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Synthesis and characterization of Tenofovir disoproxil fumarate impurities, anti HIV drug substance

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ABSTRACT

*In the process development of Tenofovir disoproxil fumarate specified known, specified unknown and unidentified impurities found. The present work describes synthesis and characterizations of USP related known and unknown impurities; mono-POC Tenofovir (**5**), mono-POC methyl Tenofovir (**6a**), mono-POC ethyl Tenofovir, (**6b**), mono-POC isopropyl Tenofovir (**6c**), Tenofovir isopropyl isoproxil (**7**) and Tenofovir disoproxil carbamate (**9**). Identification of these impurities can be useful for quality control purpose in the manufacture of Tenofovir disoproxil fumarate.*

Keywords: Antiretroviral Tenofovir disoproxil fumarate, impurity synthesis, characterization.

INTRODUCTION

Tenofovir disoproxil fumarate is a prodrug of Tenofovir and bis-isopropyloxycarbonylmethyl (bis-POC) moiety in improving the oral bioavailability of phosphonate nucleotides [1, 3]. Tenofovir disoproxil fumarate is USFDA approved potential antiretroviral agent. There is an ever increasing interest in impurities present in API. Recently, not only purity profile but also impurity profile become insist various regulatory requirements [4]. Majorly organic impurities are structurally similar with its API and comes from synthetic pathways or formulation. Due to structural similarity binds to receptor and decreases efficacy of drug and increases side effects, in order to decrease percentage of impurities it is important to identify the structures and origin of impurities [5]. According to ICH guide line, impurities in new drug products identification and quantification is mandatary, impurities above 0.1% considered as potentially toxic.

Tenofovir disoproxil fumarate, block reverse transcriptase, a crucial viral enzyme in human immunodeficiency virus 1 (HIV-1) and hepatitis B virus infections. Tenofovir is a highly potent antiviral agent, particularly for the therapy or prophylaxis of retroviral infections and belongs to a class of drugs called Nucleotide Reverse Transcriptase Inhibitors (NRTI) which blocks reverse transcriptase an enzyme crucial to viral production in HIV-infected people [6, 7]. Tenofovir is approved for commercial use as in the form of Tenofovir disoproxil fumarate (**1**), chemically known as 9-[(*R*)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy]propyl]adenine fumarate with 9-*R*-(2-hydroxypropyl) adenine as basic moiety. Tenofovir disoproxil fumarate is on the WHO's list of essential medicines, the most important medications needed in a basic health system [7, 8].

MATERIALS AND METHODS

General. The reagents and solvents were purchased from sigma aldrich and dry triethyl amine and dry pyridine used for reactions. All reaction were carried out under nitrogen atmosphere. ^{1}H NMR spectra were recorded on a Bruker Advance III 400 MHz with TMS as an internal standard. The IR spectra were recorded in the solid state as KBr dispersion using FT-IR IRAffinity-1, Shimadzu. The mass spectra were recorded on LC-MS API-2000, ABSciex.

General procedure for the synthesis of ({[(R) -1-(6-Amino-9H-purin-9-yl) propan-2-yloxy] methyl} (hydroxy) phosphoryloxy) methyl isopropyl carbonate; mono-POCPMPA (**5**) Tenofovir disoproxyl fumarate (3.0 g, 0.0058 moles) was suspended in acetonitrile (15 ml, 5.0 volumes) water (24.0 ml, 8.0 volumes) mixture at room temperature. Ammonium hydroxide was added and adjusted pH 9 at 45 ^{o}C and stirred the reaction mass for 18 hours. Monitor the reaction progress by TLC (ethyl acetate/ methanol 35:65 v/v). Concentrated the reaction mass and the crude product was purified by column chromatography using ethyl acetate/ methanol 40:60 v/v) as gradient mobile phase. Yield 85%. Melting point: 212.1 °C (decomposes). HPLC Purity 82.75%. IR (cm^{-1}): 3325, 3177, 2962, 1746, 1541, 1599, 1418, 1238, 1076, 1024, 790, 650. ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 7.18 (s, 2H, NH_2), 5.45-5.25 (t, *J* = 11.6 & 8.8, 2H, OCH_2O), 4.77-4.70 (m, 1H, $CH(CH_3)_2$), 4.27-4.23 (dd, *J* = 4.0 Hz, 1H, NCH_2), 4.17-4.11 (dd, *J* = 5.6 & 5.2 Hz, 1H, NCH_2), 3.86-3.82 (m, 1H, CH), 3.45-3.38 (dd, *J* = 8.8 Hz, 2H, CH_2), 1.20-1.18 (dd, *J* = 1.6 & 2.0 Hz, 6H, $(CH_3)_2$), 0.96-0.94 (bd, *J* = 6.0 Hz, 3H), Mass [M-H]$^-$ 402.

