2-methoxy-2-oxoethyl)-5-fluorobenzoate (43.5 g, 142 mmol), dissolved in MeCN (100 mL), was added slowly via dropping funnel as the reaction mixture was brought up to 80 °C. The reaction mixture was stirred at 80 °C for 3 hours. The reaction mixture was allowed to cool, most of the MeCN was removed *in vacuo* and the residue was partitioned between EtOAc (400 mL) and water (400 mL). The aqueous phase was re-extracted with EtOAc (100 mL), the organics were combined and washed with brine (50 mL). The organic phase was passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (45.3 g, 96%) as a pale yellow oil. ¹H NMR (400 MHz, DMSO-d₆, 30°C) 3.69 (3H, s), 3.73 (3H, s), 4.31 (1H, d), 5.04 (1H, d), 5.18 (1H, s), 6.87 – 6.94 (2H, m), 7.17 – 7.24 (2H, m), 7.50 (1H, ddd), 7.57 (1H, dd), 7.62 (1H, dd); *m/z* MH⁺ 330.

Intermediate AE: *rac*-Methyl 1-allyl-5-fluoro-2-(4-methoxybenzyl)-3-oxoisindoline-1-carboxylate



- 5 *rac*-Methyl 5-fluoro-2-(4-methoxybenzyl)-3-oxoisindoline-1-carboxylate (24.0 g, 72.9 mmol), allyl acetate (11.8 mL, 109 mmol), tris(dibenzylideneacetone)dipalladium(0) (1.67 g, 1.82 mmol) and *N,N'*-((1*R*,2*R*)-cyclohexane-1,2-diyl)bis(2-(diphenylphosphaneyl)benzamide) (2.52 g, 3.64 mmol) were stirred in THF (400 mL) at 5 °C under nitrogen. 1,1,3,3-tetramethylguanidine (13.7 mL, 109 mmol) was then added dropwise. The reaction mixture was stirred at 5 °C for 5 minutes. The THF
- 10 was removed *in vacuo*. The reaction mixture was partitioned between EtOAc (400 mL) and water (400 mL) and the organic phase was passed through a phase separating filter paper. The solvent was removed *in vacuo* to afford an orange oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (25.8 g, 96%) as a cream solid. ¹H NMR (400 MHz, DMSO-
- 15 d₆, 30°C) 3.04 – 3.20 (2H, m), 3.26 (3H, s), 3.73 (3H, s), 4.52 (1H, d), 4.71 (1H, d), 4.74 – 4.94 (3H, m), 6.82 – 6.96 (2H, m), 7.28 – 7.39 (2H, m), 7.45 – 7.58 (2H, m), 7.63 (1H, dd); *m/z* MH⁺ 370.

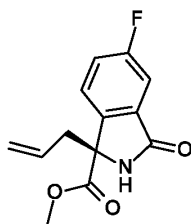
- Intermediate AF: Methyl (*S*)-1-allyl-5-fluoro-2-(4-methoxybenzyl)-3-oxoisindoline-1-**
- 20 **carboxylate**



- rac*-Methyl 1-allyl-5-fluoro-2-(4-methoxybenzyl)-3-oxoisindoline-1-carboxylate (~70:30 in favour of the desired (*S*) enantiomer) (25.8 g, 69.7 mmol) was purified by SFC chromatography (Column: Phenomenex C1, 30 x 250 mm, 5 micron, mobile phase: 10% IPA + 0.1% DEA / 90% scCO₂, flow rate: 90 ml/min, BPR: 120 bar, column temperature: 40 °C, UV max 210 nm). Pure fractions were
- 25 evaporated to dryness to afford the title compound (15.1 g, 56%) as a as a white solid. ¹H NMR

