



(51) International Patent Classification:

C07H 19/16 (2006.01) C07H 19/207 (2006.01)
C07H 19/167 (2006.01) C07H 19/213 (2006.01)
C07H 19/173 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/US2022/023247

(22) International Filing Date:

04 April 2022 (04.04.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/172,823 09 April 2021 (09.04.2021) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM,

(54) Title: NOVEL FORMS OF CYCLIC DINUCLEOTIDE COMPOUNDS

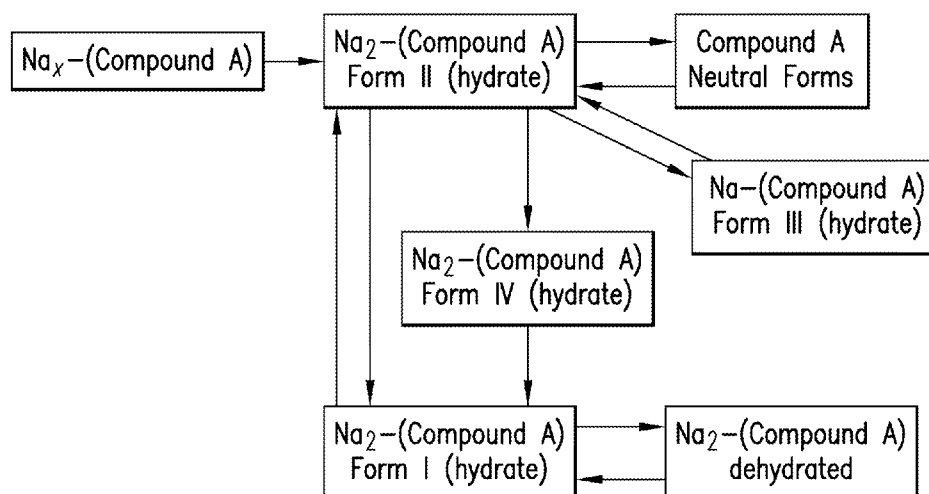


FIG. 1

(57) Abstract: Novel forms of 2-amino-9-[(2R,5R,7R,8S,10R,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfido-octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one, which include crystalline salts and hydrates of 2-amino-9-[(2R,5R,7R,8S,10R,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfido-octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one, may be useful as inducers of type I interferon production, specifically as STING active agents.



TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

TITLE OF THE APPLICATION

NOVEL FORMS OF CYCLIC DINUCLEOTIDE COMPOUNDS

FIELD OF THE INVENTION

5 The invention relates to novel forms comprising cyclic dinucleotide compounds that are STING (Stimulator of Interferon Genes) agonists that activate the STING pathway. The forms of the invention may be crystalline or amorphous.

SEQUENCE LISTING

10 The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 8, 2022, is named 25220WOPCT-SEQTXT-08FEB2022_ST25.txt and is 18,393 bytes in size.

15 BACKGROUND OF THE INVENTION

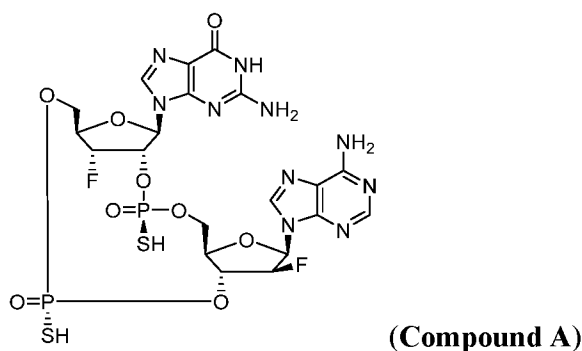
 Compounds that induce type I interferon activity have great potential as anti-viral and anti-cancer agents (*see* T.R. Vargas *et al.*, *Rationale for STING-Targeted Cancer Immunotherapy*, 75 Eur. J. Cancer 85-97 (2017); L. Corrales *et al.*, *Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity*, 20 11 Cell Reports 1018-30 (2015); Glen N. Barber, *STING: infection, inflammation and cancer*, 15 Nat. Rev. Immunol. 760-770 (2015); E. Curran *et al.*, *STING Pathway Activation Stimulates Potent Immunity Against Myeloid Leukemia*, 15 Cell Reports 2357-66 (2016). There is a growing body of data demonstrating that the cGAS-STING cGAS (cyclic GMP-AMP synthase-STING) cytosolic DNA sensory pathway has a significant capacity to induce type I interferons. 25 Thus, the development of STING activating agents is rapidly taking an important place in today's anti-tumor therapy landscape.

 Cyclic dinucleotide (CDN) compounds that are STING agonists for use in human subjects must be stored prior to use and transported to the point of administration. Reproducibly attaining a desired level of drug in a subject requires that the drug be stored in a formulation that 30 maintains the potency of the drug. The need exists for stable forms of cyclic dinucleotide STING agonist compounds that can be formulated for pharmaceutical use, *e.g.*, for treating cell proliferation disorders, such as cancers, and infectious diseases.

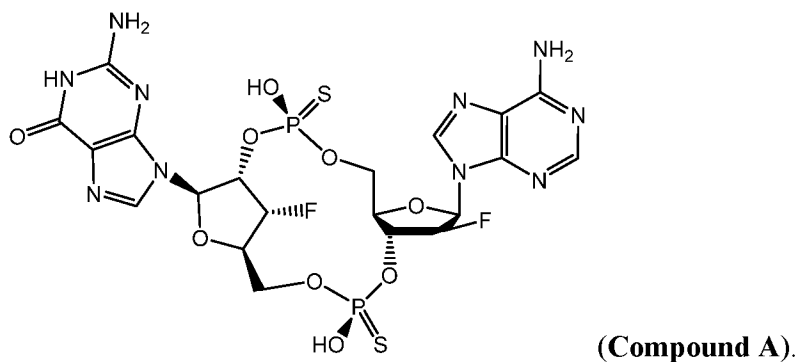
The cyclic dinucleotide STING agonist compound 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one and methods for making the same are

5 illustrated in PCT International Patent Application No. PCT/US2016/046444, which published as PCT International Patent Application Publication No. WO2017/027646, and United States Patent Application No. 15/234,182, which published as U.S. Patent Application Publication No. US2017/0044206, which are incorporated herein by reference in their entirety, as Example 247. The present invention is directed to novel forms of 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-

10 dihydro-6*H*-purin-6-one (**Compound A**):



which may also be shown as



In view of the potential of cancers and infectious diseases to cause illness and death, there remains a need for new compounds and forms that can be used to treat cell proliferation disorders, such as cancers, and infectious diseases.

20 SUMMARY OF THE INVENTION

This disclosure is directed to novel forms of 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (**Compound A**). Certain forms have advantages, such as ease of processing, handling, or stability to stress. In particular, these forms exhibit improved physicochemical properties, such as stability to stress, rendering them particularly suitable for the manufacture of various pharmaceutical dosage forms. The disclosure also concerns pharmaceutical compositions containing the novel forms thereof, as well as methods for using them as STING agonists, particularly in the treatment of cell proliferation disorders, such as cancers. In certain embodiments, described herein are pharmaceutical compositions comprising a form of 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a phase map of forms of **Compound A**.

Fig. 2 depicts an XRPD diffractogram of **Form I**, showing characteristic reflection for **Form I**.

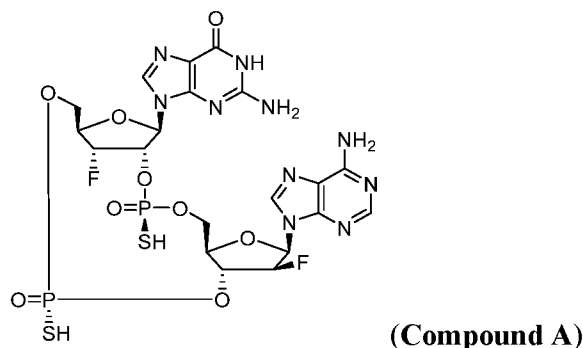
Fig. 3 depicts an XRPD diffractogram of **Form II**, showing characteristic reflection for **Form II**.

Fig. 4 depicts an XRPD diffractogram of **Form III**, showing characteristic reflection for **Form III**.

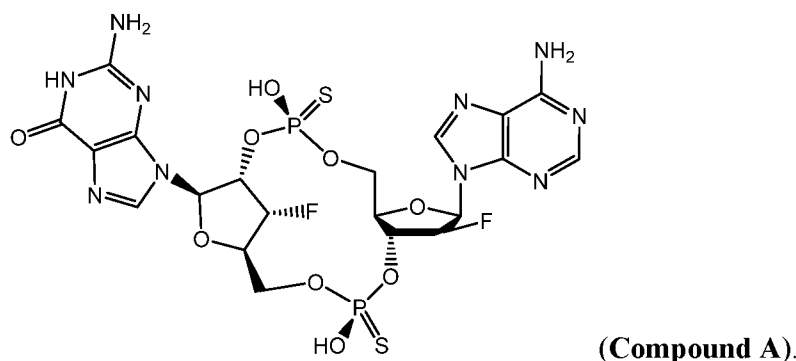
Fig. 5 depicts an XRPD diffractogram of **Form IV**, showing characteristic reflection for **Form IV**.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to forms of 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (**Compound A**):



which may also be shown as



In particular, the invention relates to crystalline and amorphous forms of **Compound A**. Unless
 5 a specific form designation is given, the term “2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,
 15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-
 12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphospha-cyclotetradecin-7-yl]-1,9-
 dihydro-6*H*-purin-6-one” includes all forms described herein.

In embodiments, forms of **Compound A** may be interconverted according to the
 10 Phase Map set forth in **Fig. 1**, and as discussed in the Examples herein.

A first embodiment of the forms of **Compound A** described herein is a 2-amino-
 9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-
 2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]
 pentaoxadi-phospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one di-sodium salt hydrate.

In a first aspect of this embodiment, the 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,
 15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-
 disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphospha-
 cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one di-sodium salt hydrate is a crystalline material
 and is designated **Form I** (hydrate). **Form I** may have better handling properties, such as higher
 20 bulk density and reduced hygroscopicity than amorphous forms of **Compound A**. For example,

it may be possible to access **Compound A** in solid form with higher purity in **Form I**, as compared to amorphous forms. **Form I** may be selectively obtained by controlled pH swing processes from crystalline **Form II** (hydrate, described hereinbelow), which can prevent gumming.

5 In instances of the first aspect, **Form I** is characterized by an X-ray powder diffraction containing at least 3 2θ values measured using $\text{CuK}\alpha$ radiation selected from the group consisting of about 4.44, about 8.87, about 9.46, about 9.72, about 10.43, about 11.33, about 12.36, about 12.96, about 13.30, about 13.69, about 14.57, about 15.21, about 16.33, about 16.91, about 17.11, about 17.54, about 18.22, about 18.73, about 19.15, about 19.45, about 10 19.76, about 20.08, about 20.30, about 20.63, about 20.90, about 21.60, about 21.96, about 22.13, about 22.59, about 22.92, about 23.20, about 23.69, about 24.31, about 24.60, about 24.85, about 25.58, about 26.09, about 26.39, about 26.74, about 27.03, about 27.45, about 28.14, about 28.53, about 28.94, about 29.12, about 30.00, about 30.69, about 30.93, about 31.31, about 31.85, about 32.39, about 33.77, about 34.38, about 34.98, about 35.28, about 15 36.05, about 36.43, about 37.30, about 37.64, about 37.93, about 38.85, about 39.48, about 40.02, and about $40.38^\circ 2\theta$. In particular instances, **Form I** is characterized by an X-ray powder diffraction containing at least 3 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 9.46, about 9.72, about 10.43, about 11.33, about 12.36, about 13.69, about 14.57, about 16.33, about 16.91, about 17.11, about 17.54, about 18.22, about 18.73, about 19.15, about 19.45, about 20 19.76, about 20.08, about 20.30, about 20.63, about 20.90, about 21.60, about 21.96, about 22.13, about 22.59, about 22.92, about 23.20, about 23.69, about 24.31, about 24.60, about 24.85, about 25.58, about 26.09, about 26.39, about 26.74, about 27.03, about 27.45, about 28.14, about 28.53, about 28.94, about 29.12, about 30.00, about 30.69, about 30.93, about 31.31, about 31.85, about 33.77, about 36.05, about 36.43, about 38.85, about 40.02, and about 25 40.38° 2θ . In further instances, **Form I** is characterized by an X-ray powder diffraction containing at least 3 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 4.44, about 8.87, about 12.96, about 13.30, about 15.21, about 32.39, about 34.38, about 34.98, about 35.28, about 37.30, about 37.64, about 37.93, and about 39.48° 2θ .

In a second aspect of this embodiment, the 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*, 30 14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one di-sodium salt hydrate is a crystalline material

and is designated **Form II** (hydrate). **Form II**, which incorporates more water than **Form I**, may also have better handling properties, such as higher bulk density and reduced hygroscopicity than amorphous forms of **Compound A**. As with **Form I**, it may be possible to access **Compound A** in solid form with higher purity in **Form II**, as compared to amorphous forms.

5 In instances of the first aspect, **Form II** is characterized by an X-ray powder diffraction containing at least 3 2θ values measured using $\text{CuK}\alpha$ radiation selected from the group consisting of about 6.87, about 7.31, about 7.73, about 8.76, about 12.54, about 13.57, about 13.85, about 14.59, about 15.21, about 16.13, about 16.35, about 16.81, about 18.26, about 18.74, about 19.23, about 20.48, about 21.04, about 21.41, about 22.07, about 22.83, about 23.46, about 24.15, about 24.96, about 25.39, about 25.7, about 26.86, about 27.21, about 27.82, and about 28.4, about 29.01, about 29.62, about 30.43, about 30.91, about 31.56, about 32.14, about 33.1, about 33.63, and about $34.06^\circ 2\theta$. In particular instances, **Form II** is characterized by an X-ray powder diffraction containing at least 3 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 12.54, about 13.57, about 13.85, about 15.21, about 16.13, about 16.35, about 16.81, about 20.48, about 21.04, about 21.41, about 22.07, about 25.7, about 27.21, about 27.82, and about $28.4^\circ 2\theta$. In other particular instances, **Form II** is characterized by an X-ray powder diffraction containing at least 2 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 29.01, about 29.62, about 30.43, about 30.91, about 31.56, about 32.14, about 33.1, about 33.63, and about $34.06^\circ 2\theta$. In further instances, **Form II** is characterized by an X-ray powder diffraction containing at least 2 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 6.87, about 7.31, about 7.73, about 8.76, about 14.59, about 18.26, about 18.74, about 19.23, about 22.83, about 23.46, about 24.15, about 24.96, about 25.39, and about $26.86^\circ 2\theta$.

A second embodiment of the forms of **Compound A** described herein is a 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one mono-sodium salt hydrate.

In aspects of this embodiment, the 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one mono-sodium salt hydrate is a crystalline material and is designated

Form III (hydrate). Like **Form I**, **Form III** may have better handling properties, such as higher bulk density and reduced hygroscopicity than amorphous forms of **Compound A**. As with **Form I** and **Form II**, it may be possible to access **Compound A** in solid form with higher purity in **Form III**, as compared to amorphous forms.

In instances of the first aspect, **Form III** is characterized by an X-ray powder diffraction containing at least 3 2θ values measured using $\text{CuK}\alpha$ radiation: selected from the group consisting of about 5.45, about 6.83, about 8.69, about 12.74, about 13.57, about 13.84, about 14.60, about 15.09, about 16.18, about 17.35, about 19.16, about 20.10, about 20.40, about 21.12, about 21.33, about 21.97, about 22.68, about 23.12, about 23.33, about 24.52, about 25.34, about 25.70, about 26.13, about 27.11, about 27.30, about 27.92, about 28.51, about 29.04, about 29.41, about 30.44, about 30.64, about 32.00, about 32.34, about 32.65, about 33.37, about 34.16, about 36.13, about 36.43, about 36.92, and about $38.20^\circ 2\theta$. In particular instances, **Form III** is characterized by an X-ray powder diffraction containing at least 3 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 21.12, about 21.33, about 21.97, about 22.68, about 23.12, about 23.33, about 24.52, about 25.34, about 25.70, about 26.13 2θ . In further particular instances, **Form III** is characterized by an X-ray powder diffraction containing at least 4 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: an X-ray powder diffraction containing at least 2 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 27.11, about 27.30, about 27.92, about 28.51, about 29.04, about 29.41, about 30.44, about 30.64, about 32.00, about 32.34, about 32.65, about 33.37, about 34.16, about 36.13, about 36.43, about 36.92, and about $38.20^\circ 2\theta$. In further instances, **Form III** is characterized by an X-ray powder diffraction containing at least 2 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 5.45, about 6.83, about 8.69, about 12.74, about 13.57, about 13.84, about 14.60, about 15.09, about 16.18, about 17.35, about 19.16, about 20.10, and about $20.40^\circ 2\theta$.

A third embodiment of the forms of **Compound A** described herein is a 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfido-octahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxa-diphospha-cyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one di-sodium salt hydrate.

In a first aspect of this embodiment, the 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfido-octahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadi-phospha-cyclotetradecin-7-

yl]-1,9-dihydro-6*H*-purin-6-one di-sodium salt hydrate is a crystalline material and is designated **Form IV** (hydrate).

In instances of the first aspect, **Form IV** is characterized by an X-ray powder diffraction containing at least 3 2θ values measured using $\text{CuK}\alpha$ radiation selected from the group consisting of about 5.52, about 6.63, about 7.22, about 7.65, about 8.56, about 11.23, about 12.73, about 13.22, about 13.46, about 14.29, about 14.61, about 15.17, about 15.52, about 15.84, about 16.32, about 17.08, about 17.39, about 17.67, about 18.35, about 18.98, about 19.20, about 19.78, about 19.99, about 20.54, about 21.25, about 21.87, about 22.04, about 22.62, about 22.97, about 23.27, about 23.77, about 24.07, about 24.58, about 25.11, about 25.56, about 25.77, about 26.47, about 26.91, about 27.30, about 27.71, about 28.18, about 28.52, about 29.15, about 29.61, about 29.92, about 30.26, about 30.92, about 31.55, and about $31.78^\circ 2\theta$. In particular instances, **Form IV** is characterized by an X-ray powder diffraction containing at least 3 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 5.52, about 7.22, about 7.65, about 8.56, about 11.23, about 12.73, about 13.46, about 14.29, about 14.61, about 15.17, about 15.52, about 15.84, about 16.32, about 17.08, about 17.39, about 17.67, about 18.35, about 20.54, about 21.81, about 21.87, about 22.04, about 22.62, about 22.97, about 23.27, about 23.77, about 24.07, about 24.58, and about $25.56^\circ 2\theta$. In still more particular instances, **Form IV** is characterized by an X-ray powder diffraction containing at least 4 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: an X-ray powder diffraction containing at least 2 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 25.77, about 26.47, about 26.91, about 27.30, about 27.71, about 28.18, about 28.52, about 29.15, about 29.61, about 29.92, about 30.26, about 30.92, about 31.55, and about $31.78^\circ 2\theta$. In further instances, **Form IV** is characterized by an X-ray powder diffraction containing at least 2 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about 6.63, about 13.22, about 18.98, about 19.20, about 19.78, about 19.99, about 21.25, and about $25.11^\circ 2\theta$.

A fourth embodiment provides a particular drug substance that comprises at least one of the forms described herein. By “drug substance” is meant the active pharmaceutical ingredient. The amount of a form in the drug substance can be detected by physical methods such as X-ray powder diffraction, fluorine-19 magic-angle spinning (MAS) nuclear magnetic resonance spectroscopy, and carbon-13 cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance spectroscopy.

Additional embodiments of the invention include pharmaceutical compositions

comprising the forms described herein and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be solid dosage forms for oral administration or sterile solutions for parenteral, intratumoral, intravenous, or intramuscular administration.

Further embodiments include the use of the forms described herein as an active ingredient in a medicament for inducing an immune response in a subject and the use of the forms described herein as an active ingredient in a medicament for inducing a STING-dependent type I interferon production in a subject. Further embodiments also include the use of the forms described herein as an active ingredient in a medicament for treatment of a cell proliferation disorder, which includes but is not limited to cancer.

Further embodiments include the use of the pharmaceutical compositions described herein as a medicament for inducing an immune response in a subject and for inducing a STING-dependent type I interferon production in a subject. Further embodiments also include the use of the pharmaceutical compositions described herein as an active ingredient in a medicament for treatment of a cell proliferation disorder, which includes but is not limited to cancer.

