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Identification, synthesis and characterization of new impurities in tenofovir

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A detailed impurity study was conducted on tenofovir, (*R*)-({[1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid (**1**), which is the key starting material of manufacturing the active pharmaceutical ingredient (API) tenofovir disoproxil fumarate (**2**) based on a recently reported procedure. The major impurities generated in the production of tenofovir (**1**) have been synthesized, characterized and confirmed. The possible formation mechanisms of these impurities were elucidated herein, which would help to understand the process. In addition, this work will also improve the quality control during manufacturing tenofovir and tenofovir disoproxil fumarate (**2**).

1. Introduction

(*R*)-({[1-(6-Amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl)-phosphonic acid (tenofovir, **1**) as a nucleoside analogue reverse transcriptase inhibitor (nRTI), exhibits *anti*-HIV effects in humans when dosed by subcutaneous injections (Deeks et al. 1998). Further research showed that the initial form of tenofovir had limited potential for widespread use because it could not be absorbed when administered orally. Tenofovir disoproxil fumarate (**2**, Viread®), was then developed as a prodrug, which could be synthesized simply from tenofovir with subsequent formation of a pharmaceutical salt (Arimilli et al. 1997; Naesens et al. 1998; Robbins et al. 1998; Arimilli et al. 1998). Tenofovir disoproxil fumarate was approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV/AIDS in 2001 and chronic hepatitis B in 2008 (FDA Label 2001, 2008).

During the preparation of tenofovir (**1**), four impurities were consistently detected in amounts of 0.1% or above determined by HPLC assay. An extensive literature survey revealed no information about these impurities. Since tenofovir (**1**) is one of the key starting materials for manufacturing tenofovir disoproxil fumarate (**2**), it is mandatory to identify and characterize all unknown impurities which are above 0.1% in the perspective of quality control. As a result, attempts were made to synthesize and then confirm these four impurities.

2. Investigations, results and discussion

2.1. Possible structures of unknown impurities

One of the most reliable preparation methods of tenofovir (**1**) (Ripin et al. 2010) is summarized in Scheme 1. Adenine (**3**) and (*R*)-propylene carbonate (**4**) react readily in the presence of catalytic amounts of sodium hydroxide in DMF to form alcohol **5** in high yield. The alcohol **5** is converted to phosphonate **7** by alkylation with (diethoxyphosphoryl)methyl 4-methylbenzenesulfonate (**6**) mediated by magnesium *tert*-butoxide. Then hydrolysis of **7** with bromotrimethylsilane (TMSBr) and sodium hydroxide in acetonitrile furnished tenofovir hydrate.

The crude mixture was analyzed on an electrospray ionization (ESI) mass spectrometer coupled with high performance liquid chromatography (HPLC), indicating that m/z of these impurities were 288.0892 [M + 1], 316.1210 [M + 1], 463.1760 [M + 1] and 302.1051 [M + 1] respectively. Based on the process route, the possible structures of these impurities were proposed as shown in the Table 1, and further syntheses, characterizations and confirmations were conducted.

2.2. Confirmation of impurity I

First of all, compounds **A** and **B** were considered as the possible structures of impurity **I** with m/z 316.1210 ([M + 1]). Compound **A** could be synthesized by partial hydrolysis of (*R*)-diethyl ({[1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl) phosphonate (**7**) under mild conditions, such as with 1.0-2.0 equiv. TMSBr or at a low temperature. Compound **B** could be prepared by treating tenofovir (**1**) with phosphorus oxychloride ($POCl_3$) or thionyl chloride ($SOCl_2$) followed by being quenched with excess dry methanol. Unfortunately, no match in HPLC could be found after comparing the retention times of compound **A** and **B** with impurity **I** in the crude mixture of tenofovir (**1**).

Next, compound **C** was proposed, which could be regarded to be formed by alkylating N^6-amino group of tenofovir (**1**) with ethyl halide or the like. The synthetic route to compound **C** is shown in Scheme 2. *N*-Ethyl-9*H*-purin-6-amine (**9**) was made in good yield from 6-chloro-9*H*-purine (**8**) substituted by ethylamine. A sequent selective alkylation at N-9 position of **9** went smoothly to afford (*R*)-1-[6-(ethylamino)-9*H*-purin-9-yl]propan-2-ol (**10**). Then, alkylation of alcohol **10** with (diethoxyphosphoryl)methyl 4-methylbenzenesulfonate (**6**) in the presence of magnesium *tert*-butoxide in dried DMF afforded phosphonate **11**, which was followed by hydrolysis with TMSBr to give (*R*)-[({1-[6-(ethylamino)-9*H*-purin-9-