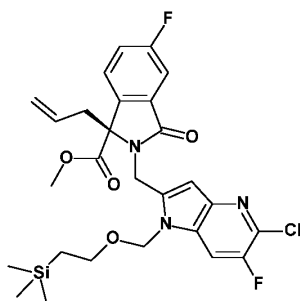
- (400 MHz, DMSO-d₆, 30°C) 3.04 – 3.20 (2H, m), 3.26 (3H, s), 3.73 (3H, s), 4.52 (1H, d), 4.71 (1H, d), 4.74 – 4.94 (3H, m), 6.82 – 6.96 (2H, m), 7.28 – 7.39 (2H, m), 7.45 – 7.58 (2H, m), 7.63 (1H, dd); *m/z* MH⁺ 370. (Presumed stereochemical assignment of this intermediate based on biological activity of bioactive compounds made using this enantiomer of the intermediate
- 5 (compared to those made using the other enantiomer), together with Xray structural evidence that the *S* enantiomer is preferred and more active than the *R* enantiomer).

Intermediate AI: Methyl (S)-1-allyl-5-fluoro-3-oxoisindoline-1-carboxylate



- 10 Methyl (S)-1-allyl-5-fluoro-2-(4-methoxybenzyl)-3-oxoisindoline-1-carboxylate (20.0 g, 54.1 mmol) was placed in a flask with MeCN (200 mL) and water (100 mL). Ammonium cerium(IV) nitrate (74.2 g, 135 mmol) was added and the reaction mixture was stirred at rt for 30 minutes. The MeCN was removed *in vacuo* and the reaction mixture was partitioned between DCM (400 mL) and water (250 mL). The aqueous phase was extracted with DCM (200 mL). The organic
- 15 phases were combined, washed with brine (100 mL), passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (12.5 g, 93%) as a cream crystalline solid. ¹H NMR (400 MHz, DMSO-d₆, 27°C) 2.79 (1H, dd), 2.94 (1H, dd), 3.68 (3H, s), 4.93 – 5.15 (2H, m), 5.35 – 5.57
- 20 (1H, m), 7.37 – 7.46 (1H, m), 7.50 (1H, ddd), 7.63 – 7.79 (1H, m), 9.32 (1H, s); *m/z* MH⁺ 250.

Intermediate BQ: Methyl (S)-1-allyl-2-((5-chloro-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-5-fluoro-3-oxoisindoline-1-carboxylate

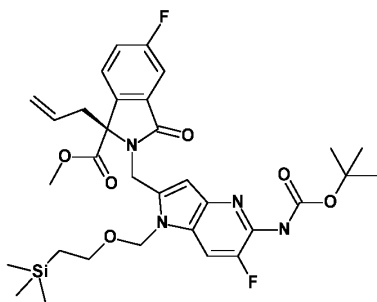


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Methyl (S)-1-allyl-5-fluoro-3-oxoisindoline-1-carboxylate (11.80 g, 47.34 mmol) and 5-chloro-2-(chloromethyl)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[3,2-*b*]pyridine (16.9 g,

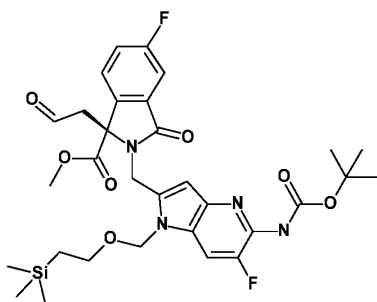
48.3 mmol) were placed in a flask with dry DMF (60 mL). Caesium carbonate (38.60 g, 118.4 mmol) was added and the reaction mixture was stirred at 60 °C for 2 hours. The reaction mixture was cooled and partitioned between water (300 mL) and EtOAc (300 mL). The aqueous phase was re-extracted with EtOAc (200 mL). The organic phases were combined, washed with water (3 x 200 mL), brine (200 mL), passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 100% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (22.2 g, 83%) as a yellow gum which slowly solidified/crystallised to give a yellow solid. ¹H NMR (400 MHz, DMSO-d₆, 27°C) -0.09 (9H, s), 0.79 – 0.88 (2H, m), 3.03 (3H, s), 3.16 – 3.29 (2H, m), 3.46 – 3.60 (2H, m), 4.73 (1H, d), 4.89 (1H, dd), 4.94 – 5.10 (2H, m), 5.26 (1H, d), 5.59 (1H, d), 5.68 (1H, d), 6.76 (1H, s), 7.49 – 7.56 (1H, m), 7.58 – 7.68 (2H, m), 8.25 (1H, dd); *m/z* MH⁺ 562.