General procedure for the synthesis of Tenofovir disoproxyl mono ether (**8a, 8b & 8c**)**; mono-POC methyl, ethyl** (**6a**) **and isopropyl ether** (**6b**)

Tenofovir mono ether (**8a, 8b & 8c**) The mixture of Tenofovir (1 equiv.) and alcohol (1.2 equiv.) was taken in dry pyridine (10 ml), DCC (2.0 equiv.). The reaction mixture was heated to reflux for 18-24 h. Reaction mass was concentrated and dissolved in methanol and dichloromethane (1:1) ratio 10 ml and filtered of undissolved material. The filtrate was concentrated under reduced pressure and the residue was applied to a short column of silica gel. Elution of the column with (dichloromethane-methanol 90:10 v/v) gradient yielded mono-POC methyl 31%, mono-POC ethyl 73%, mono-POC isopropyl 85% Tenofovir intermediate (HDP-ADV) as a white powder. Intermediates confirmed by mass mono-POC methyl 302.1 [M+H]$^+$, [M+Na]$^+$, & 299 [M-H]$^-$ and mono-POC ethyl 314.8 [M-H]$^-$.

Tenofovir disoproxyl mono ether (**6a, 6b & 6c**) In a suitable round bottom flask taken Tenofovir monoether intermediate 8a, 8b & 8c (0.0030 moles) and dissolved in *N*-methyl pyrrolidone (3.0 ml, 3.0 V) and stirred the reaction mass for 30 minutes at room temperature. Then triethylamine (0.0085 moles) was added and stirred the reaction mass and stirred for 1 h. Then chloromethyl isopropyl carbonate (0.014 moles) at 55 ºC was added and stirred for an additional 3 h. Monitored the reaction progress by TLC (ethyl acetate-methanol 9:1 v/v), upon completion ethyl acetate was added (4.0 ml) and stirred for 1 h at 10-15 ºC. Organics washed with water (3 x 25 ml), organic dried over sodium sulfate, filtered, concentrated and under reduced pressure. The crude product was purified by flash column chromatography using silica gel and ethyl acetate: methanol as gradient for column to obtain desired compounds with 10% ,31% and 35% respectively.

***O*-(Isopropoxycarbonyloxymethyl)-*O*-methyl-{(R)-[1-(6-amino-9*H*-purin-9-yl)propan-2-yloxy]}methyl phosphonate** (**6a**), Nature: Yellow liquid. IR (cm-1): 3327, 2984, 1757, 1711, 1599, 1415, 1357, 1257, 1001, 898, 789, 648. ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H, H-2), 7.90-796 (d, *J* = 5.2 Hz, 1H, H-8), 6.10 (brs, 2H, NH_2), 5.50-5.59 (m, 2H, OCH_2O), 4.80-4.91 (m, 1H, $CH(CH_3)_2$), 4.26-4.38 (tt, *J* = 3.2 & 2.0 Hz, 1H, CH_2N), 4.00-4.12 (m, 1H, CH_2N), 3.80-3.89 (m, 2H, OCH_2P), 3.88-3.86 (d, *J* = 8.8 Hz, 3H, OCH_3), 3.85-3.82 (m, 1H), 1.21-1.26 (m, 6H, $CH(CH_3)_2$), 1.19-1.67 (m, 3H). [M+H]$^+$ 417.9 [M-Na]$^-$ 416,

