

ANTIVIRAL PRODRUGS AND FORMULATIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

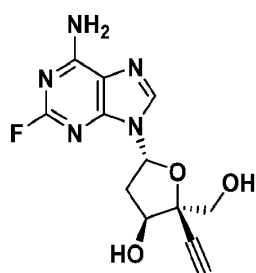
This application claims the benefit of U.S. Provisional Application Serial No. 62/898,679, filed on September 11, 2019, which is incorporated herein by reference in its entirety.

FIELD OF INVENTION

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

BACKGROUND

The compound EFdA (MK-8591) is a nucleoside analog known to be effective as an inhibitor of the enzyme reverse transcriptase (*Current Opinion in HIV and AIDS* **2018**, 13, 294-299)



MK-8591 (Islatravir, EFdA).

Certain EFdA analogs are described in a U.S. application published as US2019/185508, including phosphates, esters, carbonates, carbamates where at least one of the substitutions in the glycosyl donor ring contain a "D" atom (D = deuterium = $2H$).

Reverse transcriptase inhibitors can be effective in the treatment of viral infections caused by viruses where reverse transcriptase function is essential for viral replication and production of viral proteins, such as HIV (Human Immunodeficiency Virus) and HBV (Hepatitis B Virus). In the case of HIV, the virus is an RNA virus that uses reverse transcriptase to synthesize DNA reverse transcripts of the viral genome which are translated by the host to provide the viral proteins. In the case of HBV, a DNA virus, the DNA viral polymerase also has a reverse transcriptase function, generating viral DNA from a viral RNA

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intermediate during replication. An antiviral example of a compound that hits both HIV and HBV is lamivudine.

SUMMARY

The invention provides, in various embodiments, (1) novel compositions of chemical matter comprising bioactive prodrugs of MK-8591 or pharmaceutically acceptable salt of these prodrugs, and (2) novel formulations of these prodrugs providing therapeutic and prophylactic treatment of patients against viral infections of viruses such as HIV and HBV, wherein inhibition of a reverse transcriptase enzyme (RNA-directed DNA polymerase) slows or blocks the viral infection. The route of administration for these treatments can include, but not limited to, oral, parenteral and implants (composition and device). The formulations of this invention provide for slow or controlled or sustained release of EFdA from these prodrugs when injected as an aqueous suspension formulation.

BRIEF DESCRIPTION OF THE FIGURES

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.

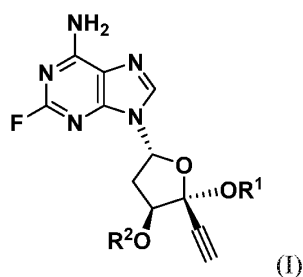
Figure 11 shows in graphic form the data provided in Table 3.

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

DETAILED DESCRIPTION

The invention provides, in various embodiments, a compound of Formula (I)

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wherein

R^1 is H or is $X-L_m$, wherein $m = 1$ or 2 , and wherein $X-L_m$ is $-C(=O)L$, $-C(=O)OL$, $-C(=O)NH(L)$, $-C(=O)N(L)_2$, $-CH(R)OC(=O)L$, $-C(=O)CH(R)-NH(L)$, $-C(=O)CH(R)-N(L)_2$, $-P(=O)(NHL)_2$, $-P(=O)(NHL)(NL_2)$, or $-P(=O)(NL_2)_2$, and each independently selected L is (C_{1-22}) alkyl, (C_{3-22}) alkenyl wherein the alkenyl can comprise 1-6 unsaturations, (C_{3-7}) cycloalkyl, $(CHR)_n$ -phenyl wherein $n=0$ or 1 , or $-CHR-N(R)_2$, or R^1 is $-OCH(R)OP(=O)(OH)_2$ or R^1 is a phosphate residue or its derivative residue comprising a monophosphate, a diphosphate, a triphosphate, a phosphonate, a phosphate polyester, a phosphate amidate (mono and di), a phosphorothioate, a phosphoroselenoate, or a phosphoroboranoate

R is H, (C_{1-22}) alkyl, or (C_{3-22}) alkenyl wherein the alkenyl can comprise 1-6 unsaturations, or is (C_{3-7}) cycloalkyl;

R^2 is H or is $X-L_m$, or R^2 is $-OCH(R)OP(=O)(OH)_2$ or R^2 is a phosphate residue or its derivative residue comprising a monophosphate, a diphosphate, a triphosphate, a phosphonate, a phosphate polyester, a phosphate amidate (mono and di), a phosphorothioate, a phosphoroselenoate, or a phosphoroboranoate;

excluding following combinations: (a) $R^1 = R^2 =$ acetyl, (b) $R^1 = R^2 =$ H, and (c) $R^2 =$ H when R^1 is a phosphate residue or its derivative residue comprising a monophosphate, a diphosphate, a triphosphate, a phosphonate, a phosphate polyester, a phosphate amidate (mono and di), a phosphorothioate, a phosphoroselenoate, or a phosphoroboranoate; or a pharmaceutically acceptable salt thereof.

In various embodiments, a compound of Formula (I) can be any of the specific compounds **2 - 51**, shown in Table 1.

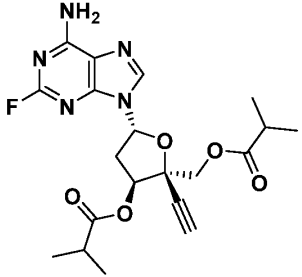
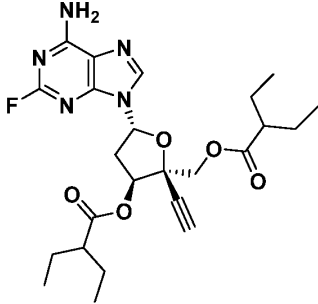
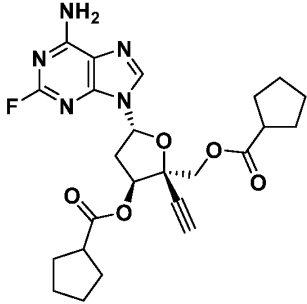
The invention further provides, in various embodiments, a method of inhibiting viral reverse transcriptase bioactivity, comprising contacting a virus expressing an enzyme with

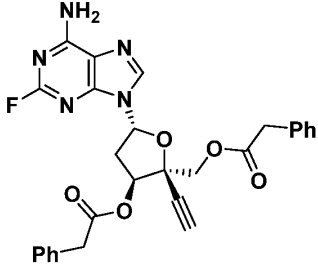
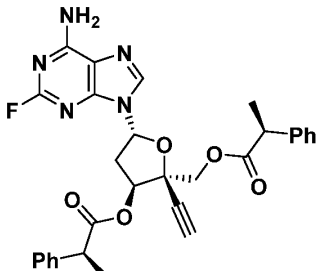
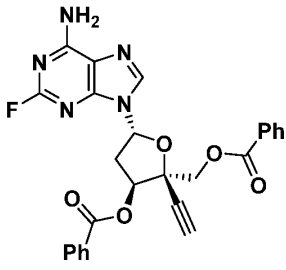
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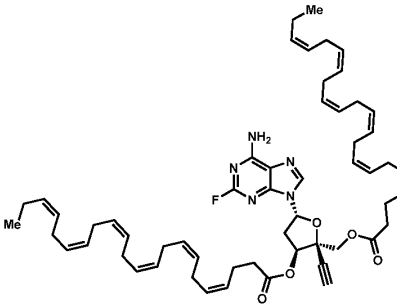
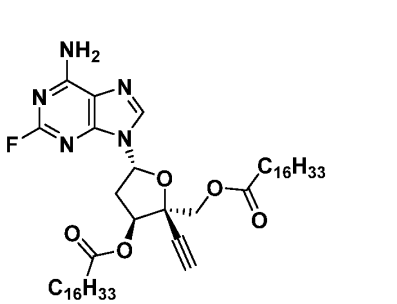
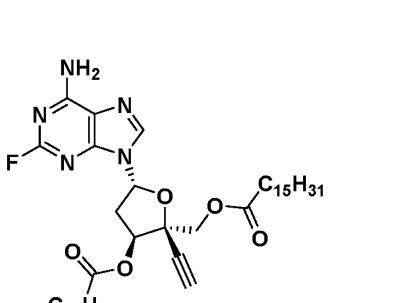
reverse transcriptase bioactivity with an effective amount or concentration of a compound of Formula (I).

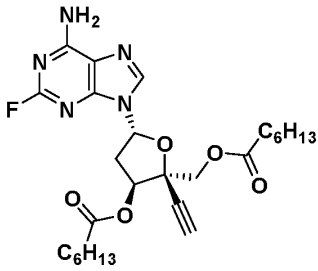
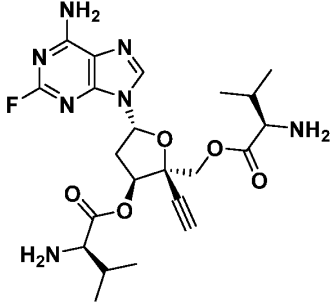
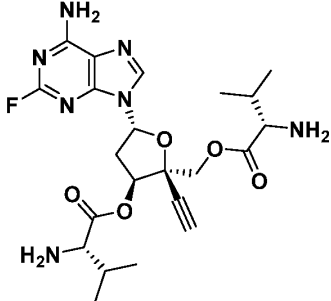
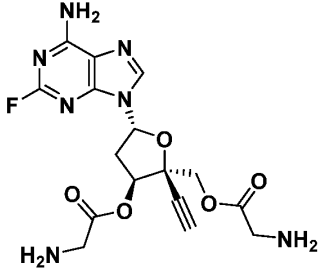
The invention further provides, in various embodiments, a method of prophylaxis of viremia or treatment of a viral infection in a patient wherein inhibition of a reverse transcriptase is medically indicated, comprising administering to the patient an effective amount or concentration of a compound of Formula (I). More specifically, the compound of Formula (I) 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 (I) can be formulated as aqueous suspension, solutions, and can be encapsulated in particles for slow-release including PLGA and those 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 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 or lamivudine.

