ISSN: 0974 - 7516 Volume 8 Issue 11

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 8(11), 2012 [432-436]

Industrially viable synthesis of tenofovir a key intermediate of tenofovir disoproxil fumarate and related impurities

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Received: 10th March, 2012 ; Accepted: 10th April, 2012

ABSTRACT

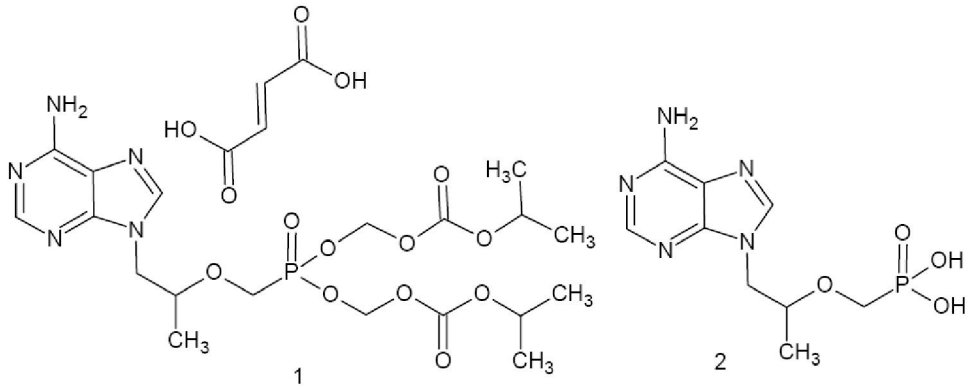
An industrially efficient method was developed to synthesize Tenofovir, a key intermediate to tenofovir disoproxil fumarate which is a highly potent antiviral agent. Tenofovir or (R)-9-[2-(phosphonomethoxy) propyl] adenine **(2)** is synthesized through by condensation of (R)-9-[2-(hydroxyl)propyl]adenine and Diethyl p-toluene sulfonyl oxymethyl phosphonate in presence of 1M dibutyl magnesium (in toluene) in NMPO solvent or mixture of NMPO and tert-butanol and followed by dealkylation with aqHBr. Three known impurities were synthesized and characterized.

KEYWORDS

1M dibutylmagnesium in toluene;
Impurity;
Antiviral;
Fumaric acid;
Tenofovir.

INTRODUCTION

Tenofovir disoproxil fumarate **(1)** is a highly potent antiviral agent, particularly for the therapy or prophylaxis of retroviral infections and belongs to a class of drugs called Nucleoside Reverse Transcriptase Inhibitors (NRTI) which blocks reverse transcriptase an enzyme crucial to viral production in HIV-infected people[1-4]. These are related to Nucleoside Reverse Transcriptase Inhibitors (NRTI). Tenofovir **(2)** or (R)-9-[2-(phosphonomethoxy) propyl] adenine **(2)** is a key intermediate of 1. (R)-9-[2-(phosphonomethoxy) propyl] adenine **(2)** is synthesized through (R)-9-[2-(hydroxyl) propyl] adenine **(3)**.