***O*-(Isopropoxycarbonyloxymethyl)-*O*-ethyl-{(R)-[1-(6-amino-9*H*-purin-9-yl)propan-2-yloxy]}methyl phosphonate** (**6b**), Nature: Yellow liquid. HPLC Purity: 87.07%. IR (cm-1): 3327, 3156, 2982, 2936, 1757, 1643, 1599, 1470, 1246, 1097, 1037, 999, 947, 891, 829, 789, 719, 648. ^{1}H NMR (DMSO-d_6) δ 8.31 (s, 1H, H-2), 8.04 (s, 1H, H-8), 7.20 (s, 2H, NH_2), 5.55-5.46 (m, 2H, OCH_2O), 4.86-4.78 (m, 1H, $CH(CH_3)_2$), 4.31-4.23 (dd, *J* = 14.4 & 3.2 Hz, 1H, NCH_2), 4.21-4.11 (m, 1H, $CH(CH_3)_2$), 4.07-3.83 (m, 4H + 1H, 2 x OCH_2 + CH_2N), 1.25-1.23 (dd, *J* = 6.4 & 2.8 Hz, 6H, 2 x CH_3), 1.19-1.12 (m, 3H, CH_2CH_3), 1.09-1.07 (dd, *J* = 6.4 & 1.6 Hz, 3H, $CHCH_3$). Mass: [M+H] 432.

***O*-(Isopropoxycarbonyloxymethyl)-*O*-isopropyl-{(R)-[1-(6-amino-9*H*-purin-9-yl)propan-2-yloxy]}methyl phosphonate** (**6c**), Nature: Pale yellow liquid. HPLC Purity: 83.15%. IR (cm^{-1}): 3329, 3166, 2981, 2935, 1757, 1643, 1599, 1417, 1247, 1143, 1099, 1028, 983, 902, 719. ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H, H-2), 8.00 (s, 1H, H-8), 6.03 (brs, 2H, NH_2), 5.71-5.58 (d, 2H, dd, *J* = 17.2 Hz, OCH_2O), 4.98-4.87 (m, 1H, $CH(CH_3)_2$), 4.83-4.68 (m, 1H, $CH(CH_3)_2$), 4.30-4.48 (dd, 1H, *J* = 15.2 & 4.4 Hz, CH_2N), 4.00-4.28 (m, 2H, OCH_2P), 3.82-3.98 (m, 1H, CH_2N), 3.51-3.66 (m, 1H, $CH(CH_3)$), 1.13-1.42 (m, 15H, CH2x$(CH_3)_2$ + CH_3). [M+H]$^+$ 446.0 [M+Na]$^+$ 468, [M+K]$^+$ 484.

5-[[(1R)-2-(6-Amino-9H-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8-tetraoxa-5-phosphanonanedethyl,propyl) Ester 5-Oxide (**7**) Triethylamine (3.53 ml, 0.0254 moles) was added to mono-POCPMPA (**5**) (3.6 g, 0.0089 moles) solution of *N*-methyl pyrrolidone (10.8 ml, 3.0 volumes) and stir the reaction mass for 30 minutes at room temperature. Reaction mixture was then heated to 54 ºC, chloromethyl *n*-propyl carbonate (1.54 ml, 0.0116 moles) was added at same temperature and maintained 5.0 hours. Monitor the reaction progress by TLC (ethyl acetate/methanol 10:90 v/v). Ethyl acetate (12.0 ml) was added in reaction mixture at then cooled to 10-15 ^{o}C and

stirred for 1 h. Filter the precipitated white solid and washed with ethyl acetate. Filtrate was concentrated and the crude product purified by flash column chromatography using methanol-ethyl acetate as gradient mobile phase to obtain pure compound, yield 11%.