The forms of the present invention exhibit different chemical and physical properties as compared to the neutral form of **Compound A** as described in Example 247 of WO2017/027646 and US2017/0044206, which may provide pharmaceutical advantages. In particular, the novel forms, which have different equilibrium solubility values as compared to sodium salts of Compound A, enhanced chemical and physical stability of the forms constitute advantageous properties in the development of solid pharmaceutical dosage forms containing the pharmacologically active ingredient.

CELL-PROLIFERATION DISORDERS

The therapies disclosed herein are potentially useful in treating diseases or disorders including, but not limited to, cell-proliferation disorders. Cell-proliferation disorders include, but are not limited to, cancers, benign papillomatosis, gestational trophoblastic diseases, and benign neoplastic diseases, such as skin papilloma (warts) and genital papilloma. The terms “cancer”, “cancerous”, or “malignant” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth.

In specific embodiments, the disease or disorder to be treated is a cell-proliferation disorder. In certain embodiments, the cell-proliferation disorder is cancer. In particular

embodiments, the cancer is selected from brain and spinal cancers, cancers of the head and neck, leukemia and cancers of the blood, skin cancers, cancers of the reproductive system, cancers of the gastrointestinal system, liver and bile duct cancers, kidney and bladder cancers, bone cancers, lung cancers, malignant mesothelioma, sarcomas, lymphomas, glandular cancers, thyroid
5 cancers, heart tumors, germ cell tumors, malignant neuroendocrine (carcinoid) tumors, midline tract cancers, and cancers of unknown primary (*i.e.*, cancers in which a metastasized cancer is found but the original cancer site is not known). In particular embodiments, the cancer is present in an adult patient; in additional embodiments, the cancer is present in a pediatric patient. In particular embodiments, the cancer is AIDS-related.

10 In specific embodiments, the cancer is selected from brain and spinal cancers. In particular embodiments, the brain and spinal cancer is selected from the group consisting of anaplastic astrocytomas, glioblastomas, astrocytomas, and esthesioneuroblastomas (also known as olfactory blastomas). In particular embodiments, the brain cancer is selected from the group consisting of astrocytic tumor (e.g., pilocytic astrocytoma, subependymal giant-cell astrocytoma,
15 diffuse astrocytoma, pleomorphic xanthoastrocytoma, anaplastic astrocytoma, astrocytoma, giant cell glioblastoma, glioblastoma, secondary glioblastoma, primary adult glioblastoma, and primary pediatric glioblastoma), oligodendroglial tumor (e.g., oligodendroglioma, and anaplastic oligodendroglioma), oligoastrocytic tumor (e.g., oligoastrocytoma, and anaplastic oligoastrocytoma), ependymoma (e.g., myxopapillary ependymoma, and anaplastic
20 ependymoma); medulloblastoma, primitive neuroectodermal tumor, schwannoma, meningioma, atypical meningioma, anaplastic meningioma, pituitary adenoma, brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, visual pathway and hypothalamic glioma, and primary central nervous system lymphoma. In specific instances of these embodiments, the brain cancer is selected from the group consisting of glioma, glioblastoma multiforme,
25 paraganglioma, and supratentorial primordial neuroectodermal tumors (sPNET).

In specific embodiments, the cancer is selected from cancers of the head and neck, including recurrent or metastatic head and neck squamous cell carcinoma (HNSCC), nasopharyngeal cancers, nasal cavity and paranasal sinus cancers, hypopharyngeal cancers, oral
30 cavity cancers (e.g., squamous cell carcinomas, lymphomas, and sarcomas), lip cancers, oropharyngeal cancers, salivary gland tumors, cancers of the larynx (e.g., laryngeal squamous cell carcinomas, rhabdomyosarcomas), and cancers of the eye or ocular cancers. In particular embodiments, the ocular cancer is selected from the group consisting of intraocular melanoma

and retinoblastoma.

In specific embodiments, the cancer is selected from skin cancers. In particular
embodiments, the skin cancer is selected from the group consisting of melanoma, squamous cell
cancers, and basal cell cancers. In specific embodiments, the skin cancer is unresectable or
5 metastatic melanoma.

In specific embodiments, the cancer is selected from cancers of the reproductive system.
In particular embodiments, the cancer is selected from the group consisting of breast cancers,
cervical cancers, vaginal cancers, ovarian cancers, endometrial cancers, prostate cancers, penile
cancers, and testicular cancers. In specific instances of these embodiments, the cancer is a breast
10 cancer selected from the group consisting of ductal carcinomas and phyllodes tumors. In
specific instances of these embodiments, the breast cancer may be male breast cancer or female
breast cancer. In more specific instances of these embodiments, the breast cancer is triple-
negative breast cancer. In specific instances of these embodiments, the cancer is a cervical
cancer selected from the group consisting of squamous cell carcinomas and adenocarcinomas. In
15 specific instances of these embodiments, the cancer is an ovarian cancer selected from the group
consisting of epithelial cancers.

In specific embodiments, the cancer is selected from cancers of the gastrointestinal
system. In particular embodiments, the cancer is selected from the group consisting of
esophageal cancers, gastric cancers (also known as stomach cancers), gastrointestinal carcinoid
20 tumors, pancreatic cancers, gallbladder cancers, colorectal cancers, and anal cancer. In instances
of these embodiments, the cancer is selected from the group consisting of esophageal squamous
cell carcinomas, esophageal adenocarcinomas, gastric adenocarcinomas, gastrointestinal
carcinoid tumors, gastrointestinal stromal tumors, gastric lymphomas, gastrointestinal
lymphomas, solid pseudopapillary tumors of the pancreas, pancreatoblastoma, islet cell tumors,
25 pancreatic carcinomas including acinar cell carcinomas and ductal adenocarcinomas, gallbladder
adenocarcinomas, colorectal adenocarcinomas, and anal squamous cell carcinomas.

In specific embodiments, the cancer is selected from liver and bile duct cancers. In
particular embodiments, the cancer is liver cancer (also known as hepatocellular carcinoma). In
particular embodiments, the cancer is bile duct cancer (also known as cholangiocarcinoma); in
30 instances of these embodiments, the bile duct cancer is selected from the group consisting of
intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma.

In specific embodiments, the cancer is selected from kidney and bladder cancers. In

particular embodiments, the cancer is a kidney cancer selected from the group consisting of renal cell cancer, Wilms tumors, and transitional cell cancers. In particular embodiments, the cancer is a bladder cancer selected from the group consisting of urothelial carcinoma (a transitional cell carcinoma), squamous cell carcinomas, and adenocarcinomas.

5 In specific embodiments, the cancer is selected from bone cancers. In particular embodiments, the bone cancer is selected from the group consisting of osteosarcoma, malignant fibrous histiocytoma of bone, Ewing sarcoma, chordoma (cancer of the bone along the spine).

10 In specific embodiments, the cancer is selected from lung cancers. In particular embodiments, the lung cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancers, bronchial tumors, and pleuropulmonary blastomas.

 In specific embodiments, the cancer is selected from malignant mesothelioma. In particular embodiments, the cancer is selected from the group consisting of epithelial mesothelioma and sarcomatoids.

15 In specific embodiments, the cancer is selected from sarcomas. In particular embodiments, the sarcoma is selected from the group consisting of central chondrosarcoma, central and periosteal chondroma, fibrosarcoma, clear cell sarcoma of tendon sheaths, and Kaposi's sarcoma.

20 In specific embodiments, the cancer is selected from glandular cancers. In particular embodiments, the cancer is selected from the group consisting of adrenocortical cancer (also known as adrenocortical carcinoma or adrenal cortical carcinoma), pheochromocytomas, paragangliomas, pituitary tumors, thymoma, and thymic carcinomas.

 In specific embodiments, the cancer is selected from thyroid cancers. In particular embodiments, the thyroid cancer is selected from the group consisting of medullary thyroid carcinomas, papillary thyroid carcinomas, and follicular thyroid carcinomas.

25 In specific embodiments, the cancer is selected from germ cell tumors. In particular embodiments, the cancer is selected from the group consisting of malignant extracranial germ cell tumors and malignant extragonadal germ cell tumors. In specific instances of these embodiments, the malignant extragonadal germ cell tumors are selected from the group consisting of nonseminomas and seminomas.

30 In specific embodiments, the cancer is selected from heart tumors. In particular embodiments, the heart tumor is selected from the group consisting of malignant teratoma, lymphoma, rhabdomyosarcoma, angiosarcoma, chondrosarcoma, infantile fibrosarcoma, and

synovial sarcoma.

In specific embodiments, the cell-proliferation disorder is selected from benign papillomatosis, benign neoplastic diseases and gestational trophoblastic diseases. In particular embodiments, the benign neoplastic disease is selected from skin papilloma (warts) and genital papilloma. In particular embodiments, the gestational trophoblastic disease is selected from the group consisting of hydatidiform moles, and gestational trophoblastic neoplasia (*e.g.*, invasive moles, choriocarcinomas, placental-site trophoblastic tumors, and epithelioid trophoblastic tumors).

In embodiments, the cell-proliferation disorder is a cancer that has metastasized, for example, liver metastases from colorectal cancer.

In embodiments, the cell-proliferation disorder is selected from the group consisting of solid tumors. In particular embodiments, the cell-proliferation disorder is selected from the group consisting of advanced or metastatic solid tumors. In more particular embodiments, the cell-proliferation disorder is selected from the group consisting of malignant melanoma, head and neck squamous cell carcinoma, and breast adenocarcinoma.

In particular embodiments, the cell-proliferation disorder is classified as stage III cancer or stage IV cancer. In instances of these embodiments, the cancer is not surgically resectable.

DOSING & FORMULATIONS

The dosage regimen is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; and the renal and hepatic function of the patient. An ordinarily skilled physician, veterinarian, or clinician can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

The forms of the present invention may be formulated and administered in solid dosage forms, such as tablets, pills, capsules, powders, or granules, which are intended for oral administration. Formulation of the compositions according to the invention can conveniently be by methods known from the art, for example, as described in Remington's Pharmaceutical Sciences, 17th ed., 1995. Furthermore, the forms of the present invention may be formulated and administered in sterile solutions for parenteral, intratumoral, intravenous, or intramuscular administration.

In the methods of the present invention, the forms described herein may be

formulated as the active pharmaceutical ingredient, and may be administered in admixture with suitable pharmaceutical diluents, excipients, or carriers (collectively referred to herein as 'carrier' materials) suitably selected with respect to the intended form of administration and consistent with conventional pharmaceutical practices, that is, oral tablets or sterile solutions for parenteral, intratumoral, intravenous, or intramuscular administration.

For instance, for oral administration in the form of a tablet or capsule, the form described herein can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier (such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like). For parenteral, intratumoral, intravenous, or intramuscular administration in the form of a sterile solution, the form described herein may be combined with suitable excipients and non-toxic, pharmaceutically acceptable, inert carrier into a formulation that may be provided as a prepared dosage form in a pre-filled injection apparatus, as a lyophilized formulation to be reconstituted for injection, or as a sterile liquid to be diluted for injection.

ABBREVIATIONS

(NH ₄) ₂ HPO ₄	Ammonium phosphate
(NH ₄) ₂ SO ₄	Ammonium sulfate
[HN(<i>n</i> -oct) ₃] ₂ [SO ₄]	Tri- <i>n</i> -octylammonium hydrogen sulfate
¹³ F-NMR	¹³ F nuclear magnetic resonance spectroscopy
¹⁹ F-NMR	¹⁹ F nuclear magnetic resonance spectroscopy
¹ H-NMR	Proton nuclear magnetic resonance spectroscopy
2,4,6-collidine	2,4,6-Trimethylpyridine
2,6-lutidine	2,6-Dimethylpyridine
2'-FA	(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i>)-5-(6-amino-9 <i>H</i> -purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol
2'-F-thio-AMP	(O-{[(2 <i>R</i> ,3 <i>R</i> ,4 <i>S</i> ,5 <i>R</i>)-5-(6-amino-9 <i>H</i> -purin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methyl} O,O-dihydrogen phosphorothioate, also known as 2'-fluoro-thio-adenosine monophosphate
2'-F-thio-ATP	2'-fluoro-thio-adenosine triphosphate
2-Me-THF	2-Methyltetrahydrofuran

2-TBS	2- <i>tert</i> -butyl(((2 <i>R</i> ,3 <i>S</i>)-3-((<i>tert</i> -butyldimethylsilyl)oxy)-2,3-dihydrofuran-2-yl)methoxy)dimethylsilane
3'-FG	9-((2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i> ,5 <i>R</i>)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-(isobutylamino)-1,9-dihydro-6 <i>H</i> -purin-6-one
3'-F-thio-GMP	(2 <i>S</i> ,3 <i>R</i> ,4 <i>S</i> ,5 <i>R</i>)-5-(2-amino-6-oxo-1,6-dihydro-9 <i>H</i> -purin-9-yl)-3-fluoro-4-hydroxy-2-(mercaptomethyl)tetrahydrofuran-3-yl dihydrogen phosphate, also known as 3'-fluoro-thio-guanosine monophosphate
3'-F-thio-GTP	3'-fluoro-thio-guanosine triphosphate
³¹ P-NMR	³¹ P nuclear magnetic resonance spectroscopy
3-TBS	<i>N</i> -((2 <i>S</i> ,3 <i>S</i> ,4 <i>R</i> ,5 <i>R</i>)-4-((<i>tert</i> -butyldimethylsilyl)oxy)-5-(((<i>tert</i> -butyldimethylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)- <i>N</i> -(phenylsulfonyl) benzenesulfonamide
Ac	Acetyl
AcP-Li/Li	Dilithium acetylphosphate
AMP	Adenosine monophosphate
aq	Aqueous
ATP	Adenosine 5'-triphosphate
BSA	Bis(trimethylsilyl) acetamide
BSTFA	Bis(trimethylsilyl) trifluoroacetamide, also referred to as trimethylsilyl 2,2,2-trifluoro- <i>N</i> -(trimethylsilyl)acetimidate
Bu	Butyl
CD ₂ Cl ₂	Deuterium-enriched dichloromethane
CD ₃ OD	Deuterium-enriched methyl alcohol, deuterium-enriched methanol
CDCl ₃	Deuterium-enriched trichloromethane
cGAS	Cyclic GMP-AMP synthase
CHCl ₃	Trichloromethane
CoSO ₄	Cobalt sulfate
CPME	Cyclopentylmethyl ether
d	Doublet
D ₂ O	Deuterium-enriched water
DBSI	<i>N,N</i> -Dibenzenesulfonimide
DCM, CH ₂ Cl ₂	Dichloromethane

DI water	Deionized water
DMAP	4-Dimethylaminopyridine
DME, Glyme	Dimethoxyethane
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
<i>eq.</i> , equiv.	Equivalents
ES	Electron spray
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethyl alcohol, ethanol
g	Grams
GMP	Guanosine monophosphate
GTP	Guanosine 5'-triphosphate
h	Hour
H ₂ O	Water
HCl	Hydrogen chloride
HDMS	Hexamethyldisilazane
hept	Heptet
HOAc, AcOH	Acetic acid
HPLC	High-performance liquid chromatography
Hz	Hertz
IPA, i-PrOH	Isopropyl alcohol
IPAc	Isopropyl acetate
IPTG	Isopropyl β-D-1-thiogalactopyranoside
<i>J</i>	NMR Coupling constant
K ₂ CO ₃	Potassium carbonate
KCl	Potassium chloride
KF	Potassium fluoride
kg	Kilogram
KOH	Potassium hydroxide
L, l	Liter
LCMS	Liquid chromatography – mass spectroscopy

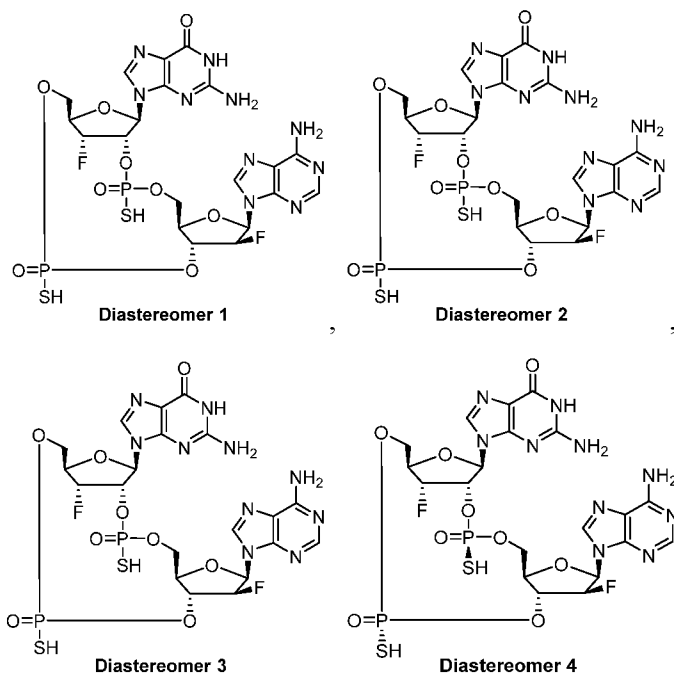
m	Multiplet
M	Molarity, number of moles of solute per liter of solution
m/z	Mass divided by charge number
Me	Methyl
MeCN, ACN	Acetonitrile
MeOH	Methanol
mg	Milligram
MgCl ₂	Magnesium chloride
MHz	Megahertz
min	Minute(s)
ML	Mother liquor
mL, ml	Milliliter
mM	Millimolar
mmol	Millimole
mol	mole
MS	Mass spectrometry
Ms	Methanesulfonyl
MTBE	Methyl <i>tert</i> -butyl ether, methyl tertiary butyl ether
N	Normality, number of mole equivalents per liter of solution
N ₂	Nitrogen (gas)
Na ₂ SO ₄	Sodium sulfate
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NFSI	N-fluorobenzenesulfonimide
Ni	Nickel
Ni-NTA	Nickel nitrilotriacetic acid
nm	Nanometer
NMI	1-Methylimidazole
NMP	<i>N</i> -Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
OD	Optical density

PBS	Phosphate-buffered saline
PG	Protecting group
PIV, Piv	Pivalate, 2,2-Dimethylpropanoate
PSCl ₃	Thiophosphoryl chloride
PTPI	<i>N,N</i> -bis(diphenylthiophosphoryl)amide
Py	Pyridine
q	Quartet
RPM, rpm	Revolutions per minute
RT, rt	Room temperature, approximately 25°C
s	Singlet
sat	Saturated
SIMS	Secondary ion mass spectrometry
STAB	Sodium triacetoxyborohydride
t	Triplet
TBS	<i>tert</i> -Butyldimethylsilyl
TBS-Cl	<i>tert</i> -Butyldimethylsilyl chloride
TES	2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino] ethanesulfonic acid, I-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid, TES free acid
TFA	Trifluoroacetic acid
TGDE, Tetraglyme	Tetraethylene glycol dimethyl ether
THF	Tetrahydrofuran
tol	Toluene
T _R	Retention time
Ts, OTs	<i>para</i> -Toluenesulfonyl or tosyl
Ts-Cl	<i>para</i> -Toluenesulfonyl chloride or tosyl chloride
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet wavelength or ultraviolet radiation
vol%	Percent by volume or volume percent
Vol., vol., V	Volumes
wt%	Percent by weight or weight percent
ZnSO ₄	Zinc sulfate

μg, ug	Microgram
μL, μl, uL, ul	Microliters
μM, uM	Micromolar

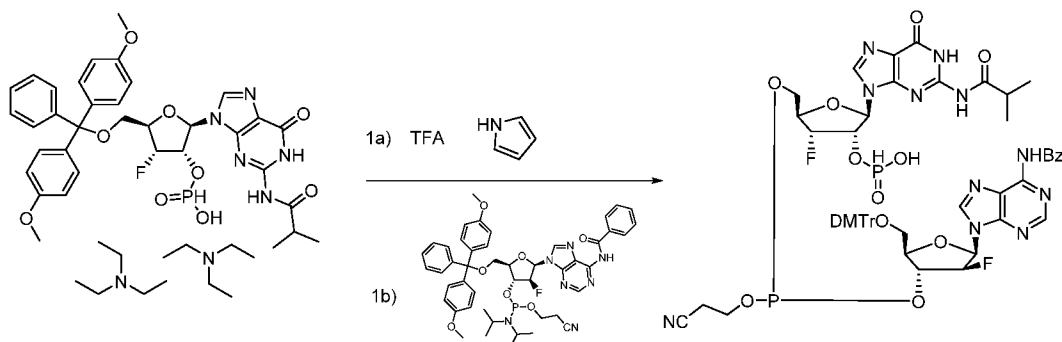
PREPARATION OF COMPOUND A

A method for preparing **Compound A**, as well as its diastereomers, is disclosed in WO2017/027646 and US2017/0044206, as Examples 244, 245, 246, and 247, 2-amino-9-[(5*R*, 7*R*, 8*S*, 12*aR*, 14*R*, 15*S*, 15*aR*, 16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidoctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaosa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomers 1 – 3) and 2-amino-9-[(5*R*, 7*R*, 8*S*, 12*aR*, 14*R*, 15*S*, 15*aR*, 16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidoctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaosa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 4), respectfully.



