(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 March 2021 (18.03.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/050956 A1

(51) International Patent Classification:
A61K 31/70 (2006.01) *C07H 19/173* (2006.01)
C07H 19/16 (2006.01)

(21) International Application Number:
PCT/US2020/050511

(22) International Filing Date:
11 September 2020 (11.09.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/898,679 11 September 2019 (11.09.2019) US

(71) Applicant: **THE SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550 North Torrey Pines Road, La Jolla, California 92037 (US).

(72) Inventors: **CHATTERJEE, Arnab Kumar**; 13268 Torrey Crest Court, San Diego, California 92129 (US). **GUPTA, Anil Kumar**; Apt. 6, 2038 Thomas Ave., San Diego, California 92109 (US). **ELIASEN, Andres Mikal**; 2468 N. Ditman Avenue, Los Angeles, California 90032 (US). **JOSEPH, Sean Barry**; 1005 Concord Street, San Diego, California 92106 (US).

(74) Agent: **CEPURITIS, Talivaldis** et al.; Olson & Cepuritis, LTD., 20 North Wacker Dr., 36th Floor, Chicago, Illinois 60606 (US).

(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, 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, 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).

(54) Title: ANTIVIRAL PRODRUGS AND PHARMACEUTICAL COMPOSITIONS THEREOF

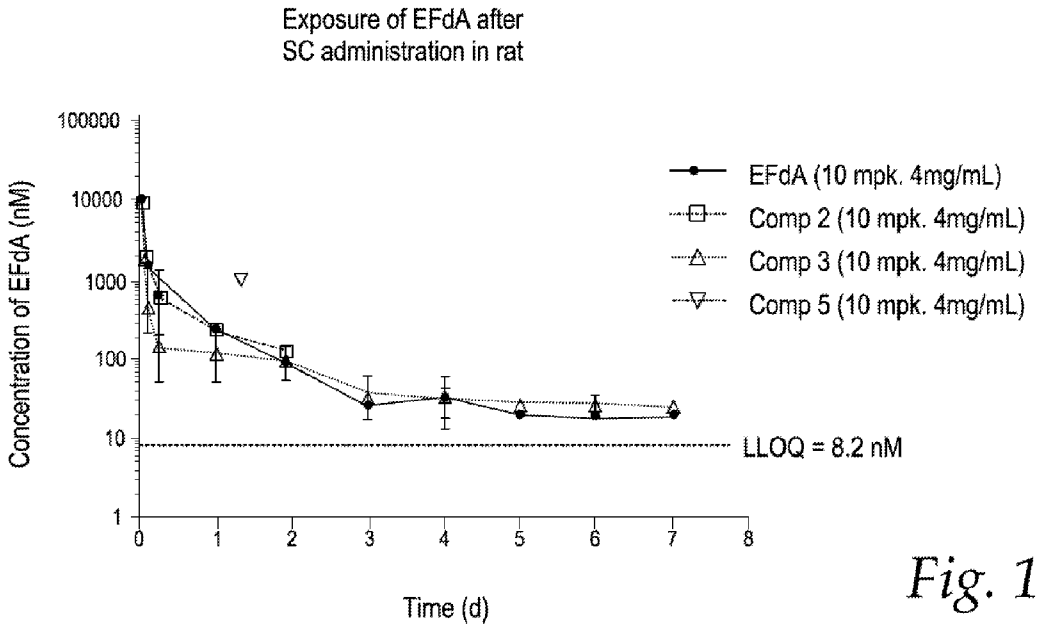


Fig. 10

(57) Abstract: Diesters of 4'-ethynyl-2-fluoro-2'-deoxyadenosine and aqueous parenteral suspensions thereof provide extended *in vivo* viral suppression of human immunodeficiency virus (HIV).

[Continued on next page]

WO 2021/050956 A1

Published:
— *with international search report (Art. 21(3))*

ANTIVIRAL PRODRUGS AND PHARMACEUTICAL COMPOSITIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

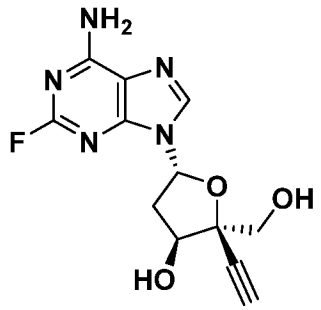
This application claims the benefit of U.S. Provisional Application Serial No. 62/898,679, filed on September 11, 2019, which is incorporated herein by reference in its entirety.

FIELD OF INVENTION

This invention relates to antiviral compounds and compositions useful for the treatment of acquired immunodeficiency syndrome (AIDS).

BACKGROUND OF INVENTION

4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) (MK-8591) represented by Formula I:



(I)

is a nucleoside analog effective as an inhibitor of nucleoside reverse transcriptase (Current Opinion in HIV and AIDS 2018, 13, 294-299) and useful as an antiretroviral in the treatment and pre-exposure prophylaxis of a HIV-1 infection. EFdA is metabolized in cells to an active triphosphate anabolite (EFdA-TP), which inhibits HIV reverse transcriptase.

EFdA, however, has a relatively high water solubility and relatively short time course of plasma concentration. As a result EFdA can provide only limited duration of viral suppression when administered to a patient for treatment of human immunodeficiency virus (HIV) infection, or for pre-exposure prophylaxis. Accordingly, there exists a need for formulations that can extend duration of viral suppression after administration. The compounds and pharmaceutical preparations of the present invention satisfy that need.

SUMMARY OF INVENTION

Antiviral compounds of the present invention are diesters of EFdA and have limited solubility in water. Whereas EFdA has an aqueous solubility of 0.877 mg/mL at physiological pH, the present diesters of EFdA have an aqueous solubility of less than 0.03 mg/mL at physiological pH, preferably less than 0.002 mg/mL. These diesters of EFdA are crystalline, are useful for providing an extended duration of suppression of HIV, and can be administered parenterally as a suspension in a pharmaceutically acceptable carrier. A preferred prophylactic dose for a human subject is in the range of about 80 mg to about 800 mg of the EFdA diester administered parenterally at about six-month intervals in a dose volume of about 0.5 to about 4 milliliters per dose. A preferred treatment dose for a human patient is in the range of about 80 mg to about 800 mg of the EFdA diester administered parenterally at about three-month intervals in a dose volume of about 0.5 to about 4 milliliters per dose

Parenteral formulations containing and antiviral compound of the present invention can be dry formulations comprising the antiviral compound together with pharmaceutically acceptable excipients or stable suspensions of the antiviral compound in an aqueous or oil-based medium.

BRIEF DESCRIPTION OF DRAWINGS

In the drawings,

Figure 1 shows an X-ray Powder Diffractogram (XPRD) of EFdA.

Figure 2 shows data provided by Differential Scanning Calorimetry (DSC) and Thermo-Gravimetric Analysis (TGA) of EFdA.

Figure 3 shows DSC and TGA data for Compound **2**.

Figure 4 shows XPRD data for Compound **3**.

Figure 5 shows DSC data for Compound **3**.

Figure 6 shows TGA data for Compound **3**.

Figure 7 shows XPRD data for Compound **5**.

Figure 8 shows DSC data for Compound **5**.

Figure 9 shows TGA data for Compound **5**.

Figure 10 shows in graphic form the data provided in Table 2.

Figure 11 shows in graphic form the data provided in Table 3.

Figure 12 shows in graphic form the data in Table 4.

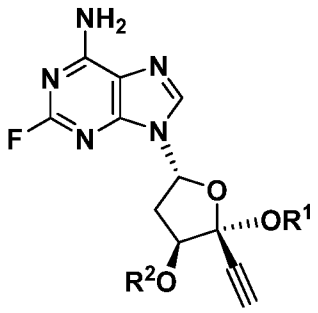
Figure 13 shows in graphic form data from Example 10.

Figure 14 shows XPRD data for Compound **2**.

Figure 15 shows XPRD data for Compound **6**.

DESCRIPTION OF PREFERRED EMBODIMENTS

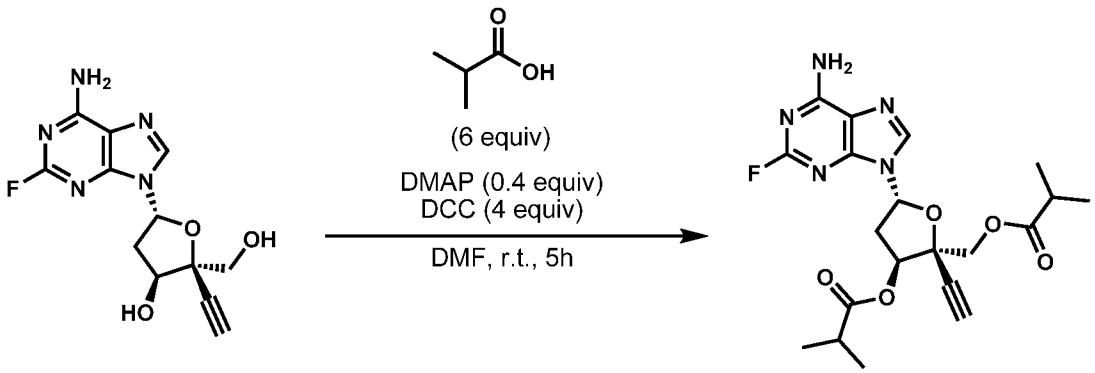
Extended *in vivo* viral suppression is achieved by diesters represented by Formula (II):



(II)

wherein R^1 and R^2 independently are -C(=O)R^3 and R^3 is a member of the group consisting of isopropyl, 3-pentyl, cyclopentyl and phenylmethyl. The foregoing diesters are prepared by reacting EFdA with the desired acid or acid anhydride and recovering the diester as a crystalline compound. The Examples below illustrate preparation of the preferred diesters.

Preparation of (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((isobutyryloxy)methyl) tetrahydrofuran-3-yl isobutyrate



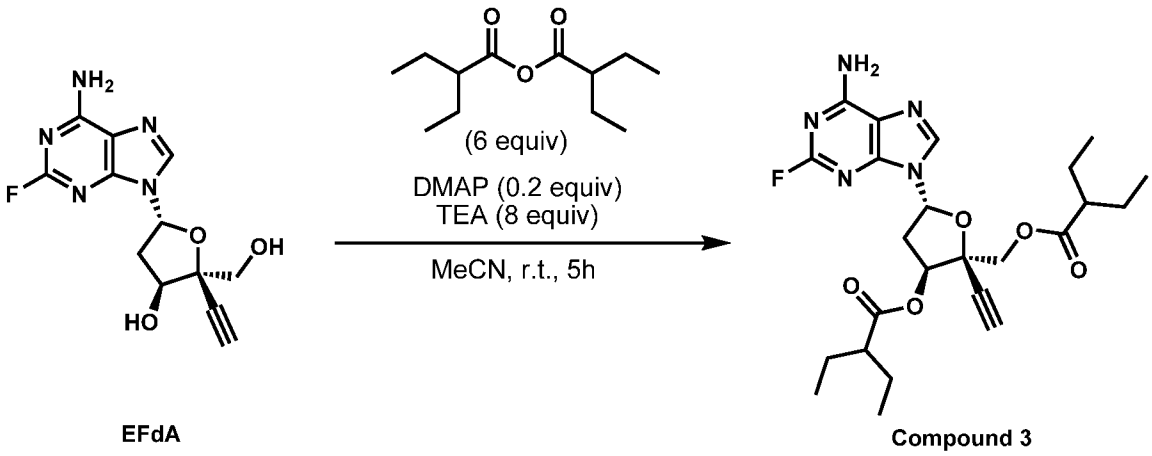
EFdA Compound 2

To a mixture of EFdA (Compund **1**) (3 g, 6.8 mmol, 1 equiv.), 4-dimethylaminopyridine (DMAP) (499 mg, 2.73 mmol, 0.4 equiv.) in anhydrous dimethyl formamide (DMF) (100 mL) isobutyric acid (8.4 g, 27.3 mmol, 6 equiv.) was added dropwise at ambient temperature. The reaction stirred for 5 h at room temperature. The reaction was monitored by LC-MS due to the occasional alkylation of NH_2 group observed for elongated reaction time. The reaction mixture was then filtered to remove byproduct urea. Acetonitrile was used to rinse the reaction mixture. Thereafter, the reaction mixture was washed twice with water and once with brine and then the solvent was dried, filtered and evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 60-70% ethyl acetate (EtOAc) in hexanes to obtain Compound **2** as a glassy solid. The obtained glassy solid was dispersed in minimum amount of isopropanol followed by its rotatory evaporation to obtain pure Compound **2** as a white solid (2.5 g, 85% yield). LC-MS (ESI+): m/z 434.49 $[M+H]^+$.

About 250 mg/g of Compound **2** was suspended in aqueous 0.25% CMC-Na/0.5% TWEEN-80 (26 gauge syringeability) and was subjected to stability for 2 weeks at 40°C/75% relative humidity .Figure 14 provides XPRD data for Compound **6** from the incubated suspension, prior to incubation (bottom plot), after one week (middle plot), and

after two weeks (top plot), showing that the compound retains good crystallinity in suspension.

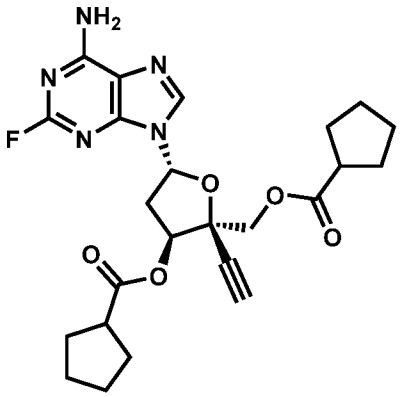
Preparation of (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((2-ethylbutanoyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-yl 2-ethylbutanoate



EFdA Compound 3

To a mixture of EFdA (1 g, 3.4 mmol, 1 equiv.), 2-ethylbutanoic anhydride (4.4 g, 20.4 mmol, 6 equiv.), triethanolamine (TEA) (3.8 mL, 27.2 mmol, 8 equiv.) in anhydrous acetonitrile (MeCN) (43 mL) and cooled to 0 °C was added 4-dimethylaminopyridine (DMAP) (83 mg, 0.68 mmol, 0.2 equiv.) at 0 °C. The resulting admixture was stirred for 0.5 h at 0 °C and then for 5 h at room temperature. The reaction was monitored by LC-MS due to the occasional alkylation of NH_2 group observed for elongated reaction time. The reaction mixture was quenched with methanol, and solvent was evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 60-70% ethyl acetate (EtOAc) in hexanes to obtain Compound **3** as a glassy solid. The obtained glassy solid was dispersed in minimum amount of isopropanol followed by its rotatory evaporation to obtain pure Compound **3** as a white solid (1.33 g, 80% yield). LC-MS (ESI+): m/z 490.56 $[M+H]^+$.

