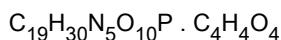
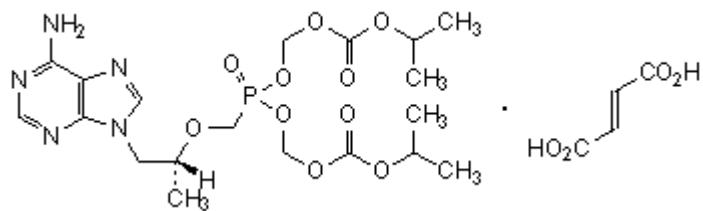


**Tenofovir disoproxil fumarate (Tenofoviri disoproxili fumaras)**

**Relative molecular mass.** 635.5.

**Chemical names.** bis(1-methylethyl) 5-[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5-λ<sup>5</sup>-phosphonanedioate (ester) hydrogen (2*E*)-but-2-enedioate (salt); bis({[(1-methylethoxy)carbonyl]oxy}methyl) {[[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl}phosphonate (ester) hydrogen (2*E*)-but-2-enedioate (salt); 5-[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8-tetraoxa-5-phosphonanedioic acid 1,9-bis(1-methylethyl) ester 5-oxide, (2*E*)-2-butenedioate (1:1);

CAS Reg. N° 202138 50 9

**Description.** White to almost-white, crystalline powder.

**Solubility.** Slightly soluble in water, soluble in methanol, very slightly soluble in dichloromethane.

**Category.** Antiretroviral (Nucleotide Reverse Transcriptase Inhibitor).

**Storage.** Tenofovir disoproxil fumarate should be kept in a tightly closed container, protected from light and stored at a temperature between 2–8 °C.

**Additional information.** Tenofovir disoproxil fumarate may exhibit polymorphism.

#### Requirements

**Definition.** Tenofovir disoproxil fumarate contains not less than 98.5% and not more than 101.0% of tenofovir disoproxil fumarate ( $\text{C}_{19}\text{H}_{30}\text{N}_5\text{O}_{10}\text{P} \cdot \text{C}_4\text{H}_4\text{O}_4$ ), calculated with reference to the anhydrous substance.

**Manufacture.** The production method is validated to ensure that the substance, if tested, would comply with:

- a limit of not more than 5 ppm for the mutagenic impurity 9-propenyladenine (impurity K), which may be a synthesis related substance, using a suitable method;
- a limit of not more than 1.0% for the tenofovir disoproxil (S)-enantiomer (impurity G) using a suitable chiral chromatographic method.

#### Identity tests

Either tests A, B and C or test D may be applied.

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under [1.14.1 Thin-layer chromatography](#) using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 10 mg of the test substance per mL and (B) 10 mg of tenofovir disoproxil fumarate RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

A.2 Carry out the test as described under [1.14.1 Thin-layer chromatography](#) using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Stain the plate with iodine vapour and examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under [1.14.1 Thin-layer chromatography](#) using silica gel R6 as the coating substance and a mixture of 50 volumes of heptane R, 30 volumes of glacial acetic acid R and 20 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 µl of each of the following 2 solutions in ethanol R. For solution (A) use 10 mg of the test substance per mL and for solution (B) use 2 mg of fumaric acid R per mL. Develop the plate in an unsaturated tank over a path of 10 cm. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

One of the principal spots obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B.2 Carry out the test as described under [1.14.1 Thin-layer chromatography](#) using the conditions described above under test B.1 but using silica gel R5 as the coating substance. Spray lightly with a 16 g/l solution of potassium permanganate R and examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

C. The absorption spectrum (1.6) of a 25 µg/mL solution, when observed between 220 nm and 320 nm, exhibits a maximum at about 261 nm; the specific absorbance ( $A_{1\text{cm}}^{1\%}$ ) is 230 to 250.

D. Carry out the examination as described under [1.7 Spectrophotometry in the infrared region](#). The infrared absorption spectrum is concordant with the spectrum obtained from tenofovir disoproxil fumarate RS or with the reference spectrum of tenofovir disoproxil fumarate. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and tenofovir disoproxil fumarate RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from tenofovir disoproxil fumarate RS.

**Specific optical rotation (1.4).** Prepare a fresh solution and perform the test without delay. Use a 10.0 mg/mL solution in hydrochloric acid (0.1 mol/l) VS and calculate with reference to the anhydrous substance;  $[\alpha]_D^{20^\circ, \text{C}} = -15^\circ$  to  $-20^\circ$ .