The compounds were prepared by the following process, as set forth in WO2017/027646 and US2017/0044206.

Step 1: (2*R*, 3*S*, 4*R*, 5*R*)-5-((((((2*R*, 3*R*, 4*S*, 5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate



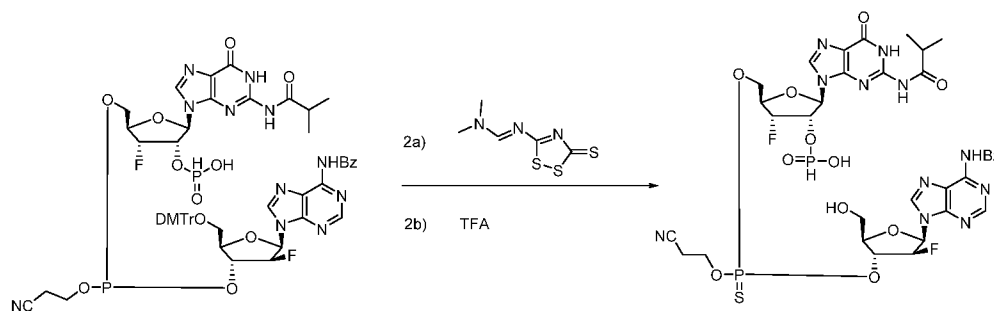
Pyrrole (0.087mL, 1.2mmol) was added to a solution of (2*R*,3*S*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate triethylamine salt (1:2) (0.34g,

0.41mmol) in acetonitrile (3.0mL) under an argon atmosphere at 0°C. After 5min, trifluoroacetic acid (0.096mL, 0.14mmol) was added, and the reaction mixture was stirred at 0°C for 30min.

Pyridine (0.13mL, 1.7mmol) was added drop wise at 0°C. The reaction mixture was then stirred for 10min at 0°C. At that time, a mixture of (2*R*,3*R*,4*S*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl (2-cyanoethyl)

diisopropylphosphoramidite (0.48g, 0.55mmol) in acetonitrile (3.0mL) was added drop wise over 5min to the reaction mixture under an argon atmosphere at 0°C. The reaction mixture was stirred at 0°C for 20min and immediately used in the next step without further manipulation.

Step 2: (2*R*,3*S*,4*R*,5*R*)-5-((((((2*R*,3*R*,4*S*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate

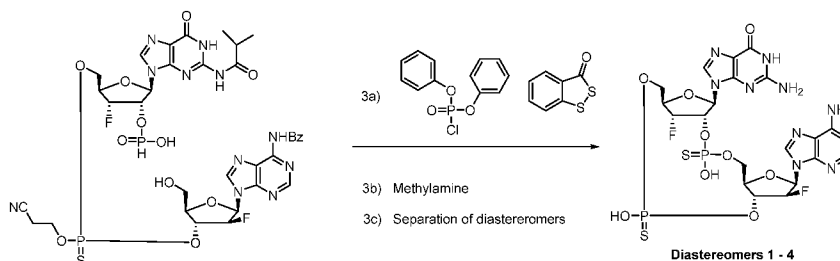


To the crude reaction mixture from Step 1 was added (*E*)-*N,N*-dimethyl-*N'*-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)formimidamide (0.10g, 0.50mmol) under an argon atmosphere at 0°C. The reaction mixture was stirred for 45 minutes at 0°C. At that time, 1-propanol (0.31mL, 4.13mmol) was added to the reaction mixture under an argon atmosphere at 0°C. The reaction

mixture was then allowed to warm to ambient temperature and stirred for 10min. TFA (0.32mL, 4.1mmol) was added to the reaction mixture, and the reaction mixture was stirred for 30min at ambient temperature. Pyridine (0.37mL, 4.6mmol) was added at ambient temperature, and the reaction mixture was stirred for 10min. The reaction mixture was concentrated under reduced pressure to approximately one-half volume. The mixture was then diluted with isopropyl acetate (20mL) and stirred for 30min at ambient temperature. The resulting suspension was filtered.

The collected solids were dried overnight under high vacuum to afford (2*R*,3*S*,4*R*,5*R*)-5-((((((2*R*,3*R*,4*S*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate. LCMS (ES, *m/z*): 922 [*M* - *H*]⁻

*Step 3: 2-amino-9-[(5*R*,7*R*,8*S*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfido-octahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one*



(2*R*,3*S*,4*R*,5*R*)-5-((((((2*R*,3*R*,4*S*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (0.30g, 0.33mmol) was azeotroped with dry pyridine (2x10mL) and then dried under high vacuum for 1h. In a separate flask, diphenyl phosphorochloridate (0.34mL, 1.6mmol) was added to a mixture of acetonitrile (15mL) and pyridine (1.0mL). The resulting solution was then cooled to -20°C. To this mixture was added drop wise over a period of 5min a mixture of (2*R*,3*S*,4*R*,5*R*)-5-((((((2*R*,3*R*,4*S*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl oxy)-methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (0.30g, 0.33mmol) in pyridine (4.0mL) at -20°C. The reaction mixture was then stirred at -20°C for 15min post-addition. 3*H*-benzo[*c*][1,2]dithiol-3-one (0.066g, 0.39mmol) and water (0.12mL, 6.5mmol) were then added to the reaction mixture at -20°C. The reaction mixture was allowed to gradually warm to ambient temperature. The reaction mixture was

stirred for 30min at ambient temperature. The reaction mixture was then concentrated under reduced pressure to approximately one quarter volume. The reaction mixture was cooled to 0°C, and methylamine (33% in ethanol) (2.63mL, 24mmol) was added drop wise. After the addition was complete, the reaction mixture was allowed to warm to ambient temperature. The reaction mixture was stirred at ambient temperature for 18h. The reaction mixture was concentrated under reduced pressure to afford the crude product residue. The crude product residue was azeotroped (3x30mL ethanol) to afford the crude product. This material was dissolved in water (5mL) and acetonitrile (1mL). The resulting mixture was purified by mass-directed reverse phase HPLC (Waters Sunfire 19x250 mm, UV 215/254 nm, fraction trigger by SIMS negative MS monitoring mass 709; mobile phase = 100mM triethylammonium acetate in water/acetonitrile gradient, 2-30% acetonitrile over 40 min) to afford the 4 diastereomers of 2-amino-9-[(5*R*,7*R*,8*S*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one.

Diastereomer 1: 2-amino-9-[(5*R*,7*R*,8*S*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one: $T_R = 17.7$ min. LCMS (ES, m/z): 709 $[M - H]^-$.

Diastereomer 2: 2-amino-9-[(5*R*,7*R*,8*S*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one: $T_R = 21.9$ min. LCMS (ES, m/z): 709 $[M - H]^-$. ^1H NMR (500MHz, DMSO- d_6) δ 8.32 (s, 1H), 8.21 – 8.09 (m, 2H), 7.46 – 7.29 (m, 2H), 6.59 – 6.43 (m, 2H), 6.40 – 6.29 (m, 1H), 5.88 (d, $J = 8.8$ Hz, 1H), 5.49 – 5.19 (m, 4H), 4.45 – 4.32 (m, 2H), 4.10 – 3.93 (m, 2H), 3.94 – 3.82 (m, 1H), 3.80 – 3.68 (m, 1H).

Diastereomer 3: 2-amino-9-[(5*R*,7*R*,8*S*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one: $T_R = 23.8$ min. LCMS (ES, m/z): 709 $[M - H]^-$. ^1H NMR (500MHz, DMSO- d_6) δ 8.18 – 8.08 (m, 3H), 7.41 – 7.33 (m, 2H), 6.59 – 6.47 (m, 2H), 6.37 – 6.27 (m, 1H), 5.84 (d, $J = 8.7$ Hz, 1H), 5.52 – 5.26 (m, 2H), 5.21 – 5.11 (m, 1H), 4.46 – 4.35 (m, 2H), 4.19 – 4.02 (m, 2H), 3.83 – 3.65 (m, 2H).

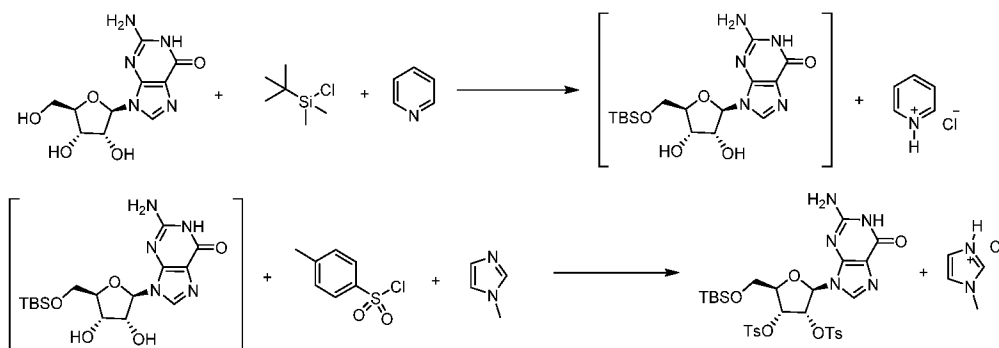
Diastereomer 4, Compound A: 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfido-octa-hydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one: $T_R = 26.4$ min. LCMS (ES, m/z): 709 $[M - H]^+$. ^1H NMR (500MHz, DMSO- d_6) δ 8.19 – 8.07 (m, 3H), 7.41 – 7.32 (m, 2H), 6.70 – 6.50 (m, 2H), 6.40 – 6.29 (m, 1H), 5.85 (d, $J = 8.7$ Hz, 1H), 5.33 – 5.25 (m, 2H), 5.23 – 5.12 (m, 1H), 4.48 – 4.35 (m, 1H), 4.33 – 4.24 (m, 1H), 4.09 – 3.93 (m, 2H), 3.92 – 3.81 (m, 1H), 3.83 – 3.70 (m, 1H).

Compound A also may be prepared from (O-{(2*R*,3*R*,4*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl)methyl} O,O-dihydrogen phosphorothioate (also known as 2'-fluoro-thio-adenosine monophosphate or 2'-F-thio-AMP) and (2*S*,3*R*,4*S*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-3-fluoro-4-hydroxy-2-(mercaptomethyl) tetrahydrofuran-3-yl dihydrogen phosphate (also known as 3'-fluoro-thio-guanosine monophosphate or 3'-F-thio-GMP) as starting materials, as disclosed in U.S. Provisional Patent Application No. 63/170,003, filed April 2, 2021. (O-{(2*R*,3*R*,4*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl)methyl} O,O-dihydrogen phosphorothioate (also known as 2'-fluoro-thio-adenosine monophosphate or 2'-F-thio-AMP) may be prepared from processes including those disclosed in United States Provisional Patent Application No. 63/080,381, filed on September 18, 2020. (2*S*,3*R*,4*S*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-3-fluoro-4-hydroxy-2-(mercaptomethyl) tetrahydrofuran-3-yl dihydrogen phosphate (also known as 3'-fluoro-thio-guanosine monophosphate or 3'-F-thio-GMP) may be prepared from processes including those disclosed in United States Provisional Patent Application No. 63/028,741, filed on May 22, 2020.

PREPARATORY EXAMPLES

Preparatory Example 1: Synthesis of (trisodium O-{(2*R*,3*S*,4*S*,5*R*)-5-(2-amino-6-oxido-9*H*-purin-9-yl)-3-fluoro-4-hydroxyoxolan-2-yl)methyl} phosphorothioate hydrate (1:6))

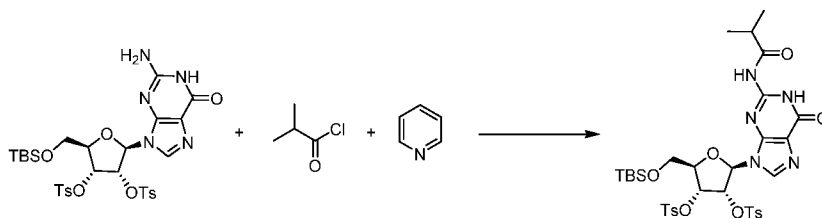
Step 1: Synthesis of (2*R*,3*R*,4*R*,5*R*)-2-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3,4-diyl bis(4-methylbenzenesulfonate)



NMP (3.5vol.) was added into a reaction vessel, and the temperature was adjusted to 48°C to 52°C. Guanosine (800g, 2824mmol) was added. The reaction mixture was stirred for 30min. to 1h, and the temperature was adjusted to 8°C to 12°C. TBS-Cl (575g, 3815mmol) (dissolved in 2vol. NMP) was added into the reaction mixture (total NMP 5.5vol.), and the reaction mixture was maintained at 8°C to 12°C. Py (670g, 8470mmol) was added to the reaction mixture, which was maintained at 8°C to 12°C and stirred for 3 to 4h. The temperature was adjusted to -20°C to -10°C and stir for 8 to 15h, after which the temperature was adjusted to -5°C to 5°C.

NMI (2319g, 28240mmol) was added to the reaction mixture, which was kept at -5°C to 5°C. To the reaction mixture, 2.1eq. Ts-Cl (1131g dissolved in 3vol. 2-Me-THF) was added, and the reaction mixture was stirred at -5°C to 5°C for 4 to 8 h. Then, 0.7eq. Ts-Cl (377g, dissolved in 1vol. 2-Me-THF) was added. The reaction mixture was stirred for 12 to 14h at -5°C to 5°C. Ts-Cl (0.16eq, 86g dissolved in 160mL 2-Me-THF) was added to the reaction mixture, which was stirred for 3 to 5h. MeOH (5.5vol.) was added to the reaction mixture at 15°C to 25°C, followed by water (8vol.). The reaction mixture was stirred at this temperature for 12 to 15h. The reaction mixture was then filtered and rinsed with 2vol. MeOH/water (1:3). The reaction product was dried under 45°C for 70h in two parts.

Step 2: Synthesis of (2R,3R,4R,5R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3,4-diyl bis(4-methylbenzenesulfonate)



The bis-tosylate (1096.00g (1185.50g x 92.45%)) was charged into a reaction

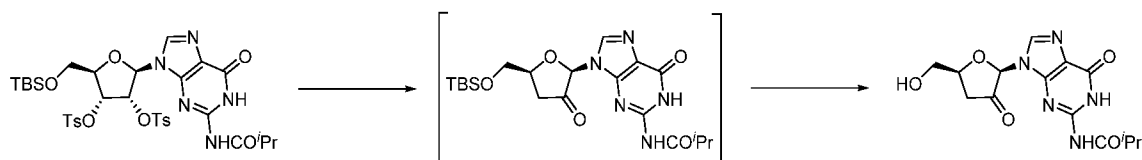
vessel. MeCN (3.3L, 3 vol.) and Py (510.52g, 4.2eq.) then were charged into the reaction vessel. The reaction mixture was cooled to -15°C to -5°C (slurry). Isobutyryl chloride (397.51g, 2.4eq.) was added by dropwise to the reaction mixture under -5°C (slurry). The reaction mixture was stirred at -15°C to -5°C for 18h. Isopropyl acetate (6L, 5vol.) was charged into the reaction mixture, and 15% K₂CO₃ liquor (6kg) was added by dropwise into the reaction mixture under -5°C. The reaction mixture was stirred at -15°C to -5°C for 30min. The reaction mixture was then warmed to 20°C to 30°C and stirred for 10 to 30min.

The reaction mixture was separated, and the aqueous layer was removed. The organic layer was concentrated to 3-4 vol. at 30°C. IPAc (6L, 5-6vol.) was charged into the concentrated organic layer, which was then stirred at 25°C to 30°C for 30min. The organic layer was then further concentrated until it reached 5-6vol. under 30°C. An additional IPAc (2L, 2-3vol.) was charged into the concentrated organic layer, and it was stirred at 25°C to 30°C for 30min. The reaction mixture was cooled to 15°C to 25°C. 3L (3vol.) n-heptane was added drop-wise at 15°C to 25°C for 6h, then the reaction mixture was stirred for 10h 25°C to 30°C. 3L n-heptane was added drop-wise at 15°C to 25°C for 6h, and the reaction mixture was stirred at 25°C to 30°C for 10h. The suspension was filtered, and the filter cake was washed with 2L mixture solution (IPAc/n-heptane = 1L/1L) to give the product, which was dried in oven under 35°C by reduce for 24h.

¹H NMR (500 MHz, DMSO-d₆) δ 11.97 (s, 1H), 11.50 (s, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.86 (s, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.00 (d, *J* = 7.9 Hz, 1H), 5.58 (dd, *J* = 7.8, 5.4 Hz, 1H), 5.05 (d, *J* = 5.3 Hz, 1H), 4.27 (t, *J* = 4.5 Hz, 1H), 3.85 (dd, *J* = 12.2, 4.1 Hz, 1H), 3.70 – 3.66 (m, 1H), 2.76 (septet, *J* = 6.8 Hz, 1H), 2.46 (s, 3H), 2.26 (s, 3H), 1.18 (t, *J* = 7.2 Hz, 6H), 0.87 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H).

¹³C NMR (125 MHz, DMSO-d₆) δ 180.4, 154.8, 148.5, 148.3, 146.3, 146.0, 137.1, 132.6, 131.6, 131.0, 130.0, 128.3, 127.3, 120.59, 83.9, 83.4, 78.5, 76.8, 62.7, 35.4, 31.7, 28.8, 26.2, 21.7, 21.4, 19.4, 19.2, 18.4, 14.4, -5.1, -5.1.

Step 3: Ketone Synthesis



A first reaction vessel was charged with i-PrOH (3.88L, 50.30mol) and THF (9.75L, 1.5vol) at RT and placed under N₂ before being cooled to -15°C. n-Butyllithium

(19.18L, 47.90mol, 2.5M in hexanes) was then added slowly, maintaining internal temperature below 25°C. A second vessel was placed under N₂ and charged with the bis-tosylate (6.5kg, 7.99mol, 96wt%) and CPME (26L, 4vol.) before being cooled to -5°C. The solution of lithium isopropoxide from the first reaction vessel was then vacuum transferred to the slurry in the second reaction vessel, and the mixture was warmed to 0°C and aged for about 18h. The slurry was cooled to -10°C, and AcOH (2.74L, 47.90mol) was added slowly, maintaining internal temperature below 5°C. To this mixture was added DI water (32.5L, 5vol.), the phases were separated, and the aqueous phase was removed from reactor. The organic layer was cooled to -10°C, and TFA (3.08L, 40.00mol) was added slowly, maintaining the internal temperature below 5°C, followed by trioctylamine (6.99L, 15.98mol). The mixture was warmed to 0°C and aged for about 16h. The slurry was cooled to -10°C, and trioctylamine (10.48L, 23.97mol) was added slowly. The resulting homogenous solution was warmed to 25°C and seeded with 1wt% of the ketone (26.7g, 0.0799mol) and aged for 18h. The slurry was filtered, and the cake was completely deliquored. The cake was then slurry washed twice with CPME (3.25L, 0.5vol.) and then dried under vacuum with N₂ sweep.

¹H NMR (500 MHz, DMSO-d₆) δ 12.12 (s, 1H), 11.47 (s, 1H), 8.11 (s, 1H), 5.99 (s, 1H), 5.05 (t, *J* = 5.6 Hz, 1H), 4.54 (app. dq, *J* = 8.6, 5.4, 4.4 Hz, 1H), 3.71-3.60 (m, 2H), 2.97 (dd, *J* = 18.5, 8.4 Hz, 1H), 2.85-2.75 (m, 2H), 1.14 (app. d, *J* = 6.8 Hz, 6H).

¹³C NMR (125 MHz, DMSO-d₆) δ 209.0, 180.6, 155.2, 149.0, 148.6, 139.4, 120.4, 81.7, 77.1, 63.8, 38.0, 35.3, 19.4, 19.3.