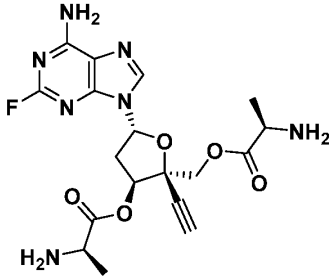
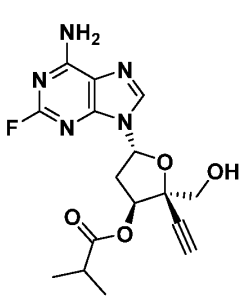
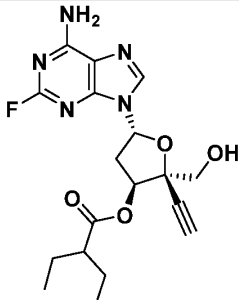
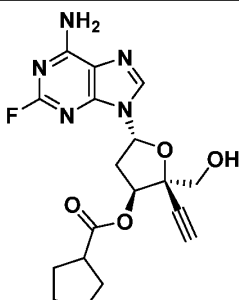
Table 1: Compounds of the Invention

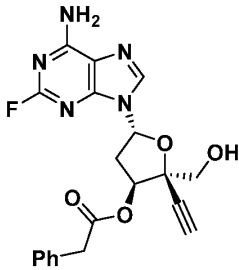
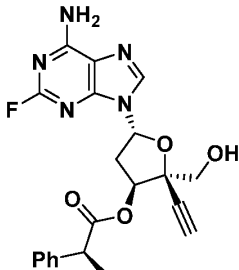
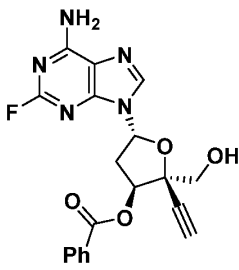
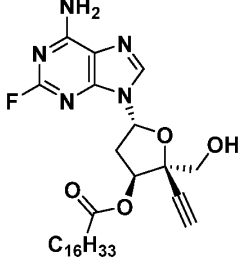
Cpd #	Structure	Characterization Data (NMR and LCMS)
2		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.34 (s, 1H), 7.96 (s, 1H), 7.87 (s, 1H), 6.35 (t, <i>J</i> = 6.6 Hz, 1H), 5.68 (t, <i>J</i> = 5.6 Hz, 1H), 4.40 (dd, <i>J</i> = 11.9, 1.5 Hz, 1H), 4.21 (d, <i>J</i> = 10.4 Hz, 1H), 3.81 (s, 1H), 3.18 (dt, <i>J</i> = 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: <i>m/z</i> 434.49 observed (M+H) ⁺ Anal calcd for C ₂₀ H ₂₄ FN ₅ O 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- <i>d</i> ₆) δ 8.35 (s, 1H), 7.91 (d, <i>J</i> = 39.8 Hz, 2H), 6.35 (t, <i>J</i> = 6.8 Hz, 1H), 5.73 (t, <i>J</i> = 6.5 Hz, 1H), 4.38 (dd, <i>J</i> = 11.8, 2.5 Hz, 1H), 4.24 (dd, <i>J</i> = 11.7, 2.5 Hz, 1H), 3.81 (s, 1H), 3.20 (dt, <i>J</i> = 13.5, 6.8 Hz, 1H), 2.61 (dt, <i>J</i> = 13.2, 6.6 Hz, 1H), 2.29 (ddd, <i>J</i> = 8.3, 5.6, 2.6 Hz, 1H), 2.13 (tt, <i>J</i> = 8.9, 5.9 Hz, 1H), 1.72 – 1.31 (m, 8H), 0.88 (td, <i>J</i> = 7.5, 2.3 Hz, 6H), 0.80 – 0.66 (m, 6H). MS-ESI: <i>m/z</i> 490.56 observed (M+H) ⁺ Aqueous solubility (pH 7.4): <0.002 mg/mL
4		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.34 (s, 1H), 7.92 (d, <i>J</i> = 36.1 Hz, 2H), 6.34 (t, <i>J</i> = 6.8 Hz, 1H), 5.68 (t, <i>J</i> = 6.2 Hz, 1H), 4.39 (dt, <i>J</i> = 11.6, 1.8 Hz, 1H), 4.21 (dd, <i>J</i> = 11.6, 2.4 Hz, 1H), 3.81 (s, 1H), 3.18 (dt, <i>J</i> = 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: <i>m/z</i> 486.44 observed (M+H) ⁺

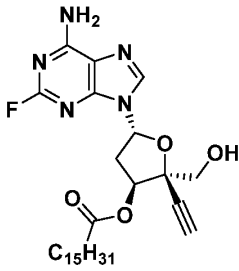
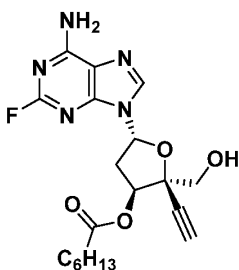
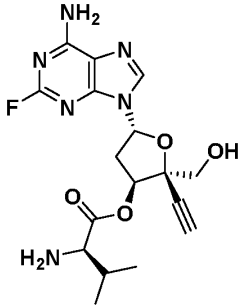
Cpd #	Structure	Characterization Data (NMR and LCMS)
5		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.31 (s, 1H), 7.93 (d, <i>J</i> = 37.3 Hz, 2H), 7.39 – 7.12 (m, 10H), 6.35 (t, <i>J</i> = 6.7 Hz, 1H), 5.71 (t, <i>J</i> = 6.0 Hz, 1H), 4.41 (d, <i>J</i> = 11.7 Hz, 1H), 4.25 (d, <i>J</i> = 11.3 Hz, 1H), 3.88 – 3.71 (m, 3H), 3.63 (q, <i>J</i> = 15.8 Hz, 2H), 3.11 (dt, <i>J</i> = 14.0, 7.0 Hz, 1H), 2.63 (dt, <i>J</i> = 12.5, 6.4 Hz, 1H). MS-ESI: <i>m/z</i> 530.52 observed (M+H) ⁺ Aqueous solubility (pH 7.4): <0.002 mg/mL
6		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.26 (s, 1H), 7.93 (d, <i>J</i> = 34.8 Hz, 2H), 7.38 – 7.13 (m, 10H), 6.35 – 6.25 (m, 1H), 5.59 (t, <i>J</i> = 6.3 Hz, 1H), 4.38 (d, <i>J</i> = 11.8 Hz, 1H), 4.09 (d, <i>J</i> = 11.4 Hz, 1H), 3.88 (q, <i>J</i> = 7.0 Hz, 1H), 3.70 (q, <i>J</i> = 7.1 Hz, 1H), 3.48 (s, 1H), 3.04 (dt, <i>J</i> = 14.3, 7.0 Hz, 1H), 2.61 – 2.53 (m, 1H), 1.47 (d, <i>J</i> = 7.4 Hz, 3H), 1.28 (d, <i>J</i> = 7.3 Hz, 3H). MS-ESI: <i>m/z</i> 558.65 observed (M+H) ⁺
7		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.38 (s, 1H), 8.09 (dt, <i>J</i> = 8.6, 1.4 Hz, 2H), 8.02 – 7.83 (m, 4H), 7.76 – 7.68 (m, 1H), 7.66 (td, <i>J</i> = 7.4, 1.5 Hz, 1H), 7.59 (td, <i>J</i> = 7.6, 7.1, 1.7 Hz, 2H), 7.53 – 7.44 (m, 2H), 6.54 (t, <i>J</i> = 6.8 Hz, 1H), 6.16 – 6.08 (m, 1H), 4.72 (d, <i>J</i> = 11.6 Hz, 1H), 4.59 (d, <i>J</i> = 11.5 Hz, 1H), 3.84 (s, 1H), 3.41 – 3.32 (m, 1H), 2.85 (dt, <i>J</i> = 13.4, 6.3 Hz, 1H). MS-ESI: <i>m/z</i> 502.41 observed (M+H) ⁺