**Water.** Determine as described under [2.8 Determination of water by the Karl Fischer method](#), Method A. Use about 1.0 g of the substance; the water content is not more than 10 mg/g.

**Heavy metals.** Use 1.0 g in 30 mL of methanol R for the preparation of the test solution as described under [2.2.3 Limit test for heavy metals](#), Procedure 2; determine the heavy metals content according to Method A; not more than 20 µg/g.

**Sulfated ash (2.3).** Not more than 2.0 mg/g.

#### Related substances

Carry out the test as described under [1.14.4 High-performance liquid chromatography](#) using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

The mobile phases for the gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

Mobile phase A: 2 volumes of acetonitrile R, 20 volumes of phosphate buffer pH 6.0 and 78 volumes of water R.

Mobile phase B: 65 volumes of acetonitrile R, 20 volumes of phosphate buffer pH 6.0 and 15 volumes of water R.

Prepare the phosphate buffer pH 6.0 by dissolving 3.50 g of potassium dihydrogen phosphate R and 1.70 g of tetrabutyl ammonium hydrogen sulfate R in 800 mL of water R, adjust the pH to 6.0 by adding sodium hydroxide (1 mol/l) VS and dilute to 1000 mL with water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–5	81	19	Isocratic

5–40	81–49	19–51	Linear gradient
40–60	49–0	51–100	Linear gradient
60–65	0	100	Isocratic
65–70	0–81	100–19	Return to initial composition
70–80	81	19	Re-equilibration

After preparation keep the solutions at about 6 °C or use an injector with cooling.

Prepare the following solutions using water R as diluent. For solution (1) use 1.0 mg of the test substance per mL. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 5 µg of tenofovir disoproxil fumarate per mL. For solution (3) use 0.2 mg of fumaric acid R per mL.

For the system suitability test prepare solution (4) by heating solution (1) carefully in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Maintain the column temperature at 30 °C.

Inject 20 µL of solution (4). The test is not valid unless the resolution between the principal peak (retention time about 40 minutes) and the peak due to tenofovir monosoproxil (impurity A) (with a relative retention of about 0.5) is not less than 25.

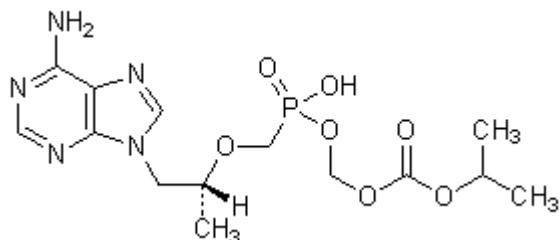
Inject alternatively 20 µL each of solutions (1) and (2) and (3). In the chromatogram obtained with solution (1) the following peak is eluted at the following relative retention, with reference to tenofovir (retention time about 40 minutes): fumarate about 0.15.

In the chromatogram obtained with solution (1) the area of any peak due to tenofovir monosoproxil (impurity A) is not greater than twice the area of the principal peak obtained with solution (2) (1.0%); the area of any other impurity peak is not greater than the area of the principal peak obtained with solution (2) (0.5%) and the areas of not more than two such peaks are greater than 0.4 times the area of the principal peak obtained with solution (2) (0.2%). The sum of the areas of all peaks, other than the principal peak, is not greater than 5 times the area of the principal peak obtained with solution (2) (2.5%). Disregard any peak corresponding to the peak obtained in the chromatogram with solution (3) and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

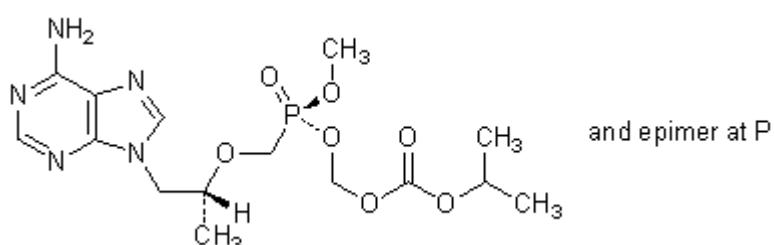
### Assay

Dissolve 0.40 g, accurately weighed, in 30 mL of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end-point potentiometrically as described under [2.6 Non-aqueous titration](#), Method A. Each mL of perchloric acid (0.1 mol/l) VS is equivalent to 63.55 mg of tenofovir disoproxil fumarate ( $C_{19}H_{30}N_5O_{10}P, C_4H_4O_4$ ).