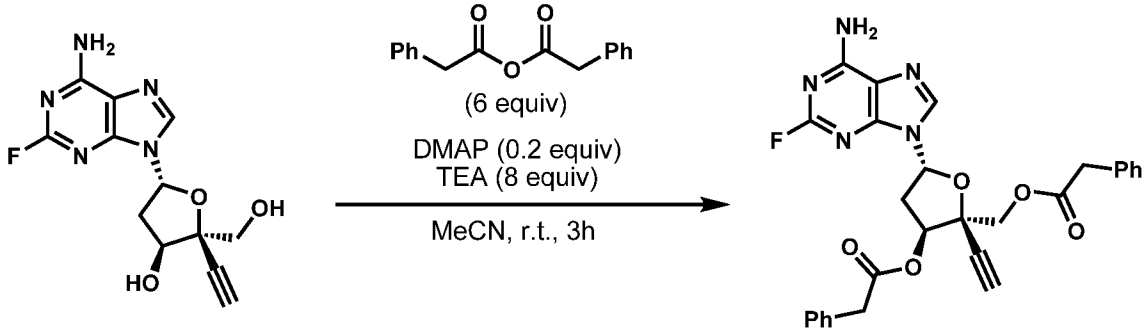
Preparation of (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((cyclopentanecarbonyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-yl cyclopentanecarboxylate



Compound 4

Compound **4** was prepared by using the procedure followed for the Compound **2** but using cyclopentanoic acid instead of isoutyric acid. LC-MS (ESI+): m/z 486.44 $[M+H]^+$.

Preparation of (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((2-phenylacetoxy)methyl) tetrahydrofuran-3-yl 2-phenylacetate



EFdA **Compound 5**

To a mixture of EFdA (499 mg, 1.7 mmol, 1 equiv.), 2-phenylacetic anhydride (2.6 g, 10.2 mmol, 6 equiv.), triethanolamine (TEA) (1.9 mL, 13.6 mmol, 8 equiv.) in

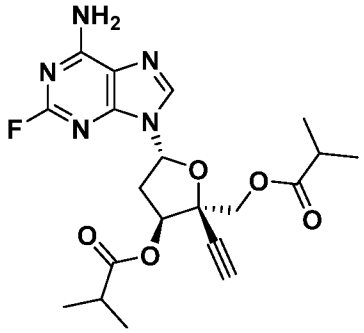
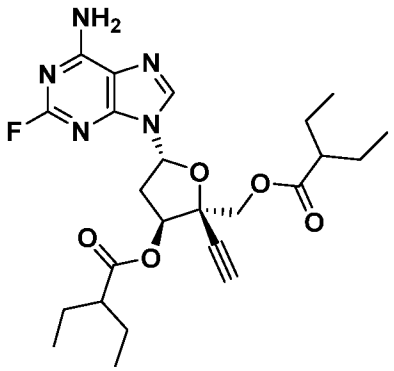
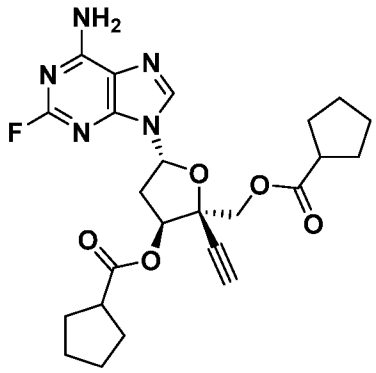
anhydrous acetonitrile (MeCN) (22 mL) and cooled to 0 °C was added 4-dimethylaminopyridine (DMAP) (42 mg, 0.34 mmol, 0.2 equiv.) at 0 °C. The reaction stirred for 0.5 h at 0 °C and then to 3 h at room temperature. The reaction is monitored by LC-MS due to the occasional alkylation of NH_2 group observed for elongated reaction time. The reaction mixture was quenched with methanol and solvent was evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 60-70% ethyl acetate (EtOAc) in hexanes to obtain Compound **5** as a glassy solid. The obtained glassy solid was dispersed in minimum amount of isopropanol followed by its rotatory evaporation to obtain pure Compound **5** as a white solid (694 mg, 77% yield). LC-MS (ESI+): m/z 530.52 $[M+H]^+$.

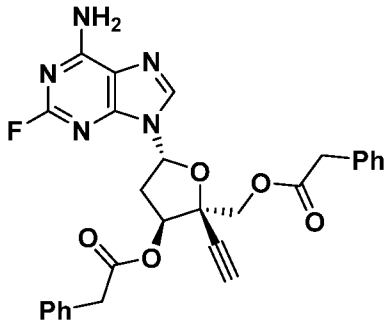
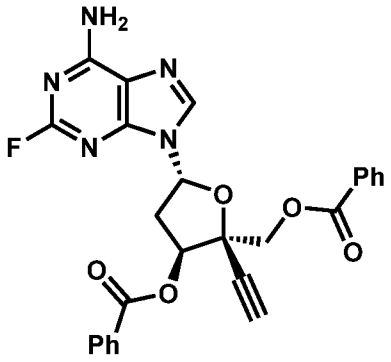
Table 1 presents compound characterization data obtained by nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS).

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((benzoyloxy)methyl)-2-ethynyl tetrahydrofuran-3-yl benzoate, Compound 6

Compound **6** was prepared by using the procedure similar to that used for Compound **2** using 4.5 equiv of corresponding acid. LC-MS (ESI+): *m/z* 502.41 $[M+H]^+$. About 275 mg/g of Compound **6** was suspended in aqueous 0.25% CMC-Na/0.5% TWEEN-80 (26 gauge syringeability) and was subjected to stability for 2 weeks at 40°C/75% relative humidity. Figure 15 provides XPRD data for Compound **6** from the incubated suspension, prior to incubation (bottom plot), after one week (middle plot), and after two weeks (top plot), showing that the compound retains good crystallinity in suspension.

Table 1: Preferred Compounds

Cpd #	Structure	Characterization Data (NMR and LC-MS)
2		^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.96 (s, 1H), 7.87 (s, 1H), 6.35 (t, J = 6.6 Hz, 1H), 5.68 (t, J = 5.6 Hz, 1H), 4.40 (dd, J = 11.9, 1.5 Hz, 1H), 4.21 (d, J = 10.4 Hz, 1H), 3.81 (s, 1H), 3.18 (dt, J = 13.6, 6.8 Hz, 1H), 2.69 – 2.57 (m, 2H), 1.2 – 1.1 (m, 6H), 1.08 – 0.98 (m, 6H). MS-ESI: m/z 434.49 observed $(M+H)^+$ Anal calcd for $C_{20}H_{24}FN_5O_5$: C, 55.42; H, 5.58; N, 16.16. Found: C, 55.48; H, 5.73; N, 15.94 Aqueous solubility (pH 7.4): 0.028 mg/mL
3		^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.91 (d, J = 39.8 Hz, 2H), 6.35 (t, J = 6.8 Hz, 1H), 5.73 (t, J = 6.5 Hz, 1H), 4.38 (dd, J = 11.8, 2.5 Hz, 1H), 4.24 (dd, J = 11.7, 2.5 Hz, 1H), 3.81 (s, 1H), 3.20 (dt, J = 13.5, 6.8 Hz, 1H), 2.61 (dt, J = 13.2, 6.6 Hz, 1H), 2.29 (ddd, J = 8.3, 5.6, 2.6 Hz, 1H), 2.13 (tt, J = 8.9, 5.9 Hz, 1H), 1.72 – 1.31 (m, 8H), 0.88 (td, J = 7.5, 2.3 Hz, 6H), 0.80 – 0.66 (m, 6H). MS-ESI: m/z 490.56 observed $(M+H)^+$ Aqueous solubility (pH 7.4): <0.002 mg/mL
4		^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.92 (d, J = 36.1 Hz, 2H), 6.34 (t, J = 6.8 Hz, 1H), 5.68 (t, J = 6.2 Hz, 1H), 4.39 (dt, J = 11.6, 1.8 Hz, 1H), 4.21 (dd, J = 11.6, 2.4 Hz, 1H), 3.81 (s, 1H), 3.18 (dt, J = 13.9, 6.9 Hz, 1H), 2.90 – 2.78 (m, 1H), 2.74 – 2.57 (m, 2H), 1.93 – 1.44 (m, 16H). MS-ESI: m/z 486.44 observed $(M+H)^+$ Aqueous solubility (pH 7.4): <0.001 mg/mL

Cpd #	Structure	Characterization Data (NMR and LC-MS)
5		^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.93 (d, J = 37.3 Hz, 2H), 7.39 – 7.12 (m, 10H), 6.35 (t, J = 6.7 Hz, 1H), 5.71 (t, J = 6.0 Hz, 1H), 4.41 (d, J = 11.7 Hz, 1H), 4.25 (d, J = 11.3 Hz, 1H), 3.88 – 3.71 (m, 3H), 3.63 (q, J = 15.8 Hz, 2H), 3.11 (dt, J = 14.0, 7.0 Hz, 1H), 2.63 (dt, J = 12.5, 6.4 Hz, 1H). MS-ESI: m/z 530.52 observed $(M+H)^+$ Aqueous solubility (pH 7.4): <0.002 mg/mL
6		^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 8.09 (dt, J = 8.6, 1.4 Hz, 2H), 8.02 – 7.83 (m, 4H), 7.76 – 7.68 (m, 1H), 7.66 (td, J = 7.4, 1.5 Hz, 1H), 7.59 (td, J = 7.6, 7.1, 1.7 Hz, 2H), 7.53 – 7.44 (m, 2H), 6.54 (t, J = 6.8 Hz, 1H), 6.16 – 6.08 (m, 1H), 4.72 (d, J = 11.6 Hz, 1H), 4.59 (d, J = 11.5 Hz, 1H), 3.84 (s, 1H), 3.41 – 3.32 (m, 1H), 2.85 (dt, J = 13.4, 6.3 Hz, 1H). MS-ESI: m/z 502.41 observed $(M+H)^+$ Aqueous solubility (pH 7.4): <0.002 mg/mL

The foregoing discussion and the Examples are illustrative, and are not to be taken as limiting. Still other variants within the spirit landscape of this invention are possible and will readily present themselves to those skilled in the art.

Synthesis of Stable Crystalline Forms

EXAMPLE 1

Compound **2** (~200 mg) was completely dissolved in minimum quantity of acetone with stirring. This was followed by slow evaporation of the solvents at ambient temperature. This resulted in a recrystallized sample as a white powder. Other solvents including ethyl acetate, methanol and tetrahydrofuran can also be used.

General Examples for the formulation of Compounds of the Invention

All formulation protocols generated a stable aqueous suspension with 26-gauge syringeability.

EFdA Formulation.

EFdA was ground and sieved through a #80 sieve. A solution of preformed 0.25% sodium carboxymethyl cellulose (Sodium CMC) & 0.1% polyoxyethlene (20) sorbitan monooleate (TWEEN-80) was added to about 300 mg of EFdA (Compound **1**) to provide a suspension of about 1 gram (300 mg of 1 + ~700 mg of polymer solution) of the final formulation (about 300 mg/g). The suspension was then bath sonicated for 10 min in an ice bath. The density of formulation of was 1.064 g/mL and provided a 319.2 mg/mL concentration of EFdA.

EXAMPLE 2

Recrystallized Compound **2** was ground and sieved through No. 80 sieve U.S. Standard Sieve Series, for Wire Cloth Screens (nominal sieve opening 0.180 mm). 250 mg of Compound **2** was taken in suitable container and a solution of preformed 0.25% Sodium Carboxymethylcellulose (CMC) and 0.1% TWEEN-80 was added to obtain 1 gram (250 mg of Compound **2** + ~750 mg of polymer solution) of the final formulation (~250 mg/g). The suspension was then probe sonicated for 5 min in an ice bath (Sonication time: 5 min; Pulse Amplitude: 20; Pulse on Time: 30 sec; Pulse off time: 20 sec).

EXAMPLE 3

Compound **3** was ground and sieved through No. 80 sieves U.S. Standard Sieve Series for Wire Cloth Screens (nominal sieve opening 0.180 mm). 250 mg of Compound **3** was taken in suitable container and a solution of preformed 0.25% Sodium CMC and 0.5% TWEEN-80 was added to obtain 1 gram (250 mg of Compound **3** + ~750 mg of polymer solution) of the final suspension (~250 mg/g). The suspension was then probe sonicated for

5 min in an ice bath (Sonication time: 5 min; Pulse Amplitude: 20; Pulse on Time: 30 sec; Pulse off time: 20 sec). The density of the suspension was of is 1.004 g/mL. The above Suspension contained 250.88 mg/mL of Compound **3** (~150 mg/mL of EFdA).

EXAMPLE 4

Compound **5** was ground and sieved through No. 80 sieve U.S. Standard Sieve Series for Wire Cloth Screens (nominal sieve opening 0.180 mm). 200 mg of Compound **5** was placed in a suitable container and a solution of preformed 0.25% Sodium CMC and 0.5% TWEEN-80 was added to obtain 1 gram (200 mg of Compound **5** + ~800 mg of polymer solution) of the final suspension (~200 mg/g). The suspension was then probe sonicated for 5 min in an ice bath (Sonication time: 5 min; Pulse Amplitude: 20; Pulse on Time: 30 sec; Pulse off time: 20 sec). The density of the suspension was 1.047 g/mL. The above suspension contained 209.4 mg/mL of Compound **5** (~115.97 mg/mL of EFdA).

Pharmacokinetic (PK) Studies

Animals: Animals (Male SD rats ~200-250 g and Male rhesus macaques ~2-3 kg) were obtained from an approved vendor (SLAC Laboratory Animal Co. Ltd., Shanghai, China and/or Topgene Biotechnology, Wuhan city, Hubei Province, China).

Acclimation/Quarantine: Following arrival, animals were assessed as to their general health by a member of the veterinary staff or other authorized personnel. Animals were acclimated for at least 3 days before being placed on study.

Animal Husbandry: Animals were group housed during acclimation and individually housed during the study. The animal room environment will be controlled (target conditions: temperature 18 to 26 °C, relative humidity 30 to 70%, 12 hours artificial light and 12 hours dark). Temperature and relative humidity were monitored daily.

Animal Cannulation: No

Animals were fasted at least 12 hours prior to the administration. All animals had access to Certified Rodent and non-Rodent Diet (Catalog # M01-F, SLAC Laboratory Animal Cl. Ltd., Shanghai, China) *ad libitum* 4 hours post dosing.

Water was autoclaved before provided to the animals *ad libitum*. Periodic analyses of the water was performed and the results archived. There were no known contaminants in the diet or water that, at the levels of detection, were expected to interfere with the purpose, conduct or outcome of the study.