Intermediate BR: Methyl (S)-1-allyl-2-((5-((*tert*-butoxycarbonyl)amino)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-5-fluoro-3-oxoisindoline-1-carboxylate



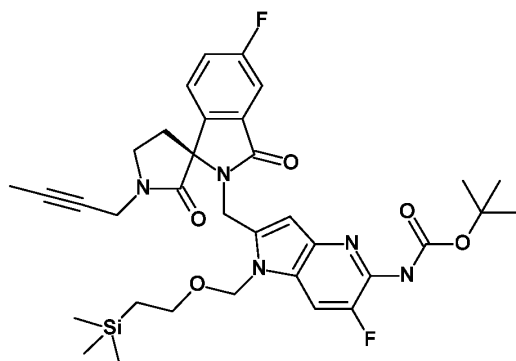
Methyl (S)-1-allyl-2-((5-chloro-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-5-fluoro-3-oxoisindoline-1-carboxylate (15 g, 26.69 mmol), caesium carbonate (21.74 g, 66.72 mmol), BrettPhos Pd G3 (2.42 g, 2.67 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphane (1.43 g, 2.67 mmol) and *tert*-butyl carbamate (6.25 g, 53.37 mmol) were placed in a flask with degassed 2-methyltetrahydrofuran (150 mL). Nitrogen was bubbled through the reaction mixture for 10 minutes and then the reaction mixture was refluxed for 3 hours. The reaction mixture was cooled, diluted with water (400 mL) and extracted with EtOAc (2 x 300 mL). The combined organic phases were washed with saturated brine (200 mL), passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (11.68 g, 68%) as a pale yellow foam. ¹H NMR (400 MHz, DMSO-d₆, 27°C) -0.08 (9H, s), 0.82 – 0.86 (2H, m), 1.42 (9H, s), 2.99 (3H, s), 3.15 – 3.30 (2H, m), 3.52 (2H, dtd), 4.71 (1H, d), 4.88 (1H, dd), 4.94 – 5.10 (2H, m), 5.26 (1H, d), 5.55 (1H, d), 5.64 (1H, d), 6.69 (1H, s), 7.46 – 7.56 (1H, m), 7.57 – 7.66 (2H, m), 7.99 (1H, d), 9.18 (1H, s); *m/z* MH⁺ 643.

Intermediate BS: Methyl (S)-2-((5-((*tert*-butoxycarbonyl)amino)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-5-fluoro-3-oxo-1-(2-oxoethyl)isoindoline-1-carboxylate carboxylate



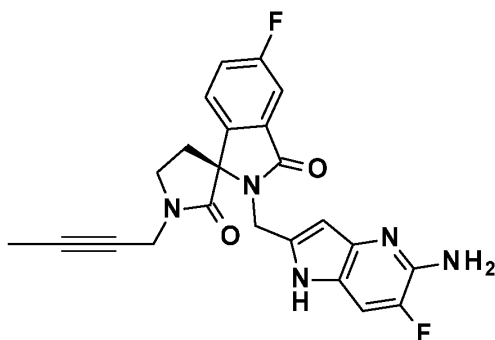
- 5 To a solution of methyl (S)-1-allyl-2-((5-((*tert*-butoxycarbonyl)amino)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-5-fluoro-3-oxoisoindoline-1-carboxylate (11.50 g, 17.89 mmol) in 1,4-dioxane (240 mL) and water (60 mL) was added osmium(VIII) oxide (4% in water) (1.14 mL, 0.18 mmol), sodium periodate (9.57 g, 44.73 mmol) and 2,6-dimethylpyridine (4.17 mL, 35.78 mmol). The reaction mixture was stirred at rt for 18
- 10 hours. The reaction mixture was partitioned between DCM (200 mL) and water (100 mL). The aqueous phase was re-extracted with DCM (100 mL) and the organic phases were combined, passed through a phase separating filter paper and the solvent was removed *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the title compound (8.70 g, 75%) as a beige
- 15 foam. ¹H NMR (400 MHz, DMSO-*d*₆, 27°C) -0.07 (9H, s), 0.82 (2H, ddd), 1.42 (9H, s), 3.25 (3H, s), 3.46 – 3.54 (2H, m), 3.67 (2H, s), 4.92 (1H, d), 5.06 (1H, d), 5.52 (1H, d), 5.61 (1H, d), 6.57 (1H, s), 7.48 – 7.57 (1H, m), 7.64 (1H, dd), 7.68 (1H, dd), 8.00 (1H, d), 9.16 (1H, s), 9.25 (1H, s); *m/z* MH⁺ 645.