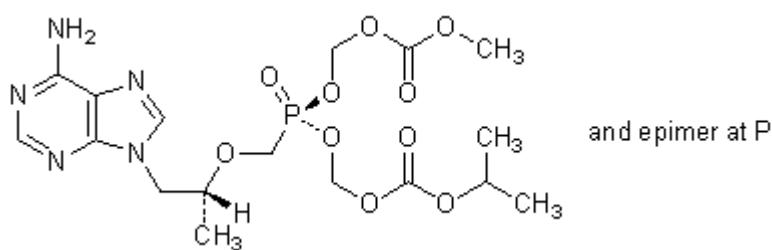
### Impurities



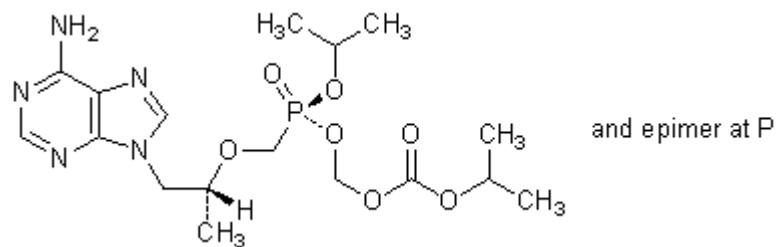
A. (1-methylethyl) (8*R*)-9-(6-amino-9*H*-purin-9-yl)-5-hydroxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ<sup>5</sup>-phosphonanoate (tenofovir monosoproxil),



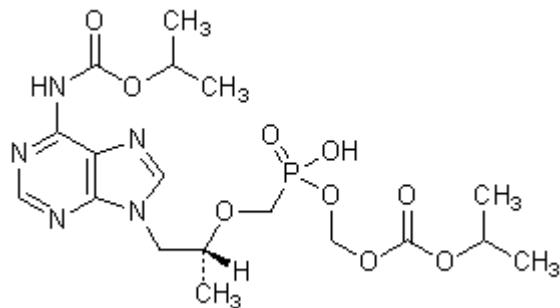
B. (1-methylethyl) (5*RS*,8*R*)-9-(6-amino-9*H*-purin-9-yl)-5-methoxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ<sup>5</sup>-phosphonanoate,



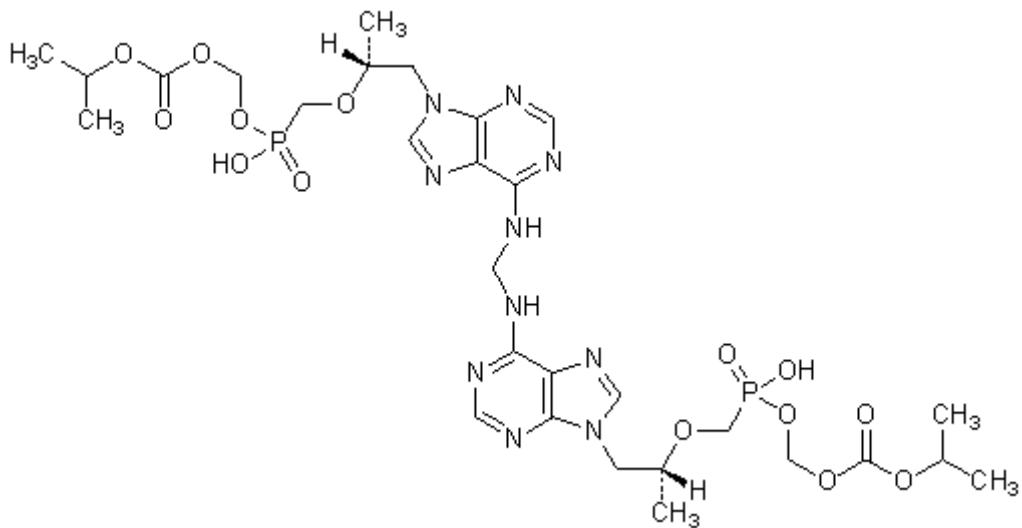
C. methyl (1-methylethyl) (5*RS*)-5-[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5- $\lambda^5$ -phosphonanedioate,