1. Dose Formulation

SC Formulation: Suspensions were prepared on the day of dosing according to the procedure described in Examples 2-5 above, and Tables 2-4. Animals were dosed within four hours of suspension preparation. Two 20 µL aliquots of each prepared suspension were transferred into 1.5 mL of polypropylene microcentrifuge tubes and dose validation was run by LC/UV or LC-MS/MS.

2. Dose Administration

The suspensions were administered via subcutaneous injection (SC) following facility standard operations procedures (SOPs).

3. Sample Collection

Approximately 200 µL blood was collected from saphenous vein at each time point for rats and 0.5 mL for rhesus macaques. All blood samples were transferred into microcentrifuge tubes containing 4 µL of K_2EDTA (0.5M) as anti-coagulant and placed on wet ice until processed for plasma.

4. Blood/Plasma processing

Blood: Blood samples were processed for plasma by centrifugation at approximately 4 °C, 3000 g 15 min within half an hour of collection. Plasma samples was stored in polypropylene tubes, quick frozen over dry ice and kept at −70±10 °C until LC/MS/MS analysis.

5. Sample Analysis

Dose concentration verification

- Aliquots of the prepared suspension were collected in the middle position of each suspension in duplicate.
- The concentration of the active ingredient in each aliquot was determined by the LC/UV or LC/MS/MS method

Bioanalytical method and sample analysis

- LC-MS/MS methods for the quantitative determination of active ingredient (test compound) in corresponding biological matrix was developed under non-GLP compliance.
- A calibration curve with 8 non-zero calibration standards was applied for the method including lower limit of quantitation (LLOQ).
- A set of quality control (QC) samples consisting of low, middle, and high concentration was applied for the method.
- The study sample analysis will be performed concurrently with a set of calibration standards and two sets of QC samples using the LC-MS/MS method (If sample numbers were more than 48, then two calibration curves with 2 sets of QC samples were applied).
- Acceptance criteria:

  Linearity: a minimum of 6 calibration standards was back calculated to within ±20% of their nominal values in plasma

  Accuracy: A minimum of 4 out of 6 QC samples was back calculated to within ±20% of their nominal values in plasma.

  Specificity: The mean calculated concentration in the single blank matrix should be 0.5 times the LLOQ.

  Sensitivity: the LLOQ will target 1~3 ng/mL.

  Carryover: the mean calculated carry-over concentration in the single blank matrix immediately after the highest standard injection should be LLOQ. If the carryover couldn't meet the criteria, then the percent of carryover should be estimated following in-house bioanalytical SOP.

6. Data Analysis

Plasma concentration versus time data was analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software program. C_{max}, T_{max}, $T_{½}$, $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$, $MRT_{(0\text{-}t)}$, $MRT_{(0\text{-}inf)}$, %F and graphs of plasma concentration versus time profile were generated.

EXAMPLE 5:

Several prodrugs including EFdA (1) as a control were subjected to a single dose rat PK studies via subcutaneous route of administration. All animals were injected with equivalent doses of 10 mg/kg and concentration of 4 mg/mL of EFdA as an aqueous suspension in 0.5% CMC-Na and 0.5% TWEEN-80. While similar exposure was observed, all compounds **2**, **3** and **5** exhibited plasma levels of EFdA above lower limit of quantitation (LLOQ) for more than a week with Cmax much lower than for EFdA.

Table 2 shows the rat PK data for Compounds **1**, **2**, **3** and **5** following subcutaneous (SC) administration at 10 mg/kg equivalent dose of EFdA. The data in graphic form are shown in Figure 10.

Table 2: SC Rat PK: EFdA Levels for EFdA, Compounds 1, 2, 3 and 5

	SC Comp 1 (EFdA)	**SC Comp 2**	**SC Comp 3**	**SC Comp 5**
Dose of EFdA (mg/kg)	10	10	10	10
Concentration of EFdA (mg/kg)	4	4	4	4
Excipients	0.5% CMC-Na / 0.5% TWEEN-80			
$T_{1/2}$ (h)	1.3 ± 0.2	17 ± 4	NA	12 ± 4
$MRT_{0\text{-last}}$ (h)	1.6 ± 0.1	12 ± 1.2	20 ± 4	13 ± 3
T_{max} (h)	0.5	2 ± 1.1	4 ± 2.3	7 ± 0
C_{max} (nM)	11,548 ± 3,173	1,795 ± 337	287 ± 177	968 ± 374
$AUC_{0\text{-last}}$ (nM*h)	22,528 ± 1,680	19,771 ± 1,219	6,475 ± 2,909	18,516 ± 4,409
$AUC_{0\text{-inf}}$ (nM*h)	23,235 ± 2,000	22,745 ± 748	NA	19,993 ± 3,525

EXAMPLE 6:

After optimization, SC rat PK studies were performed again with high equivalent dose of 100 mg/kg at equivalent concentration of 120 mg/mL for Compound **3**, 116 mg/mL for Compound **5**, and 319 mg/mL of EFdA, respectively. Compound **3** provided a delayed and 100-fold lower C_{max} than EFdA. Enhanced half life and mean residence life were also observed, making Compound **3** and Compound **5** suitable for prophylaxis.

Table 3A and Table 3B show the rat PK data for Compounds **1**, **3** and **5** following SC administration. The data for Compound **3** are shown in graphic form in Figure 11.

Table 3A: SC Rat PK: EFdA Levels for EFdA and Compound 3

	SC Comp 3	**SC Comp 3**	**SC Comp 1 (EFdA)**	**SC Comp 1 (EFdA)**
Dose of EFdA (mg/kg)	10	100	100	10
Excipients	0.5% CMC-Na / 0.5% TWEEN-80	0.25% CMC-Na / 0.1% TWEEN-80	0.25% CMC-Na / 0.1% TWEEN-80	0.5% CMC-Na / 0.5% TWEEN-80
Conc. of EFdA (mg/mL)	4	120	319	4
$T_{1/2}$ (h)	NA	474 ± 168	99 ± 13	1.3 ± 0.2
$MRT_{0\text{-last}}$ (h)	20 ± 4	456 ± 17	67 ± 24	1.6 ± 0.1
T_{max} (h)	4 ± 2.3	312 ± 0	1 ± 0	0.5
C_{max} (nM)	287 ± 177	66 ± 21	7,429 ± 1,584	11,548 ± 3,173
$AUC_{0\text{-last}}$ (nM*h)	6,475 ± 2909	38,306 ± 101	158,500 ± 17	22,528 ± 1,680
$AUC_{0\text{-inf}}$ (nM*h)	NA	52,531 ± 39	159,373 ± 16	23,235 ± 2,000

Table 3B: SC Rat PK: EFdA Levels for EFdA and Compound 5

	SC Comp 5	**SC Comp 1 (EFdA)**
Dose of EFdA (mg/kg)	100	100
Excipients	0.25% CMC-Na / 0.1% TWEEN-80	
Conc. of EFdA (mg/mL)	116	319
$T_{1/2}$ (h)	363 ± 411	99 ± 13
$MRT_{0\text{-last}}$ (h)	225 ± 64	67 ± 24
C_{max} (nM)	244 ± 54	7,429 ± 1,584
$AUC_{0\text{-last}}$ (nM*h)	68,848 ± 16,728	158,500 ± 17
$AUC_{0\text{-inf}}$ (nM*h)	129,209 ± 117,423	159,373 ± 16

EXAMPLE 7:

After rat PK analysis, the focus was shifted to non-rodents, i.e., rhesus macaque. Compound **5** and EFdA were subjected to a single dose rhesus macaque PK studies via subcutaneous route of administration with equivalent doses of 50 mg/kg of EFdA. The aqueous suspensions contained 0.25% CMC-Na and 0.1%/0.5% TWEEN-80 with equivalent concentration of 116 mg/mL and 319 mg/mL of EFdA for Compound **5** and EFdA respectively. With Compound **5** plasma levels of EFdA above LLOQ for more than a month with 24-fold lower C_{max} than EFdA itself were observed.

Table 4 shows the Rhesus PK data for Compounds **1** and **5** following SC administration at 50 mg/kg equivalent dose of EFdA. The data are shown in graphic form in Figure 12.

Table 4: SC Rhesus PK: EFdA Plasma Levels after Dosing EFdA or Compound 5 in Plasma

	SC EFdA EFdA (Plasma)	**SC Compound 5 EFdA (Plasma)**
Dose of EFdA (mg/kg)	50	50
Excipients	0.25% CMC-Na 0.1%TWEEN-80	0.25% CMC-Na 0.5%TWEEN-80
Conc. of EFdA (mg/mL)	319	116
$T_{1/2}$ (h)	165 ± 56	1,063 ± 608
$MRT_{0\text{-last}}$ (h)	79 ± 37	1,449 ± 517
T_{max} (h)	4 ± 3.6	264 ± 125
C_{max} (nM)	3,060 ± 1,058	128 ± 52
C_{42days} (nM)	0.6 ± 0.3	60 ± 16
$AUC_{0\text{-last}}$ (nM*h)	135,691 ± 22,574	143,330 ± 33,727
$AUC_{0\text{-inf}}$ (nM*h)	135,748 ± 22,594	145,643 ± 32,180

Suspending medium for the present compounds can be an aqueous vehicle such as water for injection (WFI) or a vegetable oil vehicle such as sesame oil, olive oil, and the like. The suspending medium can also contain pharmaceutically acceptable excipients such as non-ionic surfactant, suspending or flocculating agent, preservatives, buffers, toxicity adjusters, chelating agents, antioxidants, and the like.

For prophylaxis, a preferred prophylactic dose for a human subject is in the range of about 80 mg to about 800 mg of the antiviral compound administered parenterally at about six-month (semi-annual) intervals in a dose volume of about 0.5 to about 4 milliliters (mL) per dose.

For treatment of a human patient, the effective amount of the antiviral compound of this invention preferably ranges from about 80 mg to about 800 mg at a dose volume of

about 0.5 to about 4 mL per dose, more preferably an effective amount in the range of about 200 to about 400 mg, for a three-month dosage regimen. The dosage regimen can vary, however, depending on the time interval between administered doses in a particular dosing regimen.

The term "effective amount" as used herein and in the claims means an amount of the antiviral compound sufficient to inhibit HIV reverse transcriptase, inhibit HIV replication, exert a prophylactic effect and/or exert a therapeutic effect after administration.

The term "administration" and variants thereof, for example "administering a compound", with reference to the claimed method of treatment means providing the antiviral compound to the patient and includes self-administration as well as administration to the patient by another person.

Preferably, the parenteral suspensions suitable for injection contain the present antiviral compound in an amount in the range of about 3 to 45 percent by weight, based on the weight of the suspension. Preferred particle size is no greater than about 50 micrometers (μm), more preferably an average particle size in the range of about 6 μm to about 15 μm.

Preferred flocculating or suspending agents are linear polymers, particularly the substituted celluloses such as methyl cellulose, carboxymethyl cellulose (CMC), hyroxypropyl cellulose, hydroxypropylmethyl cellulose, and the like.

Preferred surfactants are non-ionic surfactants. Particularly preferred surfactant is polyoxyethlene (20) sorbitan monooleate (TWEEN-80).

In addition to parenteral dosage forms, the present compounds can also be administered in oral dosage forms and as implants.

Dosage forms containing the present compounds can also include additional anti-HIV and/or anti-HBV agents such as cabotegravir, dolutegravir, doravirine, evilegravir, lesiverine, tenofovir disoproxil fumarate, tenofovir alafenamide fumarate, lamivudine, and the like.

The invention further provides, in various embodiments, a method of prophylaxis of viremia or treatment of a viral infection in a patient wherein inhibition of a reverse transcriptase is medically indicated, comprising administering to the patient an effective

amount or concentration of a compound of Formula (II). More specifically, the compound of Formula (II) can be administered in a formulation that provides for slow or controlled or sustained release of EFdA from these prodrugs. More specifically, the compound of Formula (II) can be formulated as aqueous suspension, solutions, and can be encapsulated in particles for slow-release including PLGA and other such materials known in the art. More specifically, the viral infection can be caused by HIV or HBV. The routes of administration for these prodrugs can include, but not limited to, oral, parenteral and release from implants (drug delivery composition and device). In the method for the treatment or prevention of the viral infection, the method may further comprise an additional anti-HIV and/or anti-HBV agent including but not limited to, cabotegravir, dolutegravir, doravirine, elvitegravir, lersiverine, tenofovir disoproxil fumarate, tenofovir alafenamide fumarate, lamivudine, and the like.

EXAMPLE 8

The concentration of surfactant was optimized with 0.3% and 0.5% of methyl cellulose in a formation containing 400 mg/gm of micronized Compound **5**. Formulations were prepared with different concentrations of TWEEN-80 (0.1, 0.2 and 0.3%). No significant effect of surfactant concentration was observed in viscosity, flow and redispersion time in the formulations after 10 days of storage. The observations are reported in Table 5.

Table 5: Optimization of Surfactant Concentration with 400 mg/gm of Compound 5

Composition	Surfactant Conc. TWEEN-80	Syringeability (26G)	Viscosity	Flow	Time Taken to Resuspend
0.3 % Methyl Cellulose	0.1 %	✓	++++	++++	2.2 Min.
	0.2 %	✓	++++	++++	2.5 Min.
	0.3 %	✓	++++	++++	2.3 Min
0.5 % Methyl Cellulose	0.1 %	✓	++++	++++	2.5 Min.
	0.2 %	✓	++++	++++	2.5 Min.
	0.3 %	✓	++++	++++	3.0 Min.

Viscosity: ++++ Slightly Viscous, +++ Viscous, ++ Very Viscous, + Semisolid
Flow: + No Flow, ++ Less Flow, +++ Good Flow, ++++ Very Good Flow ✓: Syringeable

EXAMPLE 9

A bulk batch (40 gm) of Compound **5** suspension was prepared with 0.3% methyl cellulose and 0.2% TWEEN-80 concentration. Although the polymer and surfactant concentrations were optimized with 40 weight % of drug concentration, a bulk batch was also prepared with 35 weight % of drug concentration during accelerated and long term stability studies. The composition of formulation loaded in stability chamber is given in Table 6. A detailed manufacturing procedure of formulation preparation and composition is given below.

Table 6: Formulation Composition

Ingredient	Quantity (mg/g)	Quantity, g /40 g
Compound **5**	350	14
Methyl Cellulose	3	0.120
TWEEN-80	2	0.08
Water for Injection (WFI)	645	25.8

Methyl cellulose was slowly added to the requisite quantity of water for injection (WFI) in a glass bottle with continuous stirring. The resulting mixture was stirred until the solution was clear and free from any lumps using a magnetic stirrer. TWEEN-80 (0.08 g) was added to the obtained solution and stirred well.

Micronized Compound **5** (14 g; average particle size 11 μm) was slowly added to the prepared polymer, surfactant solution under vigorous stirring at 1400 rpm using a magnetic stirrer. After complete addition of Compound **5**, the obtained suspension was stirred (600 rpm) for 20 minutes to uniformly disperse the particles.