- 20 ***tert*-Butyl (S)-2-((1'-(but-2-yn-1-yl)-5-fluoro-2',3-dioxospiro[isoindoline-1,3'-pyrrolidin]-2-yl)methyl)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-5-yl)carbamate**



Methyl (S)-2-((5-((*tert*-butoxycarbonyl)amino)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-5-fluoro-3-oxo-1-(2-oxoethyl)isoindoline-1-carboxylate (9.50 g, 14.73 mmol) and but-2-yn-1-amine hydrochloride (2.33 g, 22.10 mmol) were placed in a flask with 1,2-dichloroethane (100 mL). Triethylamine (3.08 mL, 22.10 mmol) was added and the reaction mixture was stirred at rt for 30 minutes. Sodium triacetoxymethylborohydride (6.25 g, 29.47 mmol) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was diluted with DCM (250 mL) and washed with saturated NaHCO₃ (100 mL), water (100 mL) and brine (100 mL). The organic phase was passed through a phase separating filter paper and the solvent was removed *in vacuo* to afford the title compound. The crude compound was used without further purification in the next reaction assuming 100% yield. *m/z* MH⁺ 666.

(S)-2-((5-Amino-6-fluoro-1*H*-pyrrolo[3,2-*b*]pyridin-2-yl)methyl)-1'-(but-2-yn-1-yl)-5-fluorospiro[isoindoline-1,3'-pyrrolidine]-2',3-dione (Reference Form 1)



tert-Butyl (S)-2-((1'-(but-2-yn-1-yl)-5-fluoro-2',3-dioxospiro[isoindoline-1,3'-pyrrolidin]-2-yl)methyl)-6-fluoro-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrolo[3,2-*b*]pyridin-5-yl)carbamate (9.81 g, 14.73 mmol) was placed in a flask with 2,2,2-trifluoroacetic acid (22.55 mL, 294.7 mmol) and the solution was stirred for 2 hours at rt. The 2,2,2-trifluoroacetic acid was removed *in vacuo* and the residue was dissolved in MeCN (20 mL). Ammonium hydroxide (28-30% in water) (22.95 mL, 589.4 mmol) was added and the reaction mixture was stirred at 40 °C for 2 hours. The crude product was purified by reverse phase chromatography (Interchim C18-HP Flash column, 415 g), using decreasingly polar mixtures of water (containing by volume 1% NH₄OH (28-30% in H₂O)) and MeCN as eluents (30-60% gradient). Fractions containing the desired compound were combined, the MeCN was removed *in vacuo* and the resulting solid was filtered off and dried to afford the title compound (3.74 g, 58%) as a cream crystalline solid. ¹H NMR (400 MHz, DMSO-d₆, 27°C) 1.88 (3H, t), 2.36 – 2.44 (2H, m), 3.54 (1H, ddd), 3.76 (1H, dt), 4.08 (2H, qq), 4.24 (1H, d), 5.03 (1H, d), 5.49 (2H, s), 6.11 (1H, d), 7.36 (1H, dd), 7.49 – 7.64 (3H, m), 10.69 (1H, d); *m/z* MH⁺ 436.

