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<th>(51) International Patent Classification 6</th>
<th>(11) International Publication Number: WO 96/09307</th>
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<tr>
<td>C07F 9/6561, A61K 31/675</td>
<td>(43) International Publication Date: 28 March 1996 (28.03.96)</td>
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<tr>
<th>(21) International Application Number:</th>
<th>PCT/EP95/03791</th>
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<td>(22) International Filing Date:</td>
<td>25 September 1995 (25.09.95)</td>
</tr>
<tr>
<td>(30) Priority Data:</td>
<td>MI94A001939 23 September 1994 (23.09.94) IT</td>
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| (54) Title:                          | PURINE AND 8-AZAPURINE DERIVATIVES SUITABLE TO THE THERAPEUTIC TREATMENT OF AIDS |

| (57) Abstract                        | The present invention refers to new purine and 8-azapurine derivatives, to the process for their preparation and to their use in the therapy of HIV virus infections alone or together with AZT and/or ddi and/or ddc. |

**Published**

*With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*
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PURINE AND 8-AZAPURINE DERIVATIVES SUITABLE TO THE THERAPEUTIC TREATMENT OF AIDS

PRIOR ART

Since the discovery of the human immunodeficiency virus (HIV) and of its crucial role in AIDS, a considerable research effort has been aimed at studying of the virus multiplication cycle and at identifying of potential targets for clinical intervention. Among the various approaches in the search of new therapies for AIDS, the inhibition of reverse transcriptase (RT) and viral protease represents two particularly promising strategies.

Azidothymide (AZT) has been the first drug to be used for the treatment of AIDS and, more recently, dideoxyinosine (ddI) has been licenced for the treatment of patients who show intolerance to AZT. ddC and d4T are the other two drugs at present used in the clinic.

Besides the apparent selectivity for the RT, the above compounds also inhibit the mammalian DNA polymerases (α, β, γ and δ) and often show significant toxic side-effects. Moreover, the emergence of drug-resistant strains is a further problem linked to their use. There is, therefore, urgent need to find new anti-HIV agents.

Particular interest, for their anti-HIV activity, have shown some analogues of phosphonated nucleosides such as the 9-[(2-phosphonomethoxy)ethyl]adenine (PMEA), the 9-[(2-phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP) (E. De Clercq et al. Antiviral Research, 1987,8, pp. 261-272; R. Pauwels et al. Antimicrob. Agents Chemother. 1988,33, pp. 1025-1030) and the 9-[(2-phosphonomethoxy)ethyl]guanine (PMEG) (European Patent Application EP
PMEG is one of the most powerful wide spectrum antiviral agents known up to now. However, due to its cytotoxicity, it has not been possible to use it in the clinic. The search for drugs equally powerful, but endowed with a better selectivity index is therefore important.

**SUMMARY**

We have found new purine and 8-azapurine derivatives having high antiviral activity and low toxicity.

Said derivatives have the following general formula:

![Chemical Structure](image)

wherein Z is N or CH, R₁ is an amino group or hydrogen, R₂ is an halogen or a OR₅ or NR₅ group where R₅ is hydrogen or an alkyl-carbonyl group, R₃ is a CH=CH₂ or CH₂X group where X is hydrogen or halogen or N₃ and R₄ is hydrogen or methyl, and wherein R₃ and R₄ together may form also a cyclopropyl or halocyclopropyl group or may be substituted by a =CH₂ group.

The present invention refers also to pharmaceutical compositions containing at least one derivative (I) or one of its pharmacologically acceptable salts or esters and to the relative therapeutic method.

Finally the present invention refers also to a process for the preparation of the derivatives having formula (I).
DETAILED DESCRIPTION OF THE INVENTION

The characteristics and the advantages of the purine and 8-azapurine derivatives according to the present invention and the process for their preparation will be mostly pointed out during the following detailed description.