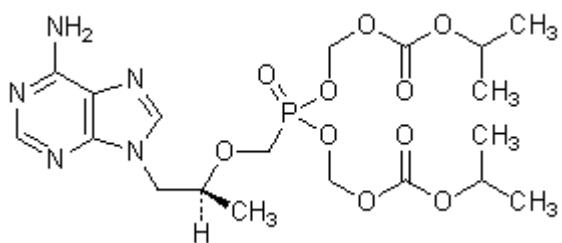
D. (1-methylethyl) (5*RS*,8*R*)-9-(6-amino-9*H*-purin-9-yl)-8-methyl-5-(1-methylethoxy)-5-oxo-2,4,7-trioxa-5- $\lambda^5$ -phosphononanoate,



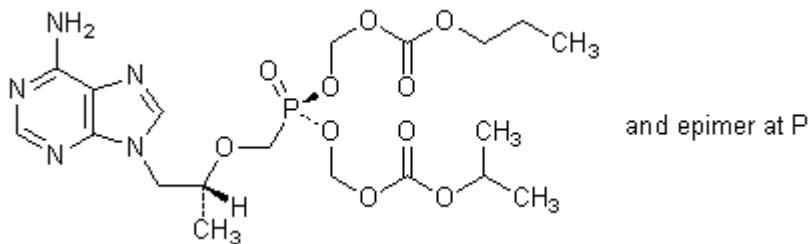
E. (1-methylethyl) (8*R*)-5-hydroxy-8-methyl-9-[(1-methylethoxy)carbonyl]amino]-9*H*-purin-9-yl)-5-oxo-2,4,7-trioxa-5- $\lambda^5$ -phosphononanoate,



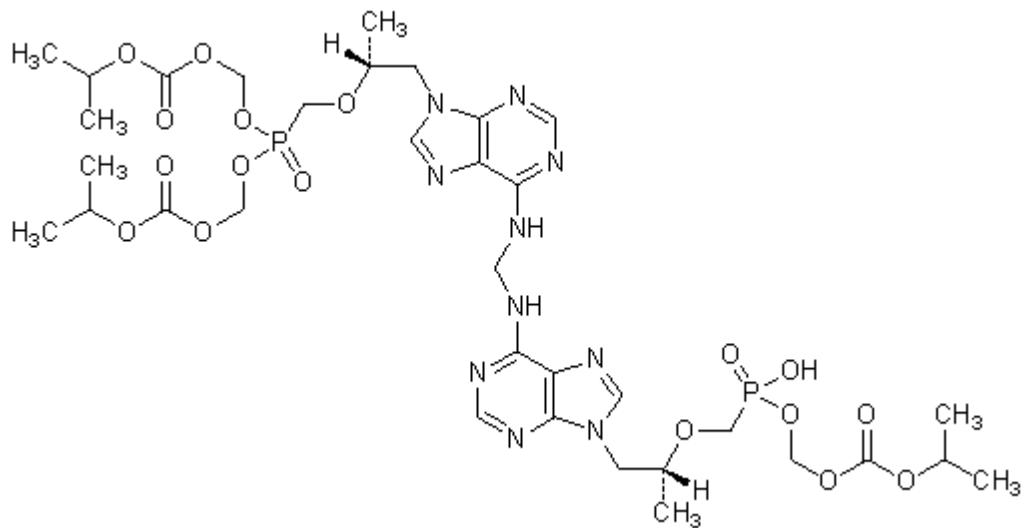
F. bis(1-methylethyl) 9,9'-[methylenebis(imino-9*H*-purine-6,9-diyl)]bis[(8*R*)-5-hydroxy-8-methyl-5-oxo-2,4,7-trioxa-5- $\lambda^5$ -phosphononanoate] (tenofovir monosoproxil dimer),