Step 4: Ketone Fluorination

NFSI (1.964kg in 5.5L THF) was charged into a first reaction vessel. The ketone (1.832kg) was then charged into a separate reaction vessel, followed by THF (5.5L), H₂O (0.932L) and L-leucine amide hydrochloride (259g). The reaction mixture in the second reaction vessel was agitated at 70rpm at RT. After 40min., the reaction temperature was 20°C, and 1.5L NFSI solution (~20%) from the first reaction vessel was added to the second reaction vessel, followed immediately by 1.371kg (NH₄)₂HPO₄. The agitation was set 80rpm. After 20min., the remainder of the NFSI from the first reaction vessel was charged into the second reaction vessel over 90min., and the reaction mixture was left for 2h at 27°C. THF (200mL) was added to rinse the bottle, and the mixture became homogenous as the temperature increased to 27.9°C. The agitation was then set to 92 rpm. The reaction mixture was then aged for 42h.

While the temperature was maintained at 27°C, H₂O (10vol, 18.32L) was charged

into the reaction mixture over 40min. The reaction mixture was concentrated by distillation, removing THF in batches. Once the distillation was completed, the slurry was allowed to de-supersaturate at 22°C overnight.

The reaction mixture was set to agitate at 47rpm. The reaction mixture was
5 filtered under vacuum. The wet cake was then washed with 11L H₂O, followed by MeCN (2x 5.5L). The wet cake was then dried under N₂ sweep for a period of two and a half days.

¹H NMR (500 MHz, DMSO-*d*₆: D₂O (5: 1)) δ 12.08 (s, 1H), 11.69 (s, 1H), 8.02 (s, 1H), 7.00 (br s, 2H), 5.85 (d, *J* = 1.9 Hz, 1H), 5.15 (br s, 1H), 4.83 (dd, *J* = 53.6, 2.7 Hz, 1H), 4.06 (dddd, *J* = 26.2, 8.3, 2.9, 2.8 Hz, 1H), 3.70 – 3.63 (m, 2H), 2.77 (sept, *J* = 6.8 Hz, 1H), 1.12
10 (d, *J* = 6.7 Hz, 6H).

¹³C NMR (126 MHz, DMSO-*d*₆: D₂O (5: 1)) δ 180.7, 155.6, 149.6, 148.3, 139.7, 119.5, 97.2 (d, *J* = 17.8 Hz), 93.4 (d, *J* = 188.7 Hz), 86.1, 81.8 (d, *J* = 23.2 Hz), 60.8 (d, *J* = 7.6 Hz), 35.3, 19.2, 19.1.

¹⁹F NMR (470 MHz, DMSO-*d*₆: D₂O (5: 1)) δ -189.1 (dd, *J* = 53.6, 26.1 Hz).

15 ***Step 5: Chemical Ketone Reduction to 3'-FG***

A first reaction vessel was charged with HOAc (2.8L, 2.0vol) and MeCN (4.2L, 3.0vol) followed by STAB (2.30kg, 3.0eq). The walls of the first reaction vessel were rinsed with MeCN (2.8L, 2.0V). The resulting solution had an internal temperature of 14°C and was heated to 22°C over 1h. The resulting solution was then stirred for 3h at RT.

20 A second reaction vessel was charged with HOAc (4.2L, 3vol.) and MeCN (6.3L, 4.5vol.) followed by the fluorinated ketone (1.40kg, 3.0eq.). The vessel walls were rinsed with MeCN (2.1L, 1.5vol.). The resulting slurry was heated to 35°C over 40min. The solution of STAB from the first reaction vessel was added to the slurry over approximately 2h. The resulting slurry was stirred for 2h at 35°C to 40°C, before the slurry was cooled to 25°C over
25 30min. MeOH (2.8L, 2vol.) was added over 1h, and the resulting solution was allowed to stir for 13.5h at RT.

The reaction vessel was placed under vacuum for distillation, and the temperature was set to 50°C, with distillation starting when the internal temperature reached to 35°C. Distillation was continued until total ~4vol. (5.6L) of the reaction mixture remained. DI water
30 (2.8L) was added over 6min when internal temperature reached 55°C. The walls were washed with water. (NH₄)₂SO₄ (2.8L, 2vol.) was added over 20min to the washed reaction solution.

The reaction mixture was aged for 40min. Following aging, (NH₄)₂SO₄ (22.4L,

16vol.) was added over 4h, and the slurry was aged again for 2h at 55°C. The reaction mixture was cooled to RT over 5h, and then aged at RT for 5.5h.

After aging, the reaction mixture was filtered, and the filter cake was washed with 4.3L of H₂O:MeOH (3:1) twice. The cake was then dried under N₂ sweep and vacuum.

¹H NMR (500M Hz, DMSO-d₆) δ 11.68 (s, 2H), 8.27 (s, 1H), 5.96 (d, J = 5.4 Hz, 1H), 5.83 (d, J = 8.1 Hz, 1H), 5.22 (t, J = 5.4 Hz, 1H), 5.07 (dd, J = 54.3, 4.1 Hz, 1H), 4.77 (dddd, 27.3, 8.1, 4.1, 4.1 Hz, 1H), 4.25 (dddd, J = 27.2, 8.1, 4.1 Hz, 4.1 Hz, 1H), 3.61 (m, 2H), 2.75 (sept, J = 6.8 Hz, 1H), 1.12 (d, J = 6.8 Hz, 6H).

¹³C NMR (125 MHz, DMSO-d₆) δ 180.2, 154.8, 149.3, 148.4, 137.4, 120.1, 92.8 (d, J = 183 Hz), 85.0, 83.6 (d, J = 21.1 Hz), 83.5, 72.6 (d, J = 16.1 Hz), 60.6 (d, J = 11.2 Hz), 34.8, 18.8, 18.8.

¹⁹F NMR (500 MHz, DMSO-d₆) δ -197.5.

Step 6: Biocatalytic Ketone Reduction to 3'-FG (alternative to Step 5)

10uL of a ketoreductase enzyme that has the amino acid sequence that is SEQ ID NO: 1, as set forth below, was inoculated into 5mL of Luria-Bretani Broth (culture media for cells), supplemented with 1% glucose and 50ug/ml of Kanamycin antibiotic and grown overnight for 20-24h at 30°C, 250 rpm, in a shaking incubator.

MHHHHHPATIVVTGGTKGIGRAIVEKFAKEGFTVLTCARTKGDNFPENV
HFFKADLSKKVEVLAFADFIKQTVNQVDILVNNTGWFLPGEINNEAEGTLEAMIE
TNLYSAYYLTRALVGDMITKKEGHIFNICSYASIVPYTSGGSYCISKTAQLGMSK
VLREELKPHHVRVTSILPGAVLNDSWAKVELPAELFIAPEDIAQIVWTAHCLPSTT
VLEEILIRPQTGDL (SEQ ID NO: 1)

5mL of the overnight culture was used to subculture 250mL of Terrific Broth growth media (commercially available from ThermoFisher Scientific as Catalog #A1374301) in a 1L flask. The subculture was allowed to grow at 30°C for 3h at 250rpm, in a shaking incubator. When the OD measures between 0.4 and 0.6, the IPTG was introduced to an IPTG final concentration of 1Mm (1mM). The subculture was allowed to grow overnight, for 18-20h.

After the growth period, the culture was transferred to a centrifuge bottle and centrifuged for 20min. at 4000rpm at 4°C. Following centrifuge, the supernatant was discarded.

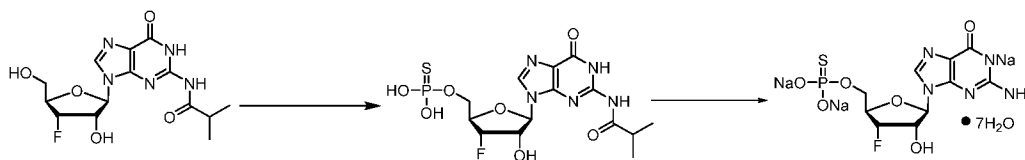
The cell pellets were resuspended in 50mM sodium phosphate buffer (pH = 7).

The cells from the resuspended cell pellets were lysed using a microfluidizer, and the cell lysate was collected and centrifuged for 60min. at 10000rpm at 4°C. The supernatant

was transferred to a petri dish and frozen at -80°C for a minimum of 2h. Samples were optionally lyophilized using a standard automated protocol.

20mg of a commercially available ketoreductase enzyme (KRED-P1 B10, commercially available from CODEXIS, Inc.) added to a reaction vessel, along with NADPH (20mg), a ketoreductase enzyme that can be represented by SEQ ID NO: 1, as set forth above (250mg, harvested from the subculture), and fluoroketone (250mg, *step 4* above). 10mL of phosphate buffer (0.1M, pH = 6.0) and 1mL IPA were added to the reaction vessel. The temperature was set at 30°C, and the reaction mixture was stirred at 350 rpm. After 20h, the mixture was cooled to 15°C. NaCl (2g) was added to the reaction vessel, and the reaction mixture was allowed to de-supersaturate overnight. The solids were filtered and washed with 2.5 mL (10 vol.) of water. The wet cake was placed into a 50°C vacuum oven to dry overnight.

Step 7: Thiophosphorylation



A reaction vessel at 22°C was charged with THF (16L). Quinine (234g, 0.72mol, 0.25equiv) was charged into the reaction vessel, followed by 2,6-lutidine (463g, 4.32mol, 1.5equiv), and 3'-FG (1100g, 2.88mol, 1.0equiv, *step 5* above). THF (6L) was used to rinse the sides of the reaction vessel, and the temperature was set to 0°C. PSCl₃ (658g, 3.89mol, 1.35equiv) was charged, maintaining the temperature below 2°C. The reaction mixture was stirred at 80rpm for approximately 40h at 0°C. The reaction progress was monitored by UPLC analysis; once 96% conversion had been obtained, the reaction temperature was adjusted to -10°C. H₂O (2.2L) was added dropwise, maintaining the temperature below 0°C. After the addition, the temperature was adjusted to 25°C, and the reaction mixture was held at this temperature for 1h.

The THF was removed *in vacuo*. After THF removal (at least 17vol.), the vacuum was broken, and the temperature was set to 25°C. MeOH (11L) was charged into the reaction vessel, and the temperature was adjusted to -10°C. Aqueous NaOH (50wt%) was diluted with H₂O (1.1L) and charged into the reaction vessel, maintaining the temperature below 25°C.

The temperature was then adjusted to 45°C, and, after 90min, the reaction mixture was seeded with 3'-F-thio-GMP (1wt%, 11g). The mixture was held at 45°C for 5h, then cooled

to 20°C over 5h, and held at 20°C. THF (1.8L, 1.6V) added over 45min at 20°C, and the mixture was agitated for 3h. The mixture was then filtered, and the wet cake was washed with 10:4:2 MeOH:THF:H₂O (10L). The cake was then washed with THF (10L), and the cake was dried under vacuum under a sweep of humidified N₂.

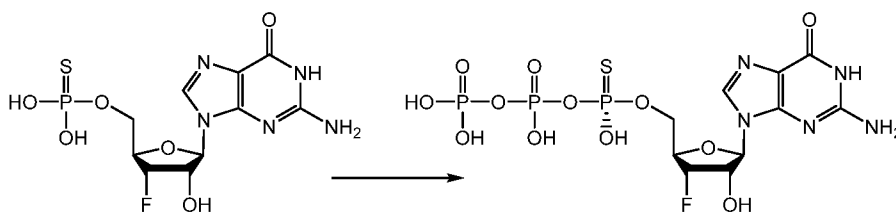
¹H NMR (500 MHz, D₂O): δ 8.17 (s, 1H), 5.94 (d, *J* = 8.4 Hz, 1H), 5.30 (dd, *J* = 54.2, 4.3 Hz, 1H), 4.93 (ddd, *J* = 25.9, 8.4, 4.3 Hz, 1H), 4.63 – 4.54 (m, 1H), 4.08 – 4.00 (m, 1H), 3.97 – 3.89 (m, 1H).

¹³C NMR (126 MHz, D₂O): 167.7, 161.0, 152.15, 135.93, 117.41, 92.53 – 93.96 (d), 84.68, 82.95 (m), 73.25 (m), 63.50 (m).

¹⁹F NMR (470 MHz, D₂O) δ – 197.9.

³¹P NMR (203 MHz, D₂O) δ 43.31.

Preparatory Example 2: 3'F-thio-GTP from 3'F-thio-GMP intermediate



To a 200mL reactor was charged 3'F-thio-GMP (2.5g, 73.5wt%, 1.0eq), followed by addition of AcP-Li/Li (1.73g, 94.9wt%, 2.5eq). Next, 2'F-thio-ATP (140mg, 81wt%, 0.05eq) was charged at 25°C, followed by addition of water (50mL) and EtOH (12.5mL), respectively. To the resulting mixture was added MgCl₂ (4.33mL, 1M, 1.0eq) and KCl (2.16mL, 3M, 1.5eq). Then, pH was adjusted to 6.4-6.6 using HCl (9M) solution while the solution was agitated 25°C. Next, a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 2 (26.5mg), as set forth below, and a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 3 (26.5mg), as set forth below, were charged to the reactor. The mixture was stirred at 25°C until completion (6-8h) using overhead stirrer. After completion (~97% conversion), HOAc was added (1.8mL) to adjust the pH to 4.3-5.0, followed by slow addition of MeCN (~250mL) to precipitate the product. At the end, the slurry was filtered, and the wet cake was washed with the same solvent ratio as mother liquor (ML) to afford the product, which was dried under vacuum to give 3'F-thio-GTP.

MALPRPVVICGPSGSGKSTLYNKLLKEFPGVFQLSVSHTTRQPRPGELNGR
EYHFINRDQFQENIKQGDFLEWAEFSGNIYGTSSKKALEEVQSNNVIPILDIDTQGV

RNVKKASLEAVYIFIKPPSIDVLEKRLRSRKTETEEALQKRLSAARNELEYGLKPG
 NFQHIITNDDLDVAYEKLKGILIKSQMPLAMATGSSSSVNSFLDKPAASATTVN
 SSSQD (SEQ ID NO: 2).

MHHHHHSSSESIRMVLIGPPGAGKGTQAPNLQERFHACHLATGDMLRS
 5 QIAKGTQLGLEAKKIMDQGGLVSDDIMVNMIDELTNNPACKNGFILDGFPRTIP
 QAEKLDQMLKEQGTPLEKAVELKIDDELLPARITGRLIHPASGRSYHKIFNPPKED
 MKDDVTGEALVQRSDDNADALKKRLAAYHKQTEPVVDFYKKTGIWAGVDASQ
 PPATVWADILNKLKGD (SEQ ID NO: 3).

The product was redissolved in water (40mL), and the pH was adjusted with
 10 KOH to pH 7.0. To the solution, KCl (800μl, 3M, 0.1eq) was added, followed by HOAc
 (1.6mL). MeCN (57mL) was added slowly to precipitate 3'F-thio-GTP. The slurry was filtered,
 and the wet cake was washed with the same solvent ratio as mother liquor (ML) and dried under
 vacuum to give 3'F-thio-GTP.

**15 Preparatory Example 3: Synthesis of (O-[(2R,3R,4S,5R)-5-(6-amino-9H-purin-9-yl)-4-
 fluoro-3-hydroxyoxolan-2-yl]methyl]O,O-dihydrogen phosphorothioate**

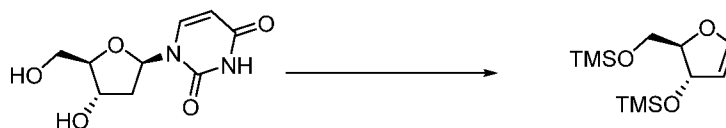
*Step 1: Synthesis of Trimethyl(((2R,3S)-3-((trimethylsilyl)oxy)-2,3-dihydrofuran-2-
 yl)methoxy) silane from Thymidine*



20 A 100L vessel was charged with toluene (14.5L), thymidine (4825g, 20mol), 2,6-
 lutidine (1081g, 0.400mol), PTPI (90g, 0.200mol) and heptane (33.8L). The mixture was heated
 to 90°C. To this, BSA (17.4kg, 85.6mol) was added over 30min. The mixture was heated to
 100°C and stirred at 100-107°C for 3h. After cooling to room temperature, the reaction mixture
 was transferred to another 100L reactor containing i-PrOH (12.3L, 161mol) slowly (204ml/min).
 25 Toluene (1L) was used to rinse and transfer any remaining material in the first reactor. The
 resulting slurry was stirred at 35°C for 2h, then cooled to 10°C and aged at that temperature
 overnight. The resulting slurry was filtered, and the filter cake was washed with heptane
 (20.0L). The combined filtrates were passed through a plug of basic alumina and transferred to a
 100L vessel. The resulting solution was concentrated under vacuum to the total volume of 24L,
 30 which was used in the subsequent reaction without further purification.

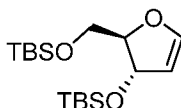
¹H NMR (400MHz, CD₂Cl₂); δ 6.50 (dd, *J*=2.7, 1.1Hz, 1H), 5.06 (t, *J*=2.7Hz, 1H), 4.84 (td, *J*=2.7, 1.0Hz, 1H), 4.28 (ddd, *J*=6.7, 6.1, 2.7Hz, 1H), 3.67 (dd, *J*=10.6, 6.1Hz, 1H), 3.47 (dd, *J*=10.6, 6.7Hz, 1H), 0.17 (s, 9H), 0.16 (s, 9H).

Step 1, alternate route: Synthesis of Trimethyl(((2*R*,3*S*)-3-((trimethylsilyl)oxy)-2,3-dihydrofuran-2-yl)methoxy) silane from 2'-Deoxyuridine



In an 8mL vial, dry 2'-deoxyuridine (1 mmol), PTPI (0.01eq, 5mg), 2,6-lutidine (0.5eq, 58μL), 1mL heptane, 1mL toluene, and 3.5eq. of BSA was added under nitrogen atmosphere. The reaction was stirred at 100°C for 3h. Reaction progress was monitored via HPLC by the presence of starting material.

Step 1, alternate route: Synthesis of 2-*tert*-butyl(((2*R*,3*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2,3-dihydrofuran-2-yl)methoxy)dimethylsilane (2-TBS)



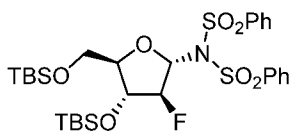
In a 2L flask was charged ammonium sulfate (5.38g, 40.7mmol), bis(*tert*-butyldimethylsilyl)thymidine (100g, 203mmol), and 2,6-di-*tert*-butyl-4-methylphenol (0.045g, 0.203mmol). HMDS (141mL, 671mmol) and heptane (1000mL) were subsequently added, and the reaction mixture was heated to reflux (140°C external bath) under nitrogen atmosphere. After 34h, the reaction mixture was cooled to ambient temperature. 2,4,6-trimethylpyridine (13.55mL, 102mmol) was added followed by ethanol (35.6mL, 610mmol) via syringe pump over 2h. The resulting slurry was then filtered, and the cake was washed with CPME (4 x 150mL). The filtrate was concentrated to provide 2-TBS (57.14 g, 166 mmol,) by quantitative NMR analysis.

¹H NMR (500MHz, CDCl₃) δ 6.47 (dd, *J* = 2.6, 0.8Hz, 1H), 5.01 (t, *J* = 2.6Hz, 1H), 4.87 (td, *J* = 2.6, 0.8Hz, 1H), 4.29 (td, *J* = 6.0, 2.8Hz, 1H), 3.69 (dd, *J* = 10.7, 5.7Hz, 1H), 3.51 (dd, *J* = 10.7, 6.3Hz, 1H), 0.90 (s, 9H), 0.89 (s, 9H), 0.09 (s, 3H), 0.09 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H).

¹³C NMR (126MHz, CDCl₃) δ 149.1, 103.6, 89.1, 76.1, 63.0, 26.1, 26.0, 18.5, 18.2, -4.1, -4.3, -5.2, -5.2.