Nature: Pale yellow liquid. HPLC Purity 91.90%. IR (cm^{-1}): 2972, 2937, 1759, 1614, 1662, 1421, 1246, 1153, 1099, 1028, 952, 893, 831, 788, 646. ^1H NMR ($CDCl_3$) δ: 8.29 (s, 1H, H-2), 7.80 (s, 1H, H-8), 6.45 (brs, 2H, NH_2), 5.80-5.58 (d, *J* = 16.8 Hz, 4H, OCH_2O), 4.98-4.80 (m, 1H, $CH(CH_3)_2$), 4.48-4.30 (d, 1H, *J* = 19.2 Hz, CH_2N), 4.21-4.15 (m, 2H, OCH_2P), 3.98-3.82 (m, 2H, OCH_2), 3.75-3.65 (dd, 1H, *J* = 14 & 4.4 Hz, CH_2N), 3.47-3.38 (m, 1H, $CHCH_3$), 1.79-1.67 (m, 2H, CH_2CH_3), 1.33-1.27 (d, 6H, *J* = 8.0 Hz, $CH(CH_3)_2$), 1.24-1.20 (d, 3H, *J* = 7.2 Hz, $CHCH_3$), 1.12-0.87 (t, 3H, *J* = 6.4 Hz, CH_3). $[M+H]^+$ 520.0 $[M+Na]^+$ 542.0, $[M+K]^+$ 558.0

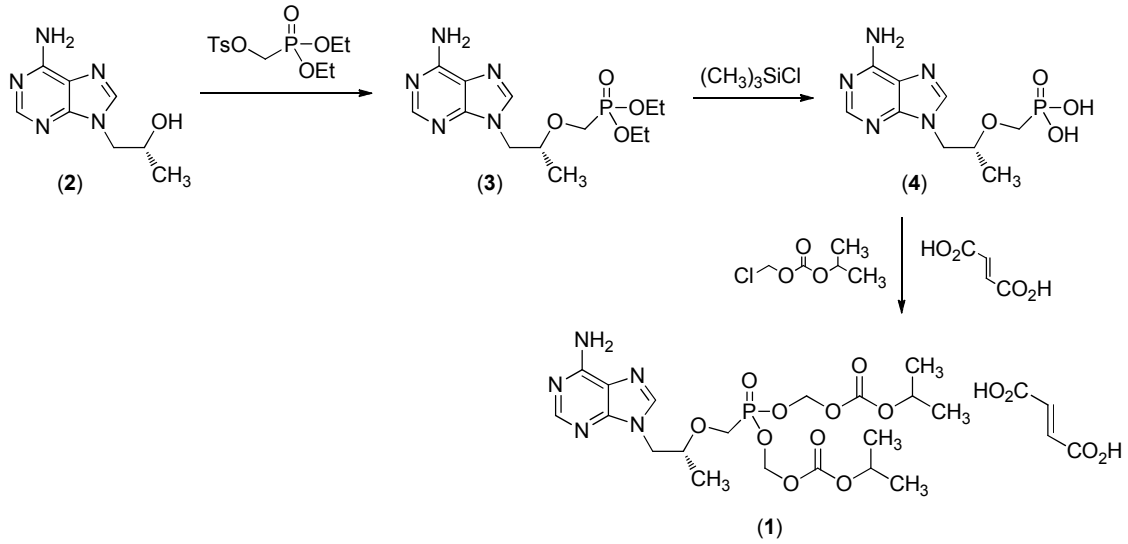
***O, O*-Bis (isopropoxycarbonyloxymethyl){(R)-1-[(6-isopropoxycarbonyl amino)-9*H*-purin-9yl] propan-2-yl oxy}methylphosphonate** (**9**)

Tenofovir disoproxil fumarate (**1**) (1.0 g, 0.0019 moles) suspended in *N*-methyl pyrrolidone (3 ml, 3.0 volumes) at room temperature then triethylamine (0.9 ml, 0.0060 moles) was added dropwise to give clear solution of reaction mass. Isopropyl chloroformate was added dropwise (0.9 ml, 0.0029 moles) and stir the reaction mass at 60 ^oC for 6 h. Monitor the reaction progress by TLC (dichloromethane-methanol 95:5 v/v). Reaction mixture was diluted in water 20 ml and extracted in ethyl acetate (3 x 25 ml), dried over sodium sulphate and concentrated to obtain sticky compound. The crude product was purified by column chromatography using gradient dichloromethane and ethylacetate to obtain pure product 0.43 g, yield 37%.