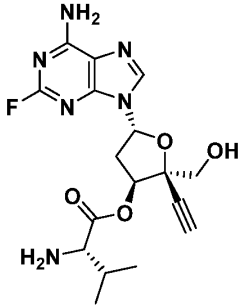
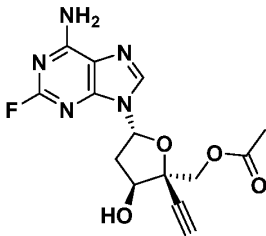
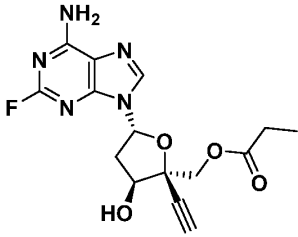
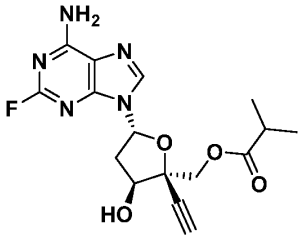
Cpd #	Structure	Characterization Data (NMR and LCMS)
8		¹H NMR (400 MHz, Chloroform-<i>d</i>) δ 8.02 (s, 1H), 6.39 (t, <i>J</i> = 6.4 Hz, 1H), 5.64 (t, <i>J</i> = 6.2 Hz, 1H), 5.49 – 5.25 (m, 24H), 4.50 (d, <i>J</i> = 12.0 Hz, 1H), 4.40 (d, <i>J</i> = 12.2 Hz, 1H), 3.00 (dt, <i>J</i> = 13.6, 6.7 Hz, 1H), 2.90 – 2.66 (m, 21H), 2.53 – 2.30 (m, 10H), 2.07 (p, <i>J</i> = 7.3 Hz, 5H), 0.97 (t, <i>J</i> = 7.5 Hz, 6H). MS-ESI: <i>m/z</i> 457.23 observed (<i>M</i>/2+<i>H</i>)⁺
9		¹H NMR (400 MHz, Chloroform-<i>d</i>) δ 7.92 (s, 1H), 6.40 (t, <i>J</i> = 6.4 Hz, 1H), 5.76 (brs, 2H), 5.65 (dd, <i>J</i> = 7.1, 5.3 Hz, 1H), 4.49 (d, <i>J</i> = 12.0 Hz, 1H), 4.38 (d, <i>J</i> = 12.1 Hz, 1H), 2.99 (dt, <i>J</i> = 13.5, 6.7 Hz, 1H), 2.75 – 2.64 (m, 2H), 2.41 (t, <i>J</i> = 7.5 Hz, 2H), 2.34 (q, <i>J</i> = 3.8 Hz, 1H), 1.71 – 1.57 (m, 9H), 1.41 – 1.17 (m, 48H), 0.88 (t, <i>J</i> = 6.8 Hz, 6H). MS-ESI: <i>m/z</i> no signal observed (<i>M</i>+<i>H</i>)⁺
10		¹H NMR (400 MHz, DMSO-<i>d</i>₆) δ 8.34 (s, 1H), 7.91 (d, <i>J</i> = 30.8 Hz, 2H), 6.34 (t, <i>J</i> = 6.9 Hz, 1H), 5.70 (t, <i>J</i> = 6.4 Hz, 1H), 4.39 (d, <i>J</i> = 11.6 Hz, 1H), 4.22 (d, <i>J</i> = 11.9 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.14 (dt, <i>J</i> = 14.1, 6.9 Hz, 1H), 2.60 (dt, <i>J</i> = 12.9, 6.0 Hz, 1H), 2.39 (td, <i>J</i> = 7.3, 2.4 Hz, 2H), 2.32 – 2.17 (m, 2H), 1.64 – 1.52 (m, 2H), 1.51 – 1.39 (m, 2H), 1.38 – 1.11 (m, 48H), 0.92 – 0.78 (m, 6H). MS-ESI: <i>m/z</i> no signal observed (<i>M</i>+<i>H</i>)⁺

Cpd #	Structure	Characterization Data (NMR and LCMS)
11		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.33 (s, 1H), 7.91 (d, <i>J</i> = 31.7 Hz, 2H), 6.34 (t, <i>J</i> = 6.7 Hz, 1H), 5.70 (t, <i>J</i> = 6.3 Hz, 1H), 4.40 (d, <i>J</i> = 11.6 Hz, 1H), 4.21 (d, <i>J</i> = 11.6 Hz, 1H), 3.82 – 3.78 (m, 1H), 3.14 (dt, <i>J</i> = 13.8, 6.9 Hz, 1H), 2.61 (dt, <i>J</i> = 13.2, 6.2 Hz, 1H), 2.39 (td, <i>J</i> = 7.4, 2.9 Hz, 2H), 2.32 – 2.15 (m, 2H), 1.63 – 1.51 (m, 2H), 1.50 – 1.37 (m, 2H), 1.35 – 1.13 (m, 12H), 0.91 – 0.76 (m, 6H). MS-ESI: <i>m/z</i> 518.25 observed (M+H) ⁺
12		MS-ESI: <i>m/z</i> 492.64 observed (M+H) ⁺
13		MS-ESI: <i>m/z</i> 492.55 observed (M+H) ⁺
14		MS-ESI: <i>m/z</i> 408.3 observed (M+H) ⁺ Aqueous solubility (pH 7.4): >106 mg/mL (HCl salt)

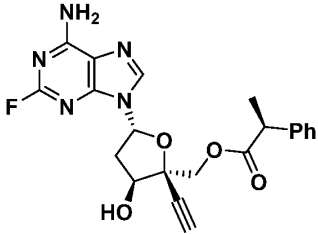
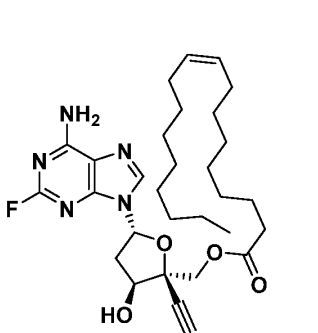
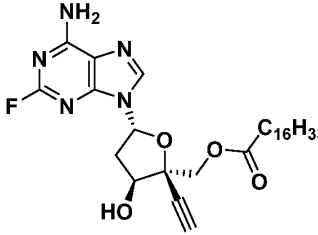
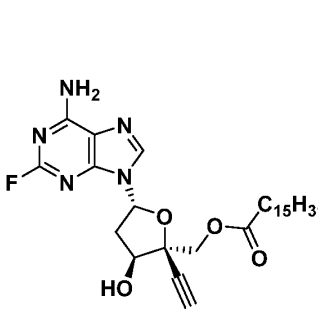
Cpd #	Structure	Characterization Data (NMR and LCMS)
15		MS-ESI: m/z 436.5 observed (M+H) ⁺
16		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.35 (s, 1H), 7.93 (d, J = 35.7 Hz, 2H), 6.32 (t, J = 7.2 Hz, 1H), 5.64 – 5.50 (m, 2H), 3.73 – 3.66 (m, 2H), 3.66 – 3.55 (m, 1H), 3.01 (dt, J = 14.4, 7.3 Hz, 1H), 2.67 – 2.57 (m, 1H), 2.55 – 2.51 (m, 1H), 1.16 (ddd, J = 11.3, 7.1, 1.7 Hz, 6H). MS-ESI: m/z 364.53 observed (M+H) ⁺
17		MS-ESI: m/z 392.47 observed (M+H) ⁺
18		¹ H NMR (400 MHz, Methanol- <i>d</i> ₄) δ 8.30 (s, 1H), 6.45 (t, J = 7.1 Hz, 1H), 5.70 (dd, J = 6.8, 3.3 Hz, 1H), 3.94 – 3.80 (m, 2H), 3.21 – 3.17 (m, 1H), 3.03 (dt, J = 14.1, 7.3 Hz, 1H), 2.90 (p, J = 8.3 Hz, 1H), 2.63 (ddt, J = 12.4, 5.8, 2.7 Hz, 1H), 2.04 – 1.87 (m, 4H), 1.79 – 1.60 (m, 4H). MS-ESI: m/z no signal observed (M+H) ⁺

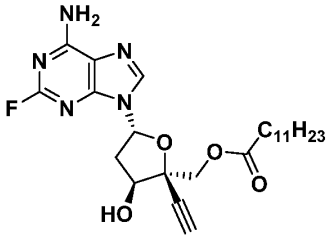
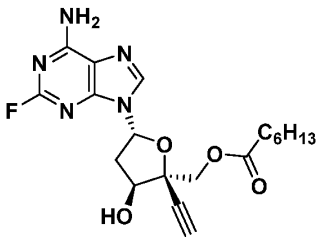
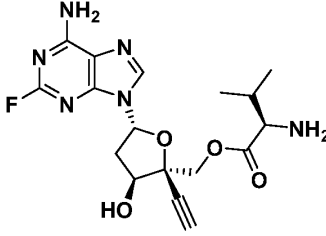
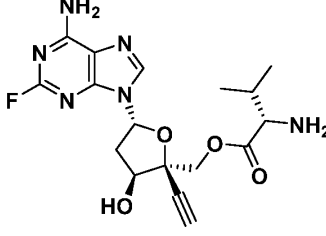
Cpd #	Structure	Characterization Data (NMR and LCMS)
19		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.35 (s, 1H), 7.93 (d, <i>J</i> = 35.1 Hz, 2H), 7.40 – 7.20 (m, 5H), 6.33 (t, <i>J</i> = 7.0 Hz, 1H), 5.59 (t, <i>J</i> = 6.3 Hz, 2H), 3.85 – 3.71 (m, 2H), 3.69 (dd, <i>J</i> = 11.8, 5.6 Hz, 1H), 3.65 – 3.63 (m, 1H), 3.59 (dd, <i>J</i> = 11.9, 7.0 Hz, 1H), 3.01 (dt, <i>J</i> = 14.4, 7.3 Hz, 1H), 2.58 – 2.52 (m, 1H). MS-ESI: <i>m/z</i> 412.43 observed (M+H) ⁺
20		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.34 (s, 1H), 7.92 (s, 2H), 7.41 – 7.23 (m, 5H), 6.30 (t, <i>J</i> = 7.0 Hz, 1H), 5.59 – 5.49 (m, 2H), 3.87 (q, <i>J</i> = 7.1 Hz, 1H), 3.64 (dd, <i>J</i> = 11.9, 5.4 Hz, 1H), 3.57 – 3.47 (m, 1H), 3.01 (dt, <i>J</i> = 14.2, 7.2 Hz, 1H), 2.58 – 2.54 (m, 1H), 1.49 (d, <i>J</i> = 7.2 Hz, 3H). MS-ESI: <i>m/z</i> 426.56 observed (M+H) ⁺
21		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.39 (s, 1H), 8.11 – 8.04 (m, 2H), 7.94 (d, <i>J</i> = 35.1 Hz, 2H), 7.71 (dddd, <i>J</i> = 8.2, 7.3, 2.7, 1.3 Hz, 1H), 7.62 – 7.51 (m, 2H), 6.45 (t, <i>J</i> = 7.2 Hz, 1H), 5.87 – 5.75 (m, 1H), 5.71 – 5.61 (m, 1H), 3.82 – 3.63 (m, 3H), 3.13 (dt, <i>J</i> = 14.2, 7.1 Hz, 1H), 2.77 – 2.65 (m, 1H). MS-ESI: <i>m/z</i> 398.47 observed (M+H) ⁺
22		¹ H NMR (400 MHz, Methanol- <i>d</i> ₄) δ 8.29 (s, 1H), 6.49 – 6.41 (m, 1H), 5.73 (dd, <i>J</i> = 6.9, 3.4 Hz, 1H), 3.90 (d, <i>J</i> = 12.1 Hz, 1H), 3.84 (d, <i>J</i> = 12.1 Hz, 1H), 3.17 (s, 1H), 3.03 (dt, <i>J</i> = 14.4, 7.3 Hz, 1H), 2.63 (ddd, <i>J</i> = 13.8, 6.2, 3.4 Hz, 1H), 2.46 (t, <i>J</i> = 7.5 Hz, 2H), 1.75 – 1.57 (m, 3H), 1.33 – 1.28 (m, 25H), 0.92 (t, <i>J</i> = 6.7 Hz, 3H).