Said derivatives have the following general formula:

\[
\begin{align*}
\text{N} & \text{N} \\
\text{O} & \text{O} \\
\text{P(OH)}_2 & \\
\end{align*}
\]

\(\text{Z} \) is N or CH, \(\text{R}_1\) is an amino group or hydrogen, \(\text{R}_2\) is an halogen or a \(\text{OR}_5\) or \(\text{NHR}_5\) group where \(\text{R}_5\) is hydrogen or an alkyl-carbonyl group, \(\text{R}_3\) is a \(\text{CH} = \text{CH}_2\) or \(\text{CH}_2\text{X}\) group where \(\text{X}\) is hydrogen or halogen or \(\text{N}_2\) and \(\text{R}_4\) is hydrogen or methyl, and wherein \(\text{R}_3\) and \(\text{R}_4\) together may form also a cyclopropyl or halocyclopropyl group or may be substituted by a \(-\text{CH}_2\) group.

The derivatives having general formula (I) according to the present invention are prepared by the process reported in the following Scheme 1 (method A) and the derivatives with formula Ia and Ib are also prepared by the process reported in the Scheme 2 (method B).

The compounds of the invention can be isolated in the form of free acids. The free acid forms can be easily transformed into physiologically acceptable salts by methods known in the art. Such salts include those of ammonium ion, \(\text{Li}^+\), \(\text{Na}^+\); the salts may be monobasic or dibasic.
Said process (method A) is realized by the following steps:

a) the compound (II) is reacted with a mesylate having formula (III) in an organic solvent in presence of cesium carbonate obtaining a
mixture of the two regioisomers (IV) and (V), respectively N8 and N9 substituted;
b) the two regioisomers (IV) and (V) are separated by silica gel chromatography;
c) the compound (V) is treated with bromotrimethylsilane in an organic solvent obtaining the derivative having formula (I). The mesylate having formula (III) is prepared with the method described by K.-L. Yu et al. (J. Med. Chem. 1992. 35. 2958-2969). The reaction of the step a) is carried out in anhydrous dimethyl sulfoxide, stirring and under nitrogen atmosphere at a temperature equal to 95-100 °C. The molar ratio between the compound (III) and the compound (II) is between 0.8:1 and 1.2:1 and preferably it is 1:1. At the end of the reaction the mixture is cooled at room temperature and filtered and the filtrate is evaporated to dryness in vacuo. The separation of the step b) is realized submitting the residue obtained by evaporation of the step a) to flash chromatography on silica gel eluting with a mixture of solvents such as CHCl₃-MeOH (95:5) or CHCl₃-MeOH-NH₄OH (90:9:1). As first fraction the compound (IV) is separated while the compound (V) is obtained as second fraction.
The reaction of the step c) is carried out in anhydrous acetonitrile at a temperature equal to 25-30 °C. The molar ratio between bromotrimethylsilane and the compound (V) is between 15:1 and 8:1 and preferably it is 10:1. Then the product (I) is separated from the reaction mixture by known technique operations. For explanatory aim of the invention the following examples concerning the preparation of the derivatives having formula (I) are reported.
EXAMPLE 1

la) Preparation of (R)-5-Amino-2-[2-(diisopropylphosphonomethoxy)propyl]-2H-1,2,3-triazolo[4,5-d] pyrimidin-7-one (IVa) and of the isomer (R)-5-amino-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d] pyrimidin-7-one (Va).

The compound (R)-2-0-[(diisopropylphosphonomethyl)-1-O-(methylsulfonyl)-1,2-propanediol (2.18 g, 6.57 mmol) prepared according to the process described by K.-L. Yu et al. (J. Med. Chem. 1992, 35, 2958-2969) in anhydrous dimethyl sulfoxide (30 ml) is added to a stirred mixture of 8-azaguanine (1 g, 6.57 mmol) in anhydrous dimethyl sulfoxide (30 ml) and cesium carbonate (2.66 g, 8.18 mmol) under nitrogen atmosphere and the reaction mixture is warmed at 95 °C for 6 hours.

After cooling at room temperature, the mixture is filtered and the filtrate is evaporated to dryness in vacuo.

lb) Separation of the isomers N8 and N9 substituted.

The residue obtained by evaporation of the step 1a) is submitted to flash chromatography on silica gel eluting with a mixture of CHCl3-MeOH (95:5). As first fraction the compound (R)-5-amino-2-[2-(diisopropylphosphonomethoxy)propyl]-2H-1,2,3-triazolo[4,5-d] pyrimidin-7-one (IVa) as yellow oil (0.5 g, 19%) is separated.