Nature: Pale yellow liquid. HPLC Purity 82.16%. IR (cm^{-1}): 2978, 2853, 1755, 1613, 1516, 1458, 1358, 1227, 1176, 1028, 954, 897, 789, 748. ^1H NMR ($CDCl_3$) δ: 8.75 (s, 1H, H-2), 7.81 (s, 1H, H-8), 5.69-5.56 (m, 4H, $2xCH_2$), 5.17-5.07 (m, 1H, CH), 4.96-4.86 (m, 2H, CH_2), 4.46-4.40 (dd, 1H, *J* = 14.4 & 2.8 Hz, CH), 4.22-4.13 (dd, 2H, *J* = 14.8 & 7.6 Hz, CH), 4.00-3.91 (m, 2H, CH_2), 3.73-3.65 (dd, 1H, *J* = 13.6 & 9.2 Hz, CH), 1.44-1.36 (d, 3H, CH_3), 1.33-1.20 (m, 18H, 4 x CH_3) $[M+Na]^+$ 628.8.

RESULTS AND DISCUSSION

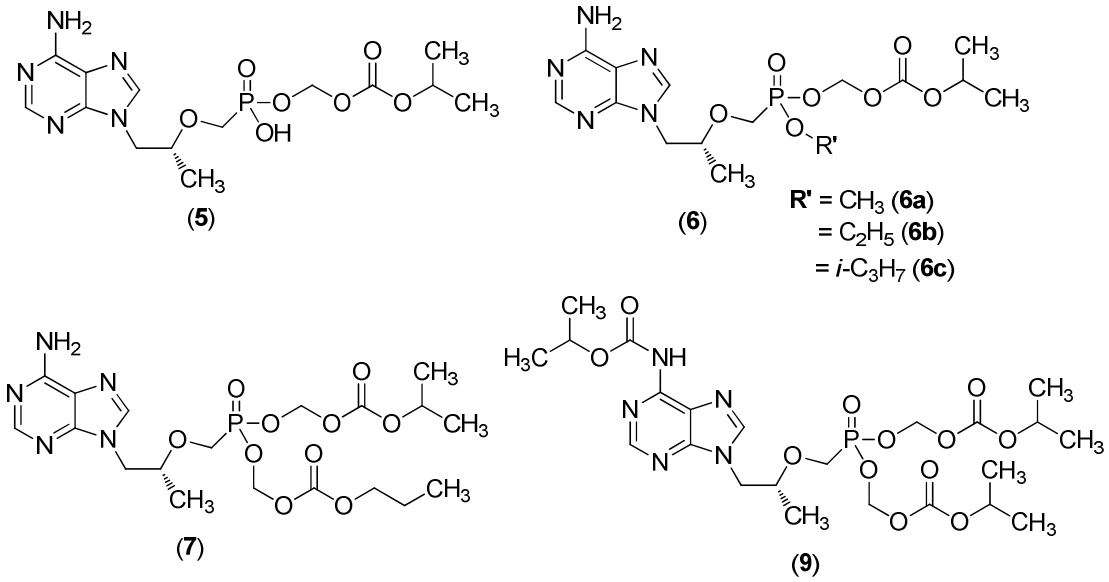
Three major steps are involved in Tenofovir disoproxil fumarate (**1**) synthesis [9, 10] (Scheme 1) are reacting 9-[2-(*R*)-(hydroxy)propyl] adenine (**2**) with diethyl-*p*-toluene sulfonyl-oxymethyl phosphonate, dealkylating the resulting compound of formula (**3**) with acid to obtain Tenofovir (**4**) which on reacting with chloromethyl isopropyl carbonate followed by treating with fumaric acid gives Tenofovir disoproxil fumarate (**1**)