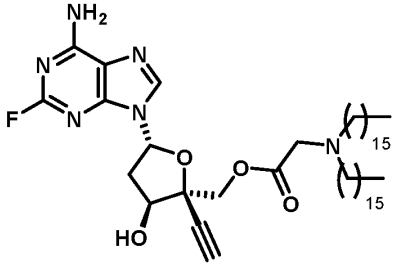
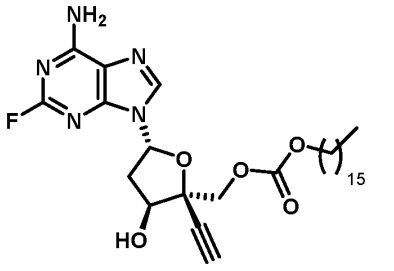
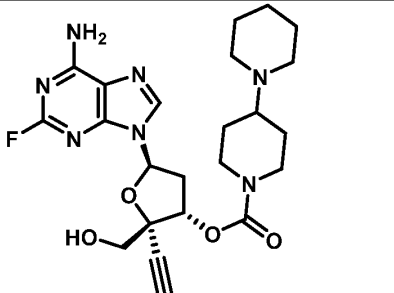
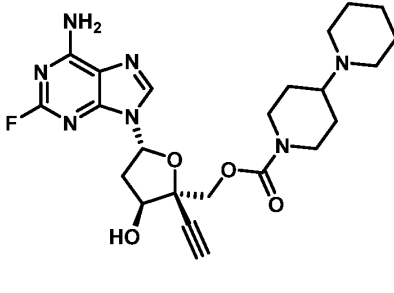
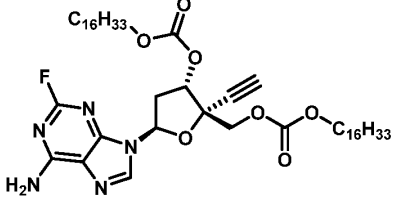
Cpd #	Structure	Characterization Data (NMR and LCMS)
		MS-ESI: <i>m/z</i> no signal observed (M+H) ⁺
23		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.35 (s, 1H), 7.93 (d, <i>J</i> = 38.4 Hz, 2H), 6.31 (t, <i>J</i> = 7.2 Hz, 1H), 5.63 – 5.54 (m, 2H), 3.75 – 3.64 (m, 2H), 3.60 (dd, <i>J</i> = 11.9, 7.1 Hz, 1H), 3.00 (dt, <i>J</i> = 14.4, 7.3 Hz, 1H), 2.55 – 2.51 (m, 1H), 2.46 – 2.31 (m, 2H), 1.64 – 1.50 (m, 2H), 1.36 – 1.16 (m, 24H), 0.89 – 0.81 (m, 3H). MS-ESI: <i>m/z</i> 532.74 observed (M+H) ⁺
24		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.35 (s, 1H), 7.93 (d, <i>J</i> = 37.5 Hz, 2H), 6.31 (t, <i>J</i> = 7.2 Hz, 1H), 5.64 – 5.54 (m, 2H), 3.74 – 3.65 (m, 2H), 3.60 (dd, <i>J</i> = 11.9, 7.0 Hz, 1H), 3.00 (dt, <i>J</i> = 14.2, 7.2 Hz, 1H), 2.56 – 2.51 (m, 1H), 2.46 – 2.31 (m, 2H), 1.64 – 1.50 (m, 2H), 1.37 – 1.21 (m, 6H), 0.92 – 0.80 (m, 3H). MS-ESI: <i>m/z</i> 406.55 observed (M+H) ⁺
25		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.35 (s, 1H), 7.93 (d, <i>J</i> = 31.1 Hz, 2H), 6.33 (t, <i>J</i> = 7.0 Hz, 1H), 5.59 (dd, <i>J</i> = 6.6, 3.8 Hz, 2H), 3.74 – 3.65 (m, 2H), 3.61 (dd, <i>J</i> = 11.9, 6.8 Hz, 1H), 3.20 (d, <i>J</i> = 5.3 Hz, 1H), 3.09 – 2.99 (m, 4H), 2.00 – 1.92 (m, 1H), 0.93 (d, <i>J</i> = 6.7 Hz, 3H), 0.88 (d, <i>J</i> = 6.7 Hz, 3H). MS-ESI: <i>m/z</i> 393.52 observed (M+H) ⁺

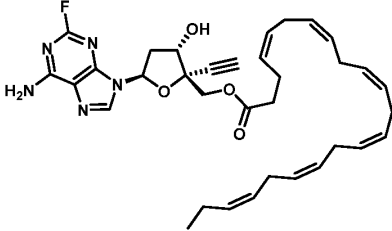
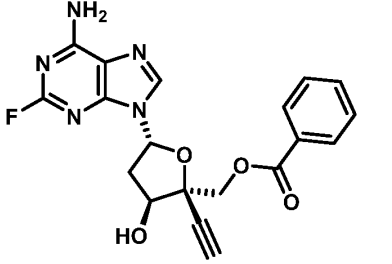
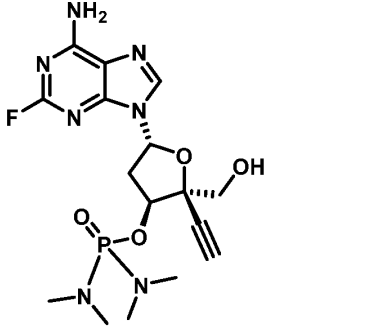
Cpd #	Structure	Characterization Data (NMR and LCMS)
26		MS-ESI: m/z 393.48 observed (M+H) ⁺
27		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.28 (s, 1H), 7.86 (s, 2H), 6.24 (dd, <i>J</i> = 8.0, 4.0 Hz, 1H), 5.80 (d, <i>J</i> = 5.4 Hz, 1H), 4.70 (q, <i>J</i> = 7.4 Hz, 1H), 4.39 (d, <i>J</i> = 11.7 Hz, 1H), 4.09 (d, <i>J</i> = 11.7 Hz, 1H), 3.65 (d, <i>J</i> = 1.0 Hz, 1H), 2.77 (ddd, <i>J</i> = 12.1, 7.2, 4.0 Hz, 1H), 2.47 – 2.43 (m, 1H), 1.94 (s, 3H). MS-ESI: m/z 336.42 observed (M+H) ⁺
28		MS-ESI: m/z 350.49 observed (M+H) ⁺
29		MS-ESI: m/z 364.57 observed (M+H) ⁺

Cpd #	Structure	Characterization Data (NMR and LCMS)
30		MS-ESI: m/z 364.49 observed (M+H) ⁺
31		MS-ESI: m/z 392.48 observed (M+H) ⁺
32		MS-ESI: m/z 390.3 observed (M+H) ⁺
33		MS-ESI: m/z 350.18 observed (M+H) ⁺
34		MS-ESI: m/z 412.43 observed (M+H) ⁺