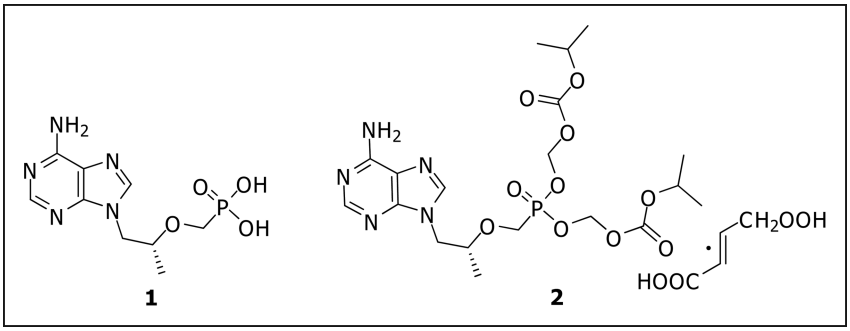


Fig. 1: Structures of tenofovir (**1**) and tenofovir disoproxil fumarate (**2**).

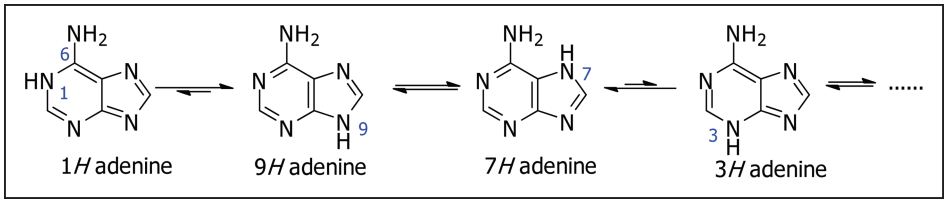
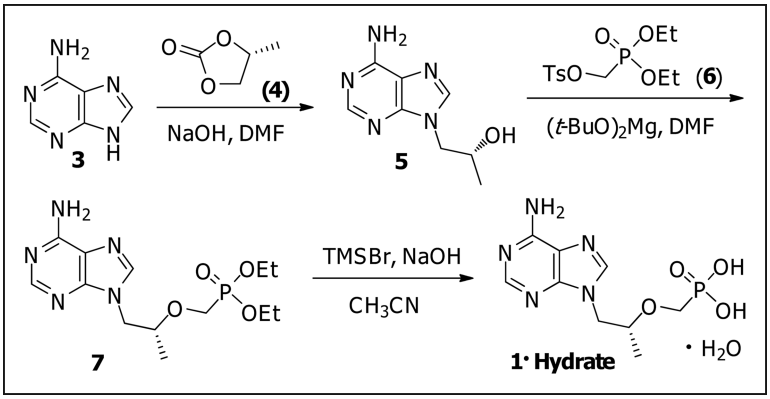
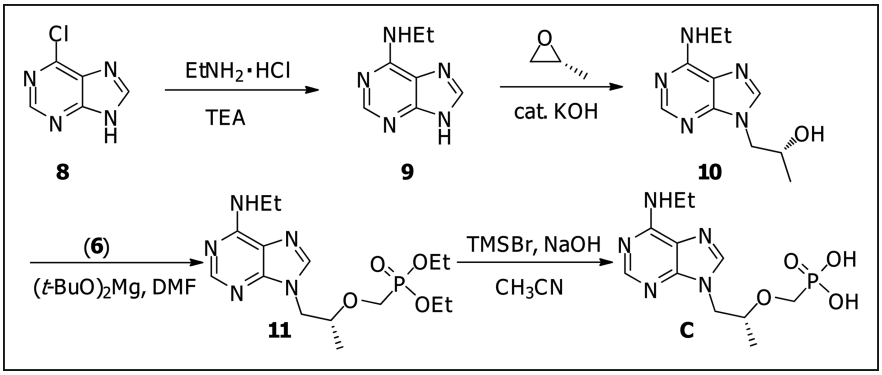


Fig. 2: Possible tautomers of adenine.



Scheme 1: Synthesis of tenofovir (**1**).