The prepared formulation was characterized for various physicochemical properties such as assay, pH, % purity, redispersability, injectability, polymorphic form and particle size. The results are presented in Table 7.

Table 7: Physicochemical Characterization of Formulation on Preparation

Test Parameter	
Appearance	White dispersion
pH	5.78
Assay by HPLC (% on label claim)	108.8 %
% Purity	99.34 %
Redispersability	Yes
Injectability	Yes
Polymorphic Form	Same as API
Average Particle Size	14 μm

The formulation shown in Table 6 was deemed acceptable based on the characteristics shown in Table 7.

EXAMPLE 10

Aqueous suspensions of EFdA and of Compounds **3** and **5** were administered subcutaneously to Rhesus Macaque, peripheral blood mononuclear cells (PBMC) periodically retrieved, and pharmaconkinetic data evaluated. The observed results are presented in Tables 8, 9, and 10, for both compounds, and in Figure 13 for Compound **5**.

Table 8: SC Rhesus PK: EFdA-TP PBMC Levels After Dosing Compound 5 or EFdA

	SC Compound 5: EFdA-TP (PBMC)	**SC EFdA: EFdA-TP (PBMC)**
EFdA Nominal Dose (mg/kg)	50	50
Formulation	0.25% CMC-Na / 0.5% TWEEN-80	0.25% CMC-Na / 0.1% TWEEN-80
Conc. of EFdA (mg/mL)	116	319
Dose volume (mL/kg)	0.43 (tot. vol. 1.39 mL)	0.16 (tot. vol. 0.47 mL)
$T_{1/2}$ (d)	31 ± 17	12 ± 5
MRT $_{0\text{-last}}$ (d)	72 ± 19	6 ± 1
MRT $_{0\text{-inf}}$ (d)	73 ± 21	6 ± 1
T_{max} (d)	21 ± 13	2 ± 0
C_{max} (µM)	192 ± 96	789 ± 154
C_{last} (µM)	0.71	0.03
$AUC_{0\text{-last}}$ (µM*h)	233,2743 ± 37,553	127,176 ± 29, 205
$AUC_{0\text{-inf}}$ (µM*h)	234,062 ± 37,077	127,201 ± 29, 225

Table 9: SC Rhesus PK: EFdA Plasma and EFdA-TP PBMC Levels After Dosing Compound 3

	SC Compound 3: EFdA (Plasma)	SC Compound 3: EFdA-TP (PBMC)
EFdA Nominal Dose (mg/kg)	50	
Formulation	0.25% CMC-Na / 0.5%TWEEN80	
Conc. of EFdA (mg/mL)	150	
Dose volume (mL/kg)	0.335 (tot. vol. 1.57 mL)	
$T_{1/2}$ (h)	566 ± 344	1,022 ± 463
$MRT_{0\text{-last}}$ (h)	1,071 ± 488	394 ± 36
T_{max} (h)	440 ± 270	784 ± 485
C_{max} (nM)	40 ± 28	627 ± 60
$AUC_{0\text{-last}}$ (nM*h)	35,385 ± 18,522	807,473 ± 519,381
$AUC_{0\text{-inf}}$ (nM*h)	45,293 ± 14,840	1,447,998 ± 576,163

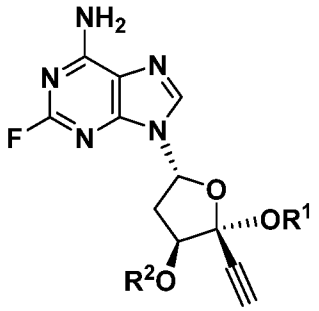
5

Table 10: SC Rhesus PK: EFdA Levels for Compounds 3 and 5 in Plasma

	SC EFdA EFdA (Plasma)	SC Compound 5: EFdA (Plasma)	SC Compound 3: EFdA (Plasma)
EFdA Nominal Dose (mg/kg)	50	50	50
Formulation	0.25% CMC-Na / 0.1%Tween 80	0.25% CMC-Na / 0.5%Tween 80	0.25% CMC-Na / 0.5%Tween 80
Conc. of EFdA (mg/mL)	319	116	150
$T_{1/2}$ (h)	165 ± 56	1,063 ± 608	566 ± 344
$MRT_{0\text{-last}}$ (h)	79 ± 37	1,449 ± 517	1,071 ± 488
T_{max} (h)	4 ± 3.6	264 ± 125	440 ± 270
C_{max} (nM)	3060 ± 1058	128 ± 52	40 ± 28
$AUC_{0\text{-last}}$ (nM*h)	135,691 ± 22,574	143,330 ± 33,727	35,385 ± 18,522
$AUC_{0\text{-inf}}$ (nM*h)	135,748 ± 22,594	145,643 ± 32,180	45,293 ± 14,840

WE CLAIM:

1. A compound of Formula (II):



(II)

wherein R^1 and R^2 independently are -C(=O)R^3 and R^3 is selected from the group consisting of isopropyl, 3-pentyl, cyclopentyl, phenyl, and phenylmethyl.

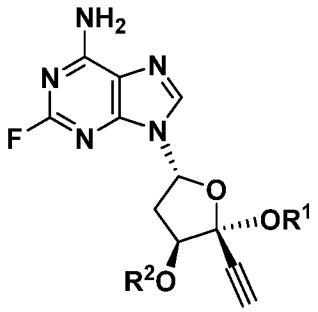
2. The compound of claim 1, which is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-(isobutyryloxy)methyl) tetrahydrofuran-3-yl isobutyrate.

3. The compound of claim 1, which is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((2-ethylbutanoyl)oxy)methyl-2-ethynyltetrahydrofuran-3-yl 2-ethylbutanoate.

4. The compound of claim 1, which is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((cyclopentanecarbonyl)oxy)methyl)-2-ethynyl-tetrahydrofuran-3-yl cyclopentanecarboxylate.

5. The compound of claim 1, which is crystaline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((2-phenlacetoxy)methyl)tetrahydrofuran-3-yl 2-phenylacetate.

6. An aqueous parenteral suspension comprising a compound of Formula (II):



(II)

wherein R^1 and R^2 independently are $-C(=O)R^3$ and R^3 is selected from the group consisting of isopropyl, 3-pentyl (1-ethoxypropyl, cyclopentyl, and phenylmethyl;

a non-ionic surfactant;

a suspending agent; and water.

7. The aqueous parenteral suspension of claim 6, wherein said compound is present in an amount in the range of about 3 to 4.5 percent by weight of the suspension, said non-ionic surfactant is present in an amount in the range of about 0.1 to about 0.5 percent by weight of the suspension, and said suspending agent is present in an amount in the range of about 0.1 to about 0.5 weight percent by weight of the suspension.

8. The aqueous parenteral suspension of claim 6, wherein the non-ionic surfactant is polyoxyethylene (20) sorbitan monooleate.

9. The aqueous parenteral suspension of claim 6, wherein the suspending agent is selected from the group consisting of methyl cellulose, carboxymethyl cellulose, hyroxypropyl cellulose, and hydroxypropylmethyl cellulose.

10. The aqueous parenteral suspension of claim 6, wherein the compound is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynl-2((2-phenylacetoxy)methyl)-tetrahydrofuran-3-yl. 2-phenylacetate.

11. The aqueous parenteral suspension of claim 6, wherein the compound is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((2-

ethylbutanoyl)oxy)methyl-2-ethynyltetrahydrofuran-3-yl 2-ethylbutanoate.

12. A method for *in vivo* suppression of human immunodeficiency virus in a patient, which comprises parenterally administering to the patient an effective amount of the compound of claim 1 in an aqueous suspension.

13. The method of claim 12, wherein the administering is a three-month dosing regimen.

14. The method of claim 12, wherein said effective amount is a dose in the range of about 80 to about 800 mg.

15. The method of claim 12, wherein said effective amount is a dose in the range of about 200 to about 400 mg.

16. The method of claim 12, wherein the compound is crystalline (2R, 3S, 5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((2-phenylacetoxy)methyl) tetrahydrofuran-3-yl 2-phenylacetate.

17. The method of claim 12, wherein the compound is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((2-ethylbutanoyl)oxy)methyl-2-ethynyltetrahydrofuran-3-yl 2-ethylbutanoate.

18. A method for prophylaxis of HIV infection in a human subject not infected with HIV which comprises parenterally administering to the human subject an effective amount of the compound of claim 1 in an aqueous suspension in a semi-annual dosing regimen.

19. The method of claim 18, wherein said compound is administered in a unit dose amount in the range of about 80 to about 800 mg.

20. The method of claim 18, wherein said compound is administered in a dose volume in the range of about 0.5 to about 4 mL per dose.

21. The aqueous parenteral suspension of claim 6 or claim 7, wherein the non-ionic surfactant is polyoxyethylene (20) sorbitan monooleate.

22. The aqueous parenteral suspension of any one of claims 6, 7 or 21, wherein the suspending agent is selected from the group consisting of methyl cellulose, carboxymethyl cellulose, hyroxypropyl cellulose, and hydroxypropylmethyl cellulose.

23. The aqueous parenteral suspension of any one of claims 6, 7, 21 or 22, wherein the compound is crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynl-2((2-phenylacetoxy)methyl)-tetrahydrofuran-3-yl. 2-phenylacetate or crystalline (2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((2-ethylbutanoyl)oxy)methyl-2-ethynyltetrahydrofuran-3-yl 2-ethylbutanoate.

24. A method for *in vivo* suppression of human immunodeficiency virus (HIV) in a patient, which comprises parenterally administering to the patient an effective amount of the compound of any one of claims 1 to 5 in an aqueous suspension.

25. The method of claim 24, wherein the administering is a three-month dosing regimen.

26. A method for prophylaxis of HIV infection in a human subject not infected with HIV which comprises parenterally administering to the human subject an effective amount of the compound of any one of claim 1 to 5 in an aqueous suspension in a semi-annual dosing regimen.

27. The method of any one of claims 24 to 26, wherein said effective amount is a dose in the range of about 80 to about 800 mg.

28. The method of claim any one of claims 24 to 27, wherein said compound is administered in a dose volume in the range of about 0.5 to about 4 mL per dose.

29. The method of any one of claims 24 to 28, wherein administration of the compound provides slow or controlled or sustained release of EFdA from the compound *in vivo*.

30. The method of any one of claims 24 to 29, wherein the route of administration for compound is selected from the group consisting of oral, parenteral, subcutaneous injections, intravenous, intramuscular, intrastemal injection, infusion, and release from an implant.

31. The method of any one of claims 24 to 30, wherein the compound is formulated as aqueous suspension, a solution, or encapsulated in particles for slow-release.

32. The method of any one of claims 24 to 31, wherein the viral infection is caused by HIV.

33. The method of any one of claims 24 to 31, wherein the viral infection is caused by HBV.

34. The method of any one of claims 24 to 33, further comprising administering an additional anti-HIV and/or anti-HBV agent.

35. The method of claim 34, wherein the additional agent is selected from the group consisting of cabotegravir, dolutegravir, doravirine, elvitegravir, lersiverine, tenofovir disoproxil fumarate, tenofovir alafenamide fumarate, and lamivudine.