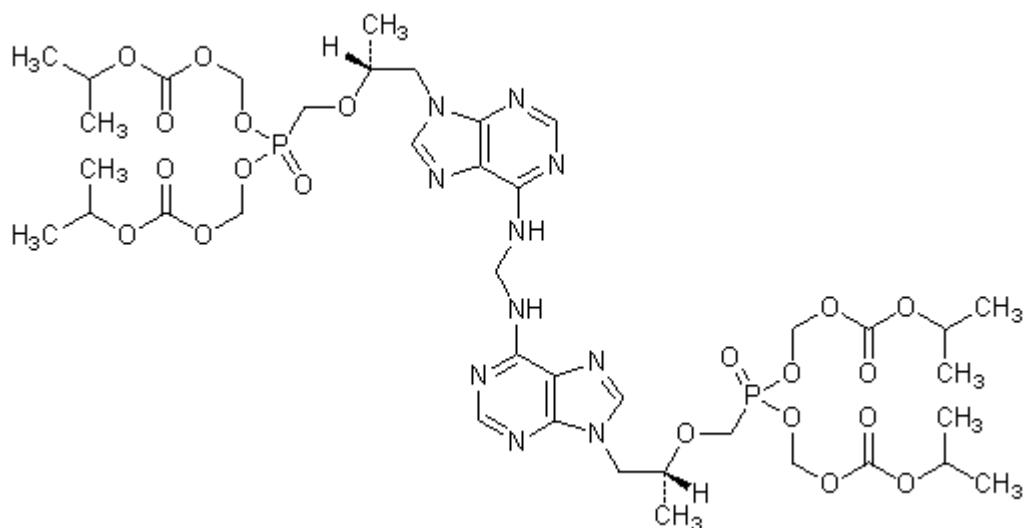
G. bis(1-methylethyl) 5-[(1S)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5- $\lambda^5$ -phosphonanediyl (tenofovir disoproxil (S)-enantiomer) [see under Manufacture],



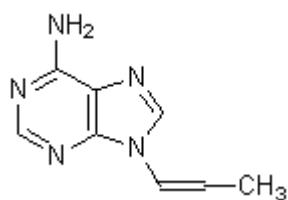
H. 1-methylethyl propyl (5*RS*)-5-[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5- $\lambda^5$ -phosphonanediyl,



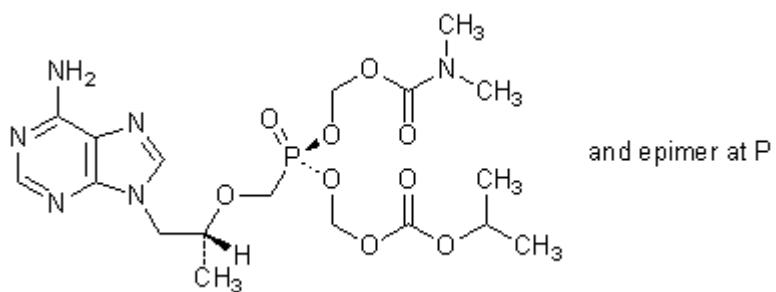
I. bis(1-methylethyl) 5-[(1*R*)-2-(6-[(9-[(2*R*)-5-hydroxy-2,11-dimethyl-5,9-dioxo-3,6,8,10-tetraoxa-5- $\lambda^5$ -phosphadodecyl]-9*H*-purin-6-yl]amino)methyl]amino]-9*H*-purin-9-yl)-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5- $\lambda^5$ -phosphonanediyl (tenofovir di- and monosoproxil heterodimer),



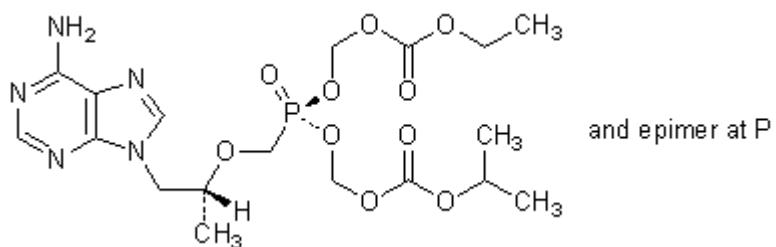
J. tetrakis(1-methylethyl) 5,5'-(methylenabis{imino-9H-purine-6,9-diyl[(2R)-propane-1,2-diyl]oxymethylene})bis[5-oxo-2,4,6,8-tetraoxa-5-λ⁵-phosphonanedioate] (tenofovir disoproxil dimer),



K. 9-(prop-1-enyl)-9H-purin-6-amine, [see under Manufacture],



L. (1-methylethyl) (5RS)-5-[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-10-methyl-5,9-dioxo-2,4,6,8-tetraoxa-10-aza-5-λ⁵-phosphaundecanoate,



M. 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-phosphonanedioate.