Cpd #	Structure	Characterization Data (NMR and LCMS)
35		MS-ESI: m/z 426.54 observed (M+H) ⁺
36		¹ H NMR (499 MHz, DMSO- <i>d</i> ₆) δ 8.26 (s, 1H), 7.84 (s, 2H), 6.24 (dd, J = 8.0, 4.0 Hz, 1H), 5.78 (d, J = 5.5 Hz, 1H), 5.35 – 5.26 (m, 2H), 4.74 – 4.66 (m, 1H), 4.41 (d, J = 11.7 Hz, 1H), 4.09 (d, J = 11.8 Hz, 1H), 3.62 (s, 1H), 2.78 (ddd, J = 13.4, 7.3, 4.0 Hz, 1H), 2.18 (ddt, J = 30.6, 15.6, 7.8 Hz, 2H), 1.96 (p, J = 6.2 Hz, 5H), 1.45 – 1.35 (m, 2H), 1.31 – 1.14 (m, 19H), 0.87 – 0.82 (m, 3H). MS-ESI: m/z no signal observed (M+H) ⁺
37		MS-ESI: m/z 546.67 observed (M+H) ⁺
38		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.27 (s, 1H), 7.84 (s, 2H), 6.23 (dd, J = 8.0, 3.9 Hz, 1H), 5.79 (d, J = 4.9 Hz, 1H), 4.70 (q, J = 7.3 Hz, 1H), 4.41 (d, J = 11.7 Hz, 1H), 4.08 (d, J = 11.7 Hz, 1H), 3.63 (s, 1H), 2.78 (ddd, J = 13.3, 7.3, 4.0 Hz, 1H), 2.44 (d, J = 8.0 Hz, 1H), 2.28 – 2.06 (m, 2H), 1.45 – 1.34 (m, 2H), 1.29 – 1.11 (m, 25H), 0.85 (t, J = 6.6 Hz, 3H). MS-ESI: m/z no signal observed (M+H) ⁺

Cpd #	Structure	Characterization Data (NMR and LCMS)
39		¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ 8.26 (s, 1H), 7.82 (s, 2H), 6.24 (dd, <i>J</i> = 8.0, 4.0 Hz, 1H), 5.77 (d, <i>J</i> = 5.4 Hz, 1H), 4.70 (td, <i>J</i> = 7.7, 5.4 Hz, 1H), 4.41 (d, <i>J</i> = 11.8 Hz, 1H), 4.09 (d, <i>J</i> = 11.8 Hz, 1H), 3.61 (s, 1H), 2.78 (ddd, <i>J</i> = 13.5, 7.3, 4.0 Hz, 1H), 2.48 – 2.43 (m, 1H), 2.18 (ddt, <i>J</i> = 30.5, 15.6, 7.8 Hz, 2H), 1.46 – 1.35 (m, 2H), 1.29 – 1.13 (m, 16H), 0.85 (t, <i>J</i> = 6.9 Hz, 3H). MS-ESI: <i>m/z</i> 476.66 observed (M+H) ⁺
40		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.27 (s, 1H), 7.84 (s, 2H), 6.24 (dd, <i>J</i> = 8.0, 4.0 Hz, 1H), 5.78 (d, <i>J</i> = 5.4 Hz, 1H), 4.71 (td, <i>J</i> = 7.8, 5.5 Hz, 1H), 4.42 (d, <i>J</i> = 11.7 Hz, 1H), 4.09 (d, <i>J</i> = 11.8 Hz, 1H), 3.63 (s, 1H), 2.79 (ddd, <i>J</i> = 13.4, 7.3, 4.0 Hz, 1H), 2.49 – 2.42 (m, 1H), 2.26 – 2.09 (m, 2H), 1.44 – 1.33 (m, 2H), 1.25 – 1.09 (m, 6H), 0.82 (t, <i>J</i> = 7.0 Hz, 3H). MS-ESI: <i>m/z</i> 406.57 observed (M+H) ⁺
41		MS-ESI: <i>m/z</i> 415.47 observed (M+Na) ⁺
42		MS-ESI: <i>m/z</i> 393.32 observed (M+H) ⁺

Cpd #	Structure	Characterization Data (NMR and LCMS)
43		MS-ESI: m/z 797.4 observed (M-H) ⁻
44		MS-ESI: m/z 562.8 observed (M+H) ⁺
45		MS-ESI: m/z 488.6 observed (M+H) ⁺
46		MS-ESI: m/z 488.7 observed (M+H) ⁺
47		MS-ESI: m/z= 828.4 observed (M-H) ⁻

Cpd #	Structure	Characterization Data (NMR and LCMS)
48		MS-ESI: m/z 604.8 observed (M+H) +
49		MS-ESI: m/z 398.5 observed (M+H) +
50		MS-ESI: m/z 428.3 observed (M+H) ⁺

EXAMPLES

Abbreviations

The following abbreviations are used: tetrahydrofuran (THF), dichloromethane (DCM), Acetonitrile (MeCN), *N,N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), trifluoroacetic acid (TFA), triethylamine (TEA), diisopropylethylamine (DIPEA), methanol (MeOH), Ethyl acetate (EtOAc), 4-Dimethylaminopyridine (DMAP), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC•HCl), *N,N'*-Dicyclohexylcarbodiimide (DCC).

General Examples for the Preparation of Compounds of the Invention

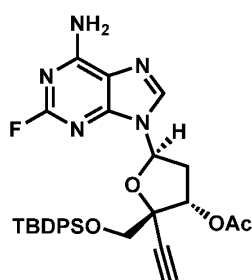
The starting materials and intermediates for the compounds of this invention may be prepared by the application or adaptation of the methods described below, their obvious

chemical equivalents, or, for example, as described in literature such as The Science of Synthesis, Volumes 1-8. Editors E. M. Carreira et al. Thieme publishers (2001-2008). Details of reagent and reaction options are also available by structure and reaction searches using commercial computer search engines such as Scifinder (www.cas.org) or Reaxys (www.reaxys.com).

Part I: Preparation of Intermediates and EFdA (MK-8591)

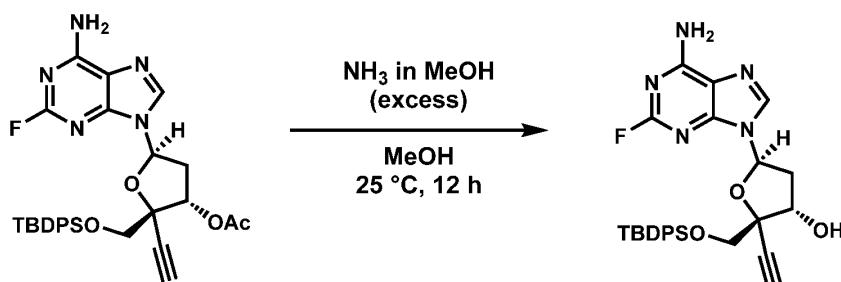
Example 1

Synthesis of intermediate-A, -B and Compound 1 (EFdA):



Intermediate -A

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-yl acetate, **A**: Intermediate A is prepared according to the reported procedure in the literature (*Org. Lett.* **2011**, *13*, 5264-5266.). LC-MS (ESI⁺): *m/z* 574.19 [M+H]⁺.



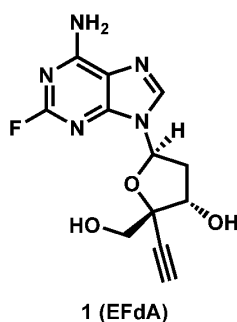
Intermediate -A

Intermediate -B

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-ol, **B**: To a solution of intermediate A (500 mg, 0.87 mmol) in MeOH (10mL) was added methanolic ammonia (10mL) at rt and stirred for 12h. The reaction mixture was concentrated under reduced pressure to obtained crude material and the resulting

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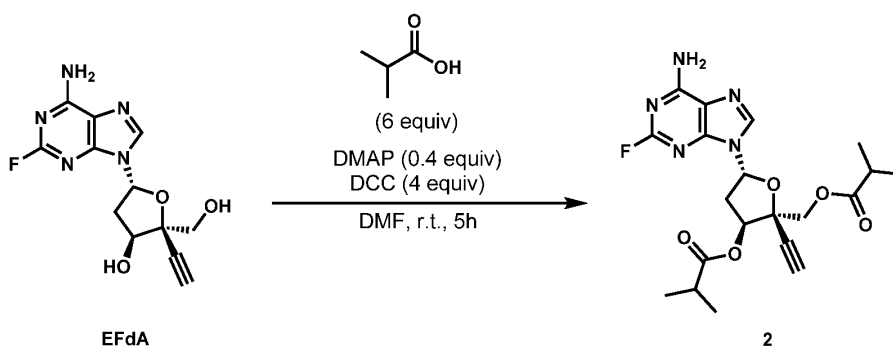
crude material was purified by silica-gel column chromatography using 2% MeOH in DCM to obtain the intermediate **B** (400 mg, 86.33%). LC-MS (ESI+): m/z 532.4 $[M+H]^+$.



(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-(hydroxymethyl) tetrahydrofuran-3-ol, 1: Compound **1** (EFdA) is prepared according to the reported procedure in the literature (*Org. Lett.* **2011**, *13*, 5264-5266.). ^1H NMR (400 MHz, DMSO- d_6) δ 8.30 (s, 1H), 7.86 (d, $J = 27.7$ Hz, 2H), 6.24 (dd, $J = 7.4, 5.0$ Hz, 1H), 5.63 – 5.54 (m, 1H), 5.35 – 5.25 (m, 1H), 4.56 (q, $J = 6.4$ Hz, 1H), 3.67 – 3.60 (m, 1H), 3.60 – 3.49 (m, 2H), 2.74 – 2.63 (m, 1H), 2.46 – 2.36 (m, 1H). LC-MS (ESI+): m/z 294.2 $[M+H]^+$.