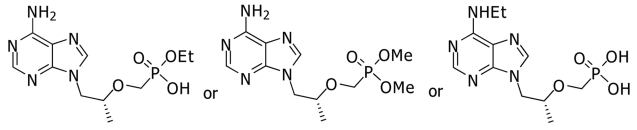
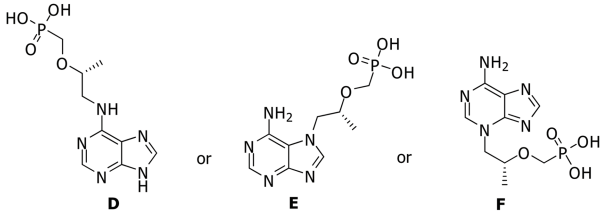
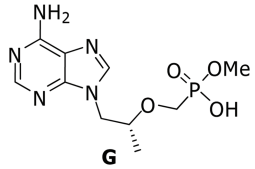
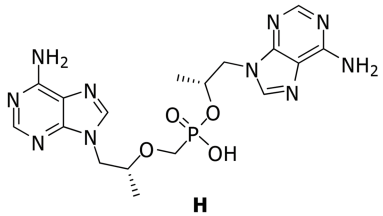
Scheme 2: Synthesis of compound **C** (impurity **I**).

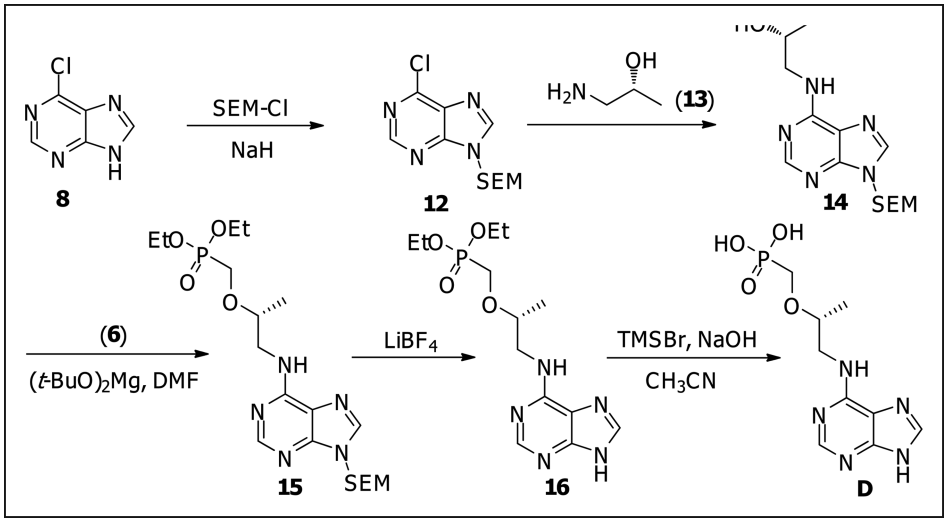
yl]propan-2-yl}oxy)methyl]phosphonic acid (**C**). A spiking study by HPLC revealed that the retention time of compound **C** was identical to impurity **I**. This impurity is possibly formed during the hydrolysis step in which the *in situ*-formed by-product ethyl bromide alkylates the N^6-amino group in the presence of the strong base NaOH.

2.3. Confirmation of impurity II

From LC-MS results, impurity **II** was supposed to be an isomer of tenofovir as both compounds have exactly the same mass. Analogous to its core structure of purine, it is known that there are a serial of tautomers for adenine, four of which are listed in (Joule and Mills 2010). The five nitrogen atoms of adenine showed different reactivities towards alkylation reagents. The N-9 position is most prone to alkylation reagents under basic conditions due to its strongest acidity. It is also known that the N-7 position can selectively respond to some alkylation reagents when the N-9 position is protected by some special functional groups, such as triphenyl methyl group *etc* (Hakimelahi et al. 2001). In addition, the N^6-amino group can easily react with some strong alkylation reagents or acylation reagents in the presence of base when the N-9 position is blocked by other groups (Joule and Mills 2010). To the best of our knowledge, there were only a few studies demonstrating alkylation at N-1 position of

Table 1: Possible structures of impurities I–IV

Name	m/z ([M+1])	Possible structures
Impurity **I**	316.1210	
Impurity **II**	288.0892	
Impurity **III**	302.1051	
Impurity **IV**	463.1760	

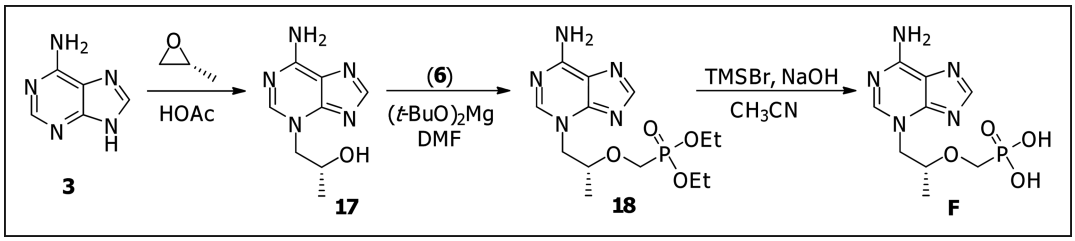


Scheme 3: Synthesis of compound **D**.

similar purines (Joule and Mills 2010). Meanwhile, very limited publication was reported about the selectivity at N-3 position (Seden et al. 1975; Lister 1971).