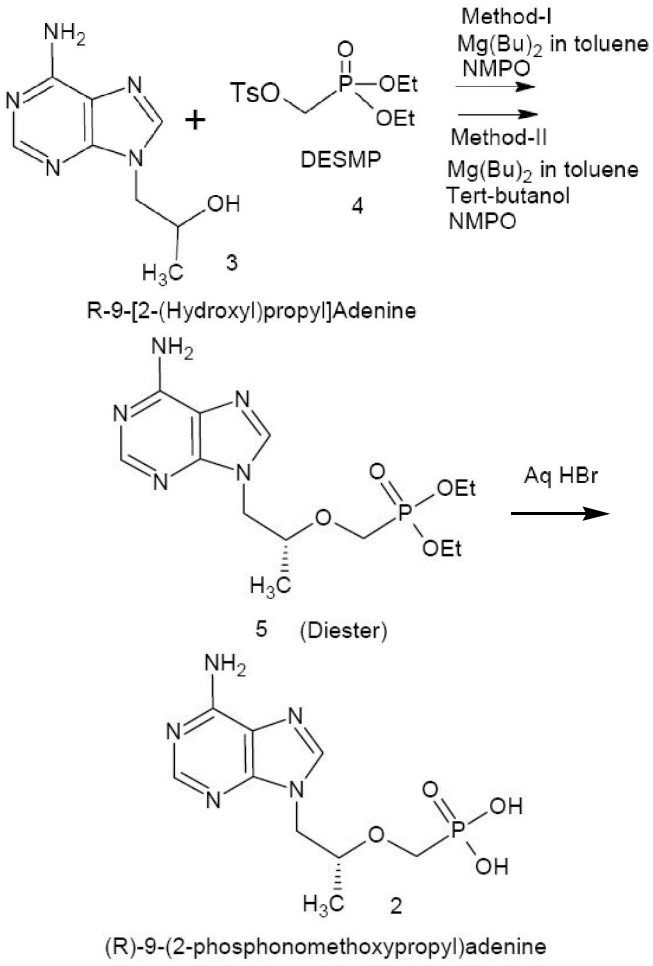
1 2

(R)-9-[2-(phosphonomethoxy) propyl] adenine **(2)** is synthesized through by condensation of (R)-9-[2-

(hydroxyl) propyl] adenine and diethyl p-toluenesulfonyloxymethylphosphonate in presence of lithium hydride or lithium tert-butoxide[5] or Magnesium tert-butoxide[6] in organic solvent followed by dealkylation with aqHBr. We wish to report to use new base 1M dibutyl magnesium in toluene, which is commercially available, easy to handle even at the production scale, and the reaction temperature reported was room temperature.

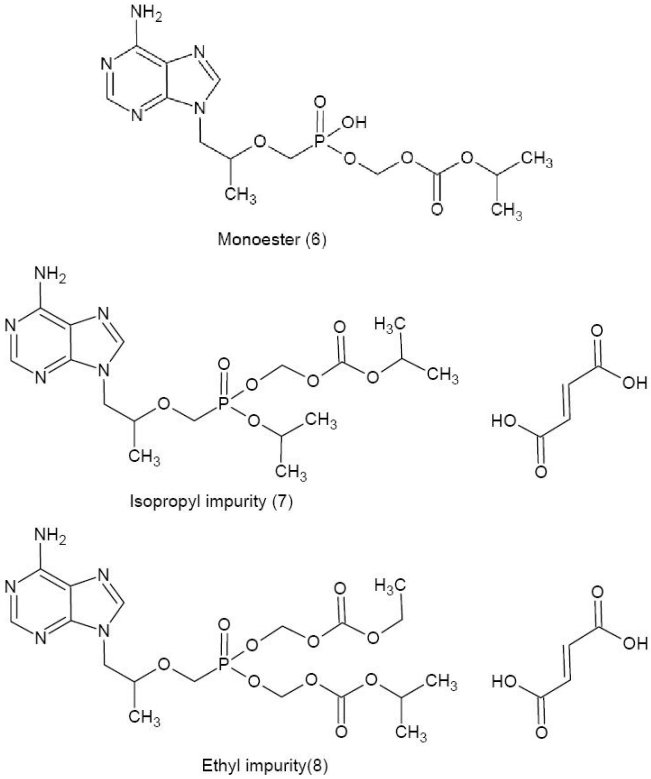
RESULTS AND DISCUSSION

(R)-9-[2-(hydroxyl)propyl]adenine **(3)**, diethyl p-toluenesulfonyloxymethylphosphonate **(4)** and 1M dibutylmagnesium in toluene are obtained from commericial source.(R)-9-[2-(hydroxyl)propyl]adenine **(3)** is condensed with diethyl p-toluene sulfonyloxymethyl phosphonate **(4)** in presence of 1M dibutylmagnesium in toluene and with tert-butanol or without tert-butanol in a polar solvent preferably NMPO at a temperature of 70° C. to 80° C. After the reaction completion the reaction mass is neutralized by adding an acid preferably acetic acid and to get (R)-9-[2(diethyl phosphonomethoxy) propyl] adenine **(5)**. Dealkylation of (R)-9-[2-(diethyl phosphonomethoxy) propyl] adenine **(5)** is carried out in presence of aq. HBr, and typically at a temperature of about 80 to 95° C.



