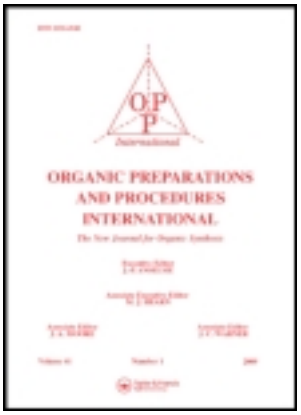

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OPPI BRIEF

Synthesis of (*E*)-9-(Propen-1-yl)-9*H*-adenine, a Mutagenic Impurity in Tenofovir Disoproxil Fumarate

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Tenofovir disoproxil fumarate 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 for the production of the virus in HIV-infected patients.[1–6] Several impurities are generated during manufacture of *tenofovir disoproxil fumarate* from adenine. The *US Pharmacopoeia* specifies that the concentration of one of those impurities, *E*-9-(propen-1-yl)adenine (**2**), should not exceed 5 ppm.[7] To the best of our knowledge, the sole reported preparation of **2** *in only milligram quantities* involves the allylation of adenine followed by base-catalyzed isomerization of **1** to **2** for which no spectroscopic data was reported (*Scheme 1*).[8] Since FDA approval requires a reliable synthesis of **2,** we investigated and now report a new and more efficient route to **2** (*Scheme 2*). This method to introduce the 9-propenyl group may be useful since it can also serve as a key blocking group for the synthesis of 1-substituted adenines.

In our approach, adenine was treated with *R,S*-propylenecarbonate in DMF at 125°C for 15 h in the presence of sodium hydroxide to afford alcohol **3.** Mesylation of **3** with methanesulfonyl chloride in presence of pyridine at 0–5°C for 6 h in chloroform gave mesylate **4** in 95% yield and 97% purity (HPLC). Treatment of the resulting mesylate with potassium *tert*-butoxide in DMF then led to (*E*)-9-(propen-1-yl)-9H-adenine **(2)** and 9-allyl adenine **(1)** in the ratio (92:8). The solid mixture was purified from water to afford pure **2** and 9-allyl adenine (**1**) was isolated, purified and characterized from the mother liquor.

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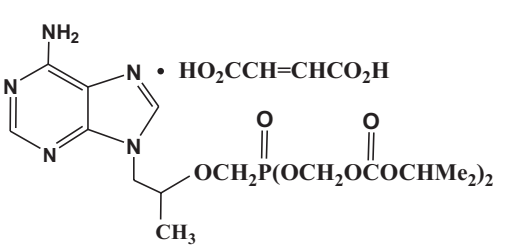
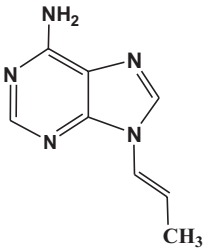
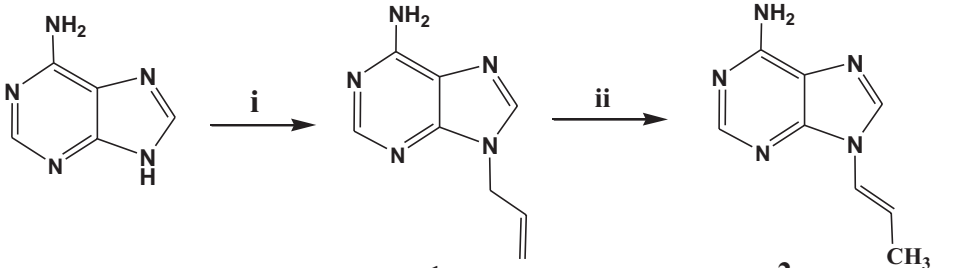