In order to confirm the structure of impurity **II,** compound **D** was first proposed and synthesized (Scheme 4). 6-Chloro-9*H*-purine (**8**) was protected by 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) to give intermediate **12**, which was then treated with (*R*)-1-aminopropan-2-ol (**13**) to form (*R*)-1-[(9-{[2-(trimethylsilyl)ethoxy]methyl}-9*H*-purin-6-yl)amino]propan-2-ol (**14**) (Ripin et al. 2010) in good selectivity. Further alkylation of the hydroxyl group with (diethoxyphosphoryl)methyl 4-methylbenzenesulfonate (**6**) furnished (*R*)-diethyl [({1-[(9-{[2-(trimethylsilyl)ethoxy]methyl}-9*H*-purin-6-yl)amino]propan-2-yl}oxy)methyl]phosphonate (**15**), which was then deprotected with $LiBF_4$, and hydrolyzed with TMSBr and NaOH to produce N^6-amino substituted compound (*R*)-[({1-[(9*H*-purin-6-yl)amino]propan-2-yl}oxy)methyl]phosphonic acid (**D**). However, the retention time of compound **D** did not match impurity **II** in HPLC.

On the other hand, the alkylation at N-7 and N-1 positions of adenine to obtain related derivatives failed even though various reagents were tried. Afterwards, compound **F**



Scheme 4: Synthesis of compound **F** (impurity **II**).

was proposed as impurity **II** and synthesized successfully as shown in Scheme 4. Adenine (**3**) and excess (*R*)-propylene oxide was heated in acetic acid overnight with alkylation occurring at N-3 position to give (*R*)-1-(6-amino-3*H*-purin-3-yl)propan-2-ol (**17**) as the major product, which could easily be isolated from (*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-ol (**5**) as the minor product. Alkylation of the hydroxyl group of intermediate **17** with (diethoxyphosphoryl)methyl 4-methylbenzenesulfonate (**6**), followed by hydrolysis and further purification afforded (*R*)-({[1-(6-amino-3*H*-purin-3-yl)propan-2-yl]oxy}methyl)phosphonic acid (compound **F**). Further HPLC analysis indicated that compound **F** matched impurity **II** exactly. This impurity was thus supposed to be formed due to the alkylation competition at N-9 position *vs.* N-3 position of adenine (**3**).

2.4. Confirmation of impurity III

Comparing m/z of impurity **III** with tenofovir (**1**), it was synthesized that mono-phosphonate ester **G** might be the desired impurity. Starting from intermediate **7** (R = Et) and excess sodium methoxide, the desired compound **G** could be prepared 30-40% yield after purification by flash column chromatography. In addition, synthesis of compound **G** could be further improved by partial hydrolysis of compound **B** (R = Me), and thus the isolated yield could easily be increased to 70% or above. Further HPLC analysis confirmed that the retention time of compound **G** conformed to impurity **III**. It should be caused due to incomplete hydrolysis of phosphonate **7** and then possible methanol exchange during the following recrystallization step in which MeOH was employed as the solvent.

2.5. Confirmation of impurity IV

In the manufacturing process of tenofovir (**1**), it was found that the incomplete conversion of intermediate **5** could leave residue in phosphonate **7**. Consequently it was supposed that small amount of unreacted **5** could react with tenofovir (**1**) to give monophosphonate compound **H**, which was proposed to be impurity **IV**.

The synthetic route to compound **H** is shown in Scheme 6. A direct esterification between alcohol **5** and tenofovir (**1**) was firstly conducted with heating in the presence of TMSBr. However, decomposition of tenofovir (**1**) was found to be seriously overwhelming the formation of compound **H** during the reaction. In the meanwhile, a milder esterification reaction of these two substrates was realized by employing a dehydration reagent such as *N*, *N*'-dicyclohexylcarbodiimide (DCC) (Burger and Anderson 1957; Gilmore and McBribe 1974). Fortunately as expected, the mono coupling product was found as the major product, probably due to the weak acidity of the second P-OH bond. This compound was later confirmed to be impurity **IV** by HPLC analysis. It was assumed that in the production process, excess amount of TMSBr as a strong Lewis acid might facilitate the esterification from phosphonic acid **1** and alcohol **5**.