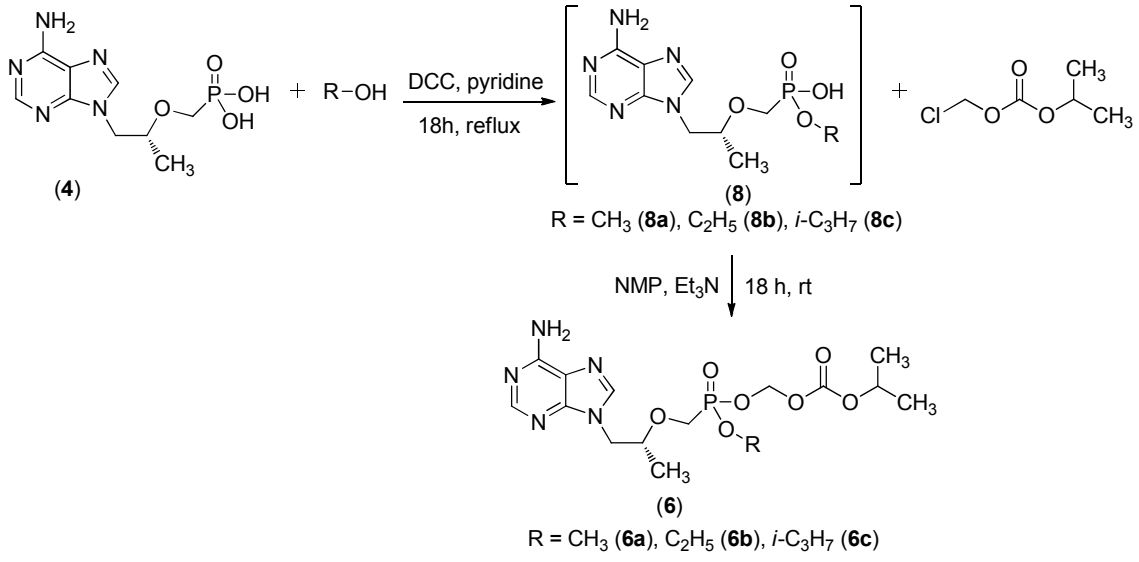
Scheme 1. Tenofovir disoproxil fumarate (1) synthesis

Corresponding impurities of Tenofovir disoproxil fumarate likewise **5** Tenofovir isoproxil monoester chemically known as ({[(R) -1-(6-Amino-9*H*-purin-9-yl) propan-2-yloxy] methyl} (hydroxy) phosphoryloxy) methyl isopropyl carbonate [11] synthesized treating with ammonium hydroxide solution at pH 9. Impurity **6a** Tenoforvir methyl isoproxil or Mono-POC methyl impurity, chemically known as *O*-(Isopropoxycarbonyloxymethyl)-*O*-methyl-{(R)-[1-(6-amino-9*H*-purin-9-yl) propan-2-yloxy]}methylphosphonate. Impurity **6b** Tenoforvir ethyl isoproxil or Mono-POC ethyl impurity, chemically known as *O*-(Isopropoxycarbonyloxymethyl)-*O*-ethyl-{(R)-[1-(6-amino-9*H*-purin-