Scheme 1

Tenofovir disoproxil fumarate **(1)** was prepared from above key intermediate **(2)** by esterification with Chloro methyl isopropylCarbonate in presence of base and further treated with fumaric acid in isopropyl alcohol according to the literature[6]. Three known impurities in Tenofovir disoproxil fumarate bulk drug at level 0.2% (ranging from 0.05-0.2%) were detected by gradientreverse phase high performance liquid chromatography. These impurities were synthesized and characterized.



Structure elucidation of monoester impurity (6)

Sample was analyzed by HPLC and its purity was found to be 93.14%, molecular weight of monoester impurity is 403.34. The protonated molecular ion at m/z 404 (M+1) confirms the mass as 403 corresponding to molecular formula of $C_{14}H_{22}N_5O_7P$. IR spectrum displayed characteristic absorptions at 3328 & 3082, 2946

cm-1 corresponding to >NH and aromatic >CH stretching. The peaks at 1509.95 & 1452.92 cm^{-1} in IR spectrum is indicative of >C=C< ring stretching.

^{1}H NMR (300 MHz, DMSO-d_6): 1.12 (d, 3H); 1.20 (d,6H); 3.60-4.0 (m,3H); 4.1-4.3 (dd,1H); 4.3-4.6 (dd,1H); 4.7-4.8 (m,1H); 5.4-5.5 (s,2H); 7.94 (brs,2H); 8.17 (s,1H); 8.22 (s,1H).

^{13}C NMR (70 MHz, DMSO-d_6): 16.7, 21.3, 47.0, 64.0, 72.0, 75.0, 84.5, 118.0, 142.5, 148.5,149.6, 153.0, 154.1

Structure elucidation of isopropyl ester impurity (7)

Sample was analyzed by HPLC and its purity was found to be 93.14%, molecular weight of isopropyl ester base impurity is 445.. The protonated molecular ion at m/z 446 (M+1) confirms the mass as 445 corresponding to molecular formula of $C_{17}H_{28}N_5O_7P$. IR spectrum displayed characteristic absorptions at 3338 & 3072, 2936 cm-1 corresponding to >NH and aromatic >CH stretching. The peaks at 1518.65 & 1432.52 cm^{-1} in IR spectrum is indicative of >C=C< ring stretching..

^{1}H NMR (300 MHz, DMSO-d_6): 1.02 (d, 6H); 1.20 (d,9H); 3.70-4.0 (m,3H); 4.1-4.3 (dd,2H); 4.5-4.6 (dd,1H); 4.8-4.9 (m,1H); 5.4-5.5 (s,2H); 6.64 (s,2H, Fumarate protons); 7.20 (brs,2H); 8.02 (s,1H); 8.15 (s,1H).

^{13}C NMR (70 MHz, DMSO-d_6): 16.6, 21.3, 23.4, 46.7, 62.3, 71.3, 72.8, 75.5, 84.3, 118.2, 134.2 (Fumarate carbons), 141.3, 149.6, 152.3.0, 152.7, 156.0, 166.3 (Fumaric acid carbonyl groups).

Structure elucidation of ethyl ester impurity (8)

Sample was analyzed by HPLC and its purity was found to be 92.15%, molecular weight of ethyl ester base impurity is 505.. The protonated molecular ion at m/z 506 (M+1) confirms the mass as 505 corresponding to molecular formula of $C_{18}H_{28}N_5O_{10}P$. IR spectrum displayed characteristic absorptions at 3342 & 3062, 2955 cm-1 corresponding to >NH and aromatic >CH stretching. The peaks at 1512 & 1442.32 cm^{-1} in IR spectrum is indicative of >C=C< ring stretching..