3. Experimental

3.1. Samples and reagents

The investigated samples of tenofovir and its unknown impurities were prepared in Zhejiang Jiuzhou Pharmaceutical Science & Technology Co., Ltd. (Part of *R&D* Centre of Zhejiang Jiuzhou pharmaceutical Co., Ltd.). Reagents used for HPLC analysis were ammonium acetate (HPLC grade, Scharlab S. L., Spain), acetonitrile and methanol (HPLC grade, Merck, Germany) and milliQ water. Deuterated reagents for NMR were from Cambridge Isotope Laboratories, Inc., USA. Other reagents and solvents were of industry or analytical grade and were used as received from commercial sources without further purification.

3.2. LC-MS analysis

LC-MS analysis was carried out with an Agilent time of flight mass spectrometer coupled with Agilent G1312B binary pumps, Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm) and G1315D DAD detector. The capillary voltage was maintained at 4 KV, the drying gas temperature and flow rate was kept at 350 °C and 5 L/min respectively. High pure nitrogen was used as drying gas and nebulizing gas. The pressure of nebulizing gas was set at 45 psig. The mobile phase consisted of mixture of acetonitrile, methanol and 0.01 M ammonium acetate solution with the flow rate set at 1.0 mL/min. Masshunter workstation software was employed for data acquisition and data processing. LC-MS spectra were acquired from m/z 100-1700.

3.3. NMR Spectroscopy

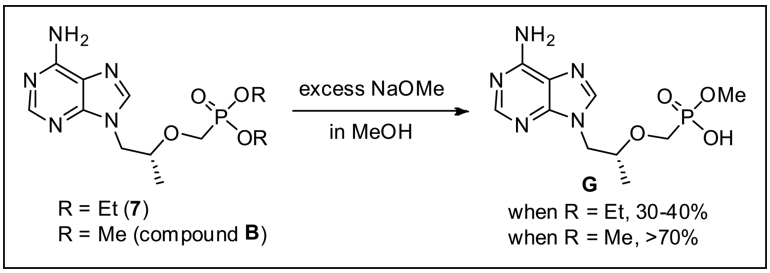
^{1}H, ^{13}C, ^{31}P NMR spectra were recorded in deuterated reagents on a Bruker AVANCE III 400 spectrometer (400 MHz for ^{1}H NMR) with tetramethylsilane (TMS) as the internal standard.

3.4. Mass spectrometry

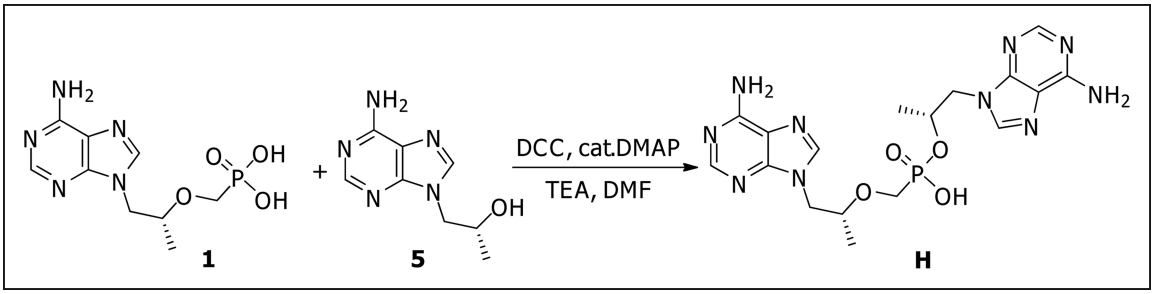
Mass spectra were recorded on an Agilent Technologies 6530 Accurate-Mass Q-TOF mass spectrometer equipped with an electrospray ion source.