Part II: Preparation of Example Compounds

Example 2

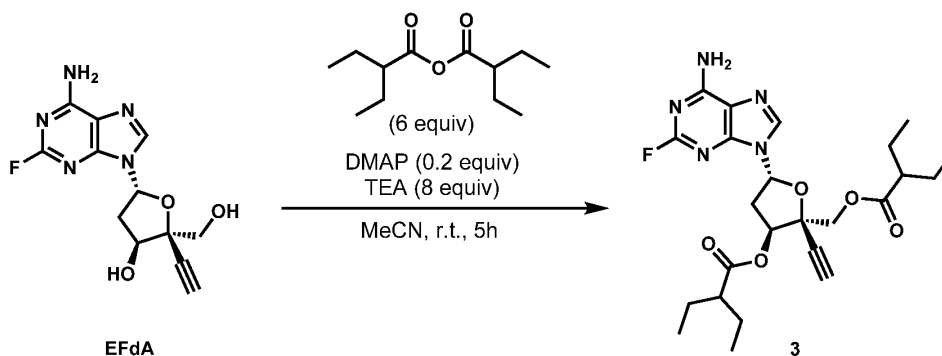


(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((isobutyryloxy)methyl) tetrahydrofuran-3-yl isobutyrate, 2: To a mixture of EFdA (3 g, 6.8 mmol, 1 equiv.), DMAP (499 mg, 2.73 mmol, 0.4 equiv.) in anhydrous DMF (100 mL) was added isobutyric acid (8.4 g, 27.3 mmol, 6 equiv.) dropwise at ambient temperature. The reaction stirred for 5 h at rt. The reaction is monitored by LCMS due to the occasional alkylation of NH_2 group

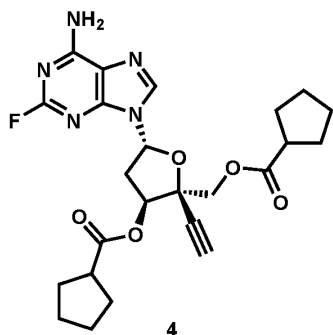
- 20 -

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 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% EtOAc in Hexanes to obtain the compound **2** as a glassy solid. The obtained solid was dispersed in minimum amount of isopropanol followed by its rotatory evaporation to obtain pure compound as a white solid (2.5 g, 85% yield). LC-MS (ESI+): m/z 434.49 [M+H]⁺.

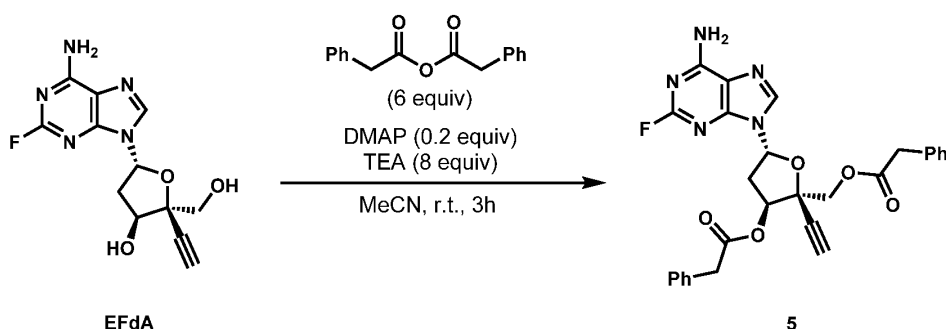
Example 3



(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((2-ethylbutanoyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-yl 2-ethylbutanoate, 3: To a mixture of EFdA (1 g, 3.4 mmol, 1 equiv.), 2-ethylbutanoic anhydride (4.4 g, 20.4 mmol, 6 equiv.), TEA (3.8 ml, 27.2 mmol, 8 equiv.) in anhydrous MeCN (43 mL) and cooled to 0 °C was added DMAP (83 mg, 0.68 mmol, 0.2 equiv.) at 0 °C. The reaction stirred for 0.5 h at 0 °C and then to 5 h at rt. The reaction is monitored by LCMS due to the occasional alkylation of NH₂ group observed for elongated reaction time. The reaction was further quenched with methanol and solvent was evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 60-70% EtOAc in Hexanes to obtain the compound **3** as a glassy solid. The obtained solid was dispersed in minimum amount of isopropanol followed by its rotatory evaporation to obtain pure compound as a white solid (1.33 g, 80% yield). LC-MS (ESI+): m/z 490.56 [M+H]⁺.

Example 4

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-(((cyclopentanecarbonyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-yl cyclopentanecarboxylate, 4: Compound **4** was prepared by using the procedure followed for the compound **2**. LC-MS (ESI⁺): m/z 486.44 [M+H]⁺.

Example 5

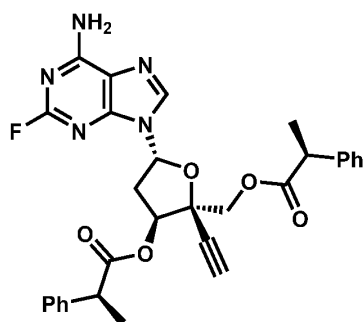
(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((2-phenylacetoxy)methyl)tetrahydrofuran-3-yl 2-phenylacetate, 5: To a mixture of EFdA (499 mg, 1.7 mmol, 1 equiv.), 2-phenylacetic anhydride (2.6 g, 10.2 mmol, 6 equiv.), TEA (1.9 ml, 13.6 mmol, 8 equiv.) in anhydrous MeCN (22 mL) and cooled to 0 °C was added 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 rt. The reaction is monitored by LCMS due to the occasional alkylation of NH₂ group observed for elongated reaction time. The reaction was further quenched with methanol and solvent was evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 60-70% EtOAc in Hexanes to obtain the compound **5** as a glassy solid. The obtained solid was dispersed in minimum amount of isopropanol followed

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by its rotatory evaporation to obtain pure compound as a white solid (694 mg, 77% yield).

LC-MS (ESI+): m/z 530.52 $[M+H]^+$.

Example 6

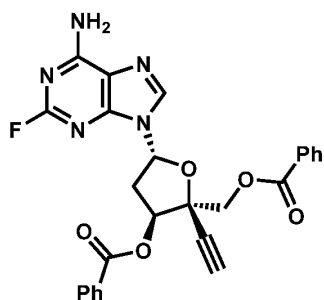


6

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-(((S)-2-phenylpropanoyl)oxy) methyl)tetrahydrofuran-3-yl (S)-2-phenylpropanoate, 6:

Compound 6 was prepared by using the procedure followed for the compound 2 with following differences: a) 4 equiv of corresponding acid used instead of 6; b) After remove urea via filtration, the solvent was evaporated under reduced pressure and resulting white solid was then suspended in minimum amount of isopropanol and stirred for an hour. The suspension was then filtered to obtain pure compound as a white solid in 75 % yield. LC-MS (ESI+): m/z 558.65 $[M+H]^+$.

Example 7

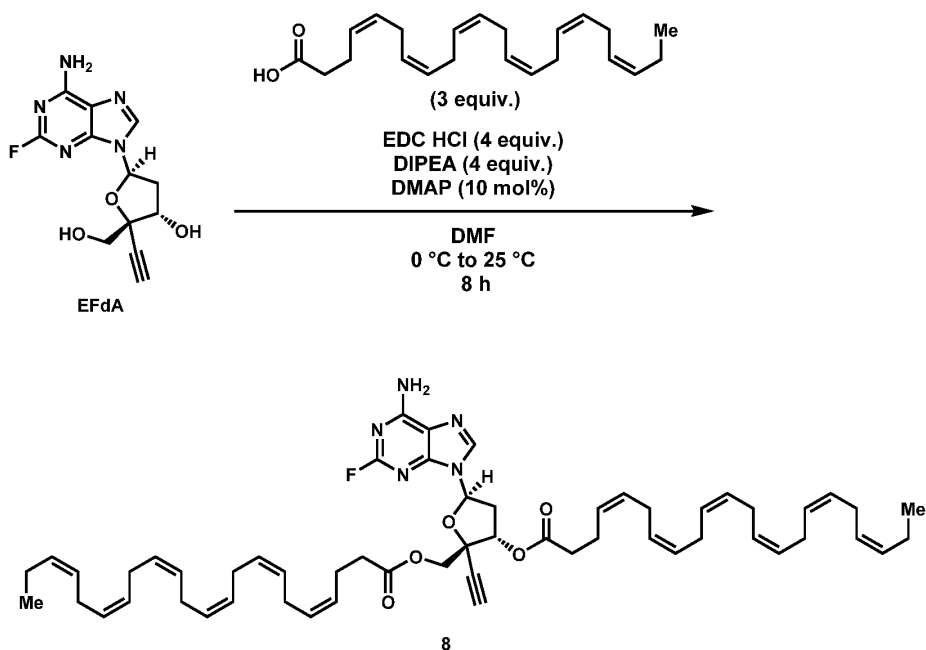


7

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((benzoyloxy)methyl)-2-ethynyl tetrahydrofuran-3-yl benzoate, 7: Compound 7 was prepared by using the procedure

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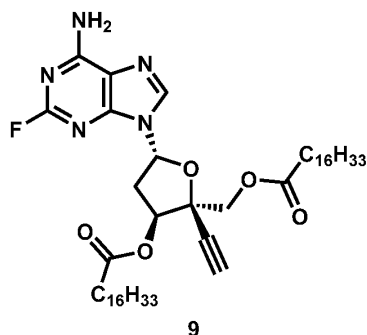
followed for the compound **2** using 4.5 equiv of corresponding acid. LC-MS (ESI+): m/z 502.41 $[M+H]^+$.