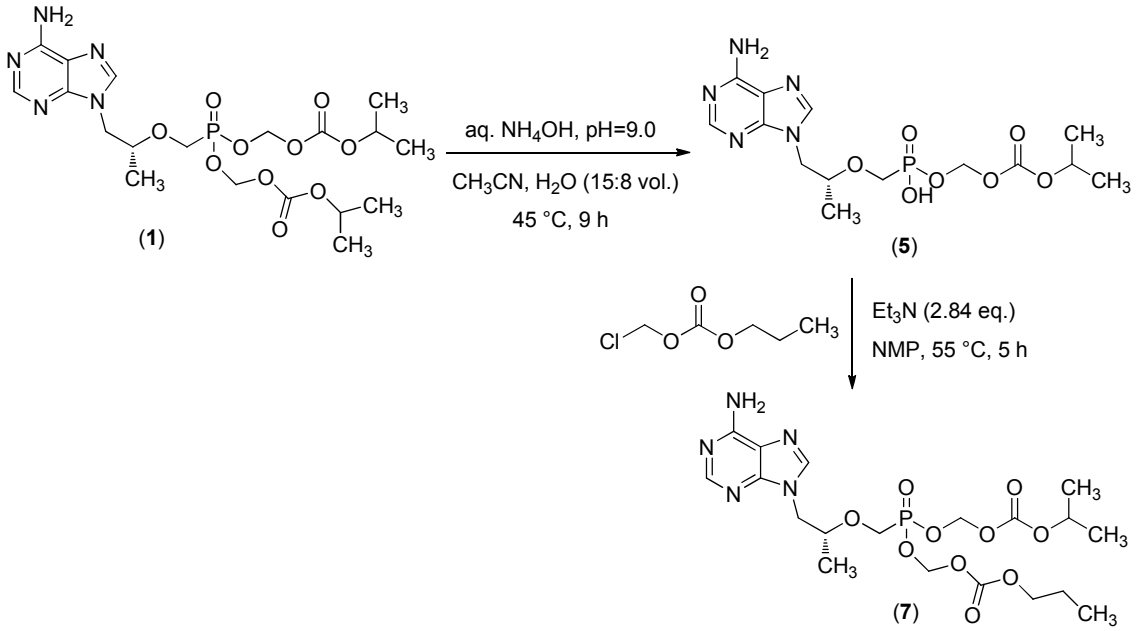
9-yl) propan-2-yloxy]}methylphosphonate and Impurity **6c** Tenoforvir isopropyl isoproxil or mono-POC isopropyl impurity,*O*-(Isopropoxycarbonyloxymethyl)-*O*-isopropyl-{(R)-[1-(6-amino-9*H*-purin-9-yl) propan-2-yloxy]}methyl phosphonate are monoether impurities derived from process related impurities. Impurities **6a**, **6b** and **6c** synthesized from intermediate **5**. Impurity **7** n-POC-POC Tenofovir chemically known as 5-[[(1R)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8-Tetraoxa-5-phosphanonanedioic acid 1-(1-methylethyl) 9-propyl ester 5-oxide synthesized from intermediate **5** and chloromethyl n-propyl carbonate [12]. Impurity **8** Tenofovir disoproxil carbamate, chemically known as *O*, *O*-Bis (isopropoxycarbonyloxymethyl){(R)-1-[(6-isopropoxycarbonyl amino)-9*H*-purin-9yl] propan-2-yloxy]}methylphosphonate and are process related impurities synthesized treating Tenofovir disoproxil API with isopropyl chloroformate in basic condition. All impurities are as listed in **Scheme 2**.



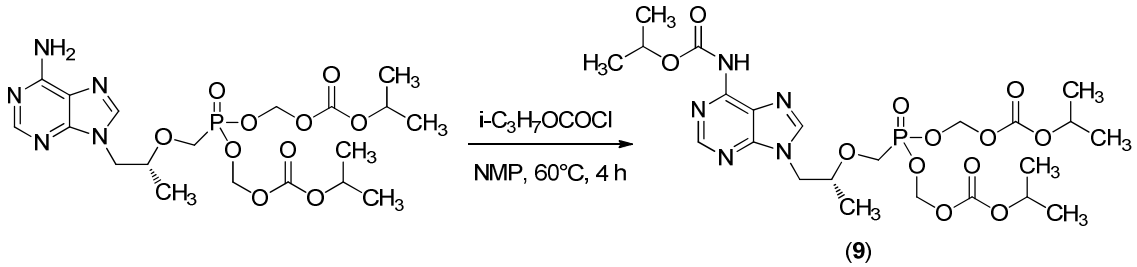
Scheme 2. Tenofovir disoproxil fumarate (1) impurities



Scheme 3. Synthesis of Tenofovir disoproxyl mono ether; mono-POC methyl(6a), mono-POC ethyl (6b) and mono-POC *i*-propyl ether (6c)



Scheme 4. Synthesis of n-POC-POC Tenofovir (7)



Scheme 5. Tenofovir disoproxil carbamate (9)

CONCLUSION

All of the above synthesized impurities Tenofovir isoproxil monoester, mono-POC methyl (**6a**), mono-POC ethyl (**6b**) and mono-POC *i*-propyl ether (**6c**), n-POC-POC Tenofovir (**7**) and Tenofovir disoproxil carbamate (**9**) can be used for impurity profiling of Tenofovir disoproxil fumarate.

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