Step 2: Synthesis of *N*-((2*S*,3*S*,4*R*,5*R*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-

butyldimethylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)-N-(phenylsulfonyl) benzenesulfonamide (3-TBS)



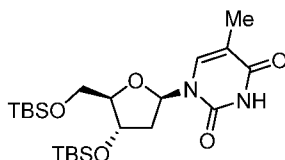
In a 1L flask was charged the crude 2-TBS (1.0equiv, 82.95g, 166mmol) and CPME (263mL). Additionally, 2,4,6-trimethylpyridine (4.53mL, 34.2mmol), BSTFA (22.02mL, 83mmol), and NFSI (68.0g, 216mmol) were added to the reaction mixture. The resulting mixture was warmed to 65°C and stirred for 20h. After cooling to ambient temperature, heptane (286mL) was added, and the reaction mixture was stirred for 1.75h at ambient temperature. The resulting slurry was filtered, and the cake was washed with CPME:heptane (1:1, 286mL). The filtrate was subsequently concentrated under vacuum. Heptane (286mL) was added to the concentrated crude material, and the mixture was heated to 70°C. The mixture was filtered while hot into a 1L flask, and the filtrate was crystallized while being slowly cooled to ambient temperature. The resulting slurry was further cooled to -30 to -35°C and filtered. After drying under vacuum, the desired DBSI adduct 3-TBS (94.63g, 138mmol) was collected.

¹H NMR (500MHz, CDCl₃) δ 8.04 (dd, *J* = 8.5, 1.1Hz, 4H), 7.64 (t, *J* = 7.5Hz, 2H), 7.54 (t, *J* = 7.9Hz, 4H), 6.00 (dd, *J* = 16.5, 5.9 Hz, 1H), 5.67 (ddd, *J* = 57.2, 6.3, 6.3Hz, 1H), 4.48 (ddd, *J* = 21.3, 8.9, 6.7Hz, 1H), 4.39 – 4.24 (m, 1H), 3.78 (ddd, *J* = 12.0, 1.8, 1.8Hz, 1H), 3.65 (dd, *J* = 12.0, 3.1Hz, 1H), 0.92 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H), 0.05 (s, 3H), 0.05 (s, 3H).

¹³C NMR (126MHz, CDCl₃) δ 140.6, 134.0, 129.1, 128.4, 99.7 (d, *J* = 188.1Hz), 92.2 (d, *J* = 36.8Hz), 83.4 (d, *J* = 9.9Hz), 73.0 (d, *J* = 20.9Hz), 61.3, 26.0, 25.7, 18.5, 18.0, -4.5, -5.0, -5.1, -5.3.

¹⁹F NMR (500MHz, CDCl₃) δ -195.0.

Step 3: Synthesis of 1-((2R,4S,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione



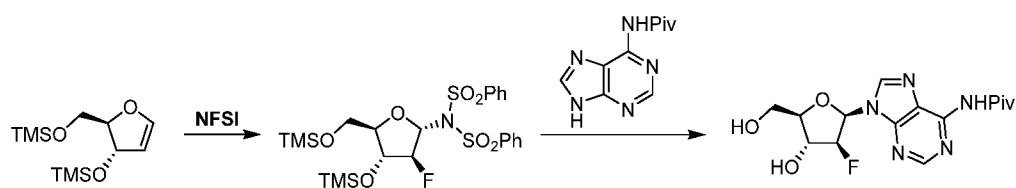
While under a nitrogen atmosphere, thymidine (12.1g, 50mmol), imidazole (2.5equiv, 8.5g, 125mmol), *tert*-butyldimethylsilyl chloride (2.2equiv, 16.6g, 110mmol), DMF

(20mL), and DMAP (0.01equiv, 0.061g, 0.5mmol) were added to a 200mL round-bottom flask, and the resulting mixture was stirred for 1h at ambient temperature. The reaction was determined to be complete by HPLC. Subsequent addition of 100mL water was followed by stirring at ambient temperature for 1h. Filtration of the slurry was performed, and the cake was washed with 200mL water. The cake was dissolved in 100 mL MTBE, and the solution was washed with 100mL water and dried over magnesium sulfate. The filtered MTBE solution was evaporated to approximately 30mL, diluted with 30mL hexanes and 80mL heptane and evaporated to approximately 100mL. The residue was cooled to 0°C over 2h, and crystallization was observed to occur. The slurry was filtered and washed with 30 mL 9:1 hexanes:MTBE and subsequently with 50mL hexanes. The solid was dried under a nitrogen stream to provide 1-((2*R*,4*S*,5*R*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (21g, 44.6mmol).

¹H NMR (500MHz, CDCl₃) δ 8.35 (s, 1H), 7.47 (d, *J* = 1.2Hz, 1H), 6.33 (dd, *J* = 7.9, 5.8Hz, 1H), 4.40 (ddd, *J* = 5.6, 2.5, 2.5Hz, 1H), 3.93 (ddd, *J* = 2.5, 2.5, 2.5Hz, 1H), 3.87 (dd, *J* = 11.4, 2.6Hz, 1H), 3.76 (dd, *J* = 11.4, 2.4Hz, 1H), 2.25 (ddd, *J* = 13.1, 5.8, 2.6Hz, 1H), 2.00 (ddd, *J* = 13.3, 7.9, 6.1Hz, 1H), 1.92 (d, *J* = 1.1Hz, 3H), 0.93 (s, 9H), 0.89 (s, 9H), 0.11 (s, 3H), 0.11 (s, 3H), 0.08 (s, 3H), 0.07 (s, 3H),

¹³C NMR (126MHz, CDCl₃) δ 164.3, 150.7, 135.8, 111.2, 88.2, 85.2, 72.6, 63.4, 41.8, 26.3, 26.1, 18.8, 18.4, 12.9, -4.3, -4.5, -5.0, -5.1.

Step 4: Synthesis of Piv-protected (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol



To a 100L reactor was charged the crude toluene stream for trimethyl(((2*R*,3*S*)-3-((trimethylsilyl)oxy)-2,3-dihydrofuran-2-yl)methoxy)silane (10.2kg, 13.4mol), which contained 0.36 equivalents lutidine and 2 vol toluene. To this was added toluene (8.75L), 2,6-lutidine (0.563L, 4.84mol), and BSTFA (0.178L, 0.672mol), and the resulting mixture was warmed to 65°C. *N*-fluorobenzenesulfonimide (NFSI) (4.66kg, 14.77mol) was added portionwise, then toluene (1.75L) was used to rinse the sides of the reactor. The reaction mixture was stirred at 65°C until trimethyl(((2*R*,3*S*)-3-((trimethylsilyl)oxy)-2,3-dihydrofuran-2-yl)methoxy)silane was

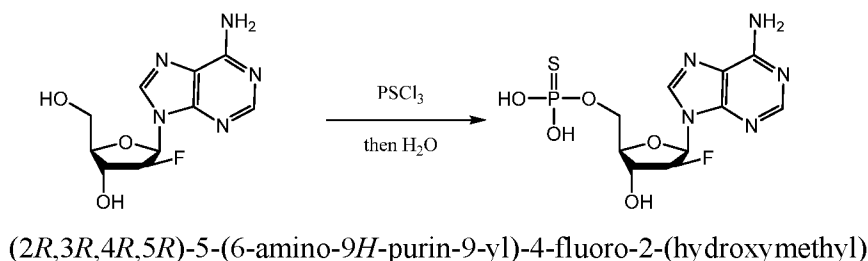
consumed judged by NMR analysis, after which 2,6-lutidine (0.782L, 6.72mol), ethyl acetate (50.75L) and *N*-(9*H*-purin-6-yl)pivalamide (2.88kg, 12.76mol) were added. An additional 1.75L of ethyl acetate was used to rinse the sides of the reactor. The resulting mixture was warmed to 75°C and stirred for overnight. The crude reaction mixture was then concentrated under vacuum to a total volume of 35L. The resulting slurry was filtered, and the filter cake was washed with ethyl acetate (17.5L, 5vol). The filtrate was transferred to a 50L reactor while continuously evaporating under vacuum to a total volume of 17.5L. To this, ethanol (5.25L), 2,6-lutidine (0.313L, 2.69mol), and TFA (103ml, 1.34mol) were added to start desilylation. Vacuum distillation while feeding 21L of 3.8:1 v/v EtOAc:EtOH was conducted to aid the desilylation. When the desilylation was achieved with > 90% conversion 17.5L EtOAc was fed into the reactor while distilling away excess EtOH. An additional continuous vacuum distillation was performed with 3.5L toluene feed while the mixture was concentrated to the total volume of 17.5L. After the distillation was completed, the reaction mixture was stirred at room temperature overnight to crystallize. The product was collected by filtration rinsing with 21L of 2:10:88 v/v/v EtOH:tol:EtOAc. Total mass was 3.16kg.

¹H NMR (500MHz, DMSO-*d*₆) δ 10.19 (s, 1H), 8.72 (s, 1H), 8.56 (d, *J* = 1.9Hz, 1H), 7.30-7.10 (toluene, m, 5H), 6.55 (dd, *J* = 13.4, 4.7Hz, 1H), 5.99 (bs, 1H), 5.29 (ddd, *J* = 52.6, 4.3, 4.3Hz, 1H), 4.49 (ddd, *J* = 19.1, 4.6, 4.6, 1H), 3.89 (ddd, *J* = 4.8, 4.8, 4.8Hz, 1H), 3.72 (dd, *J* = 12.1, 3.1Hz, 1H), 3.66 (dd, *J* = 12.0, 5.1Hz, 1H), 2.30 (toluene, s, 3H), 1.28 (s, 9H).

¹³C NMR (124MHz, DMSO-*d*₆) δ 176.3, 151.8, 151.7, 150.4, 142.8 (d, *J* = 3.8Hz), 137.4 (toluene), 128.9 (toluene), 128.2 (toluene), 125.2 (toluene), 125.1, 95.4 (d, *J* = 192.4Hz), 83.5 (d, *J* = 5.7Hz), 81.5 (d, *J* = 17.0Hz), 72.5 (d, *J* = 23.0Hz), 60.3, 26.9, 21.0 (toluene).

¹⁹F NMR (470MHz, DMSO-*d*₆) δ 197.9.

Step 5: Synthesis of (O-[(2*R*,3*R*,4*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methyl}O,O-dihydrogen phosphorothioate from (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol



tetrahydrofuran-3-ol (50g, 186mmol) was azeotroped with 3x100mL of dry pyridine and was dissolved in 500mL (10vol) of dry pyridine (KF = 128ppm). The pyridine solution was cooled to 0°C. for 1h. Thiophosphoryl chloride (1.04eq) was added dropwise at 0°C over 10min. The reaction was stirred at 0°C for 80min, with constant monitoring by UPLC. The reaction was filtered to remove the excess starting material. Water (10eq) was then added to the filtrate at 0°C and was slowly warmed to room temperature. The reaction was allowed to stir for an additional 30min at room temperature. The volatiles were removed *in vacuo*, and the product was dissolved in 500mL of water. The solution pH was 4. The solution was filtered, and the filtrate was stirred while 12M HCl was added until the pH of the solution was 0 (about 35mL). The resulting slurry was allowed to stir at room temperature overnight (~16h). Then the slurry was allowed to settle for 1h. The slurry was then filtered, and the filter cake was washed with 200mL of water. The washed cake was allowed to dry over a stream of nitrogen overnight (29.9g).

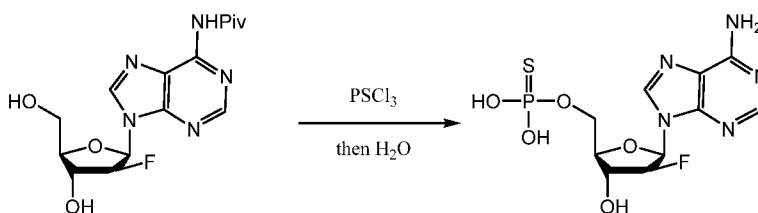
¹H NMR (500MHz, DMSO-*d*₆) δ = 8.26 (d, *J* = 1.9Hz, 1H), 8.21 (s, 1H), 7.55 (br s, 2H), 6.46 (dd, *J* = 15.0, 4.5Hz, 1H), 5.24 (dt, *J* = 52.4, 4.1Hz, 1H), 4.51 (dt, *J* = 18.1, 4.2Hz, 1H), 4.22 – 4.04 (m, 3H).

¹³C NMR (125MHz, DMSO-*d*₆): δ = 155.9, 152.6, 149.5, 140.3 (d, *J* = 4.1Hz), 118.7, 95.4 (d, *J* = 191.9Hz), 82.1 (d, *J* = 16.7Hz), 81.8 (dd, *J* = 9.3, 5.3Hz), 73.5 (d, *J* = 23.7Hz), 65.50 (dd, *J* = 4.6, 1.8Hz).

¹⁹F NMR (470MHz, DMSO-*d*₆): δ = -197.80 (ddd, *J* = 52.7, 16.6, 16.6Hz, 1F).

³¹P NMR (202MHz, DMSO-*d*₆): δ = 59.49 (dd, *J* = 7.4, 7.4Hz, 1P).

Step 5, alternate route: Synthesis of (O-[(2*R*,3*R*,4*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methyl)O,O-dihydrogen phosphorothioate from Piv-protected (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol



In a 50L reactor was charged triethylphosphate (4vol, 8.58L), Piv-protected (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (2.5kg, 85.75wt%) followed by the remaining triethylphosphate (1vol, 2.14L) washing the sides of the vessel. To this, 2,6-lutidine (3eq, 1.97kg) and pyridine (0.3eq, 144g) were charged, and the resulting mixture was cooled to -20°C. Then thiophosphoryl chloride (1.835kg, 1.75eq.) was

added slowly over 1h. The reaction mixture was aged at -20°C for overnight, after which additional thiophosphoryl chloride (32mL, 0.05eq.) was added. Water (8eq, 0.87L) was added dropwise over 1h to quench the reaction. Additional water (32eq, 3.5L) was added dropwise over 1h, then the resulting mixture was warmed to 50°C and aged at that temperature for 3h.

After pivaloyl group was removed judged by HPLC analysis, the mixture was cooled to 30°C, and added water (9vol, 19.3L) to crystallize the product while cooling to 0°C slowly. The product was collected by filtration rinsing with water (12.5L) then dried under vacuum with nitrogen sweep. The resulting product (1.971kg, 88.65wt%) was then collected and stored under ambient temperature.

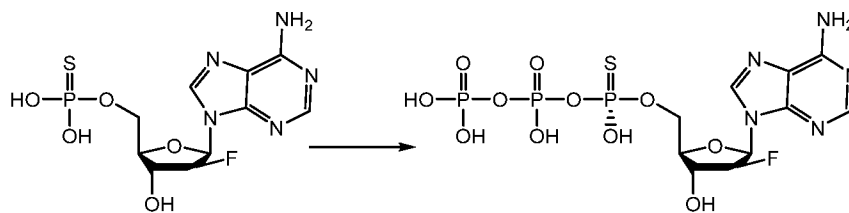
¹H NMR (500MHz, DMSO-*d*₆) δ = 8.26 (d, *J* = 1.9Hz, 1H), 8.21 (s, 1H), 7.55 (br s, 2H), 6.46 (dd, *J* = 15.0, 4.5Hz, 1H), 5.24 (dt, *J* = 52.4, 4.1Hz, 1H), 4.51 (dt, *J* = 18.1, 4.2Hz, 1H), 4.22 – 4.04 (m, 3H).

¹³C NMR (125MHz, DMSO-*d*₆): δ = 155.9, 152.6, 149.5, 140.3 (d, *J* = 4.1Hz), 118.7, 95.4 (d, *J* = 191.9Hz), 82.1 (d, *J* = 16.7Hz), 81.8 (dd, *J* = 9.3, 5.3Hz), 73.5 (d, *J* = 23.7Hz), 65.50 (dd, *J* = 4.6, 1.8Hz).

¹⁹F NMR (470MHz, DMSO-*d*₆): δ = -197.80 (ddd, *J* = 52.7, 16.6, 16.6Hz, 1F).

³¹P NMR (202MHz, DMSO-*d*₆): δ = 59.49 (dd, *J* = 7.4, 7.4Hz, 1P).

Preparatory Example 4: Synthesis of 2'F-Thio-ATP from (O-[(2*R*,3*R*,4*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methyl}O,O-dihydrogen phosphorothioate



To a 1L reactor was charged 2'F-thio-AMP (23.3g, 86wt%), 2'F-thio-ATP disodium salt, tetrahydrate (1g, 85wt%) and 0.9M aq. solution of acetyl phosphate, disodium (3.6eq, 219mL). The reaction solution was added 1M aq solution of MgCl₂•(H₂O)₆ solution (0.125eq, 6.9mL), and the pH of the reaction mixture was adjusted to 6.5 with addition of NaOH. The reaction volume was diluted to 500 mL with water. An adenylate kinase enzyme that has the amino acid sequence that is SEQ ID NO: 4 as set forth below (100mg) and a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 5 as set forth below (200mg) were charged to

the reaction vessel, and the reaction mixture was stirred at 500rpm at ambient temperature. After 6h, the reaction was quenched with 37% aq. solution of HCl (40mL) to bring the pH to 2. The resulting slurry was filtered, and the filtrate was transferred into 3L vessel with an overhead stirrer rate of 270rpm. The filtered solution was charged sodium chloride (2.0eq, 6.41g). EtOH (505mL) was charged to the reaction mixture, and 2'-F-Thio-ATP, disodium salt, tetrahydrate was added as seeds. Once seed bed is formed, the crystal slurry was stirred overnight at 270rpm. After overnight aging, the slurry was charged another portion of EtOH (130mL) over 2h via addition funnel. The reaction vessel was cooled to 4°C. Another portion of EtOH (500mL) was charged over 4h via addition funnel to reach EtOH/water ratio of approximately 2:1. The slurry was filtered, and the wet cake was washed with cold 2:1 EtOH/water solution (4 x 20mL), cold EtOH (3 x 20mL). The resulting wet cake was dried under vacuum with positive nitrogen pressure overnight to yield 2'-F-Thio-ATP (31.3g).

MHHHHHSSSESIRMVLIGPPGAGKGTQSPNLQERFHACHLGTGDMRLS
QHAKGTQLGLEAKKIMDQGGLVSDDIMVNMKDELTTNPCKNGFILDGFPRTI
PQAMKLEQMLKEQGTPLEKAVELKIDDELLPARITGRLIHPASGRSYHKIFNPPKE
DMKDDVTGEALVQRSDDNADALKKRLAAYHKQTEPVVDFYKKAGIWAGVDA
SQPVATVWADILNKLKGD (SEQ ID NO: 4)

MGSHHHHHSRVLVINSGSSSIKYQLIEMEKEKVLCKGIAERIGIEGSRLV
HRVGDEKHVIERELPDHEEALKLILNTLVDEKLGVIKDLKEIDAVGHRVVHGGE
RFKESVLVDEEVLKAIEEVSPLAPLHNPNANLGMKIVAMKLLPGVPNVAVFDTA
HQTIPQKAYLYAIPYEYKYKIRRYGFHGTSHRYVSKRAAEILGKKLEELKIITC
HIGNGASVA AVKYGKCVDTSMGFTPTGLVMGTRSGDLDP AIPFFIMEKEGISPQ
EMYDILNKKSGVYGLSKGFSSDLRDIEEAALKGDEWCKLVLEIYDYRIAKYIGAY
AAAMNGVDAIVFTAGVCENSPITREDVC SYLEFLGVKLDKQKNEETIDGKEGIIS
TPDSRVKVLVVPTNEELMIARDTKEIVEKIGR (SEQ ID NO: 5)

¹H NMR (500 MHz, Deuterium Oxide) δ 8.67 (d, J = 1.8 Hz, 1H), 8.49 (s, 1H), 6.60 (dd, J = 11.7, 4.8 Hz, 1H), 5.40 (dt, J = 51.7, 4.6 Hz, 1H), 4.81 (m, 1H), 4.45 – 4.38 (m, 2H), 4.35 (m, 1H).

¹³C NMR (126 MHz, Deuterium Oxide) δ 151.0 147.9, 146.8, 142.7 (d, J = 3.7 Hz), 117.9, 94.3 (d, J = 193.8 Hz), 82.5 (d, J = 17.1 Hz), 81.7 (dd, J = 9.4, 5.8 Hz), 72.2 (d, J = 24.6 Hz), 64.3 (d, J = 6.3 Hz).