3.5. Preparation of impurity I

Step 1: 26.00 g (318.9 mmol) ethylamine hydrochloride was dissolved in 20 mL water and 20 mL ethanol under N_2 atmosphere. The mixture was stirred and then cooled to 10 °C followed by addition of triethylamine 38.85 g (381.6 mmol) mixed with 20 mL ethanol. 9.88 g (62.7 mmol) 6-chloro-9*H*-purine (**8**) was charged and the reaction was refluxed until disappearance of **8** from TLC (DCM/MeOH = 4:1). The mixture was evaporated under reduced pressure. The resulting solid was further purified by reslurry in water and drying in vacuum to furnish 9.02 g compound **9** as a white solid (88% yield).
Step 2: 6.30 g compound **9** (38.6 mmol), 0.11 g KOH (5% equiv.) and 11.16 g (193.0 mmol) *R*-2-methyloxiran were dissolved in 50 mL DMF in a sealed tube. The reaction was stirred at 100 °C until from disappearance of compound **9** from TLC (DCM/MeOH = 5:1). The mixture was evaporated under reduced pressure, and the residue was directly used for the next step without further purification.
Step 3: The residue above was dissolved in 75 mL DMF in N_2 atmosphere followed by addition of 19.70 g (115.8 mmol) magnesium *t*-butoxide. The mixture was stirred, heated to 70 °C and then compound **6** (24.80 g, 77.2 mmol) was charged. The reaction was kept at the same temperature until the endpoint controlled by TLC (DCM/MeOH = 10:1). The mixture was cooled down to 20∼25 °C and 150 mL water was charged, followed by adjusting pH to 7 and extracting with DCM for three times. The combined organic layer was washed with water and concentrated under reduced pressure. 9.24 g compound **11** was obtained as a brown solid after purification by flash column chromatography on silica gel (64% for 2 steps).
Step 4: 7.70 g (21.0 mmol) compound **11** was dissolved in 70 mL MeCN in N_2 atmosphere. The mixture was stirred, cooled and maintained at −5∼0 °C when TMSBr (12.86 g, 84.0 mmol) was added. Afterwards, the reaction was heated to 70 °C and kept at the same temperature until disappearance



Scheme 5: Synthesis of compound **G** (impurity **III**).



Scheme 6: Synthesis of compound **H** (impurity **IV**).

of compound **11** from TLC (DCM/MeOH = 5:1). The mixture was concentrated under reduced pressure. The resulting residue was then mixed with 100 mL ethyl acetate to furnish a pale-yellow solid. The crude solid was dissolved and stirred in 10 mL EtOH by heating and then was cooled down to −10 °C, followed by slow addition of 10 mL toluene. The solid was filtered, washed with toluene and dried in vacuum to furnish the title compound as an off-white solid 5.27 g (81%).
^{1}H NMR (DMSO-*d6*) δ (ppm) = 8.30 (s, 1H), 8.27 (s, 1H), 4.40 (dd, *J* = 14.4, 2.8 Hz, 1H), 4.21 (dd, *J* = 14.8, 8.0 Hz, 1H), 3.91-3.94 (m, 1H), 3.75 (dd, *J* = 14.0, 9.2 Hz, 1H), 3.49-3.55 (m, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.14 (d, *J* = 6.0 Hz, 3H).
^{13}C NMR (DMSO-*d6*) δ (ppm) = 148.2, 147.0, 144.7, 144.2, 117.8, 76.6, 76.5, 64.1, 62.5, 48.4, 37.4, 15.8, 12.7.
^{31}P NMR (DMSO-*d6*) δ (ppm) = 19.52.
HRMS (+ESI): m/z 316.1175 (100%, $[M+H]^+$), Calculated: 316.1175 for ($[C_{11}H_{18}N_5O_4P+H]^+$).