Example 8

(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-((((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)methyl)-2-ethynyltetrahydrofuran-3-yl

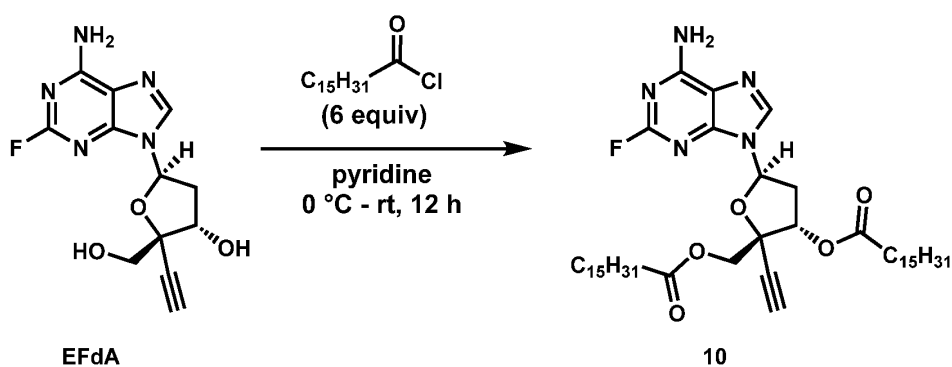
(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate, 8: To a mixture of EFdA (117 mg, 0.4 mmol, 1 equiv.), Docosahexaenoic Acid (0.39 g, 1.2 mmol, 3 equiv.), DMAP (4.9 mg, 0.04 mmol, 0.1 equiv.), EDC•HCl (307 mg, 1.6 mmol, 4 equiv.) in anhydrous DMF (4 mL) and cooled to 0 °C was added DIPEA (0.3 mL, 1.6 mmol, 4 equiv.) at 0 °C. The reaction stirred for 8 h at rt. The reaction is monitored by LCMS due to the occasional alkylation of NH_2 group observed for elongated reaction time. Thereafter, the reaction was diluted with EtOAc and washed thrice with 2.5% NaCl, thrice with buffer pH~7 solution 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 5% DCM in MeOH to obtain the compound **8** as gel-like semi solid (183 mg, 50% yield). LC-MS (ESI+): m/z 457.23 $[M/2+H]^+$.

Example 9



(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((heptadecanoyloxy)methyl)tetrahydrofuran-3-yl heptadecanoate, 9: Compound **9** was prepared by using the procedure followed for the compound **2** using 8 equiv of corresponding acid and 6 equiv of DCC. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.92 (s, 1H), 6.40 (t, *J* = 6.4 Hz, 1H), 5.76 (brs, 2H), 5.65 (dd, *J* = 7.1, 5.3 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.38 (d, *J* = 12.1 Hz, 1H), 2.99 (dt, *J* = 13.5, 6.7 Hz, 1H), 2.75 – 2.64 (m, 2H), 2.41 (t, *J* = 7.5 Hz, 2H), 2.34 (q, *J* = 3.8 Hz, 1H), 1.71 – 1.57 (m, 9H), 1.41 – 1.17 (m, 48H), 0.88 (t, *J* = 6.8 Hz, 6H). LC-MS (ESI⁺): *m/z* no signal observed.

Example 10

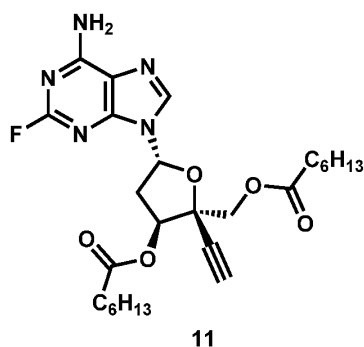


(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((palmitoyloxy)methyl)tetrahydrofuran-3-yl palmitate, 10: To a flask containing EFdA (202 mg, 0.7 mmol, 1 equiv.) was added anhydrous pyridine (7 mL). The mixture was stirred for 1-2 min and cooled to 0 °C. To this mixture was added palmitoyl chloride (1.3 mL, 4.2 mmol, 6 equiv.)

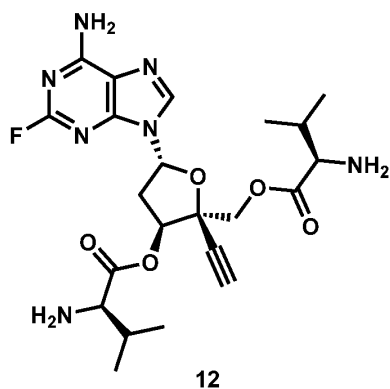
- 25 -

dropwise at 0 °C. The reaction stirred for overnight at rt. The reaction is monitored by LCMS due to the occasional alkylation of NH₂ group observed for elongated reaction time. Thereafter, the reaction was diluted with EtOAc and washed thrice with water, once with NaHCO₃ 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% EtOAc in Hexanes to obtain the compound **10** as a glassy solid. The obtained solid was dispersed in minimum amount of isopropanol followed by its rotatory evaporation to obtain pure compound as a white solid (372 mg, 70% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.34 (s, 1H), 7.91 (d, *J* = 30.8 Hz, 2H), 6.34 (t, *J* = 6.9 Hz, 1H), 5.70 (t, *J* = 6.4 Hz, 1H), 4.39 (d, *J* = 11.6 Hz, 1H), 4.22 (d, *J* = 11.9 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.14 (dt, *J* = 14.1, 6.9 Hz, 1H), 2.60 (dt, *J* = 12.9, 6.0 Hz, 1H), 2.39 (td, *J* = 7.3, 2.4 Hz, 2H), 2.32 – 2.17 (m, 2H), 1.64 – 1.52 (m, 2H), 1.51 – 1.39 (m, 2H), 1.38 – 1.11 (m, 48H), 0.92 – 0.78 (m, 6H). LC-MS (ESI+): *m/z* no signal observed.

Example 11



(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-((heptanoyloxy)methyl)tetrahydrofuran-3-yl heptanoate, 11: Compound **11** was prepared by using the procedure followed for the compound **2** using 8 equiv of corresponding acid and 6 equiv of DCC. LC-MS (ESI+): *m/z* 518.25 [M+H]⁺.

Example 12

((2R,3S,5R)-3-((D-valyl)oxy)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyltetrahydrofuran-2-yl)methyl D-valinate, 12: Compound **12** was prepared by using the procedure followed for the compound **2** using 7 equiv of corresponding boc protected amino acid and 7 equiv of DCC followed by typical deprotection of Boc group using HCl in dioxane. LC-MS (ESI+); m/z 492.64 $[M+H]^+$.

Examples 13-15

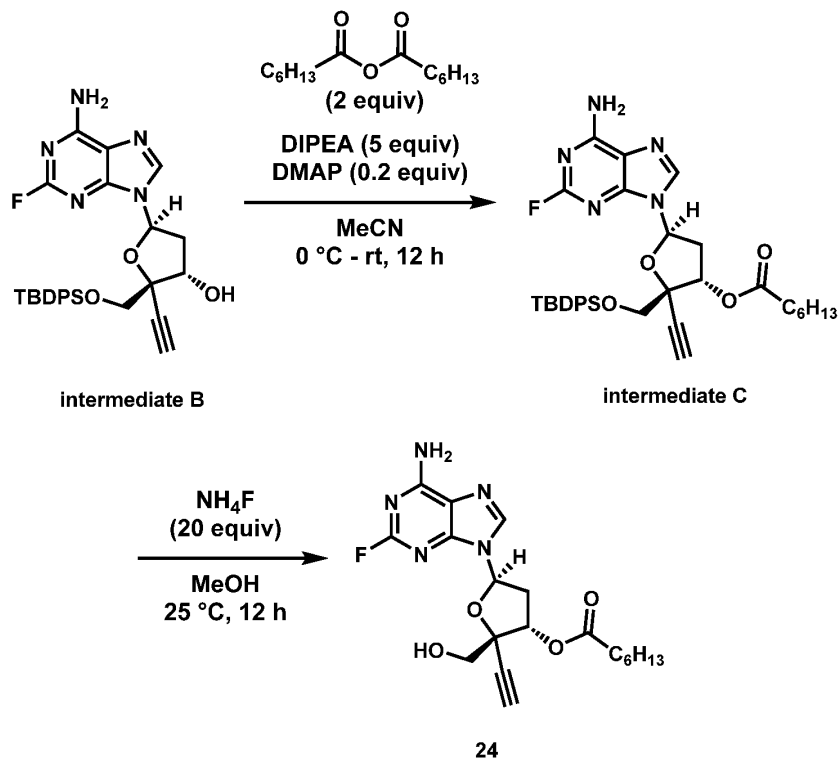
Compounds **13-15** were prepared by using the procedure followed for the compound **12**.

Examples 16-23

Compounds **16-23** were prepared by using the procedure followed for the compound **24** described below in Example 24.

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Example 24



(2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-yl heptanoate, 24:

Step-1: To a solution of intermediate **B** (400 mg, 0.75 mmol, 1 equiv.) in anhydrous MeCN (12 mL) was added DIPEA (0.6 mL, 3.77 mmol, 5 equiv.) and DMAP (18.4 mg, 0.15 mmol, 0.2 equiv.). To this stirred solution at 0 °C was added dropwise heptanoic anhydride (365 mg, 1.5 mmol, 2 equiv.) in MeCN (5 mL). The resulting mixture was then stirred at rt for 1 h. The reaction is monitored by LCMS. After completion of reaction, reaction mixture was quenched in ice cold water (20ml) and was extracted with ethyl acetate (3 X 20 mL). The combined organic layer was dried, filtered and evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 1.5% DCM in MeOH to obtain the intermediate **C** (430 mg, 88.8% yield). LC-MS (ESI+): m/z 645.00 [M+H]⁺.