Example 1: Preparation of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isoindoline-3,3'-pyrrolidine]-1,2'-dione:hemi-adipic acid co:crystal ("Compound (I) Form A")

5 Method A: Single solvent co-crystallisation

Approximately 27.6 mg of adipic acid was dissolved in 500 µL methanol and heated, separately approximately 150 mg of Reference Form 1 was dissolved in 2 mL methanol with heating, subsequently these two solutions were combined and left stirring with a magnetic stirrer bar at ambient temperature for 8 days with a slightly loosened lid on the vial to allow slow evaporation.

- 10 The resultant material was filtered, the solid recovered and dried under a stream of air overnight resulting in Compound (I) Form A at a yield of approximately 53%.

Method B: Anti-solvent/cooling co-crystallisation (not seeded)

- Reference Form 1 (501.51 mg, 1.0942 mmol, 95 mass%) was charged into a 8 mL vial, adipic acid (166.13 mg, 1.1368 mmol, 100 mass%) and dimethyl sulfoxide (3.54 g, 45.3 mmol, 100 mass%) were charged and the contents agitated using a doubly 3-pitched blade impeller at 600 rpm, heated to 25°C and held at this temperature for 5 minutes. The contents of the vial were then heated at a rate of 5°C/minute to 80°C and the temperature held for 20 minutes. Water (1.4 mL, 78 mmol, 100 mass%) was charged to the vial at 80°C over 2 hours at a rate of 0.012 mL/minute and the temperature held for a further 30 minutes. The contents of the vessel were then cooled to 20°C over 8 hours at a rate of 0.125°C/minute and the temperature held for 80 hours. The contents of the vial were further cooled to 5°C over 15 minutes and the temperature held for 3 hours, then heated to 20°C and water (0.75 mL, 42 mmol, 100 mass%) was charged to the vial during which crystallisation occurred. The contents of the vial were then discharged to a filter (30 mm diameter filter paper) under ambient conditions and deliquored under vacuum. Pre-mixed dimethyl sulfoxide (0.525 mL, 7.4 mmol, 100 mass%) and water (0.225 mL, 12.5 mmol, 100 mass%) were charged to the vessel under ambient conditions for washing. The contents of the vial were agitated at 20°C then discharged to the filter under ambient conditions and deliquored under vacuum. This step was repeated for a second wash, then 2-propanol (1.00 mL, 13.1 mmol, 100 mass%) was charged to the filter and the contents deliquored under vacuum. This step was repeated for the final wash, after which the contents of the filter were discharged to a drying dish and dried under vacuum in an oven at 45-50°C for 24 hours, resulting in Compound (I) Form A at a yield of approximately 92%.

35 Method C: Cooling/anti-solvent co-crystallisation (seeded)

Reference Form 1 (8.01 g, 18.2 mmol, 99 mass%) was charged into a 100 mL vessel, adipic acid (2.57 g, 17.6 mmol, 100 mass%) and dimethyl sulfoxide (54.88 g, 702.4 mmol, 100 mass%) were charged and the contents agitated using a 4-pitched blade impeller at 400 rpm,

heated to 25°C and held at this temperature for 30 minutes. The contents of the vessel were then heated at a rate of 5°C/minute to 80°C and the temperature held for 20 minutes. Water (21.6 mL, 1200 mmol, 100 mass%) was charged to the vessel at 80°C over 2 hours at a rate of 0.1125 mL/minute and the temperature held for a further 5 minutes. The contents of the vessel were then cooled to 65°C over 30 minutes at a rate of 0.5°C/minute and the temperature held for 30 minutes. The seed (prepared according to Method B; 8.7 mg, 0.017 mmol, 100 mass%) was then charged to the vessel at 65°C and the temperature held for 3 hours. The contents of the vessel were then cooled to 20°C over 6 hours at a rate of 0.125°C/minute and the temperature held for a further 3 hours. The anti-solvent, water (12 mL, 666.119 mmol, 100 mass%) was charged to the vessel at 20°C over 6 hours and the temperature held for a further 6 hours. The contents of the vessel were then discharged to a filter (63 mm diameter) under ambient conditions and deliquored under vacuum resulting in a cake height of 8 mm. Pre-mixed dimethyl sulfoxide (7.2 mL, 100 mmol, 100 mass%) and water (4.8 mL, 270 mmol, 100 mass%) were charged to the vessel under ambient conditions for washing. The contents of the vessel were agitated at 20°C then discharged to the filter under ambient conditions and deliquored under vacuum. This step was repeated for a second wash, then 2-propanol (16 mL, 209 mmol, 100 mass%) was charged to the filter and the contents deliquored under vacuum. This step was repeated for the final wash, after which the contents of the filter were discharged to a drying dish and dried under vacuum in an oven at 45-50°C for 17 hours, resulting in Compound (I) Form A at a yield of approximately 85%.