36. Use of a compound of any one of claims 1 to 11 for treatment and prevention of a viral infection of HIV or HBV.

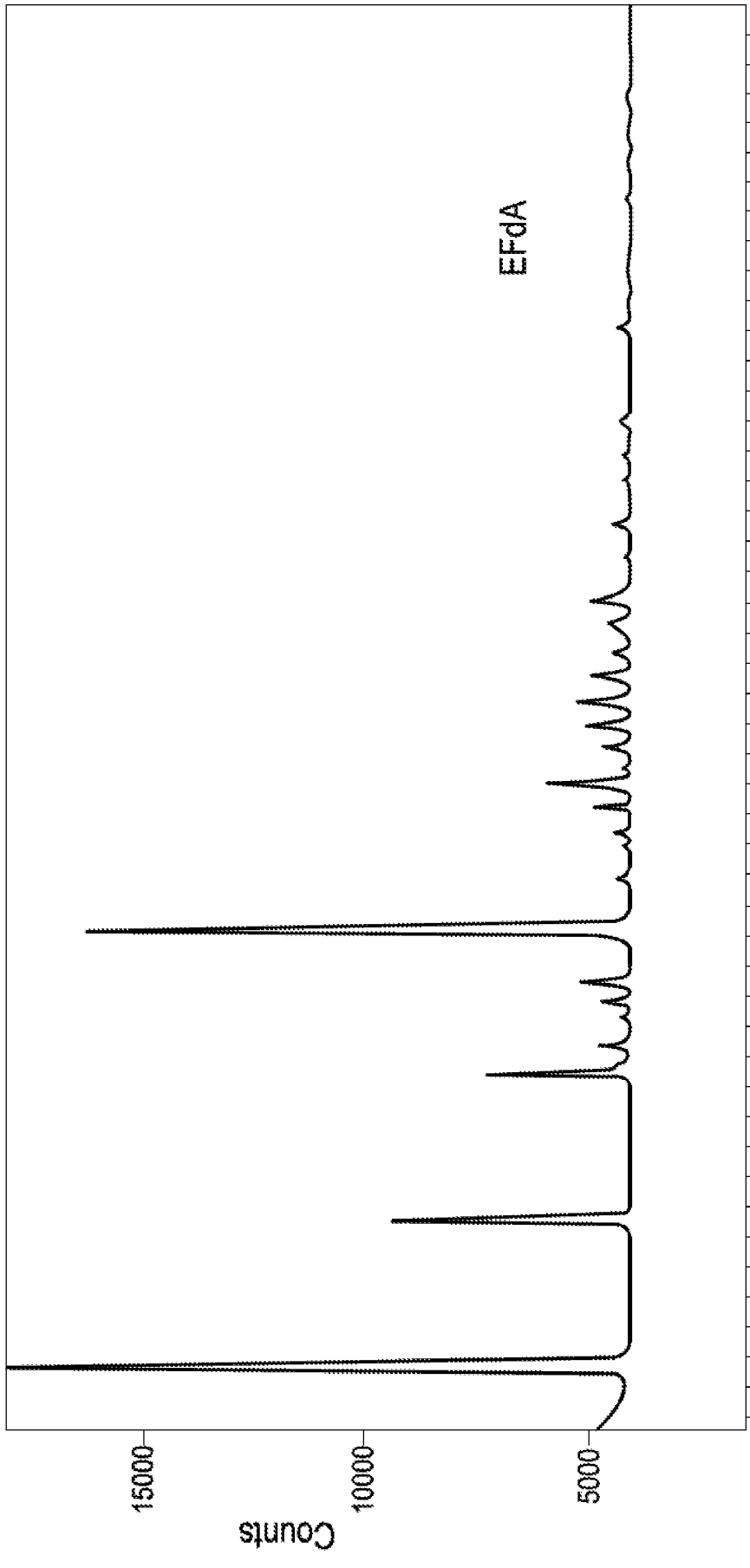
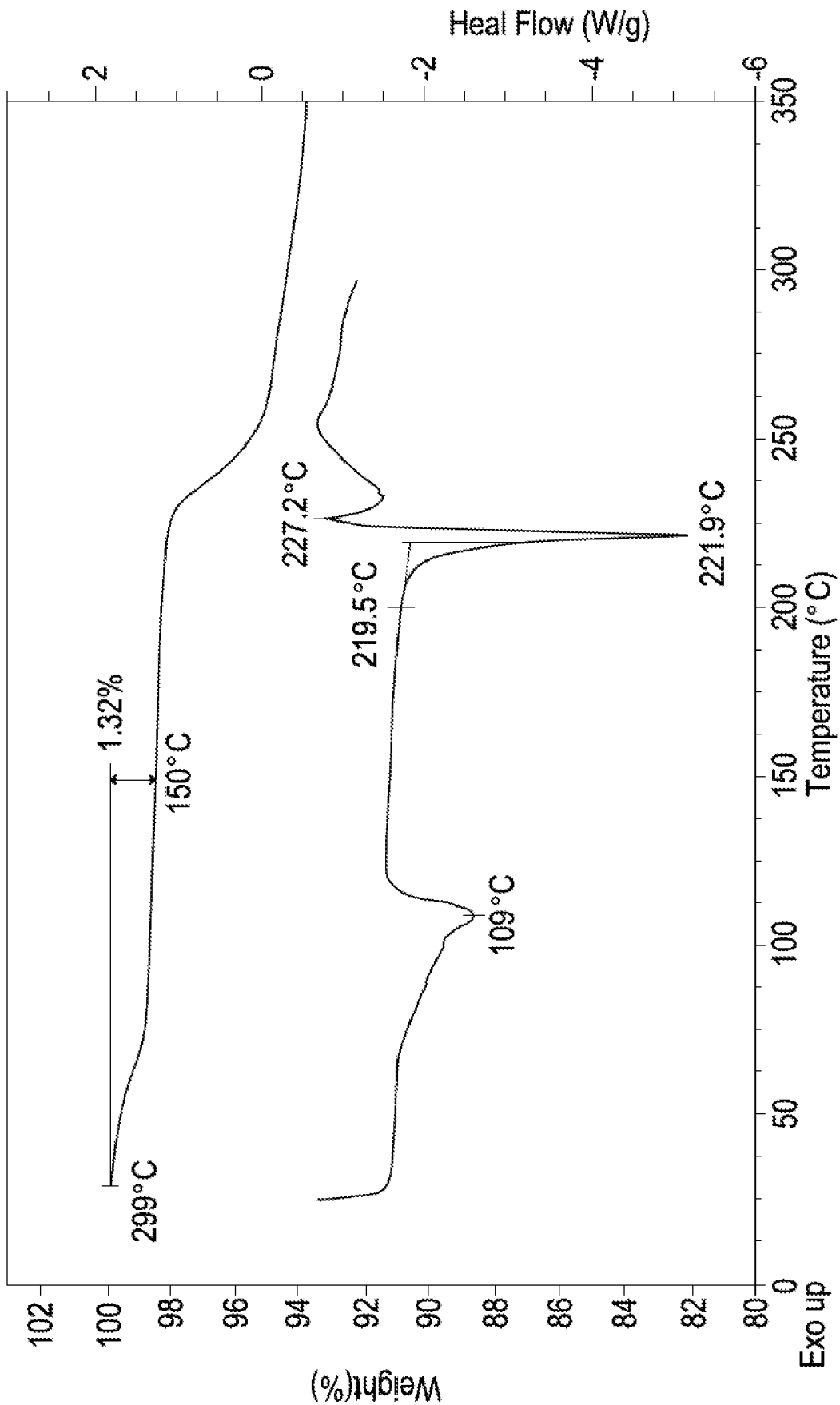

EFdA
Counts
15000
10000
5000
10
20
30
40
Position (°2θ) (Copper (Cu))

Fig. 1

Fig. 2


Heal Flow (W/g)
2
0
-2
-4
-6
350
300
250
200
150
100
50
0
Temperature (°C)
Exo up
Weight(%)
102
100
98
96
94
92
90
88
86
84
82
80
1.32%
150°C
299°C
227.2°C
219.5°C
221.9°C
109°C

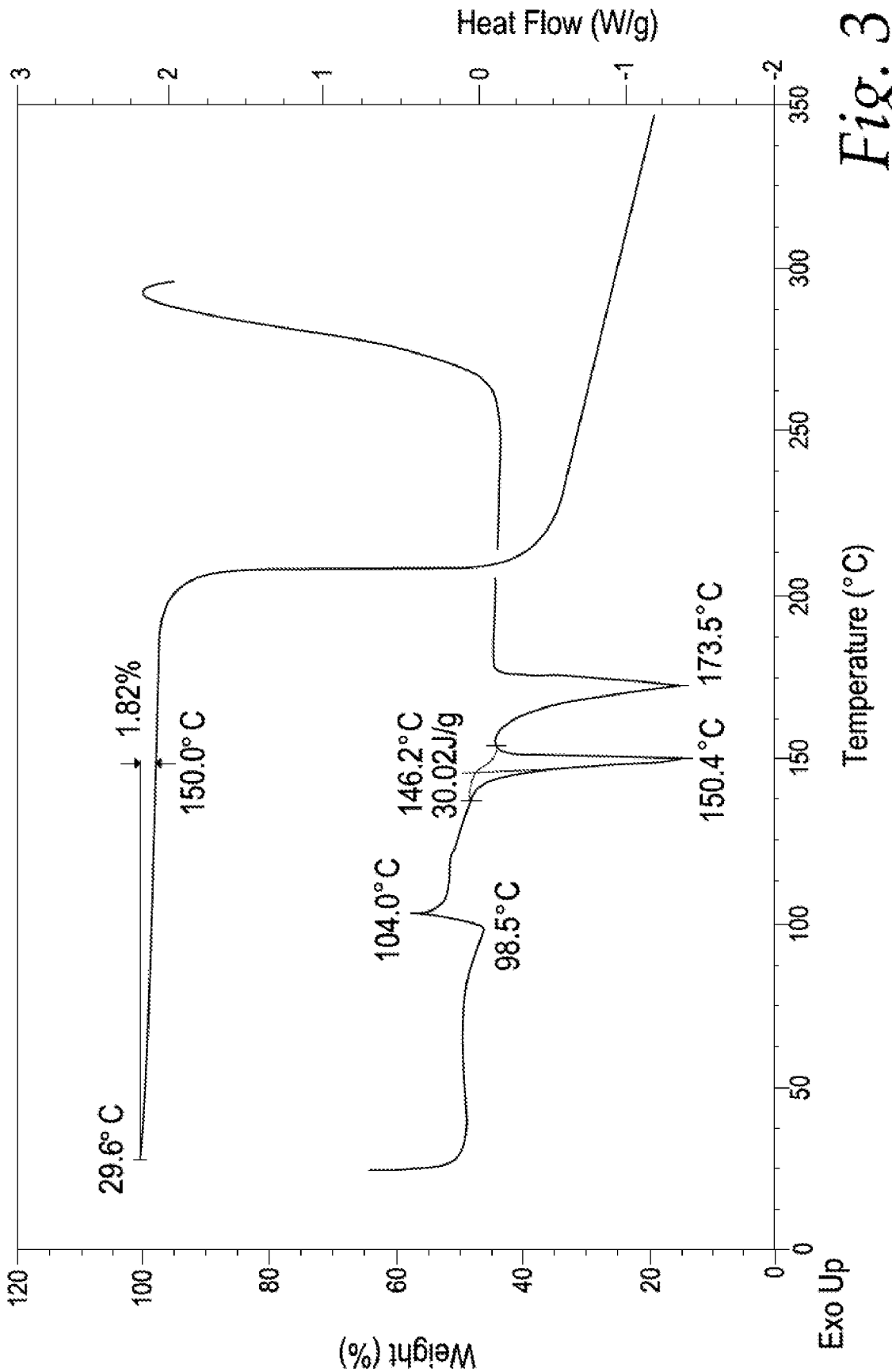

Heat Flow (W/g)
Weight (%)
Temperature (°C)
Exo Up
29.6° C
1.82%
150.0° C
104.0° C
98.5° C
146.2° C
30.02J/g
150.4° C
173.5° C

Fig. 3

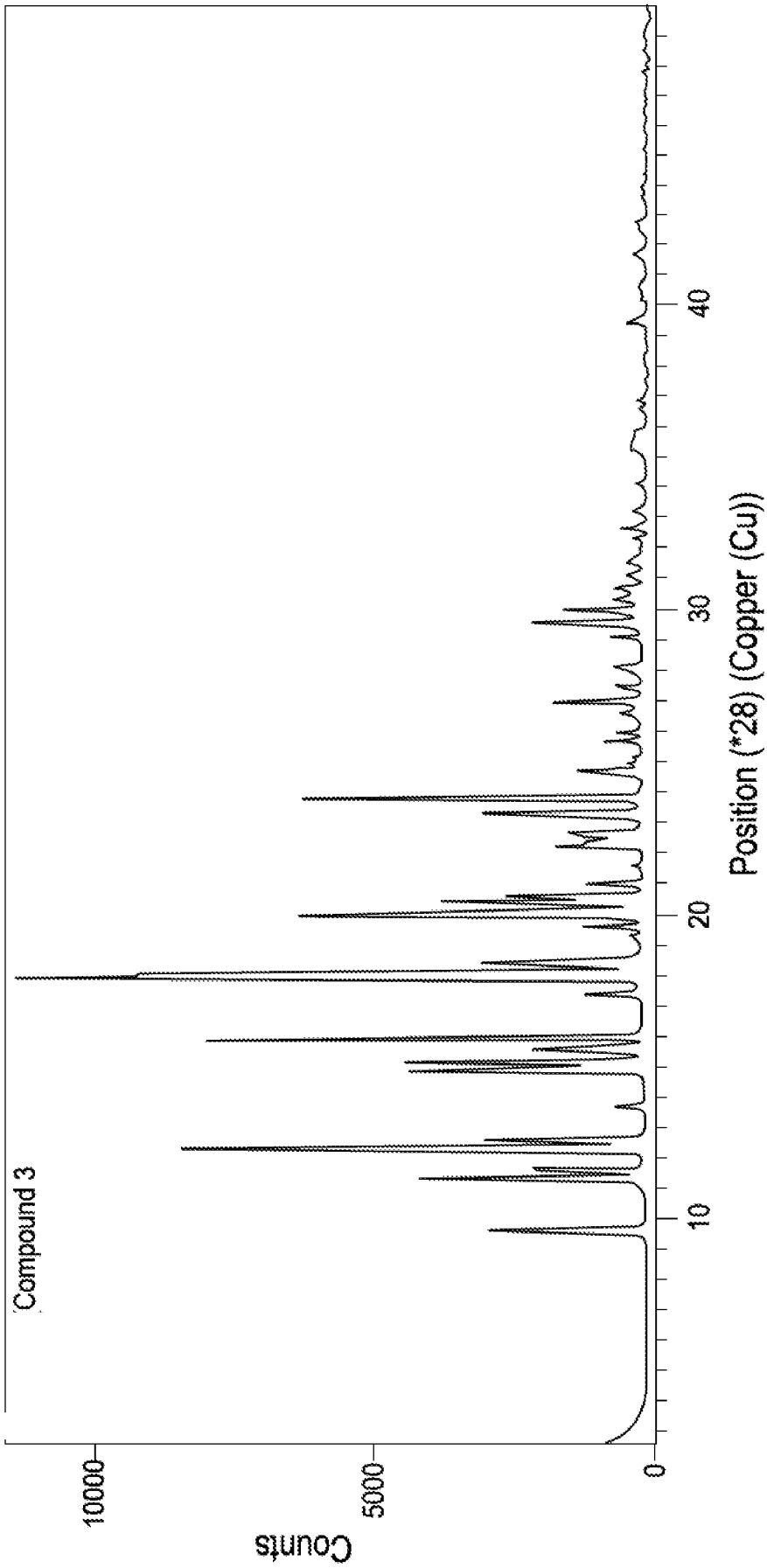

Compound 3
Counts
10000
5000
0
10
20
30
40
Position (*28) (Copper (Cu))

Fig. 4

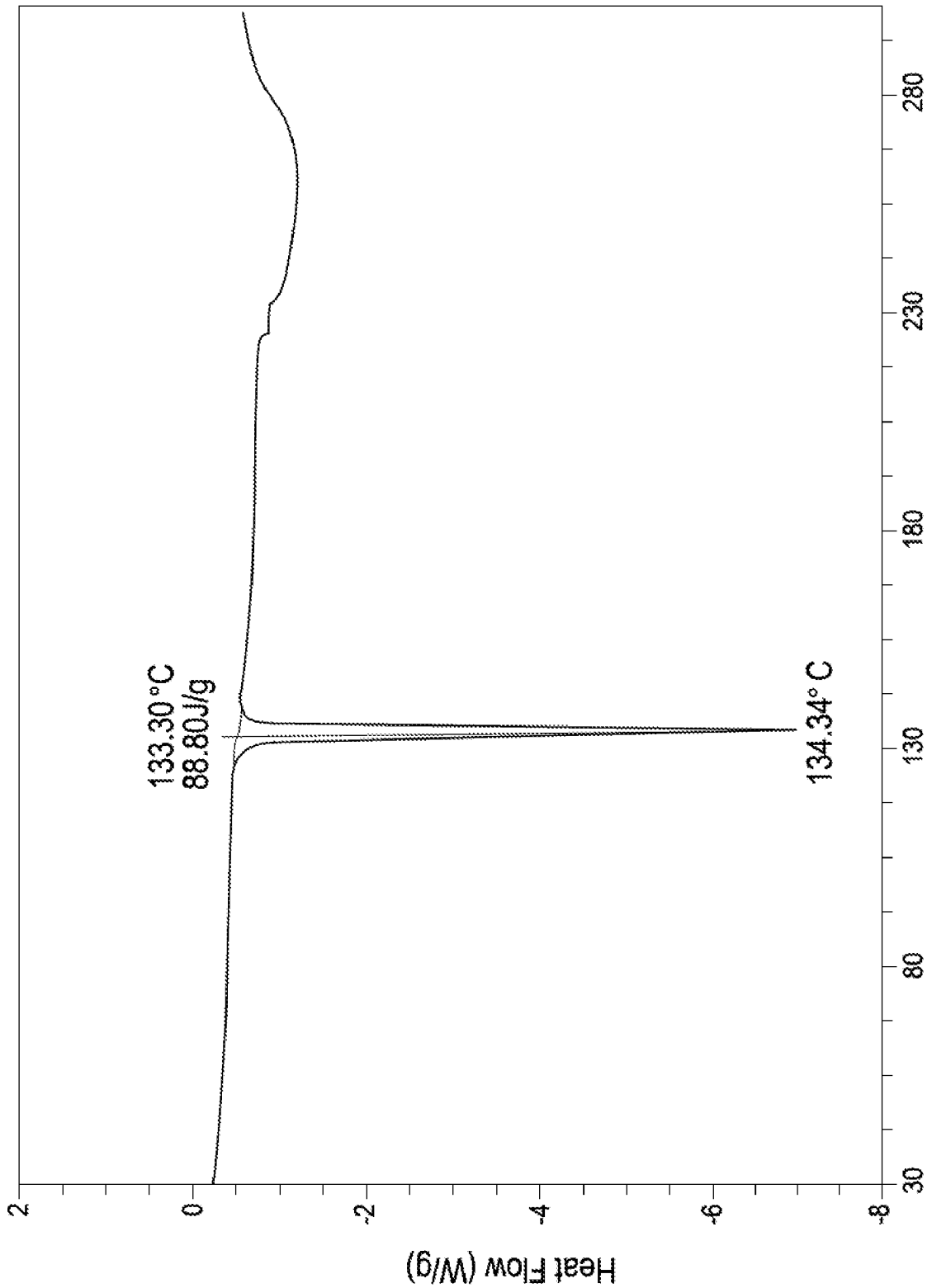

Heat Flow (W/g)
2
0
-2
-4
-6
-8
30
80
130
180
230
280
133.30°C
88.80J/g
134.34° C