3.6. *Preparation of impurity II*

Step 1: Adenine (13.51 g, 100.0 mmol) and (*R*)-2-methyloxirane (23.21 g, 400.0 mmol) were mixed in AcOH (75 mL). The mixture was heated to 100 °C and kept at the same temperature until adenine (**3**) disappeared. The solution was concentrated on a rotary evaporator and the residue was purified by flash column chromatography on silica gel (DCM/MeOH = 12:1) to afford alcohol **17** (10.22 g) in 53% yield.
Step 2: To intermediate **17** (3.10 g, 16.0 mmol) dissolved in DMF (50 mL) under N_2 atmosphere, was added magnesium *tert*-butoxide (5.47 g, 32.1 mmol). The resulting mixture was heated to 70 °C, and then (diethoxyphosphoryl)methyl 4-methylbenzenesulfonate (**6**, 7.76 g, 24.1 mmol) was added over 10 min. The reaction was kept at the same temperature until compound **17** disappeared. The mixture was concentrated on a rotary evaporator and the residue was purified by flash column chromatography on silica gel to afford the phosphonate product **18**, followed by reslurry overnight in petroleum ether and ethyl acetate (3:1, 20 mL). Filtration and drying in vacuum gave **18** as a white solid (2.89 g, 42%).
Step 3: To a mixture compound of **18** (1.76 g, 5.13 mmol) in 20 mL MeCN under N_2 atmosphere bromotrimethylsilane (1.57 g, 10.25 mmol) was added within 5 min. The mixture was then heated to 70 °C and maintained at this temperature overnight. The reaction was monitored by ^{31}P NMR until **18** disappeared. Then 20 mL ethyl acetate was added to the mixture and stirred overnight. Filtration and drying in vacuum gave the title compound as an off-white solid (1.10 g, 75%).
^{1}H NMR (DMSO-*d6*) δ (ppm) = 9.35 (s, 1H), 8.80 (s, 1H), 8.68 (s, 1H), 8.67 (s, 1H), 4.55 (dd, *J* = 14.0, 3.2 Hz, 1H), 4.42 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.02-4.06 (m, 1H), 3.63 (dd, *J* = 13.2, 8.8 Hz, 1H), 3.63 (dd, *J* = 13.2, 9.2 Hz, 1H), 1.16 (d, *J* = 4.8 Hz, 3H).
^{13}C NMR (DMSO-*d6*) δ (ppm) = 153.5, 148.8, 147.7, 144.7, 110.5, 74.4, 74.3, 64.9, 63.3, 53.1, 16.7.
^{31}P NMR (DMSO-*d6*) δ (ppm) = 16.73.
HRMS (+ESI): m/z 288.0860 (100%, $[M+H]^+$), Calculated: 288.0862 for $[C_9H_{14}N_5O_4P+H]^+$.

3.7. *Preparation of impurity III*

Step 1: (*R*)-({[1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid (**1**, 17.23 g, 60.0 mmol) was added to thionyl chloride (40 mL) under N_2 atmosphere and the resulting mixture was heated to reflux overnight. The mixture was then cooled to 20∼25 °C and evaporated under reduced pressure followed by slow addition of 20 mL dry MeOH at 0 °C. After stirring for 30 min, the mixture was evaporated under reduced pressure. Then it was partitioned between ethyl acetate (300 mL) and water (300 mL) with vigorous stirring. The organic layer was collected and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layer was dried over Na_2SO_4 and then filtered. Concentration under reduced pressure furnished compound **B** (17.34 g, 92%) as a yellow liquid which could be used for the next step without purification.
Step 2: Compound **B** (7.06 g, 22.4 mmol) was dissolved in 100 mL MeOH, and NaOMe (26%, 14.52 g, 69.9 mmol) was added. The reaction was refluxed overnight and then cooled to 20∼25 °C followed by evaporation. The residue was diluted with water and extracted with ethyl acetate. The aqueous layer was adjusted to pH 6-7 with 3N HCl aq. and evaporated under reduced pressure to remove water. To the residue above, was added 100 mL EtOH and the mixture was stirred overnight, followed by filtration and concentration to obtain the title compound (5.01 g, 74%) as a pale-yellow liquid.
^{1}H NMR (DMSO-*d6*) δ (ppm) = 8.29 (s, 1H), 8.28 (s, 1H), 4.40 (dd, *J* = 14.8, 2.8 Hz, 1H), 4.22 (dd, *J* = 14.8, 7.6 Hz, 1H), 3.93-3.95 (m, 1H), 3.67-3.73 (m, 1H), 3.45-3.50 (m, 1H), 3.36-3.38 (d, *J* = 6.4 Hz, 3H), 1.16 (d, *J* = 6.0 Hz, 3H).
^{13}C NMR (DMSO-*d6*) δ (ppm) = 150.7, 148.6, 145.6, 145.1, 117.5, 75.9, 75.8, 63.3, 61.7, 51.6, 51.5, 48.2, 15.8.
^{31}P NMR (DMSO-*d6*) δ (ppm) = 18.87.
HRMS (+ESI): m/z 302.1010 (100%, $[M+H]^+$), Calculated: 302.1018 for ($[C_{10}H_{16}N_5O_4P+H]^+$).