Step-2: To a solution of intermediate **C** (430 mg, 0.67 mmol, 1 equiv.) in anhydrous MeOH (5 mL) was added NH₄F (495 mL, 13.35 mmol, 20 equiv.). The resulting mixture was then stirred at rt for 12 h. The reaction is monitored by LCMS. After completion of reaction,

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reaction mixture was quenched in ice cold water (20ml) and was extracted with ethyl acetate (3 X 20 mL). The combined organic layer was dried, filtered and evaporated under reduced pressure. The resulting crude material was purified by silica-gel column chromatography using 2.5% DCM in MeOH to obtain the compound **24** as an off-white solid (187 mg, 69.1% yield). LC-MS (ESI+): m/z 406.55 $[M+H]^+$.

Note: If reaction not completed after 12h stirring at rt then stirred for additional 2 h at 60 °C for other compounds including compound **24**.

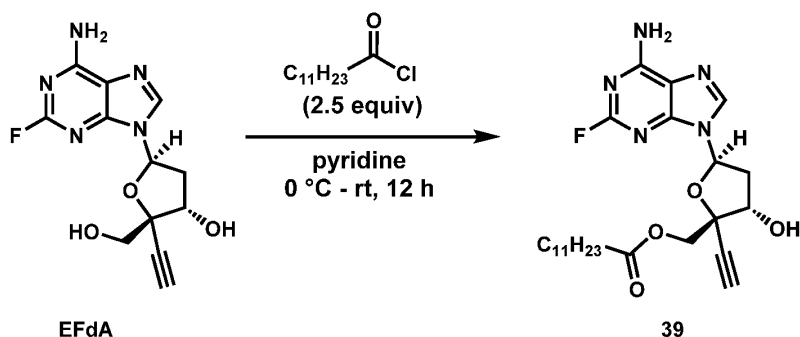
Examples 25-26

Compounds **25-26** were prepared by using the procedure followed for the compound **24** described above in Example 24.

Examples 27-38

Compounds **27-38** were prepared by using the procedure followed for the compound **39** described below in Example 39.

Example 39



((2R,3S,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl dodecanoate, 39: To a flask containing EFdA (997 mg, 3.4 mmol, 1 equiv.) was added anhydrous pyridine (34 mL). The mixture was stirred for 1-2 min and cooled to 0 °C. To this mixture was added lauroyl chloride (2.0 mL, 8.5 mmol, 2.5 equiv.) dropwise at 0 °C. The reaction stirred for overnight at rt. The reaction is monitored by LCMS due to the occasional alkylation of NH_2 group observed for elongated reaction time. Thereafter, the reaction was diluted with EtOAc and washed thrice with water, once with $NaHCO_3$ and once with brine and then the solvent was dried, filtered and evaporated under reduced pressure.

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The resulting crude material was purified by silica-gel column chromatography using using 1.5% DCM in MeOH to obtain the compound **39** as an off-white solid (1.0 g, 65% yield).

LC-MS (ESI+): m/z 476.66 [M+H]⁺.

Examples 40-42

Compounds **40-42** were prepared by using the procedure followed for the compound **39** described above in Example 39.

Examples 43-50

Compound **43** can be prepared by using the procedure followed for the compound **39** using the acid chloride of known acid dihexadecylglycine.

Compound **44** can be prepared by the reaction between EFdA and the corresponding chloroformate in the pyridine as the solvent.

Compounds **45-50** can be prepared following procedures similar to those of the other examples described herein.

Synthesis of Stable Crystalline Forms

Example 51

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 EtOAc, MeOH and THF 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.

Example 52

Compound **1** (EFdA) was grinded and sieved through #80. 300 mg of **1** was taken in suitable container and a solution of preformed 0.25% sodium carboxymethyl cellulose (Sodium CMC) and 0.1% polyoxyethylene (20) sorbitan monooleate (TWEEN-80) was added to obtain 1 gram (300 mg of **1** + ~700 mg of polymer solution) of the final formulation (~300 mg/g). The suspension was then bath sonicated for 10 min in an Ice bath. The density of

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formulation of is 1.064 g/mL. Henceforth, the above formulation suspension will be 319.2 mg/mL concentration of compound **1** (EFdA).

Example 53

Recrystallized Compound **2** was grinded and sieved through #80. 250 mg of **1** was taken in suitable container and a solution of preformed 0.25% Sodium CMC and 0.1% TWEEN-80 was added to obtain 1 gram (250 mg of **1** + ~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 54

Compound **3** was grinded and sieved through #80. 250 mg of **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 **3** + ~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). The density of formulation of is 1.004 g/mL. Henceforth, the above formulation suspension will be 250.88 mg/mL concentration of compound **3** (~ 150 mg/mL of EFdA).

Example 55

Compound **5** was grinded and sieved through #80. 200 mg of **5** 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 (200 mg of **5** + ~800 mg of polymer solution) of the final formulation (~ 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 formulation of is 1.047 g/mL. Henceforth, the above formulation suspension will be 209.4 mg/mL concentration of compound **5** (~ 115.97 mg/mL of EFdA).

Pharmacokinetic Studies

1. 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 Co. 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 are no known contaminants in the diet or water that, at the levels of detection, is expected to interfere with the purpose, conduct or outcome of the study.

2. Dose Formulation

SC Formulation: The formulations were prepared according to the procedure given in Examples 52-55 and Table 2-4. The formulations were prepared on the day of dosing.

Animals were dosed within four hours after the formulation is prepared. Two 20 µL aliquots of each formulation were removed from each of the formulation solutions, transferred into 1.5 mL of polypropylene microcentrifuge tubes and run dose validation by LC/UV or LC-MS/MS.

3. Dose Administration

For SC dosing, the dose formulation was administered via subcutaneous injection following facility SOPs.

4. 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 4µL of K₂EDTA (0.5M) as anti-coagulant and placed on wet ice until processed for plasma.

5. 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.

6. Sample Analysis

Dose formulation concentration verification

- Aliquots of the formulations were collected in the middle position of each dose formulation in duplicate
- The concentrations of the test compound in dose formulation samples were determined by the LC/UV or LC/MS/MS method

Bioanalytical method and sample analysis

- LC-MS/MS methods for the quantitative determination of test compound in corresponded biological matrix was developed under non-GLP compliance.
- A calibration curve with 8 non-zero calibration standards was applied for the method including LLOQ.
- A set of 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.

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Sensitivity: the LLOQ will be tried to 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 $\leq$ LLOQ. If the carryover couldn't meet the criteria, then the percent of carryover should be estimated following in-house bioanalytical SOP.

7. 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} , $T_{1/2}$, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, $MRT_{(0-t)}$, $MRT_{(0-inf)}$, %F and graphs of plasma concentration versus time profile were reported.

Example 56

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

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

Table 2

	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
Formulation	0.5% CMC- Na/ 0.5%TWEEN- 80	0.5% CMC- Na/ 0.5%TWEEN- 80	0.5% CMC- Na/ 0.5%TWEEN- 80	0.5% CMC- Na/ 0.5%TWEEN- 80
T_{1/2} (h)	1.3 ± 0.2	17 ± 4	NA	12 ± 4
MRT_{0-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)	11548 ± 3173	1795 ± 337	287 ± 177	968 ± 374
AUC_{0-last} (nM*h)	22528 ± 1680	19771 ± 1219	6475 ± 2909	18516 ± 4409
AUC_{0-inf} (nM*h)	23235 ± 2000	22745 ± 748	NA	19993 ± 3525

Example 57

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

Table 3 shows the rat PK data for compounds **1** and **3** following SC administration at 10 mg/kg and 100 mg/kg equivalent dose of EFdA at high concentration formulation. The data are shown in graphic form in Figure 11.

Table 3

	SC Comp 3	SC Comp 3	SC Comp 1 (EFdA)	SC Comp 1 (EFdA)
Dose of EFdA (mg/kg)	10	100	100	10
Formulation	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	53 ± 8	1.3 ± 0.2
MRT_{0-last} (h)	20 ± 4	456 ± 17	59 ± 20	1.6 ± 0.1
T_{max} (h)	4 ± 2.3	312 ± 0	1 ± 0	0.5
C_{max} (nM)	287 ± 177	66 ± 21	7429 ± 1584	11548 ± 3173
AUC_{0-last} (nM*h)	6,475 ± 2909	38306 ± 101	156462 ± 19	22528 ± 1680
AUC_{0-inf} (nM*h)	NA	52531 ± 39	156462 ± 19	23235 ± 2000

Example 58

After rat PK analysis, the focus was shifted to non-rodents i.e. rhesus macaque. Prodrug **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 suspension formulations were derived from 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 prodrug **5** and EFdA respectively. Prodrugs **5** exhibited plasma levels of EFdA above LLOQ for more than a month with 24-fold lower C_{max} than EFdA itself.

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

Table 4

	SC Comp 1 (EFdA)	SC Comp 5
Dose of EFdA (mg/kg)	50	50
Formulation	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)	69 ± 14	ND
MRT_{0-last} (h)	76 ± 36	350 ± 25
T_{max} (h)	4 ± 3.6	264 ± 125
C_{max} (nM)	3060 ± 1058	128 ± 52
C_{28days} (nM)	1.4 ± 0.3	73 ± 29
AUC_{0-last} (nM*h)	135294 ± 22629	61934 ± 27834
AUC_{0-inf} (nM*h)	135429 ± 22675	ND