Example 2: XRPD analysis of Compound (I) Form A polymorph

The powder X-ray diffractogram was recorded with a two theta scan axis and in one dimensional scan with a Rigaku SmartLab (wavelength of X-rays 1.5418 Å nickel-Filtered Cu K α radiation, 40 kV, 50 mA) equipped with a D/tex Ultra 250 detector and CBO-E optic. The sample was rotated at 30 revolutions per minute during measurement. The sample was scanned from 3-40° 2-theta using a 0.01° and 0.1°/min step width and scan speed, respectively. The powder sample was packed in a long glass capillary with 0.9 mm outer diameter.

The 2-theta values (in degrees) and relative intensities of peaks derived from the XRPD trace which characterise Form A are shown in Table 3 below:

Table 3. Complete list of peaks in X-ray powder diffraction pattern of Form A, in the range 3-30° 2 θ .

2 θ , °	Relative intensity	2 θ , °	Relative intensity
8.9	very strong	20.5	medium
11.8	medium	20.6	medium

2 θ , °	Relative intensity	2 θ , °	Relative intensity
12.2	medium	21.2	strong
14.5	strong	22.2	weak
15.4	weak	22.9	very strong
15.5	weak	23.4	medium
15.8	medium	23.7	strong
16.1	weak	24.7	weak
16.9	strong	25.1	medium
17.4	strong	25.8	strong
17.6	medium	26.4	weak
17.8	weak	26.6	medium
17.9	weak	27.0	strong
17.9	weak	27.3	medium
18.2	medium	27.9	weak
18.6	weak	28.1	strong
19.1	very strong	28.6	medium
19.4	medium	28.8	weak
19.7	very strong	29.2	strong

Relative intensity refers to integrated peak size normalised to that of the largest peak with the categories defined as in Table 1.

5 Results

The powder X-ray diffraction pattern shown in Figure 1 demonstrates that Compound (I) Form A is highly crystalline.

Example 3: Properties of Reference Form 1 and Compound (I) Form A

10 Differential Scanning Calorimetry (DSC)

Thermal events were analysed by standard mode differential scanning calorimetry on a TA Discovery DSC instrument. Approximately 1.5-2.0 mg of material contained in a standard closed aluminium pan was measured over the temperature range 25°C to 300°C at a constant heating rate of 10°C/minute. Nitrogen was used as a purge gas at a flow rate of 50 mL/minute.

15

Thermogravimetric Analysis (TGA)

Weight loss was analysed by standard thermogravimetric analysis on a TA Discovery TGA instrument. Approximately 3-5 mg of material placed on a 100 μ L platinum pan was heated to 300°C from instrument ambient temperature at a constant heating rate of 10°C/minute. Nitrogen

20

was used as a purge gas at a flow rate of 25 mL/minute.

Dynamic Vapour Solvation (DVS)

Approximately 5-10mg of material was weighed into an aluminium sample pan and subjected to the following relative humidity profile using a Surface Measurement Systems DVS Resolution instrument: 40-90-0-90-0 % relative humidity with 10% relative humidity steps and dm/dt threshold of 0.002%, and maximum step time limit of six hours. The temperature was maintained at approximately 25°C throughout.