3.8. *Preparation of impurity IV*

To the mixture of (*R*)-({[1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid (**1**, 14.42 g, 50 mmol), (*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-ol (**5**, 11.64 g, 60 mmol) and DCC (41.25 g, 200 mmol) suspended in 150 mL DMF under N_2 atmosphere, was added Et_3N (10.12 g, 100 mmol) and DMAP (0.61 g, 5.0 mmol). The resulting mixture was heated to 90 °C and kept at the same temperature overnight. The mixture was cooled to 20∼25 °C and filtered. The solid was removed and the liquor was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM/MeOH = 1:1) to afford the title compound (12.70 g, 55%).
^{1}H NMR (DMSO-*d6*) δ (ppm) = 8.27 (s, 1H), 8.17 (s, 1H), 8.15 (s, 2H), 7.56 (br, 1H), 7.36 (br, 2H), 7.32 (br, 2H), 4.58-4.64 (m, 1H), 4.10-4.22 (m,

4H), 3.77-3.81 (m, 1H), 3.28-3.44 (m, 2H), 1.01 (d, $J = 6.0$ Hz, 3H), 0.93 (d, $J = 6.0$ Hz, 3H).
^{13}C NMR (DMSO-*d6*) δ (ppm) = 155.73, 155.68, 152.1, 152.0, 149.81, 149.76, 141.9, 141.8, 118.3, 118.2, 74.8, 74.7, 68.32, 68.26, 65.8, 64.3, 48.6, 46.5, 19.59, 19.57, 16.8.
^{31}P NMR (DMSO-*d6*) δ (ppm) = 12.77.
HRMS (+ESI): m/z 232.0897 $[M + 2H]^{2+}/2$ (100%), Calculated: 232.0899 for $[C_{17}H_{23}N_{10}O_4P + 2H]^{2+}/2$.

References

Arimilli MN, Cundy KC, Dougherty JP, Kim CU, Oliyai R, Stella VJ (1998) Antiviral phosphonomethoxy nucleotide analogs having increased oral bioavailability US5977089.

Arimilli MN, Kim CU, Dougherty J, Mulato A, Oliyai R, Shaw JP, Cundy KC, Bischofberger N (1997) Synthesis, *in vitro* biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs. Antivir Chem Chemoth 8: 557–564.

Burger A, Anderson JJ (1957) Monoesters and ester-amidates of aromatic phosphonic acids. J Am Chem Soc 79: 3575–3579.

Deeks SG, Barditch-Crovo P, Lietman PS, Hwang F, Cundy KC, Rooney JF, Hellmann NS, Safrin S, Kahn JO (1998). Safety, pharmacokinetics, and antiretroviral activity of intravenous 9-[2-(*R*)-(Phosphonomethoxy)propyl]adenine, a novel anti-human immunodeficiency virus (HIV) therapy, in HIV-infected adults. Antimicrob Agents Chemother 42: 2380–2384.

Label, FDA Label (2001, 2008) Approved for VIREAD, NDA no. 021356.

Gilmore WF, McBribe HA (1974) New compounds: monoesters of α-aminobenzylphosphonic acid. J Pharm Sci 63: 965–966.

Hakimelahi GH, Ly TW, Moosavi-Movahedi AA, Jain ML, Zakerinia M, Davari H, Mei HC, Sambaiah T, Moshfegh AA, Hakimelahi S (2001) Design, synthesis, and biological evaluation of novel nucleoside and nucleotide analogues as agents against DNA viruses and/or retroviruses. J Med Chem 44: 3710–3720.

Joule JA, Mills K (2010) Heterocyclic Chemistry 5th Ed., Oxford, p. 515–538.

Lister JH (1971) in Weissberger A, Taylor EC (eds.): The chemistry of heterocyclic compounds", Vol 24 (part II), New York, p. 342.

Naesens L, Bischofberger N, Augustijns P, Annaert P, Van den Mooter G, Arimilli MN, Kim CU, De Clercq E (1998) Antiretroviral efficacy and pharmacokinetics of oral *bis*(isopropyloxycarbonyloxymethyl)-9-(2-phosphonylmethoxypropyl)adenine in mice. Antimicrob Agents Chemother 42: 1568–1573.

Ripin DHB, Teager DS, Fortunak J, Basha SM, Bivins N, Boddy CN, Byrn S, Catlin KK, Houghton SR, Jagadeesh ST, Kumar KA, Melton J, Muneer S, Rao LN, Rao RV, Ray PC, Reddy NG, Reddy RM, Shekar KC, Silverton T, Smith DT, Stringham RW, Subbaraju GV, Talley F, Williams A (2010) Process improvements for the manufacture of tenofovir disoproxil fumarate at commercial scale. Org Process Res Dev 14: 1194–1201.

Robbins BL, Srinivas RV, Kim C, Bischofberger N, Fridland A (1998) Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-*R*-(2-phosphonomethoxypropyl)adenine (PMPA), *Bis*(isopropyloxymethylcarbonyl)PMPA. Antimicrob Agents Chemother 42: 612–617.

Seden TP, Turner RW (1975) The reaction of adenine and epichlorohydrin. J Heterocyclic Chem 12: 1045–1046.