³¹P NMR (203 MHz, Deuterium Oxide) δ 43.92 (d, J = 27.0 Hz), -10.88 (d, J =

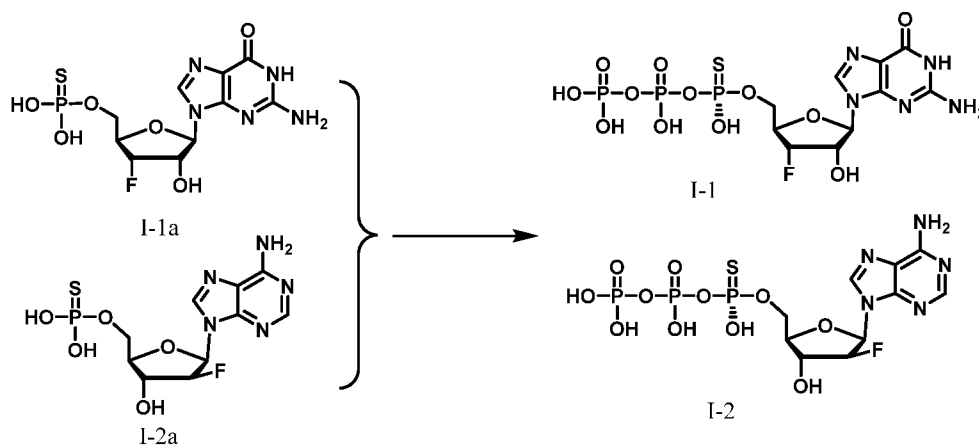
19.4 Hz), -23.94.

Preparatory Example 5: Preparation of Cobalt-Treated cGAS

500mL of cGAS whole cell lysate was spun at 5000 G-force at 4°C for 20min.

- 5 The supernatant was discarded, and the insoluble fraction was suspended with 500mL (1vol) of ultrapure, deionized, biology-grade water. The resulting mixture was spun at 5000 G-force at 4°C for 20min. The resulting supernatant was discarded, and the insoluble fraction was suspended with 500mL of 0.1M CoSO₄ (1vol, pH 4-8). The mixture was incubated for 1h at RT. The resulting mixture was spun at 5000 G-force at 4°C for 20min. The resulting supernatant was discarded, and the insoluble fraction was suspended with 500mL of ultrapure, deionized, biology-grade water (1vol). The resulting mixture was spun at 5000 G-force at 4°C for 20min. The resulting supernatant was discarded, and the insoluble fraction is Co-treated cGAS, which was stored at 4°C and used directly for the cGAS reaction.

- 15 **Preparatory Example 6: Tandem synthesis of 2'F-thio-ATP and 3'F-thio-GTP from monophosphate precursors using co-immobilized enzymes**



Step 1: Immobilization

- Ni-functionalized chelating resin suspension (commercially available as Bio-rad Nuvia IMAC Ni, 1.8L, 53vol% resin solids in 20%/80% EtOH/water) was added to a filter and washed (10L) with binding buffer (50 mM sodium phosphate buffer; 500 mM NaCl, pH 8) to remove the resin storage solution. The resin was isolated as a cake by filtration, and then re-suspended in the binding buffer (0.75L) and transferred by funnel into a first reactor (10L). An addition 0.25L of binding buffer was used to rinse the transfer vessel, and this liquid was also

transferred into the first reactor.

In a second vessel, lyophilized crude cell-free extracts were charged at a pre-determined ratio: a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 6 (21.20g), as set forth below, a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 4 (16.90g), as set forth above, and a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 5 (12.70g), as set forth above, and the extracts were dissolved in binding buffer (1.0L). Following complete dissolution, the contents of the second vessel were charged into the first reactor and aged overnight at 4°C with overhead agitation. The resulting mixture was filtered over vacuum yielding a wet cake of immobilized-enzyme on resin. The resulting cake was subsequently washed with 10L of a modified binding buffer containing imidazole (50mM sodium phosphate buffer; 500mM NaCl; 15mM imidazole, pH 8) and then washed with water (10L). The washed resin was isolated as a wet cake by filtration, re-suspended in water (1.0L), and stored at 4°C prior to use.

MHHHHHHALPTPVVICGPGSGKTTLYNKLLKEFPGVFQLTASHTTRQPR
PGEENGREFHFINRDQFQENIKQGDFLEWAEHSGNLYGTSSKKALEEVQANNVIPI
LDIDTQGVRTVKKASLEAVYIFIKPPSIEVLEERLRSRGTTETEEALQKRLSAAPNEL
EYGLKPGNFQHIITNDDLDVAYEKLKGILIDSQMPLAGATGSSSSVVNSFLDKPA
ASATTVNSSSQD (SEQ ID NO: 6)

Step 2: Reaction

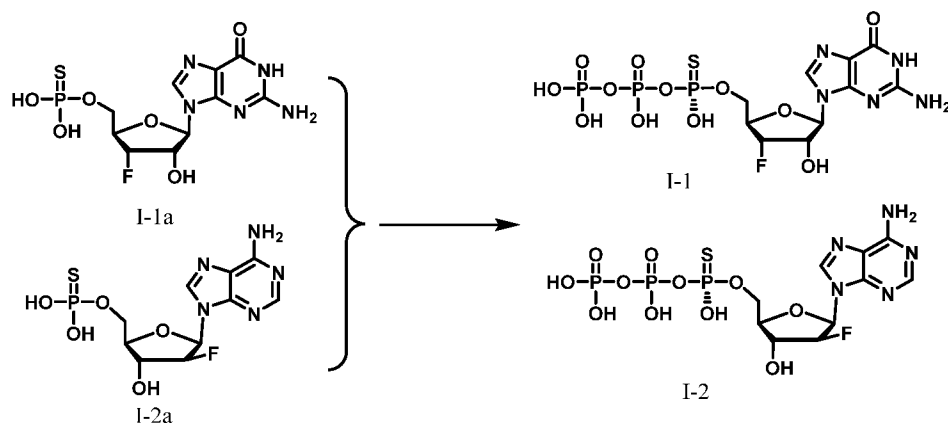
In a third reactor (100L), the following material was charged and held at 25°C: 25L water, followed by 3'-thio-GMP (600g, 1.0 eq), followed by 1.0L water to rinse the vessel walls. The mixture was dissolved with overhead agitation and subsequently cooled to 10°C. To the third reactor, 2'-thio-AMP (283g, 0.87eq), 2'-thio-ATP (5.29g, 0.01 eq), dilithium acetylphosphate (609g, 88wt% purity, 4.5eq), MgCl₂·5H₂O (374g, 2.0eq), and KCl (68.6g, 1.0eq) were charged, followed by water (1.0L) to rinse the walls of the vessel. The resulting mixture was held at 10°C and briefly agitated. The pH of the solution was then adjusted to approximately 7.3 - 7.4 using conc. KOH and HCl (5.0 N). Water was added to adjust the final fill volume to 28.15L.

While continuing to agitate the third reactor, 15% of the immobilized enzyme prepared in Step 1 was aliquoted into a bottle and stored at 4°C, while the remaining 85% of the immobilized enzyme was added to the 50L reactor, including 500mL water used to rinse the

vessel in which the immobilized enzyme was stored. An additional 500mL water was added to the reactor to rinse the vessel walls. The mixture was aged for 22h at 10°C. After the reaction was judged complete by HPLC analysis, the vessel contents were emptied into a filter, and the reaction filtrate was isolated under gentle vacuum and stored at 4°C or -20°C for subsequent use.

5

Preparatory Example 7: Tandem synthesis of 2'F-thio-ATP and 3'F-thio-GTP from monophosphate precursors using independently immobilized enzymes.



Step 1: Immobilization

10 Ni-NTA resin (commercially available as Bio-rad Nuvia IMAC Ni, 2.14mL of 70vol% resin slurry) was transferred to a filter, and the storage solution removed by vacuum filtration. Subsequently, the resin was displacement washed with a total of 15mL binding buffer (50mM sodium phosphate buffer; 500mM NaCl, pH 8), resuspended in 3.0mL binding buffer and transferred to a centrifuge tube, yielding a 50vol% suspension of resin in binding buffer.

15 Lyophilized CFE powders of a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 6 (as set forth above), a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 4 (as set forth above), and a kinase enzyme that has the amino acid sequence that is SEQ ID NO: 5 (as set forth above) were separately immobilized as follows: 25mg of the respective lyophilized CFE was weighed into a vial and resuspended in 0.5mL binding buffer.

20 To each 1.0mL of the 50v% suspension of Ni-NTA resin prepared above was added, followed by an additional 1.0mL binding buffer. Each vial was closed and mixed at RT for 1h to complete the immobilization.

Subsequently, the immobilized enzyme-resin from each vial was isolated as follows: the supernatant was decanted, and the resin was washed with a total of 5.0mL of a

modified binding buffer (50mM sodium phosphate buffer; 500mM NaCl, 15mM imidazole, pH 8) followed by 5.0mL of 1X PBS, the supernatant was decanted, and the resin was resuspended in 1.5mL water to obtain a 33vol% slurry of immobilized enzyme resin in water.

Step 2: Reaction

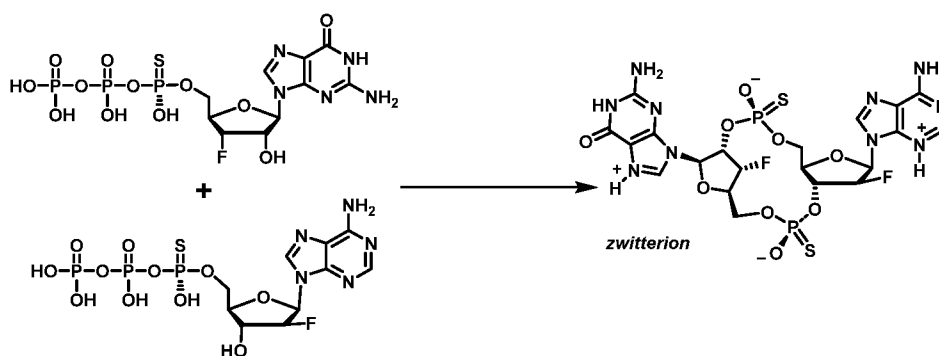
A reaction master mix was created by charging the following to a vessel: 2'F-Thio-ATP (9.45mg, 0.05eq), 2'F-Thio-AMP (111mg, 0.87eq), 3'F-Thio-GMP (200mg, 1.0eq), dilithium acetyl phosphate (207mg, 4.25eq), water (8.0mL), 1M MgCl₂·6H₂O (604μL, 2eq). The pH was adjusted to 7.47 by addition of 2N KOH (145μL, 0.98eq) and brought up to 10.0mL with water. The stock solution was stored at 4°C until ready for use.

Reactions were performed in a 96-well deep well microplate. To each well, 500μL of the reaction master mix was added. The reaction stoichiometry for each experiment was varied by changing the volume of each immobilized enzyme resin charged into the wells, between 0.1μL and 5.0μL of each resin.

The plate was sealed and mixed on a thermomixer at 10°C. The reaction progress was assessed at both 16h and 24h time points. For each, the reaction mixture was sampled, diluted volumetrically 20x with an aqueous solution containing 25% acetonitrile, and the conversion was analyzed by UPLC.

Preparatory Example 8: Synthesis of [P(R)]-2'-deoxy-2'-fluoro-5'-O-[(R)-

hydroxymercaptophosphinyl]-P-thio-β-D-arabino-adenylyl-(3'→5')-3'-deoxy-3'-fluoroguanosine cyclic nucleotide using isolated 2'F-thio-ATP and 3'F-thio-GTP

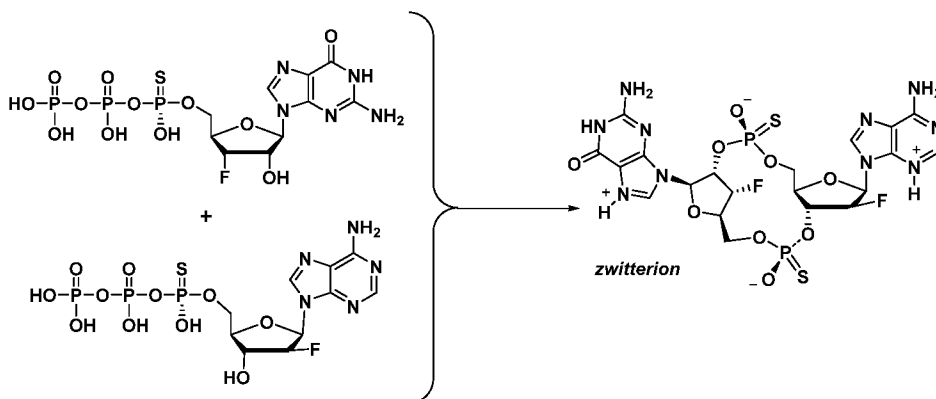


To a flask at RT under N₂ were charged 10mL of TES (1M, 5mmol, pH = 7.5), 6mL of 3'F-thio-GTP (0.1M, 0.3mmol, pH = 7), 1.82mL of 2'F-thio-ATP (0.33M, 0.3mmol, pH = 7), 3mL of CoSO₄ (0.5M, 0.75mmol), 3mL of ZnSO₄ (0.5M, 0.75mmol), 10mL of TGDE.

This solution was warmed up to 35°C, and the pH was adjusted to 7.4 via 0.1N KOH solution. A

wet cGAS pellet (872mg, 15wt% cGAS) in 8mL of DI water was charged, and reaction mixture was aged at 35°C for 24h. The reaction was then quenched with NaH₂PO₄ and cooled down to RT.

5 **Preparatory Example 9: Synthesis of [P(R)]-2'-deoxy-2'-fluoro-5'-O-[(R)-hydroxymercaptophosphinyl]-P-thio-β-D-arabino-adenylyl-(3'→5')-3'-deoxy-3'-fluoroguanosine cyclic nucleotide from 2'F-thio-AMP and 3'F-thio-GMP prepared using immobilized kinases**

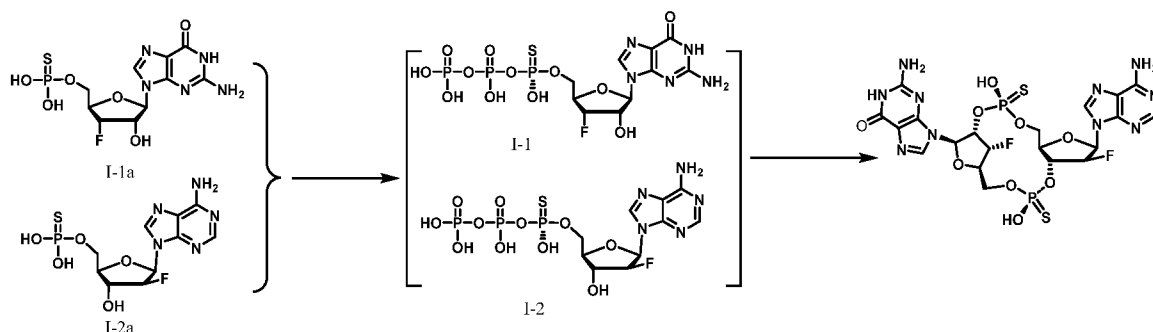


10 To a 100L vessel was charged 31L of a solution containing 2'F-thio-ATP (0.58mol) and 3'F-thio-GTP (0.64mol), followed by water used to rinse the container that was used to store the solution (6L). The jacket temperature of the vessel was set to 45°C, and the agitation set to 80RPM. *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES, 2.148kg, 9.37mol) and water used to rinse the TES container (4L) were added, giving a pH of

15 6.1. The pH was then adjusted to 8.0 via addition of potassium hydroxide (0.5L, 45wt%). TGDE (16L) was then added, followed by cobalt sulfate solution (1.5M, 1.1L) and zinc sulfate solution (1.1M, 2L), along with water used to rinse both containers (2L). Addition of metal solutions reduced the pH to 7.4. At this time, the jacket temperature was reduced to 42°C; the reaction temperature was 37°C. Next, cGAS enzyme slurry (8L) was then added to initiate

20 reaction. The reaction was aged at 35°C for an additional 13h until the reaction was judged to have completed (<2% 2'F-thio-ATP remaining).

25 **Preparatory Example 10: Synthesis of [P(R)]-2'-deoxy-2'-fluoro-5'-O-[(R)-hydroxymercaptophosphinyl]-P-thio-β-D-arabino-adenylyl-(3'→5')-3'-deoxy-3'-fluoroguanosine cyclic nucleotide from 2'F-thio-AMP and 3'F-thio-GMP**



Step 1

To a 100L reactor was charged 2'-F-thio-AMP (382.2g, 1.0eq) and 3'-F-thio-GMP (564.7g, 0.97eq). The resulting mixture was then cooled down to 10°C-15°C followed by addition of water (33.3L). ATP (57mg, 0.0001eq) was dissolved in water (60mL) and charged to the reactor. To this, MgCl₂·6H₂O (369.2g, 2.0eq) was added at 10°C-15°C, followed by addition of TES (1.041kg, 5.0eq). To adjust the pH of the reaction mixture from 5.20 to 5.98 (10°C-15°C), around 70.0mL of KOH (45wt%) was utilized. Next, AcP-Li/Li (752.4g, 5.2eq) was charged at 10°C-12°C. Once AcP-Li/Li was fully dissolved, around 150mL-160mL of KOH (45wt%) was added to adjust the pH to 7.42 at 9.5°C-10.5°C. To this clear solution, a solution of a kinase enzyme that can be represented by SEQ ID NO: 5 (2.10g dissolved in 0.20L water) (as set forth above) was charged, followed by a solution of a kinase enzyme that can be represented by SEQ ID NO: 4 (2.87g dissolved in 0.25L water) (as set forth above) and a solution of a kinase enzyme that can be represented by SEQ ID NO: 6 (3.44g dissolved in 0.35L water) (as set forth above) at 9.0°C-11°C, respectively. The reaction mixture was aged at 10°C under nitrogen for 17h - 24h until completion (1-3% 2'-F-thio-AMP and 3'-F-thio-GMP leftover).

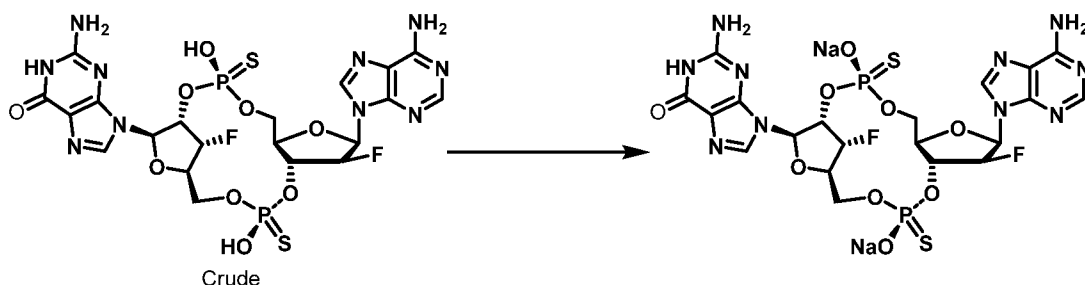
Step 2

Next, Na₃VO₄ (50.1g, 0.3eq) was charged to the reactor, followed by slow addition of a pre-cooled mixture of TGDE (15.3L) and water (11.0L), while maintaining the temperature below 15°C. To this, ZnSO₄·7H₂O (784.0g, 3.0eq) was added in one portion. Around 270ml - 285mL of KOH (45wt%) was charged to adjust the pH from 6.98 to 7.8 at 10°C. Then, cobalt-treated cGAS enzyme slurry that can be represented by SEQ ID NO: 7 (as set forth below) in water (22.1kg) was charged at 10°C. Temperature was increased to 35°C, and reaction was aged at 35°C for 15h-24h, until completion (<2% 3'-F-thio-ATP remaining).

MHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKEMDSLTFLYDGIEIQADQTPEDLDMEDNDIIEAHREQIGGE

NLYFQGGGPRLREVL SRLSLGRQDVSEASGLVNQVVSQLIQAIRSQEGSFGSIERL
 NTGSYYEHVKISEPNEFDIMLVMPVSRLQLDECDDTGAFYYLTFKRNSKDKHLF
 KFLDEEDGKLSAFKMLQALRDIIKREVKNKNAEVTVKRKKAGSPAITLQIKNPPA
 VISVDIILTLEPQQSWPPSTQDGLKIEKWLGKVRGQFRNKSLYLVAQNPREKV
 5 LRGNTWRLSFSHIEKDM LNNHGSSKTCCESDGLKCCRKG CYKLLKYLLERLKM
 KYPHQLEKRSSSYEVKTAFFHSCVMWPNDLDWHLSDLDYCFQKYLGYFLDCLQK
 SELPHFFIPQYNLLSLEDKASNDFLSRQINYELNNRFPIFQERY (SEQ ID NO: 7)

Preparatory Example 11: Extraction Isolation of [P(R)]-2'-deoxy-2'-fluoro-5'-O-[(R)-hydroxymercaptophosphinyl]-P-thio-β-D-arabino-adenylyl-(3'→5')-3'-deoxy-3'-fluoroguanosine cyclic nucleotide



To a 100L reactor containing 89.00kg of the cGAS reaction mixture of Example 10 at 49.0°C was added Na₂SO₄ (1.628kg, 20eq). After 2.5h stirring at 49°C, the reactor was cooled to 10°C, and 43.88kg of the reaction mixture was removed and stored at 0°C. To the remaining 45.12kg of reaction mixture was added 65.4mM [HN(*n*-oct)₃]₂[SO₄] in 2-Me-THF (21.9L, 108vol., 5.0eq), and the reaction mixture was stirred for 25min. The reaction mixture was cooled to -20°C, and 1-propanol (19L, 93.3vol.) was added. The reaction mixture was then stirred for 17h. The reaction mixture was warmed to 50°C and stirred for 2h. The cell debris-rich aqueous phase was removed, and the organic phase was filtered to remove residual cell debris. The filtered organic extracts were charged into a 100L reaction, and 0.25wt% Na₂SO₄ in water (40L, 196vol.) was added. The reaction mixture was stirred for 2h at RT. The aqueous phase was removed, and the organic phase was stored at 0°C. This step was repeated to recover additional crude product.

The organic extracts were combined in a 100L reactor at 23°C, and water (6.6L, 16.2vol) was added. The mixture was stirred for 25min. After 25min, the aqueous phase was removed, water (6.6L, 16.2vol) was added, and the mixture was stirred for 25min. After 25min,

the aqueous phase was removed, and 10% NaCl in water (4L, 9.8vol.) was added. The reaction mixture was stirred for 5min, and the aqueous phase was removed.

The organic extracts were combined in a 30L reactor at 23°C, and water (500mL, 1.23vol) and 10N NaOH (585mL, 1.43vol., 10.2eq) were added, until the mixture reached pH 13.15, over 20min while stirring. The aqueous phase was removed, and 1N NaOH (400mL, 0.98vol., 0.70eq) was added. The reaction mixture was stirred for 10min, and the aqueous extracts were removed and combined.

The aqueous extracts were filtered through a 1µm filter and added to a 10L reactor. The aqueous extracts were heated to 55°C. 2N HCl (400mL, 0.98vol., 1.40eq) was added dropwise over 2h to pH 7.30. The resulting slurry was cooled to 25°C and stirred for 12h. The product was collected by filtration and washed once with 93% EtOH:7% water (4L, 9.82vol.), and again with 93% EtOH:7% water (1.5L, 3.68vol.). The product was dried under air flow for 90min then under vacuum, at a relative humidity of 32.9% to 45.0%, over 41h.

¹H NMR (600 MHz, Deuterium Oxide) δ 8.41 (s, 1H), 8.37 (s, 1H), 8.11 (s, 1H), 6.68 (dd, *J* = 15.0, 4.1 Hz, 1H), 6.18 (d, *J* = 8.6 Hz, 1H), 5.90 – 5.66 (m, 2H), 5.58 (dd, *J* = 53.3, 3.4 Hz, 1H), 5.41 (ddt, *J* = 13.6, 8.3, 3.9 Hz, 1H), 4.86 (d, *J* = 26.0 Hz, 1H), 4.61 (d, *J* = 4.9 Hz, 1H), 4.58 (d, *J* = 8.5 Hz, 1H), 4.31 (t, *J* = 5.8 Hz, 2H), 4.27 (d, *J* = 11.9 Hz, 1H).

EXAMPLES

Example 1: Form I (hydrate)

Form I was produced by crystallization from the aqueous extracts in Preparatory Example 12. The aqueous extracts were filtered through a 1µm filter and added to a 10L reactor. The aqueous extracts were heated to 55°C. 2N HCl (400mL, 0.98vol., 1.40eq) was added dropwise over 2h to pH 7.30. The resulting slurry was cooled to 25°C and stirred for 12h. The product was collected by filtration and washed once with 93% EtOH:7% water (4L, 9.82vol.), and again with 93% EtOH:7% water (1.5L, 3.68vol.). The product was first dried under air flow and vacuum for 90min and then under vacuum, with nitrogen flow at a relative humidity of 32.9% to 45.0%, over 41h. **Form I** was generated.

Example 1: Alternate method 1

Form I also was produced by phase conversion, through humid drying of **Form IV** (hydrate). A wet cake of **Form IV** (hydrate) (from **Example 4**, 3.8g) was placed in vacuum

oven to dry under humid drying conditions (25°C, 70-75%RH). After 7 hours, **Form I** (hydrate) was generated.

Example 2: Form II (hydrate)

Form II was produced by dissolving **Form I** (hydrate) (from **Example 1**, 2.0g) in water at pH 11.5. the solution pH was adjusted using 1N NaOH and 1N HCl. The pH was then decreased to 10.0-10.5 with 1N HCl to reach seed point, at room temperature. **Form I** (hydrate) (from **Example 1**, 25mg) was charged as seed. The pH was further decreased to 6.5-7.0 by adding 1N HCl added over 4-6 hours to crystallize the batch. **Form II** (hydrate) was generated.

Example 3: Form III (hydrate)

Form III was produced by dissolving amorphous form of **Compound A** (from **Preparatory Example 12**, 2.5g) with 2 equivalents of Na in water at pH = 11.5, by adjusting the solution pH using 1N NaOH and 1N HCl. The solution pH was then decreased to 10.0-10.5 with 1N HCl to reach seed point. **Form I** (hydrate) (from **Example 1**, 25mg) was charged as seed. The pH was further decreased with 1N HCl over 6 hours to crystallize the batch; the pH dropped to 6.2 at mid-point of addition of HCl. The batch was cooled to 5°C and aged overnight. **Form III** (hydrate) was formed following filtration and a water wash.

Example 4: Form IV (hydrate)

Form IV was produced by adding **Form I** (hydrate) (from **Example 1**, 4g) to 54.6 ml water and 132.8 ml of ethanol to form a slurry. The slurry was stirred at 50°C for 6 days. The wet cake then was filtered out, resulting in **Form IV** (hydrate).

Example 5: X-Ray Powder Diffraction Characterization

X-ray powder diffraction (XRPD) studies are widely used to characterize molecular structures, crystallinity, and polymorphism. The X-ray powder diffraction patterns for the solid phases of **Compound A** were generated on a Philips Analytical X'Pert PRO X-ray Diffraction System. A Cu K-Alpha radiation source with a pressed powder sample in Bragg Brentano mode was used. The diffraction peak positions were referenced by silicon (internal standard), which has a 2 theta (2 θ) value of 28.4409 degree. The experiments were analyzed at ambient condition.

Analysis was performed on **Form I**, as provided in the primary procedure of **Example 1**. **Fig. 2** shows characteristic peaks for **Form I**, in the range of 2° - 40° 2θ . The X-ray powder diffraction pattern was generated to characterize **Form I**, as shown in **Fig. 2**, which exhibited characteristic reflections corresponding to d-spacings ($\pm 0.3^{\circ}$ 2θ) as shown in

5 **Table 1**.

Table 1

Pos. [$^{\circ}2\theta$]	d-spacing [Å]	Rel. Int. [%]
4.44	19.90	3.29
8.87	9.96	0.66
9.46	9.35	32.77
9.72	9.10	30.6
10.43	8.49	22.81
11.33	7.81	13.42
12.36	7.16	48.23
12.96	6.83	1.09
13.30	6.65	3.03
13.69	6.47	16.31
14.57	6.08	34.52
15.21	5.82	2.86
16.33	5.43	25.47
16.91	5.24	14.75
17.11	5.18	17.04
17.54	5.06	8.75
18.22	4.87	17.65
18.73	4.74	100
19.15	4.63	13.89
19.45	4.56	8.12
19.76	4.49	8.69
20.08	4.42	30.64
20.30	4.38	29.86
20.63	4.31	48.47
20.90	4.25	17.72
21.60	4.11	69.89
21.96	4.05	21.24
22.13	4.02	40.13
22.59	3.94	23.37
22.92	3.88	28.21
23.20	3.83	41.91
23.69	3.76	25.99

Pos. [$^{\circ}2\theta$]	d-spacing [Å]	Rel. Int. [%]
24.31	3.66	39.07
24.60	3.62	9.46
24.85	3.58	21.77
25.58	3.48	7.72
26.09	3.41	39.11
26.39	3.38	14.81
26.74	3.33	8.82
27.03	3.30	11.03
27.45	3.25	17.61
28.14	3.17	10.52
28.53	3.13	7.89
28.94	3.09	15.79
29.12	3.07	8.55
30.00	2.98	10.14
30.69	2.91	18.4
30.93	2.89	22.23
31.31	2.86	23.61
31.85	2.81	8.02
32.39	2.76	3.43
33.77	2.65	10.83
34.38	2.61	4.94
34.98	2.57	4.97
35.28	2.54	4.23
36.05	2.49	9.56
36.43	2.47	6.96
37.30	2.41	3.9
37.64	2.39	4.84
37.93	2.37	3
38.85	2.32	5.07
39.48	2.28	3.21
40.02	2.25	6.55
40.38	2.23	5.16

Analysis was performed on **Form II**, as provided in **Example 2**. **Fig. 3** shows characteristic peaks for **Form II**, in the range of 2° - 40° 2θ . The X-ray powder diffraction pattern was generated to characterize **Form II**, as shown in **Fig. 3**, which exhibited characteristic reflections corresponding to d-spacings ($\pm 0.3^{\circ}$ 2θ) as shown in **Table 2**.

5

Table 2

Pos. [2θ]	d-spacing [Å]	Rel. Int. [%]
6.87	12.87	52
7.31	12.10	40
7.73	11.44	16
8.76	10.10	12
12.54	7.06	7
13.57	6.52	9
13.85	6.39	24
14.59	6.07	31
15.21	5.83	6
16.13	5.49	8
16.35	5.42	21
16.81	5.27	8
18.26	4.86	25
18.74	4.74	34
19.23	4.62	100
20.48	4.34	47
21.04	4.22	54
21.41	4.15	39
22.07	4.03	43

Pos. [2θ]	d-spacing [Å]	Rel. Int. [%]
22.83	3.90	26
23.46	3.79	32
24.15	3.69	8
24.96	3.57	11
25.39	3.51	35
25.70	3.46	13
26.86	3.32	32
27.21	3.28	17
27.82	3.21	18
28.40	3.14	13
29.01	3.08	19
29.62	3.02	20
30.43	2.94	22
30.91	2.89	11
31.56	2.84	11
32.14	2.79	25
33.10	2.71	9
33.63	2.66	13
34.06	2.63	13

Analysis was performed on **Form III**, as provided in **Example 3**. **Fig. 4** shows characteristic peaks for **Form III**, in the range of 2° - 40° 2θ . The X-ray powder diffraction pattern was generated to characterize **Form III**, as shown in **Fig. 4**, which exhibited characteristic reflections corresponding to d-spacings ($\pm 0.3^{\circ}$ 2θ) as shown in **Table 3**.

5

Table 3

Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
5.45	16.22	24	24.52	3.63	4
6.83	12.95	100	25.34	3.52	1
8.69	10.17	12	25.70	3.47	1
12.74	6.95	3	26.13	3.41	3
13.57	6.52	8	27.11	3.29	5
13.84	6.40	10	27.30	3.27	5
14.60	6.07	15	27.92	3.20	2
15.09	5.87	12	28.51	3.13	2
16.18	5.48	24	29.04	3.07	1
17.35	5.11	2	29.41	3.04	3
19.16	4.63	36	30.44	2.94	4
20.10	4.42	15	30.64	2.92	9
20.40	4.35	5	32.00	2.80	2
20.58	4.32	18	32.34	2.77	2
21.12	4.21	14	32.65	2.74	3
21.33	4.17	1	33.37	2.69	5
21.97	4.05	7	34.16	2.62	1
22.68	3.92	6	36.13	2.49	5
23.12	3.84	3	36.43	2.47	1
23.33	3.81	32	38.20	2.36	2

Analysis was performed on **Form IV**, as provided in **Example 4**. **Fig. 5** shows characteristic peaks for **Form IV**, in the range of 2° - 40° 2θ . The X-ray powder diffraction pattern was generated to characterize **Form IV**, as shown in **Fig. 5**, which exhibited characteristic reflections corresponding to d-spacings ($\pm 0.3^{\circ}$ 2θ) as shown in **Table 4**.

5

Table 4

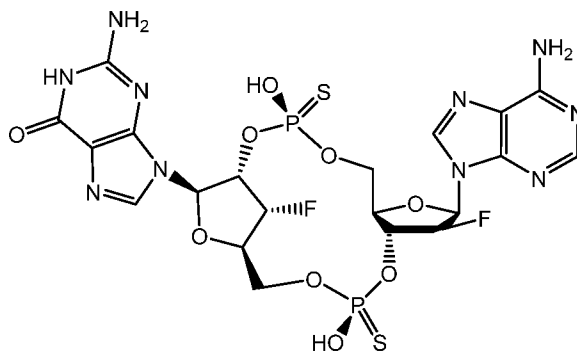
Pos. [$^{\circ}2\theta$]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	Pos. [$^{\circ}2\theta$]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
5.52	16.00	2	21.81	4.07	24
6.63	13.34	100	21.87	4.06	3
7.22	12.25	7	22.04	4.03	5
7.65	11.56	2	22.62	3.93	31
8.56	10.33	10	22.97	3.87	9
11.23	7.88	2	23.27	3.82	41
12.73	6.95	11	23.77	3.74	11
13.22	6.69	6	24.07	3.70	9
13.46	6.58	35	24.58	3.62	6
14.29	6.20	11	25.11	3.55	34
14.61	6.06	10	25.56	3.48	16
15.17	5.84	9	25.77	3.46	19
15.52	5.71	9	26.47	3.37	17
15.84	5.59	5	26.91	3.31	9
16.32	5.43	8	27.30	3.27	9
17.08	5.19	9	27.71	3.22	10
17.39	5.10	9	28.18	3.17	7
17.67	5.02	10	28.52	3.13	7
18.35	4.83	3	29.15	3.06	8
18.98	4.68	67	29.61	3.02	9
19.20	4.62	14	29.92	2.99	14
19.78	4.49	65	30.26	2.95	14
19.99	4.44	26	30.92	2.89	15
20.54	4.32	25	31.55	2.84	18
21.25	4.18	28	31.78	2.82	11

It will be appreciated that various of the above-discussed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also, that various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the art that are also intended to be encompassed by the following claims.

10

WHAT IS CLAIMED IS:

1. A crystalline compound having the structural formula:



- 5 or a pharmaceutically acceptable salt hydrate thereof.

2. The crystalline compound of claim 1, wherein the crystalline compound is a 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]penta-oxa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one di-sodium salt hydrate.

3. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 3 2θ values measured using $\text{CuK}\alpha$ radiation selected from the group consisting of about 4.44, about 8.87, about 9.46, about 9.72, about 10.43, about 11.33, about 12.36, about 12.96, about 13.30, about 13.69, about 14.57, about 15.21, about 16.33, about 16.91, about 17.11, about 17.54, about 18.22, about 18.73, about 19.15, about 19.45, about 19.76, about 20.08, about 20.30, about 20.63, about 20.90, about 21.60, about 21.96, about 22.13, about 22.59, about 22.92, about 23.20, about 23.69, about 24.31, about 24.60, about 24.85, about 25.58, about 26.09, about 26.39, about 26.74, about 27.03, about 27.45, about 28.14, about 28.53, about 28.94, about 29.12, about 30.00, about 30.69, about 30.93, about 31.31, about 31.85, about 32.39, about 33.77, about 34.38, about 34.98, about 35.28, about 36.05, about 36.43, about 37.30, about 37.64, about 37.93, about 38.85, about 39.48, about 40.02, and about $40.38^\circ 2\theta$.

4. The crystalline compound of claim 3, characterized by an X-ray powder diffraction containing at least 3 of the following 2θ values measured using $\text{CuK}\alpha$ radiation: about

9.46, about 9.72, about 10.43, about 11.33, about 12.36, about 13.69, about 14.57, about 16.33, about 16.91, about 17.11, about 17.54, about 18.22, about 18.73, about 19.15, about 19.45, about 19.76, about 20.08, about 20.30, about 20.63, about 20.90, about 21.60, about 21.96, about 22.13, about 22.59, about 22.92, about 23.20, about 23.69, about 24.31, about 24.60, about 24.85, about 25.58, about 26.09, about 26.39, about 26.74, about 27.03, about 27.45, about 28.14, about 28.53, about 28.94, about 29.12, about 30.00, about 30.69, about 30.93, about 31.31, about 31.85, about 33.77, about 36.05, about 36.43, about 38.85, about 40.02, and about 40.38° 2 θ .

5. The crystalline compound of claim 3, characterized by an X-ray powder diffraction containing at least 3 of the following 2 θ values measured using CuK α radiation: about 4.44, about 8.87, about 12.96, about 13.30, about 15.21, about 32.39, about 34.38, about 34.98, about 35.28, about 37.30, about 37.64, about 37.93, and about 39.48° 2 θ .

6. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 3 2 θ values measured using CuK α radiation selected from the group consisting of about 6.87, about 7.31, about 7.73, about 8.76, about 12.54, about 13.57, about 13.85, about 14.59, about 15.21, about 16.13, about 16.35, about 16.81, about 18.26, about 18.74, about 19.23, about 20.48, about 21.04, about 21.41, about 22.07, about 22.83, about 23.46, about 24.15, about 24.96, about 25.39, about 25.7, about 26.86, about 27.21, about 27.82, and about 28.4, about 29.01, about 29.62, about 30.43, about 30.91, about 31.56, about 32.14, about 33.1, about 33.63, and about 34.06° 2 θ .

7. The crystalline compound of claim 6, characterized by an X-ray powder diffraction containing at least 3 of the following 2 θ values measured using CuK α radiation: about 12.54, about 13.57, about 13.85, about 15.21, about 16.13, about 16.35, about 16.81, about 20.48, about 21.04, about 21.41, about 22.07, about 25.7, about 27.21, about 27.82, and about 28.4° 2 θ .

8. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 2 of the following 2 θ values measured using CuK α radiation: about 29.01, about 29.62, about 30.43, about 30.91, about 31.56, about 32.14, about 33.1, about 33.63,

and about 34.06° 2 θ .

9. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 2 of the following 2 θ values measured using CuK α radiation: about 6.87, about 7.31, about 7.73, about 8.76, about 14.59, about 18.26, about 18.74, about 19.23, about 22.83, about 23.46, about 24.15, about 24.96, about 25.39, and about 26.86° 2 θ .

10. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 3 2 θ values measured using CuK α radiation selected from the group consisting of about 5.52, about 6.63, about 7.22, about 7.65, about 8.56, about 11.23, about 12.73, about 13.22, about 13.46, about 14.29, about 14.61, about 15.17, about 15.52, about 15.84, about 16.32, about 17.08, about 17.39, about 17.67, about 18.35, about 18.98, about 19.20, about 19.78, about 19.99, about 20.54, about 21.25, about 21.87, about 22.04, about 22.62, about 22.97, about 23.27, about 23.77, about 24.07, about 24.58, about 25.11, about 25.56, about 25.77, about 26.47, about 26.91, about 27.30, about 27.71, about 28.18, about 28.52, about 29.15, about 29.61, about 29.92, about 30.26, about 30.92, about 31.55, and about 31.78° 2 θ .

11. The crystalline compound of claim 10, characterized by an X-ray powder diffraction containing at least 3 of the following 2 θ values measured using CuK α radiation: about 5.52, about 7.22, about 7.65, about 8.56, about 11.23, about 12.73, about 13.46, about 14.29, about 14.61, about 15.17, about 15.52, about 15.84, about 16.32, about 17.08, about 17.39, about 17.67, about 18.35, about 20.54, about 21.81, about 21.87, about 22.04, about 22.62, about 22.97, about 23.27, about 23.77, about 24.07, about 24.58, and about 25.56° 2 θ .

12. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 2 of the following 2 θ values measured using CuK α radiation: about 25.77, about 26.47, about 26.91, about 27.30, about 27.71, about 28.18, about 28.52, about 29.15, about 29.61, about 29.92, about 30.26, about 30.92, about 31.55, and about 31.78° 2 θ .