Fig. 5

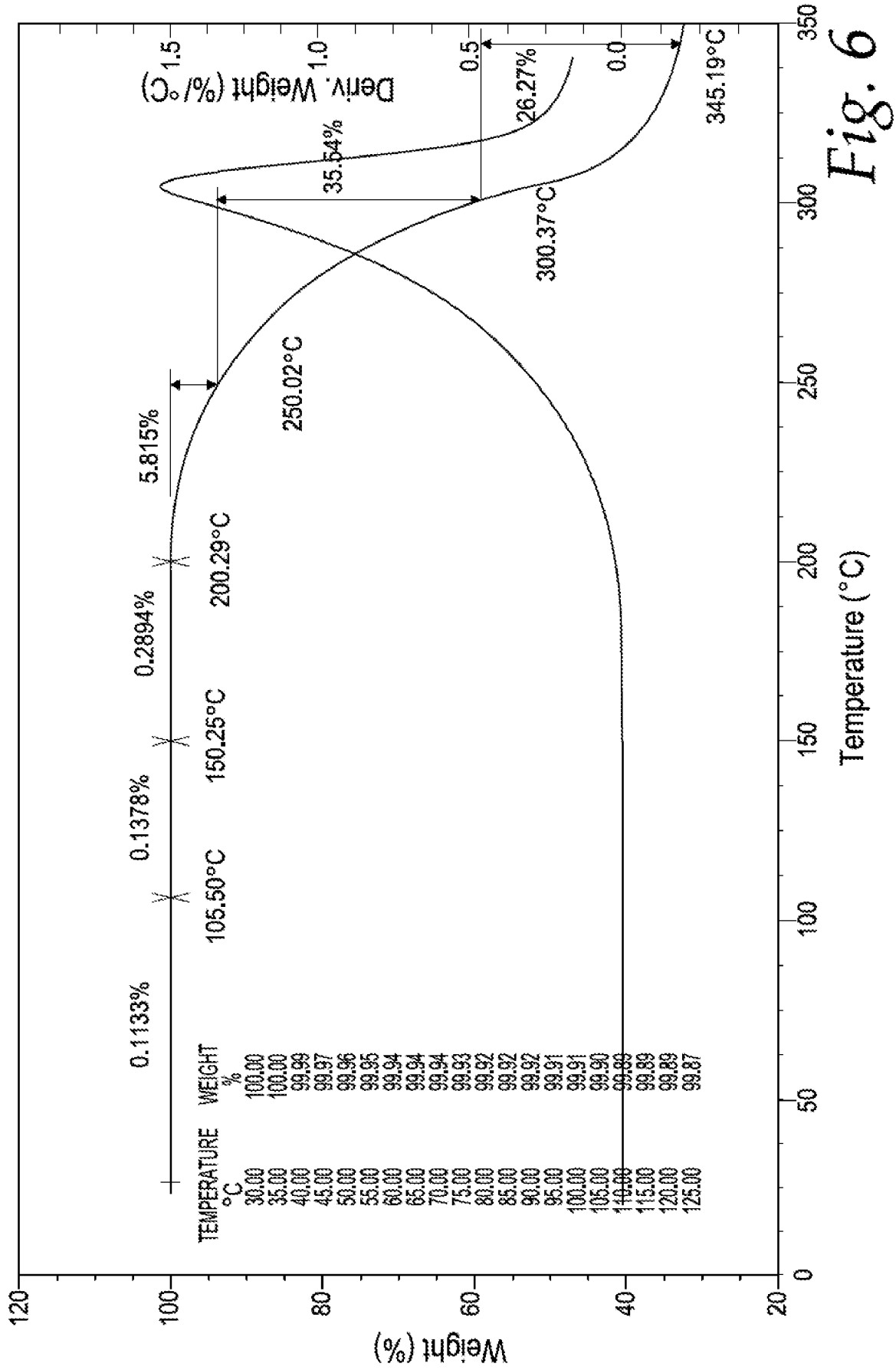
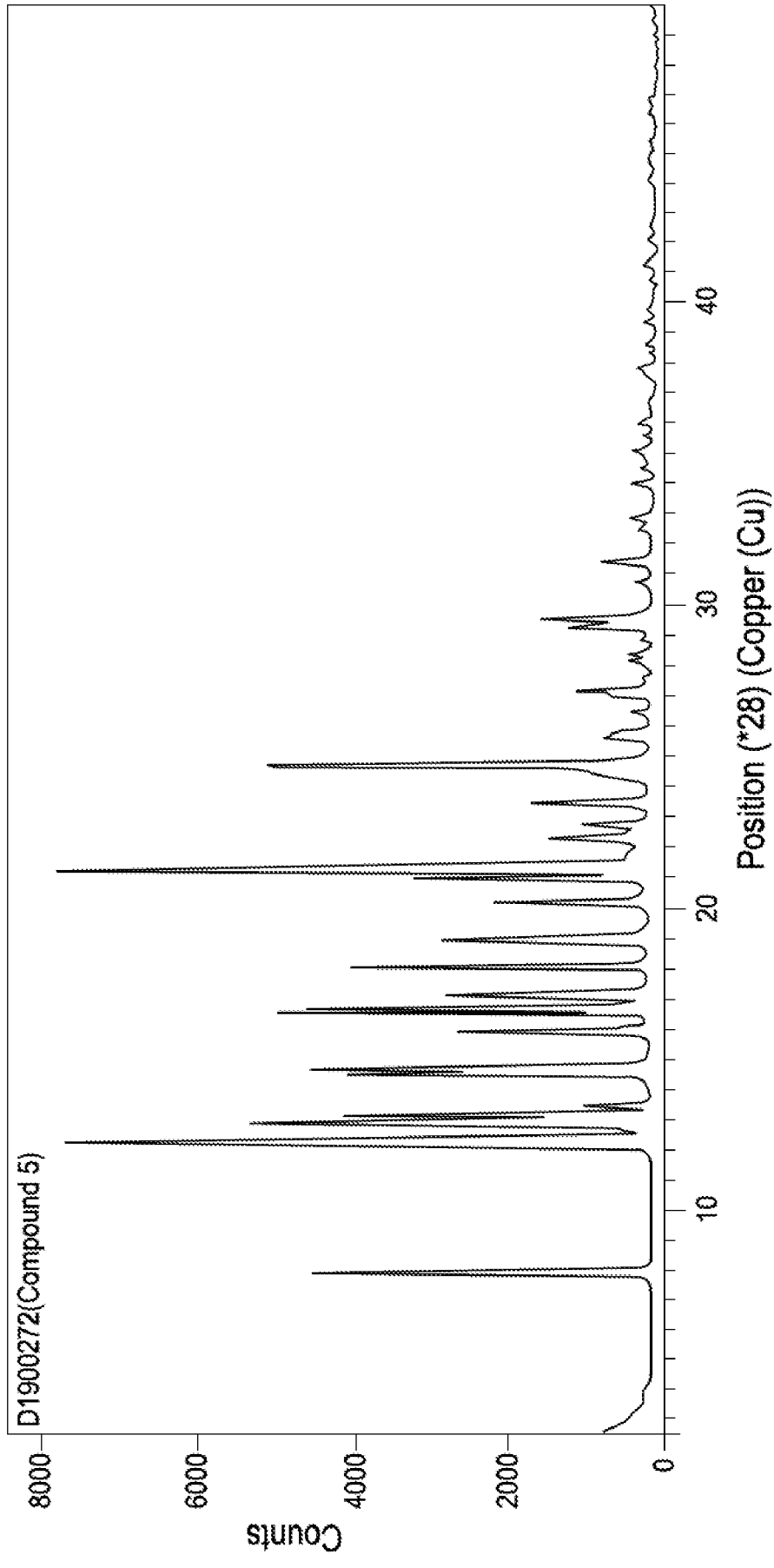

Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
0.1133%
0.1378%
0.2894%
5.815%
35.54%
26.27%
105.50°C
150.25°C
200.29°C
250.02°C
300.37°C
345.19°C
TEMPERATURE °C
WEIGHT %
30.00 100.00
35.00 100.00
40.00 99.99
45.00 99.97
50.00 99.96
55.00 99.95
60.00 99.94
65.00 99.94
70.00 99.94
75.00 99.93
80.00 99.92
85.00 99.92
90.00 99.92
95.00 99.91
100.00 99.91
105.00 99.90
110.00 99.89
115.00 99.89
120.00 99.89
125.00 99.87

Fig. 6

Fig. 7


D1900272(Compound 5)
Counts
8000
6000
4000
2000
0
10
20
30
40
Position (*2θ) (Copper (Cu))

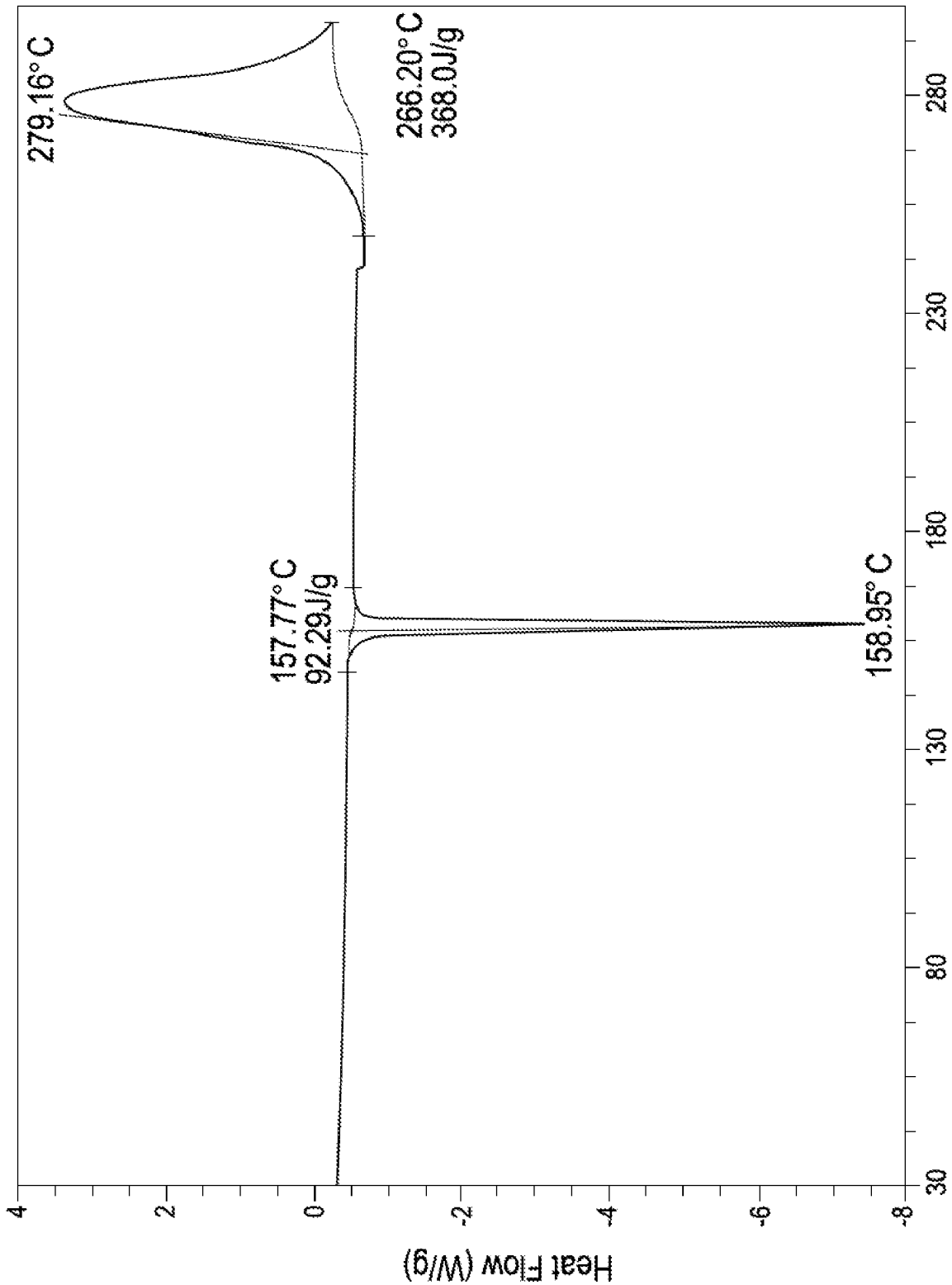

279.16°C
266.20°C
368.0J/g
157.77°C
92.29J/g
158.95°C
Heat Flow (W/g)
4
2
0
-2
-4
-6
-8
30
80
130
180
230
280