Results

The results of the DSC and TGA experiments for Reference Form 1 and Compound (I) Form A are shown in Figures 2 and 3, respectively. The results of the DVS experiments for Reference Form 1 and Compound (I) Form A are shown in Figures 4 and 5, respectively.

Thermal analysis of Reference Example 1 by DSC shows a broad endotherm indicative of de-solvation followed by a single melting endotherm (melting peak: endothermic, onset 164.08 °C, peak 168.52 °C, enthalpy 44.746 J/g) (Figure 2). A single melting endotherm for Compound (I) Form A was observed (melting peak: Endothermic, onset 216.63 °C, peak 217.70 °C, enthalpy 122.48 J/g) (Figure 3).

TGA of Reference Form 1 shows weight loss of 1.617% to 100°C due to de-solvation, indicating an initial hydrated form (Figure 2). TGA of Compound (I) Form A showed no significant weight loss prior to the melting point, indicating an anhydrous form (Figure 3).

DVS analysis shows that Reference Form 1 undergoes reversible hydration from the hemi-hydrate to the mono-hydrate at high relative humidity, and reversible dehydration from the hemi-hydrate to the an-hydrate at low relative humidity (Figure 4). DVS analysis also reveals that Reference Form 1 has a moisture uptake of 3.37% at 80% relative humidity (Figure 4).

In contrast, Compound (I) Form A has very low hygroscopicity, with a moisture uptake of 0.50% at 80% relative humidity (Figure 5).

Therefore, the results show that Compound (I) Form A displays high thermal stability, is unsolvated and has low hygroscopicity.

Example 4: Solubility of Reference Form 1 and Compound (I) Form A

The results of preliminary solubility testing of Reference Form (I) and Compound (I) Form A over 24 hours at 37°C are shown in Table 4.

Table 4. Solubility in biorelevant media of Compound (I) Form A and Reference Form 1.

Media	Solubility (μM)	
	Compound (I) Form A	Reference Form 1
SGF	21152*	42704*
FaSSIFv2	1161	747
Blank FaSSIF	369	250
FeSSIF	1376	830
Blank FeSSIF	649	659

*Unsaturated samples; SGF = simulated gastric fluid; FaSSIF = fasted state simulated intestinal fluid; FeSSIF = fed state simulated intestinal fluid

5 Results

The results show that Compound (I) Form A is more soluble than Reference Form 1 in a variety of biorelevant media (Table 4). The solubility testing in SGF was inconclusive as both samples were unsaturated.

10 **Example 5: Physical stability of Compound (I) Form A**

Physical stability of Compound (I) Form A was assessed by a water slurry method.

Approximately 15 mg of Compound (I) Form A was weighed into a vial and 250 μL water added.

This was stirred using a magnetic stirrer bar at ambient temperature for 10 days, then filtered using a 0.45 μm centrifuge filter (13,000 rpm for 5 minutes) and analysed by XRPD, which

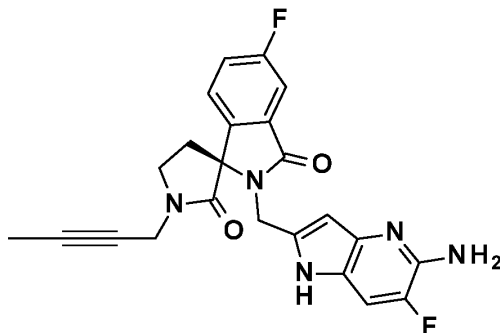
15 showed no form change in the Compound (I) Form A material, indicating that Compound (I) Form A appears to be physically stable.

Conclusions

The results of the experiments above demonstrate that Compound (I) Form A is a discrete
 20 crystalline form which is more thermally stable than Reference Form 1. Compound (I) Form A is anhydrous, unlike Reference Form 1 which displays variable (de)hydration due to its sensitivity to temperature and/or humidity. Compound (I) Form A is less hygroscopic than Reference Form 1 and preliminary solubility data suggest that Compound (I) Form A is more soluble than Reference Form 1 in a variety of biorelevant media. Compound (I) Form A also appears to be
 25 physically stable.