Tenofovir disoproxil fumarate



2

Figure 1



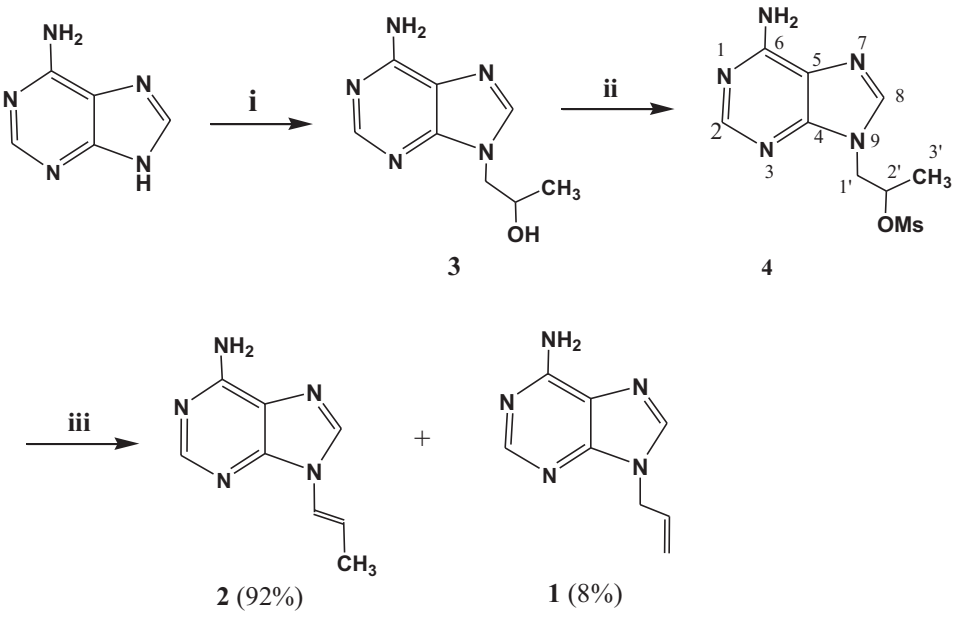
1 **2**

i) Allyl bromide, KOH, dimethylacetamide ii) KOtBu, DMSO

Scheme 1

Experimental Section

Unless otherwise stated, all melting points are uncorrected and were determined on a Electothermal melting point apparatus. Electrospray ionization mass spectroscopy was performed using an ion trap mass spectrometer (Model 6310 Agilent). The NMR spectra were performed on Bruker Avance II 400 MH_Z. The ^1H chemical shift values are reported in the δ scale relative to TMS (δ 0.00) and the ^{13}C chemical shift values are given relative to $CDCl_3$ (δ 77.00) and DMSO-d_6 (δ 39.50) as internal standards. The IR spectra were recorded as KBr pellets using a Perkin-Elmer 100 FT-IR spectrophotometer. The purity /impurity ratios of compounds **3** and **4** were determined by *HPLC method A* with an Shimadzu Class-VP system at UVmax = 260 nm, using a Hypersil - BDS C_{18} (2) column (4.6 mm ID X 250 mm, 5.0 micron) at 30°C with flow rate of 1.0 mL/min and run time of 30.0 min. Solvents: A H_2O + 0.01% disodium hydrogen orthophosphate and adjust pH to 6.0 ± 0.05 with orthophosphoric acid, B A+ Acetonitrile (20:80); Gradient: B 5%/0.0 min, B 5%/7.50 min, B 15%/15.0 min, B 40%/20.0 min, B 5%/25.0 min, B 5%/30.0 min. HPLC samples were prepared as 0.5 mg in 1.0 mL of 5% aqeous acetonitrile. The purity/impurity ratios of compounds **1** and **2** were determined by *HPLC method B* with an Shmadzu Class-VP system at UVmax = 260 nm, using a Hypersil – ODS C_{18} (2) column (4.6 mm ID X 250 mm, 5.0 micron) at 30°C with flow rate of 1.0 mL/min and run time of 60.0 min. Mobile



i) R, S-Propylene carbonate, NaOH, DMF ii) $MeSO_2Cl$, Pyridine, $CHCl_3$ iii) KOtBu, DMF

Scheme 2

phase: 0.01 M ammonium acetate + acetonitrile (60:40). HPLC samples were prepared as 0.1 mg in 1.0 mL of mobile phase.

Preparation of (R,S)-9-(2-Hydroxypropyl)adenine (3)

Adenine (100.0 g, 0.74 mol) and NaOH (2.40 g, 0.06 mol) were mixed with DMF (250 mL) at 25–30°C and stirred for 10–15 min. *R,S*-Propylenecarbonate (93.0 g, 0.97 mol) was added to the reaction mixture over 10–15 min at 25–30°C. The mixture was then heated and maintained at 125°C for 15 h; completion of reaction was monitored by HPLC. After cooling to 90°C, toluene (600 mL) was added. The suspension containing the precipitated solid was cooled to 0–5°C and stirred for 1 h. The product was collected and washed with 250 mL of chilled toluene to yield 130.0 g, (92%) of a pale yellow solid, mp. 191–193°C, *lit.*[9] mp. 191–193°C.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.07 (d, 3H), 3.98 (m, 1H), 4.06 (m, 2H), 5.05 (d, 1H), 7.19 (brs, 2H), 8.05 (s, 1H), 8.14 (s, 1H).