Fig. 8

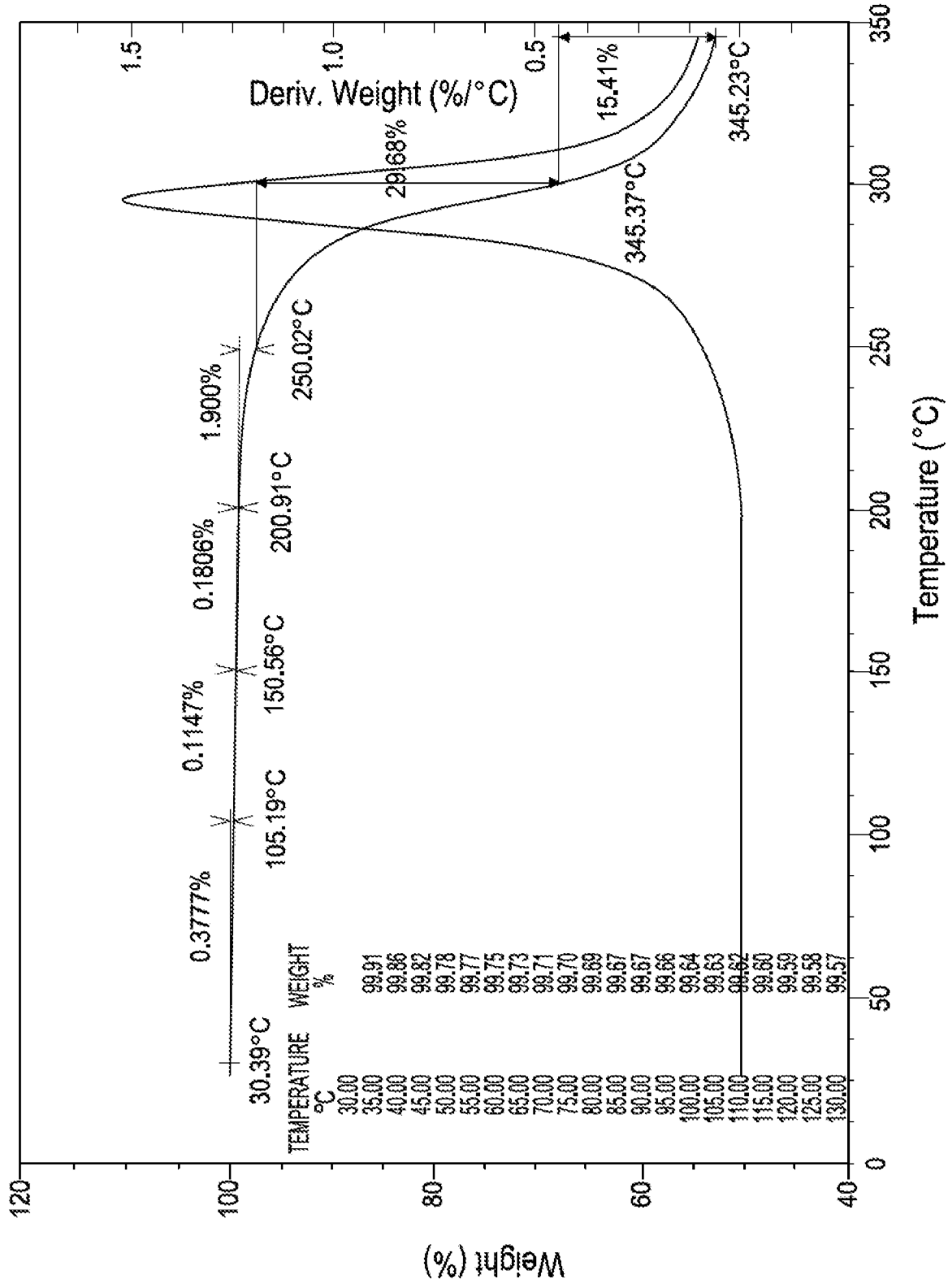

Deriv. Weight (%/°C)
Weight (%)
Temperature (°C)
30.39°C
0.3777%
105.19°C
0.1147%
150.56°C
0.1806%
200.91°C
1.900%
250.02°C
29.68%
345.37°C
15.41%
345.23°C
TEMPERATURE °C
WEIGHT %
30.00 99.91
35.00 99.86
40.00 99.82
45.00 99.78
50.00 99.77
55.00 99.75
60.00 99.73
65.00 99.71
70.00 99.70
75.00 99.69
80.00 99.67
85.00 99.67
90.00 99.66
95.00 99.64
100.00 99.63
105.00 99.62
110.00 99.60
115.00 99.59
120.00 99.58
125.00 99.57
130.00

Fig. 9

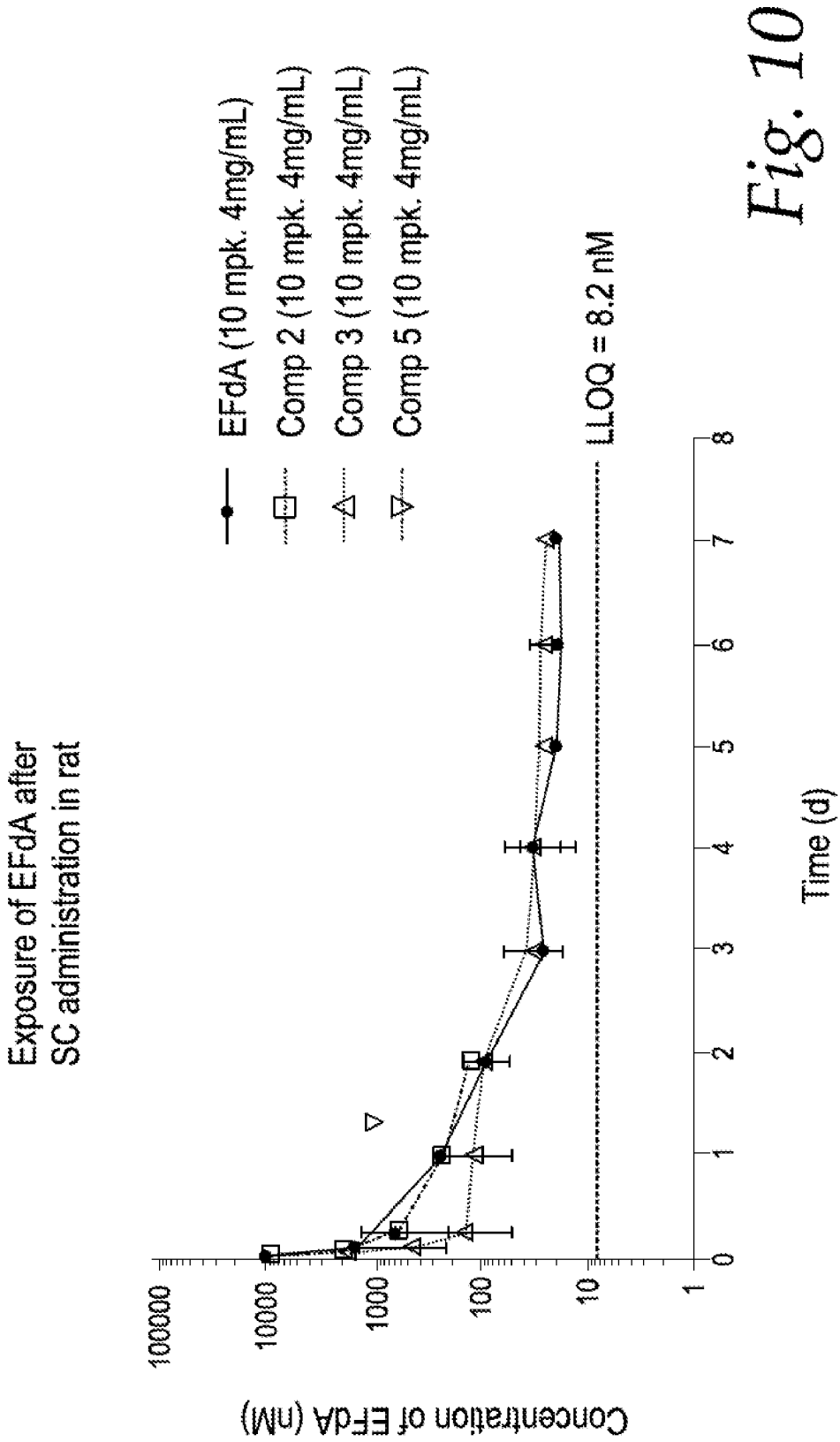

Exposure of EFdA after
SC administration in rat
EFdA (10 mpk. 4mg/mL)
Comp 2 (10 mpk. 4mg/mL)
Comp 3 (10 mpk. 4mg/mL)
Comp 5 (10 mpk. 4mg/mL)
LLOQ = 8.2 nM
Concentration of EFdA (nM)
100000
10000
1000
100
10
1
0
1
2
3
4
5
6
7
8
Time (d)

Fig. 10

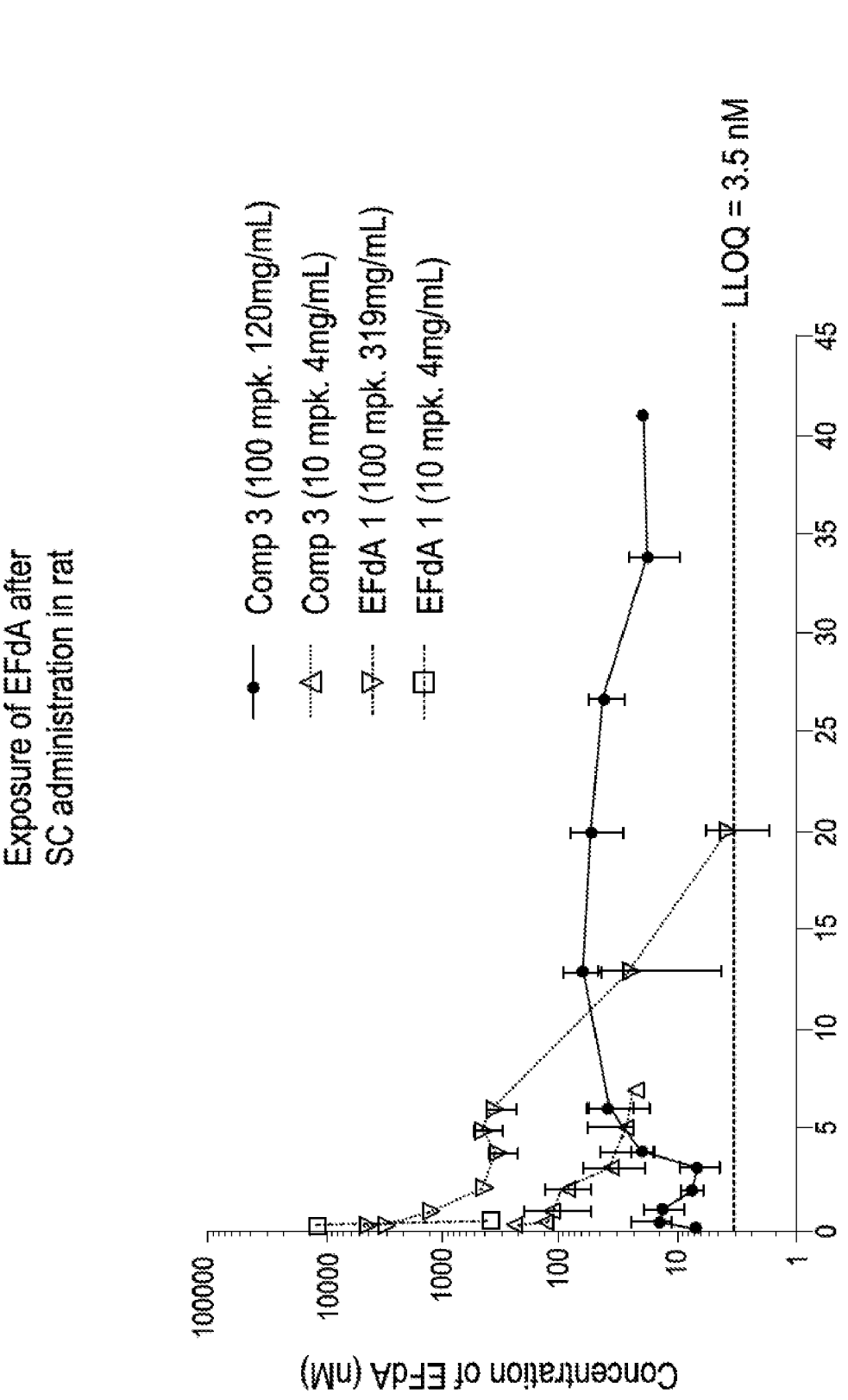

Exposure of EFdA after
SC administration in rat
Comp 3 (100 mpk. 120mg/mL)
Comp 3 (10 mpk. 4mg/mL)
EFdA 1 (100 mpk. 319mg/mL)
EFdA 1 (10 mpk. 4mg/mL)
LLOQ = 3.5 nM
Concentration of EFdA (nM)
100000
10000
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
Time (d)

Fig. 11

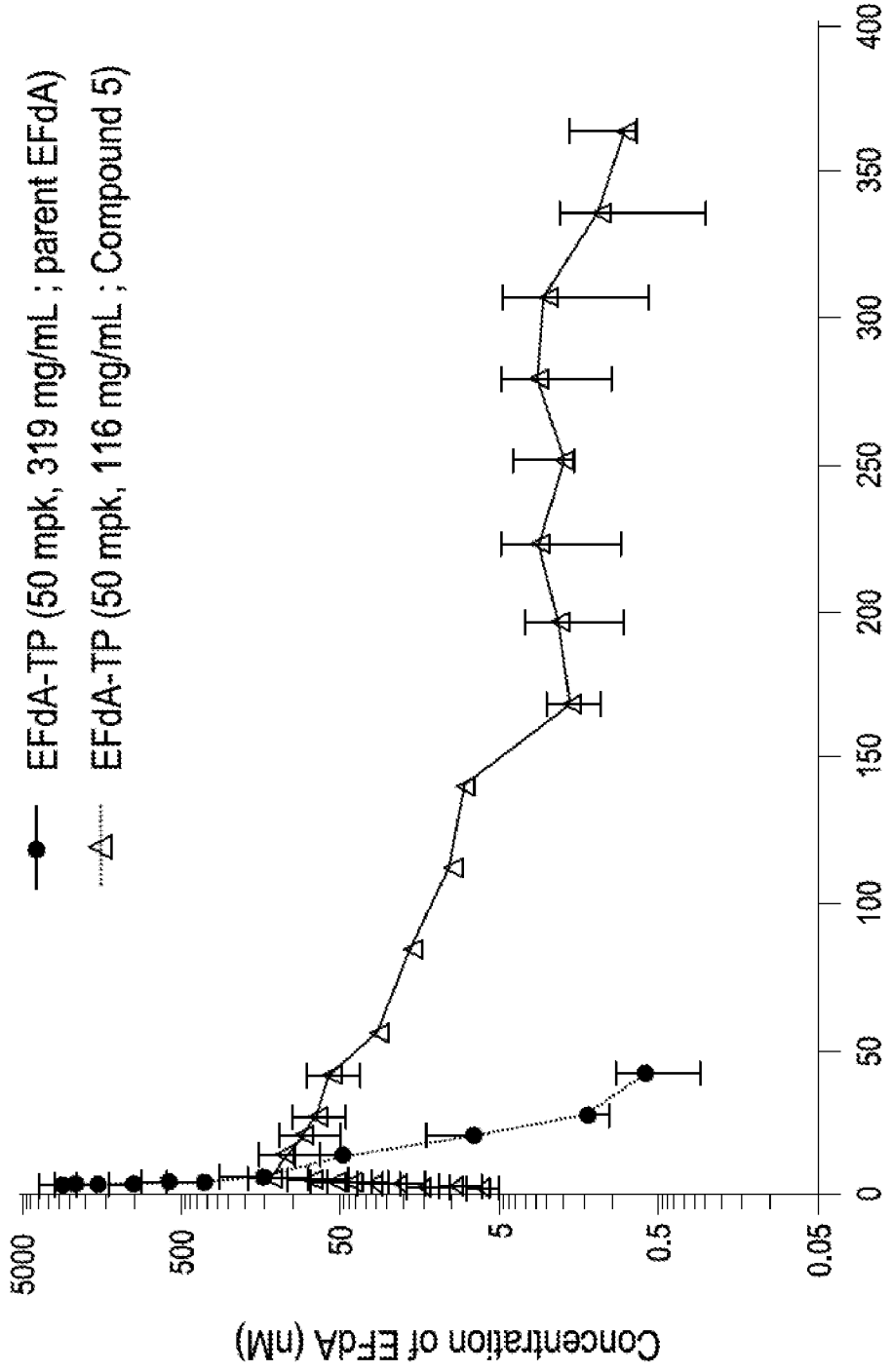

EFdA-TP (50 mpk, 319 mg/mL ; parent EFdA)
EFdA-TP (50 mpk, 116 mg/mL ; Compound 5)
Concentration of EFdA (nM)
5000
500
50
5
0.5
0.05
0
50
100
150
200
250
300
350
400
Time (d)