CLAIMS

1. A crystalline form which is a co-crystal of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione:



5

Compound (I),

and adipic acid in a 2:1 ratio (Compound (I) Form A).

2. The crystalline form according to claim 1, wherein the crystalline form has an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation.

10

3. The crystalline form according to claim 1 or 2, wherein the crystalline form has an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation.

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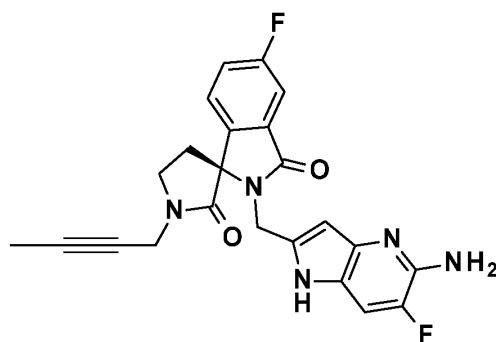
4. The crystalline form according to any one of claims 1 to 3, wherein the crystalline form has an X-ray powder diffraction substantially as shown in Figure 1 when measured using CuK α radiation.

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5. The crystalline form according to any one of claims 1 to 4, wherein the crystalline form has a DSC thermogram substantially as shown in Figure 3.

6. The crystalline form according to claim 1, wherein the crystalline form is a co-crystal of (3S)-2-[(5-amino-6-fluoro-1H-pyrrolo[3,2-b]pyridin-2-yl)methyl]-1'-but-2-ynyl-6-fluoro-spiro[isindoline-3,3'-pyrrolidine]-1,2'-dione:

25



Compound (I),

and adipic acid in a 2:1 ratio (Compound (I) Form A), having at least one of the following:

- a) an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 19.1, 19.7 and 22.9 degrees 2-theta when measured using CuK α radiation;
 - 5 b) an X-ray powder diffraction pattern containing specific peaks at (± 0.2) 8.9, 14.5, 16.9, 17.4, 19.1, 19.7, 21.2, 22.9, 23.7 and 25.8 degrees 2-theta when measured using CuK α radiation;
 - c) an X-ray powder diffraction pattern substantially as shown in Figure 1;
 - d) a DSC thermogram with an onset melting at 216.6°C plus or minus 5°C and a peak at
10 217.7°C plus or minus 5°C;
 - e) a DSC thermogram and optionally a TGA thermogram substantially as shown in Figure 3.
7. A pharmaceutical composition comprising the crystalline form according to any one of
15 claims 1 to 6 and at least one pharmaceutically acceptable excipient.
 8. The crystalline form according to any one of claims 1 to 6 or the pharmaceutical composition according to claim 7, for use as a medicament, for example in the prophylaxis or treatment of a disorder mediated by PRMT5.
20
 9. Use of the crystalline form according to any one of claims 1 to 6 or the pharmaceutical composition according to claim 7, in the manufacture of a medicament, for example for the prophylaxis or treatment of a disorder mediated by PRMT5.
 - 25 10. A method of prevention or treatment, for example preventing or treating a disorder mediated by PRMT5, which comprises administering the crystalline form according to any one of claims 1 to 6, or the pharmaceutical composition according to claim 7.
 11. The crystalline form for use, pharmaceutical composition for use, use or method
30 according to any one of claims 8 to 10, wherein the disorder is cancer.

12. The crystalline form for use, pharmaceutical composition for use, use or method according to claim 11, wherein the cancer is a MTAP deleted cancer.

- 5 13. The crystalline form for use, pharmaceutical composition for use, use or method according to claim 11 or 12, wherein the cancer is selected from gastric, pancreatic, colorectal, uterine, bile duct, stomach, bladder, cervical, testicular germ cell, lung (e.g. non-small cell lung cancer), multiple myeloma, lymphoma (e.g. diffuse large B cell lymphoma or Hodgkin's lymphoma), rhabdomyosarcoma and cutaneous squamous cell carcinoma.

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