^{1}H NMR (300 MHz, DMSO-d_6): 1.0 (d, 3H); 1.22 (d,9H); 3.90-4.0 (m,3H); 4.1-4.3 (m,4H); 4.8-4.9 (m,1H); 4.7-4.8; 5.5-5.6 (m,4H); 6.70 (s,2H, Fumarate protons); 7.3 (brs,2H); 8.0 (s,1H); 8.15 (s,1H).

^{13}C NMR (70 MHz, DMSO-d_6): 14.0, 16.7, 21.3, 47.0, 62.0, 64.7, 73.0, 76.0, 84.1, 84.3, 118.2, 134.0 (Fumarate carbons), 141.5, 149.6, 151.8, 152.7.0, 153.4, 155.4, 166.0.(Fumaric acid carbonyl groups).

EXPERIMENTAL

(R)-9-[2-(hydroxyl)propyl]adenine **(3)**, diethyl p-toluene sulfonyloxymethylphosphonate **(4)** and 1M dibutylmagnesium in toluene are obtained from commericial source..

Mass spectrometry

Electrospray ionization mass spectroscopy was performed using an ion trap mass spectrometer (Model 6310 Agilent). The positive and negative eletrospray MS data was obtained by switching the capillary voltage between n+5000 and -4500V respectively.

NMR spectroscopy

The NMR experiments were performed on Brukeravance II 400 MH_Z. The ^{1}H chemical shift values were reported in the δ scale in ppm, relative to TMS (δ=0.00) and the ^{13}C chemical shift values were reported relative to $CDCl_3$ (δ = 77.00 ppm) and DMSO, d6 (δ=39.50 ppm) as internal standards.

FT-IR spectroscopy

The IR spectra were recorded in the solid state as KBr dispersion medium using perkin Elmer spectrum 100 FT-IR spectrophotometer.

Preparation of (R)-9-[2-(phosphonomethoxy)propyl] adenine (2) (Method-I)

(R)-9-[2-(hydroxyl)propyl]adenine (100 gm, 0.518 mol) was suspended in NMPO (200ml) at 25-35° C. Added dibutylmagnesium (400ml, 1M solution in toluene, 0.4338 mol) for 2-3 hrs at 25-35°C. Added Diethyl p-toluenesulfonyloxymethylphosphonate (300 gm, 0.9324 mol)and stirred for 10 min. Heated to 76-80°C and maintained for 10 hr. Reaction was monitored by HPLC. The reaction mixture was cooled to 25-30^{0}C. Charged acetic acid (63gm, 1.05mol) and stir for 30min. Added aq. HBr (721 gm), heated to 95°C and maintained for 4 hrs. Reaction was monitored by HPLC. The reaction mixture was cooled to 0-5^{0}C and the precipitated salts were filtered. The reaction mixture was

cooled to 20-25^{0}C, diluted with water (600 ml) and washed twice by extraction with methylene dichloride (2 X 100ml). The aqueous layer was cooled to about 10-15^{0}C, and the p^H was adjusted to between 2.8 and 3.2 with 40% NaOH solution at 3-6^{0}C, crystallizing the product. The resulting mixture was stirred at 5-8^{0}C for 2.0 h, and the product isolated by filtration. The solids were washed with chilled water (~5^{0}C, 100 ml) and dried under vaccum below 65^{0}C to yield 45 g of 2 (PMPA.H_2O, 28.5%) The solids had an HPLC purity of 98.9%, with a moisture content of 6 % w/w.