Fig. 12

Fig. 13

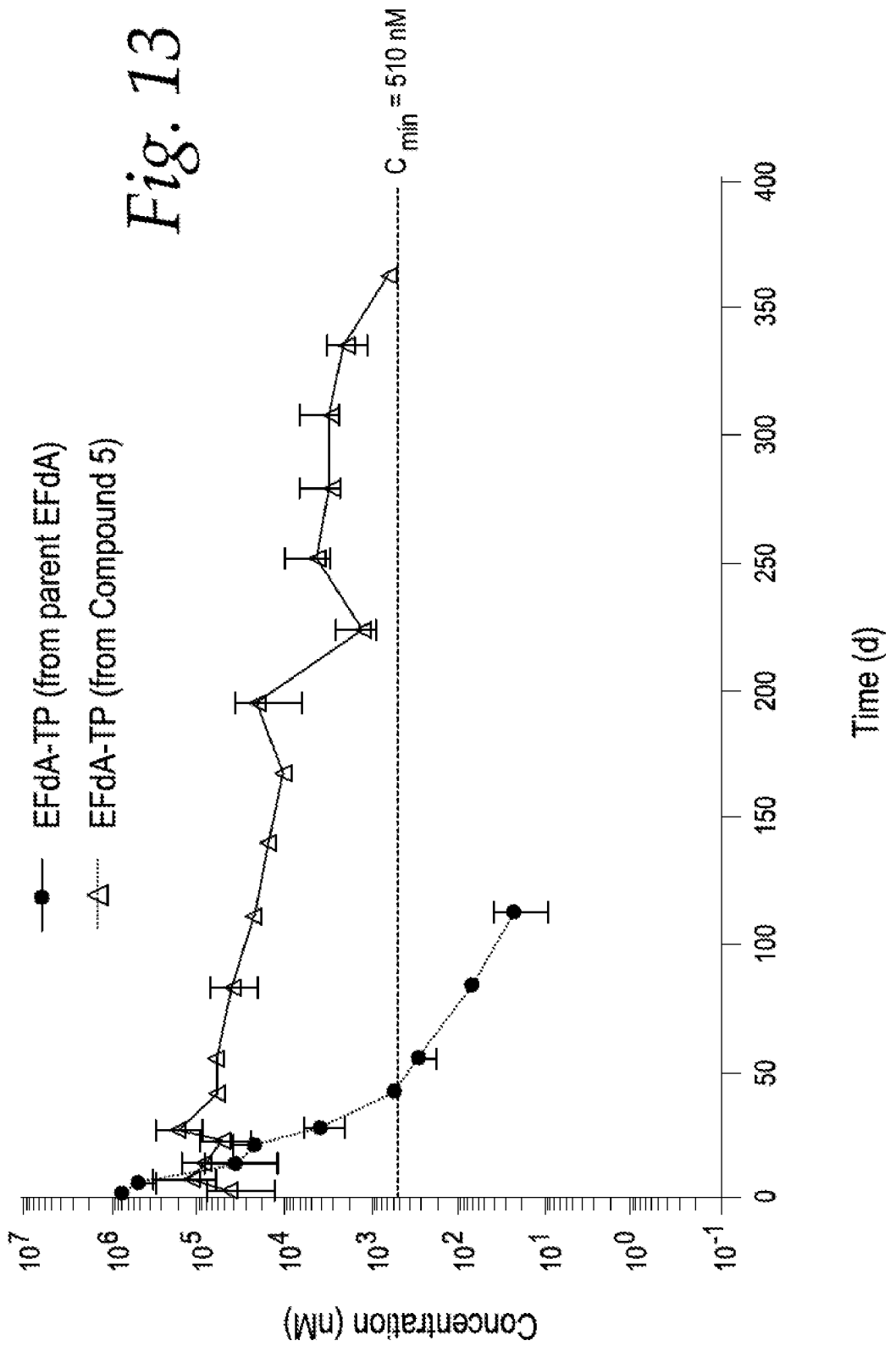

EFdA-TP (from parent EFdA)
EFdA-TP (from Compound 5)
C min = 510 nM
Concentration (nM)
Time (d)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
0
50
100
150
200
250
300
350
400

Fig. 14

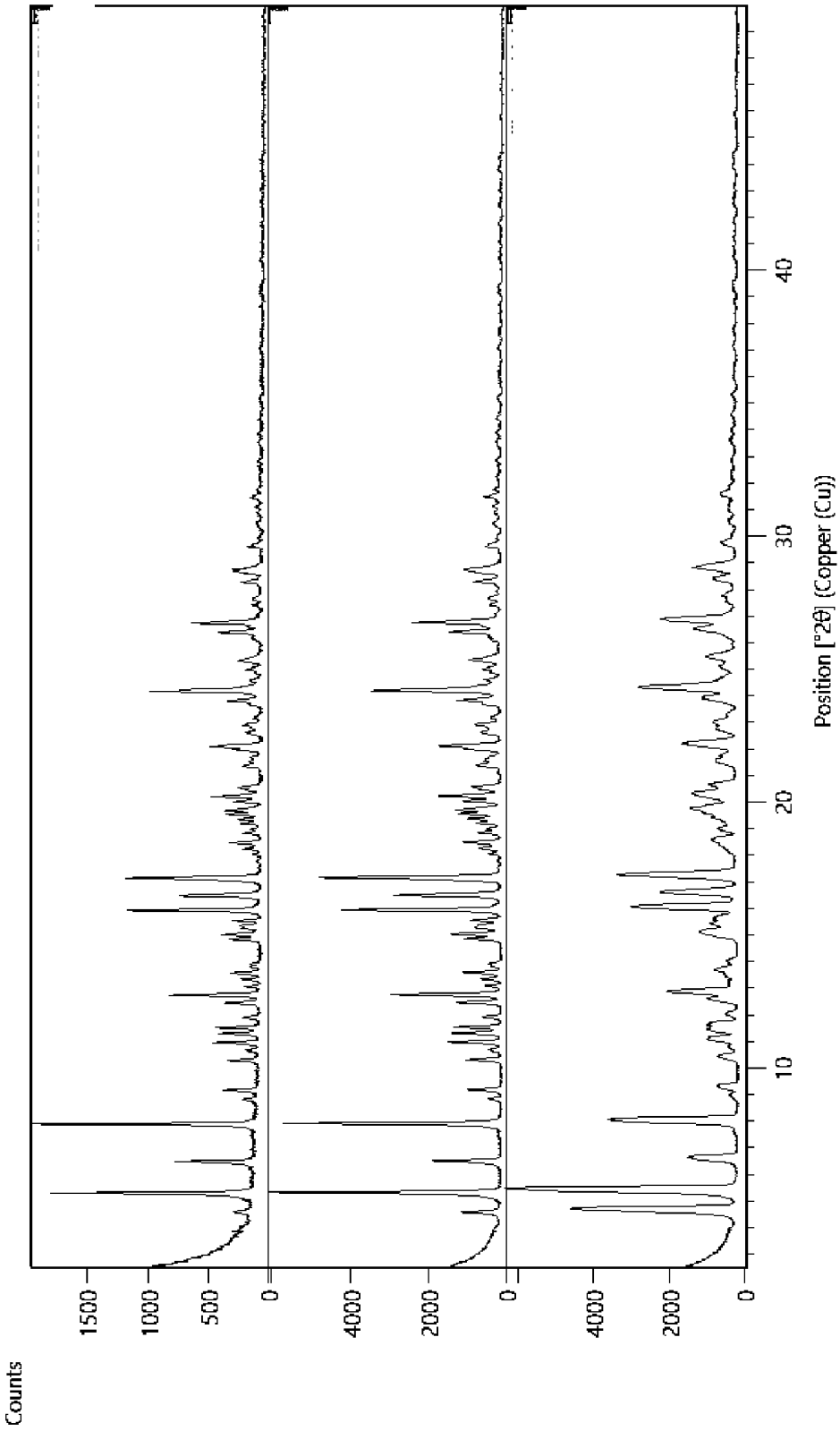
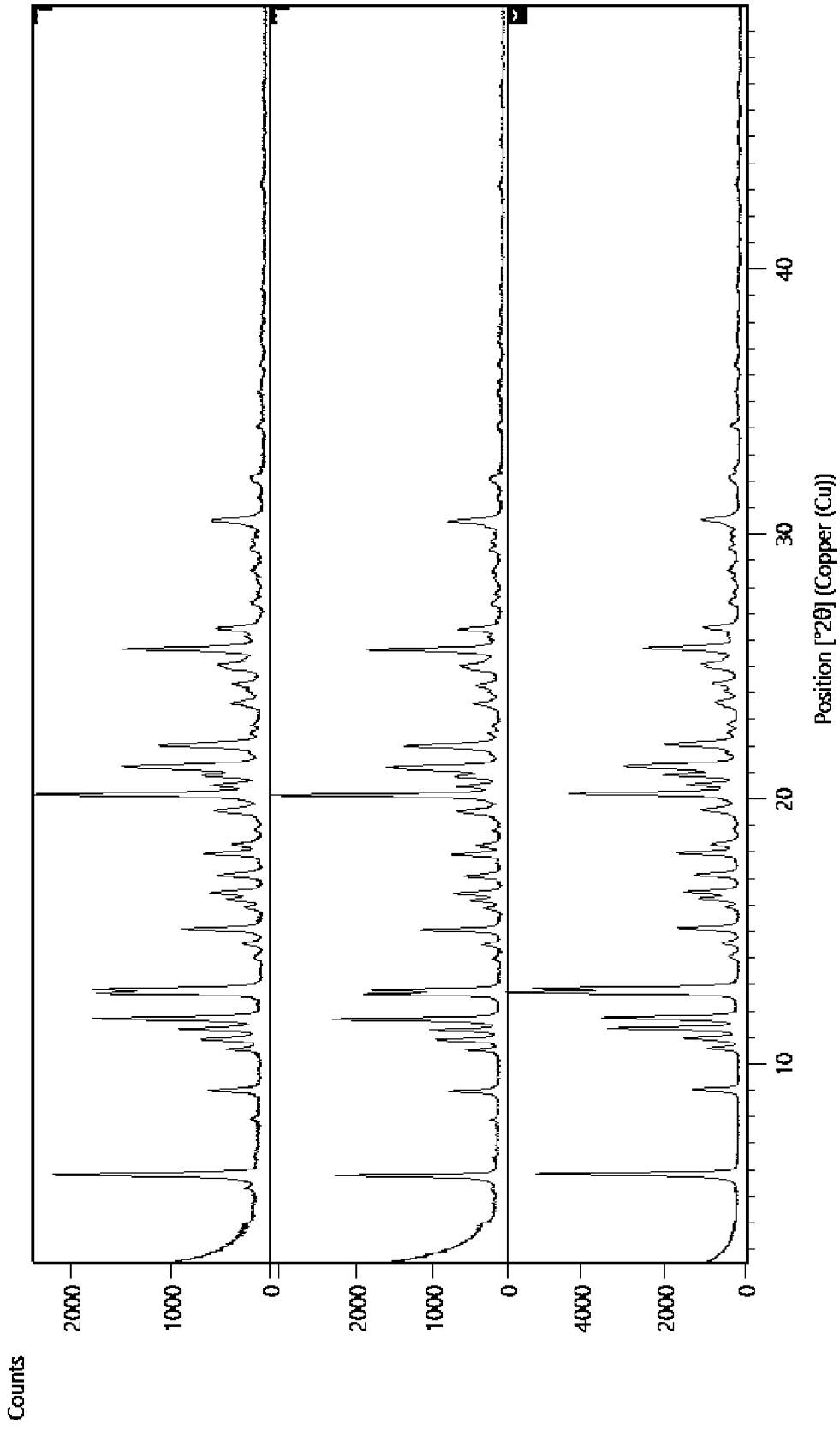

Counts
1500
1000
500
0
4000
2000
0
4000
2000
0
10
20
30
40
Position [°2θ] (Copper (Cu))

Fig. 15


Counts
2000
1000
0
2000
1000
0
4000
2000
0
10
20
30
40
Position [°2θ] (Copper (Cu))

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/050511

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/70; C07H 19/16; C07H 19/173 (2020.01)
CPC - A61K 31/70; C07H 19/16; C07H 19/173 (2020.08)

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.
A	US 7,339,053 B2 (KOHGO et al) 04 March 2008 (04.03.2008) entire document	1-21, 24-26, 36
A	US 8,835,615 B2 (CHANG) 16 September 2014 (16.09.2014) entire document	1-21, 24-26, 36
A	US 2018/0002366 A1 (MERCK SHARP & DOHME CORP) 04 January 2018 (04.01.2018) entire document	1-21, 24-26, 36
A	Kageyama et al. 'Enantioselective Total Synthesis of the Potent Anti-HIV Nucleoside EFdA'; 02 September 2011, Organic Letters, Volume 13, Number 19, Pages 5264-5266; abstract	1-21, 24-26, 36

☐ 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	Date of mailing of the international search report
06 November 2020	08 DEC 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/050511

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.: 22, 23, 27-35
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.