13. The crystalline compound of claim 2, characterized by an X-ray powder diffraction containing at least 2 of the following 2 θ values measured using CuK α radiation: about

6.63, about 13.22, about 18.98, about 19.20, about 19.78, about 19.99, about 21.25, and about 25.11° 2 θ .

14. The crystalline compound of claim 1, wherein the crystalline compound is a 2-amino-9-[(2*R*,5*R*,7*R*,8*S*,10*R*,12*aR*,14*R*,15*S*,15*aR*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidoctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]penta-oxa-diphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one mono-sodium salt hydrate.

15. The crystalline compound of claim 14, characterized by an X-ray powder diffraction containing at least 3 2 θ values measured using CuK α radiation: selected from the group consisting of about 5.45, about 6.83, about 8.69, about 12.74, about 13.57, about 13.84, about 14.60, about 15.09, about 16.18, about 17.35, about 19.16, about 20.10, about 20.40, about 21.12, about 21.33, about 21.97, about 22.68, about 23.12, about 23.33, about 24.52, about 25.34, about 25.70, about 26.13, about 27.11, about 27.30, about 27.92, about 28.51, about 29.04, about 29.41, about 30.44, about 30.64, about 32.00, about 32.34, about 32.65, about 33.37, about 34.16, about 36.13, about 36.43, about 36.92, and about 38.20° 2 θ .

16. The crystalline compound of claim 15, characterized by an X-ray powder diffraction containing at least 3 of the following 2 θ values measured using CuK α radiation: about 21.12, about 21.33, about 21.97, about 22.68, about 23.12, about 23.33, about 24.52, about 25.34, about 25.70, about 26.13° 2 θ .

17. The crystalline compound of claim 15, characterized by an X-ray powder diffraction containing at least 4 of the following 2 θ values measured using CuK α radiation: an X-ray powder diffraction containing at least 2 of the following 2 θ values measured using CuK α radiation: about 27.11, about 27.30, about 27.92, about 28.51, about 29.04, about 29.41, about 30.44, about 30.64, about 32.00, about 32.34, about 32.65, about 33.37, about 34.16, about 36.13, about 36.43, about 36.92, and about 38.20° 2 θ .

18. The crystalline compound of claim 14, characterized by an X-ray powder diffraction containing at least 2 of the following 2 θ values measured using CuK α radiation: about

5.45, about 6.83, about 8.69, about 12.74, about 13.57, about 13.84, about 14.60, about 15.09, about 16.18, about 17.35, about 19.16, about 20.10, and about 20.40° 20.

5 19. A pharmaceutical composition comprising a crystalline compound of any one of claim 1 to claim 18 and a pharmaceutically acceptable carrier.

 20. The pharmaceutical composition of claim 20, wherein the pharmaceutical composition is a solid dosage form for oral administration.

10 21. The pharmaceutical composition of claim 20, wherein the pharmaceutical composition is a sterile solution for parenteral, intratumoral, intravenous, or intramuscular administration.

15 22. Use of the crystalline compound of any one of claim 1 to claim 18 as an active ingredient in a medicament for inducing an immune response in a subject.

 23. Use of the pharmaceutical composition of any one of claim 19 to claim 21 as a medicament for inducing an immune response in a subject.

20 24. Use of the crystalline compound of any one of claim 1 to claim 18 as an active ingredient in a medicament for inducing a STING-dependent type I interferon production in a subject.

25 25. Use of the pharmaceutical composition of any one of claim 19 to claim 21 as a medicament for inducing a STING-dependent type I interferon production in a subject.

 26. Use of the crystalline compound of any one of claim 1 to claim 18 as an active ingredient in a medicament for treatment of a cell proliferation disorder.

30 27. Use of claim 26, wherein the cell proliferation disorder is cancer.

 28. Use of the pharmaceutical composition of any one of claim 20 to claim 22

as a medicament for treatment of a cell proliferation disorder.

29. Use of claim 28, wherein the cell proliferation disorder is cancer.

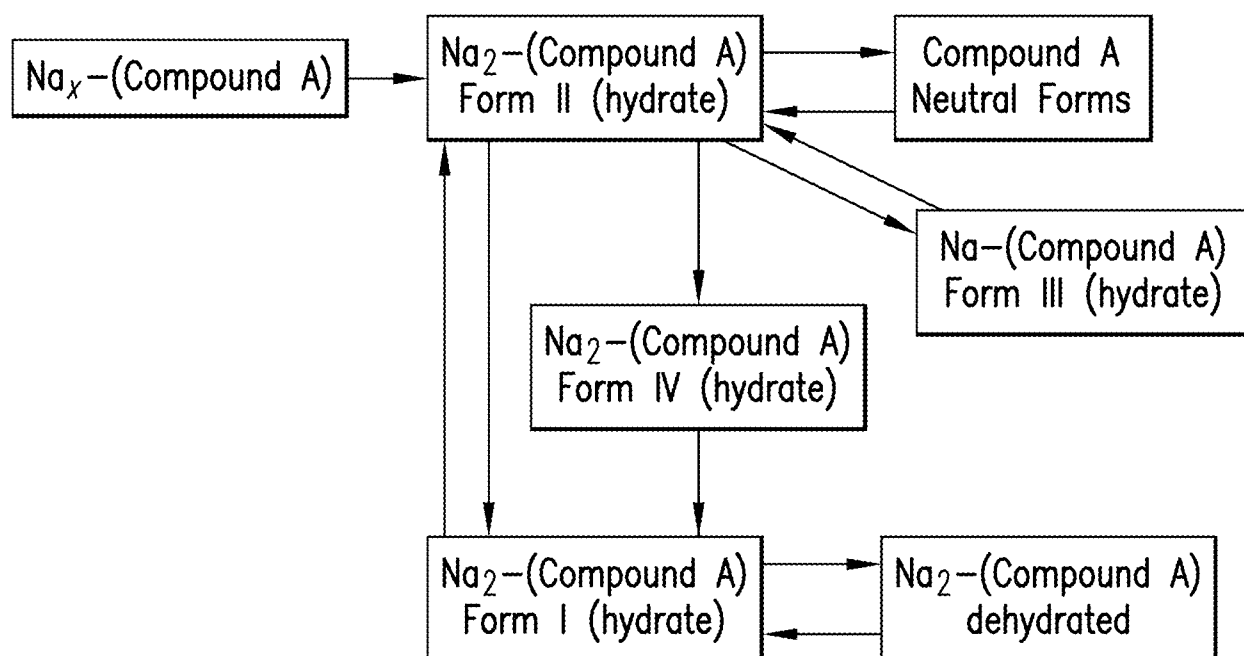


FIG. 1

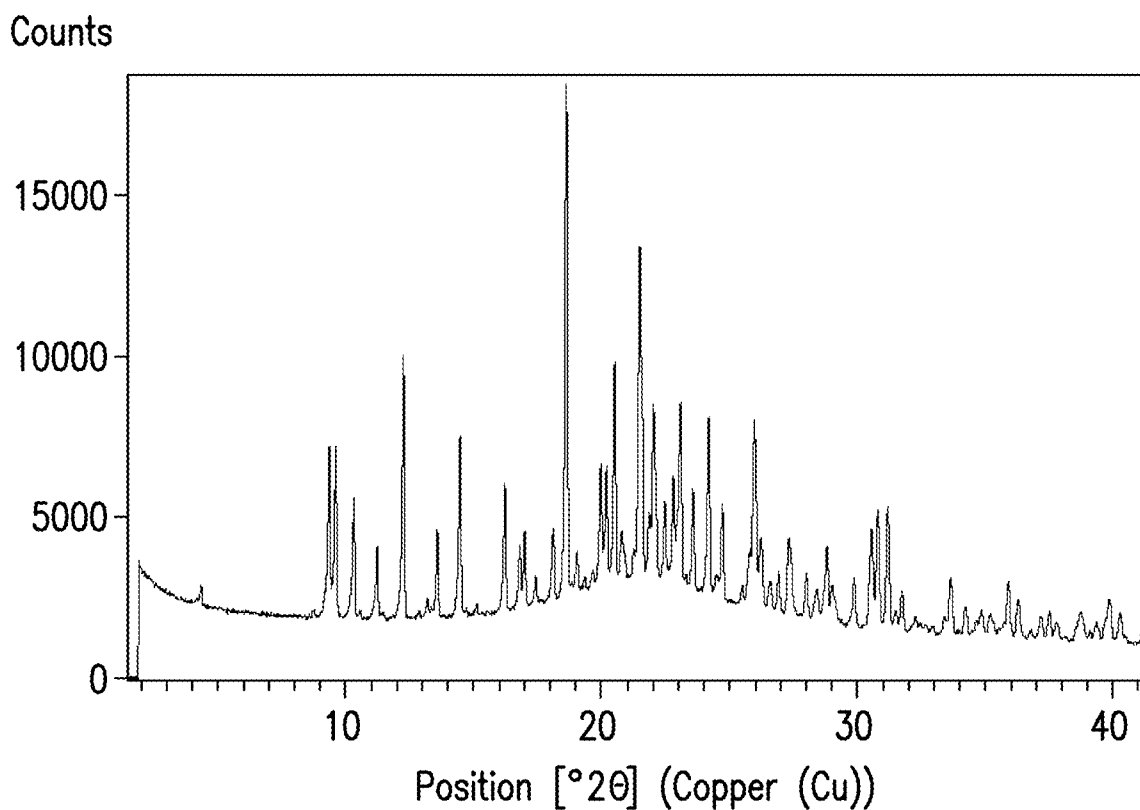
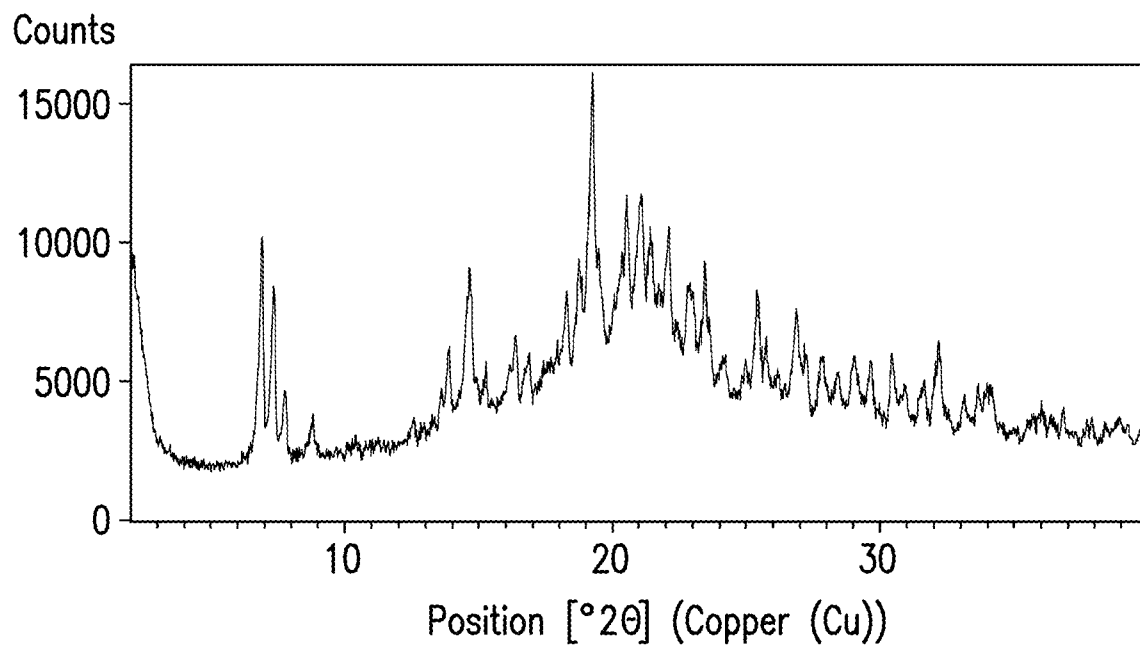
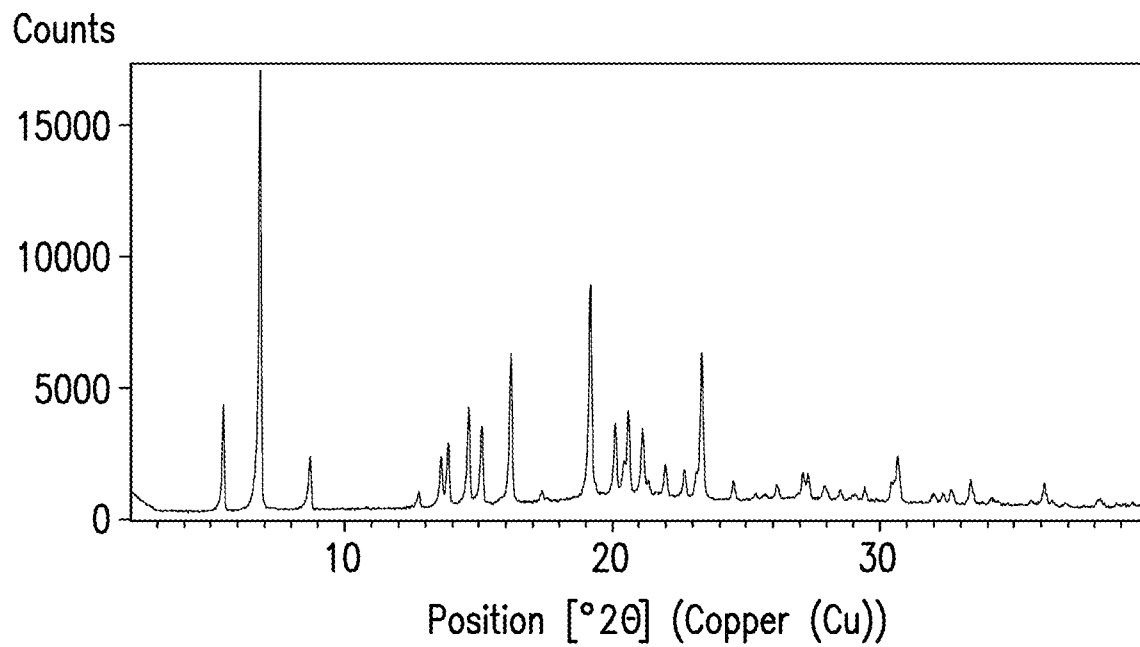


FIG. 2

**FIG.3****FIG.4**

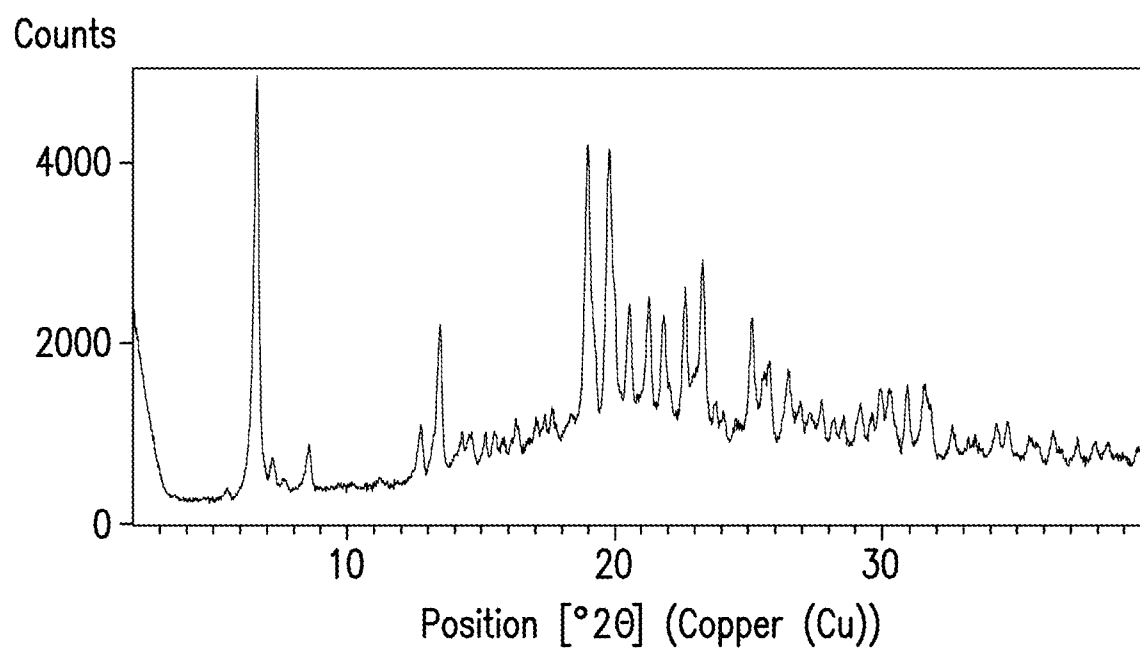


FIG.5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23247

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07H 19/16; C07H 19/167; C07H 19/173; C07H 19/207; C07H 19/213; A61P 35/00 (2022.01)

CPC - C07H 19/16; C07H 19/167; C07H 19/173; C07H 19/207; C07H 19/213; A61P 35/00

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)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 10,106,574 B2 (MERCK SHARP AND DOHME CORP, et al.) 23 October 2018; column 4, lines 5-15; column 29, lines 65-67; column 30, lines 1-10, 15-20; column 345, lines 25-35, 40-45; column 348, lines 35-40; column 358, lines 54-58; column 359, lines 50-57; column 360, lines 50-57; column 362, lines 15-25; column 701, lines 10-20; column 702, lines 45-65; column 708, lines 5-10, 30-55; column 710, lines 20-35; claims 27-28	1-2, 14, 19/1-2, 19/14, 20/19/1-2, 20/19/1-14, 21/20/19/1-2, 21/20/19/14, 24/1-2, 24/14, 26/1-2, 26/14, 27/26/1-2, 27/26/14 --- 3-13, 15-18, 19/3-13, 19/15-18, 20/19/3-13, 20/19/15-18, 21/20/19/3-13, 21/20/19/15-18, 22/3-13, 22/15-18, 24/3-13, 24/15-18, 26/3-13, 26/15-18, 27/26/3-13, 27/26/15-18
Y --- A	US 2019/0169221 A1 (JANSSEN BIOPHARMA, INC.) 6 June 2019; paragraph [0091]	1-2, 14, 19/1-2, 19/14, 20/19/1-2, 20/19/1-14, 21/20/19/1-2, 21/20/19/14, 24/1-2, 24/14, 26/1-2, 26/14, 27/26/1-2, 27/26/14 --- 3-13, 15-18, 19/3-13,

☒ 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

"D" document cited by the applicant in the international application

"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

09 July 2022 (09.07.2022)

Date of mailing of the international search report

AUG 03 2022

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Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23247

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0260878 A1 (HARANO, F et al.) 23 October 2008; paragraph [0034]	19/15-18, 20/19/3-13, 20/19/15-18, 21/20/19/3-13, 21/20/19/15-18, 22/3-13, 22/15-18, 24/3-13, 24/15-18, 26/3-13, 26/15-18, 27/26/3-13, 27/26/15-18 2, 14, 19/2, 19/14, 20/19/2, 20/19/14, 21/20/19/2, 21/20/19/14, 24/2, 24/14, 26/2, 26/14, 27/26/2, 27/26/14
A	US 7,427,675 B2 (CAPALDI, DC et al.) 23 September 2008; column 79, lines 1-30; column 80, lines 1-30	3-13, 15-18, 19/3-13, 19/15-18, 20/19/3-13, 20/19/15-18, 21/20/19/3-13, 21/20/19/15-18, 22/3-13, 22/15-18, 24/3-13, 24/15-18, 26/3-13, 26/15-18, 27/26/3-13, 27/26/15-18
A	US 2008/0293665 A1 (UNDHEIM, K et al.) 27 November 2008; paragraph [0036]	3-13, 15-18, 19/3-13, 19/15-18, 20/19/3-13, 20/19/15-18, 21/20/19/3-13, 21/20/19/15-18, 22/3-13, 22/15-18, 24/3-13, 24/15-18, 26/3-13, 26/15-18, 27/26/3-13, 27/26/15-18
A	(SUHADOLNIK, RJ et al.) Phosphorothioate Analogues of 2',5'-Oligoadenylate. Enzymatic Synthesis, Properties, and Biological Activities of 2',5'-Phosphorothioates from Adenosine 5'-O-(2-Thiotriphosphate) and Adenosine 5'-O-4 3-Thiotriphosphate). Biochemistry, Vol. 26, No. 22, 3 November 1987, doi: 10.1021/bi00396a041, pages 7143-1149; entire publication	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23247

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23247

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 23, 25, 28-29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.