Preparation of (R)-9-[2-(phosphonomethoxy)propyl] adenine (2) (Method-II)

(R)-9-[2-(hydroxyl)propyl]adenine (100 gm, 0.518 mol) was suspended in NMPO (30ml) and tert-butanol (70gm,0.944 mol) at 25-35° C. Charged Diethyl p-toluenesulfonyloxymethylphosphonate (300 gm, 0.9324 mol)and stirred for 10 min. Added Di-n-butyl-Magnesium (400ml, 1M solution in toluene, 0.4338 mol) for 2-3 hrs at 25-35°C. Heated to 76-80°C and maintained for 10 hr. Reaction was monitored by HPLC. The reaction mixture was cooled to 25-30^{0}C. Charged acetic acid (63gm, 1.05mol) and stir for 30min. Added aq. HBr (721 gm), heated to 95°C and maintained for 4 hrs. Reaction was monitored by HPLC. The reaction mixture was cooled to 0-5^{0}C and the precipitated salts were filtered. The reaction mixture was cooled to 20-25^{0}C, diluted with water (600 ml) and washed twice by extraction with methylene dichloride (2 X 100ml). The aqueous layer was cooled to about 10-15^{0}C, and the p^H was adjusted to between 2.8 and 3.2 with 40% NaOH solution at 3-6^{0}C, crystallizing the product. The resulting mixture was stirred at 5-8^{0}C for 2.0 h, and the product isolated by filtration. The solids were washed with chilled water (~5^{0}C, 100 ml) and dried under vaccumbelow 65^{0}C to yield 90 g of 2 (PMPA.H_2O, 57%) The solids had an HPLC purity of 99.0% with a moisture content of 6 % w/w

Impurities synthesis

(1-methylethyl) (8R)-9-(6-amino-9H-purin-9-yl)-5-hydroxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ5-phosphanonanoate (tenofovirmonosoproxil), or Monoester Impurity (6)

TenofovirDisoproxil (2 g) was charged into one neck round bottom flask with magnetic stirrer. Aqammonia (10 ml) was added. The contents were stirred slowly at room temperature. The sample was checked using HPLC and purification by preparative HPLC.

(1-methylethyl)(5RS,8R)-9-(6-amino-9H-purin-9-yl)-8-methyl-5-(1-methylethoxy)-5-oxo-2,4,7-trioxa-5-λ5-phosphanonanoate,Fumarate salt or Isopropyl Impurity (7)

(R)-9-[2-(Hydroxyl)Propyl] Adenine compound is reacted with Diisopropylpara toluene sulfonyloxy methyl phosphonate in presence of Dibutylmagnesium in toluene and NMPO as solvent to get diester and further hydrolysis with aqHBr and work-up with MDC and NaOH to yield (R)-9-[2-(isopropyl Phosphonomethoxy) propyl]adenine.. (R)-9-[2-(isopropyl Phosphonomethoxy)propyl]adenine on esterification with chloromethyl isopropyl carbonate in presence of triethylamine in NMPO yields (1-methylethyl) (5RS,8R)-9-(6-amino-9H-purin-9-yl)-8-methyl-5-(1-methylethoxy)-5-oxo-2,4,7-trioxa-5-λ5-phosphanonanoate, which on salt formation with fumaric acid in isopropyl alcohol affords fumarate.

Ethyl 1-methylethyl (5RS)-5-{[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl}-5-oxo-2,4,6,8-tetraoxa-5-λ5-phosphanonanedioate., fumarate salt or ethyl impurity (8)

(R)-9-[2-(Phosphonomethoxy)propyl]adenine on esterification with mixture ofchloromethyl isopropyl carbonate and chloromethyl ethyl carbonate in presence of triethylamine in NMPO yieldsethyl 1-methylethyl (5RS)-5-{[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl}-5-oxo-2,4,6,8-tetraoxa-5-λ5-phosphanonanedioate, which on salt formation with fumaric acid in isopropyl alcohol affords fumarate

CONCLUSION

The research paper describes the synthesis of Tenofovir disoproxil fumarate and related impurities structure elucidation. The synthesis of impurities was also discussed in brief.

ACKNOWLEDGEMENT

The authors are thankful to tyche industries limited

for financial assistance.

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