Preparation of 1-(Adenin-9-yl)propan-2-yl Methanesulfonate (4)

(*R,S*)-9-(2-Hydroxypropyl)adenine (100.0 g, 0.517 mol) and pyridine (61.0 g, 0.77 mol) were mixed with chloroform (500 mL) at 25–30°C and stirred for 10–15 min. Methanesulfonyl chloride (65.0 g, 0.567 mol) was then added to the reaction mixture over 1 h at 0 to –5°C. The mixture was heated to 25–30°C for 6 h; completion of reaction was monitored

by HPLC. Water (500 mL) was added and the suspension containing the precipitated solid was maintained at 20–25°C for 1 h. The product was collected and washed with 250 mL of chilled water to yield (133.0 g, 95%) of a colorless solid, mp. 177–180°C.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.36 (d, 3H), 2.94 (s, 3H), 4.41 (m, 2H), 5.2 (m, 1H), 7.3 (brs, 2H), 8.12 (s, 1H), 8.0 (s, 1H); ^{13}C NMR (70 MHz, DMSO-d_6) : δ 156.0 (C-6), 152.6 (C-2), 149.8 (C-4), 141.2 (C-8), 118.5 (C-5), 47.0 (C-1′), 76.8 (C-2′), 18.4 (C-3′), 37.5 (C-mesylate methyl); MS : m/z 271.8 ($M^+ + 1$).

Anal. Calcd for C_9 H_{13} N_5 O_3 S : C, 39.84; H, 4.83. Found: C, 39.75; H, 4.95

Preparation of (E)-9-(Prop-1-enyl)-9H-adenine (2)

1-(Adenin-9-yl)propan-2-yl methanesulfonate **(4)** (20.0 g, 0.073 mol) and potassium *tert*-butoxide (8.5 g, 0.075 mol) were mixed with DMF (150 mL) at 25–30°C. The reaction mass was stirred at 25–30°C for 3 h, completion of reaction was monitored by HPLC, the reaction mixture was poured slowly into water (1500 mL) and the suspension obtained was stirred at 25–30°C for 1 h. The product mixture was extracted into chloroform (3 × 100 mL), washed with water and concentrated to afford a mixture of **1** and **2** as a thick solid residue (9.50 g, 95%). It was dissolved in water (25 mL) at 70–75°C and stirred for 30 min. The clear solution was cooled slowly to 0–5°C and stirred for 1 h and the resulting solid was collected, washed with chilled water (10 mL), dried under vacuum to afford pure **2** (8.50 g, 85%) as a white solid, mp. 203–205°C, lit.[7] mp. 197°C. IR (cm^{-1}): 3370, 3312, 3140, 1645, 1590; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (dd, 3H), 6.55 (m, 1H), 7.1 (dd, 1H), 7.35 (brs, 2H), 8.36 (s, 1H), 8.42 (s, 1H); ^{13}C NMR (70 MHz, DMSO-d_6) : δ 156.0 (C-6), 152.9 (C-2), 148.4 (C-4), 138.5 (C-8),

121.6 (C-1′), 118.9 (C-5), 115.9 (C-2′), 15.06 (C-3′); MS : m/z 176.26 ($M^+ + 1$).

The combined mother liquors (35 mL) from the isolation of **2** was distilled under vacuum (660 mm Hg) to give a crude solid which was purified by crystallization from acetonitrile to yield (0.50 g, 5%) of **1** as a pale yellow solid, mp. 143–145°C, lit.[8] mp. 143–145°C. ^{1}H NMR (300 MHz, DMSO-d_6) : δ 4.83 (dd, 2H), 5.18 (bd, 1H), 5.33 (bd, 1H), 6.05 (m, 1H), 6.2 (bs, 2H), 7.82 (s, 1H), 8.38 (s, 1H)

Acknowledgment

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