TLC (CHCl3-MeOH 80:20); Rf 0.67.

1H NMR (Me2SO-d6): 6 1.11-1.17 (m, 12H, POCH3); 1.19 (d, J=6.0 Hz, 3H, CH3); 3.65 (dd, J=9.3, 13.7 Hz, 1H, OCH2P); 3.80 (dd, J=9.3, 13.7 Hz, 1H, OCH2P); 4.16 (m, 1H, H-2'); 4.42-4.58 (m, 4H, 2H-1', 2POCH); 6.52 (s, 2H, NH2); 10.98 (s, 1H, NH).
Analysis: calc. for C_{14}H_{25}N_{6}O_{5}P: C 43.3; H 6.49; N 21.64;
Found: C 43.27; H 6.38; N 21.68.

The compound (R)-5-amino-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Va) has been obtained as a yellow foam (0.67 g, 26%).

TLC (CHCl_{3}-MeOH 80:20); Rf 0.64.

{\textsuperscript{1}H NMR (Me_{2}SO-d_{6})): 6 1.16 (m, 12 H, POCH}_{3}; 1.20 (d, J = 5.7 Hz, 3 H, CH}_{3}; 3.65 (dd, J = 9.3, 13.7 Hz, 1 H, OCH}_{2}P); 3.78 (dd, J = 9.3, 13.7 Hz, 1 H, OCH}_{2}P); 4.10 (q, 1 H, H-2'); 4.31 (d, J = 5.9 Hz, 2 H, H-1'); 4.49 (m, 2H, POCH); 6.95 (s, 2 H, NH); 10.98 (s, 1 H, NH).

Analysis: calc. for C_{14}H_{25}N_{6}O_{5}P: C 43.3; H 6.49; N 21.64;
Found: C 43.33; H 6.40; N 21.70.

1c) Preparation of (R)-5-Amino-3-[2-(phosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Ia).

A solution of (R)-5-amino-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Va) (0.65 g, 1.68 mmol) in anhydrous acetonitrile (20 ml) is treated with bromotrimethylsilane (2.56 g, 16.8 mmol) at 28 °C for 24 hours. The mixture is evaporated to dryness in vacuo and the residual oil is treated with a mixture of H_{2}O-acetone (5:30 ml) for 30 min. at 28-30 °C and left at -20 °C for 14 hours. The obtained precipitate is filtered and washed with acetone obtaining the compound (R)-5-amino-3-[2-(phosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Ia) as a white crystalline solid (0.33 g, 64%).

M.p. 252-254 °C; TLC (H_{2}O-CH_{3}CN 80:20); Rf 0.83.
UV (pH 12) λ max 256 nm (ε 8300); 280 (ε 13400).

$[\alpha]^{20}_{D}$ -4.85° (c 0.62 H$_2$O).

$^{31}$P NMR (Me$_2$SO-d$_6$): 16.3.

$^1$H NMR (Me$_2$SO-d$_6$): δ 1.15 (pseudo t, 3H, CH$_3$); 3.49 (d, J=9.1 Hz, 2H, OCH$_2$P); 4.10 (m, 1H, H-2'); 4.43-4.65 (m, 2H, 2H-1'); 6.50 (s, 2H, NH$_2$); 11.05 (s, 1H, NH).

$^{13}$C NMR (Me$_2$SO-d$_6$): δ 156.0, 155.8, 151.8, 124.3, 74.8 (d, $^3$J$_{cp}$ = 10.5 Hz, C-2'); 65.5 (d, $^1$J$_{cp}$=160 Hz, OCH$_2$P), 49.9 (C-1'), 17.9 (C-3').

Analysis: calc. for C$_8$H$_{13}$N$_6$O$_2$P: C 31.59; H 4.31; N 27.63;

Found: C 31.50; H 4.40; N 27.68.

**EXAMPLE 2**

2a) Preparation of (S)-5-Amino-2-[2-(diisoproplyphosphonomethoxy)propyl]-2H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (IVb) and of the isomer (S)-5-amino-3-[2-(diisoproplyphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Vb).

The (S)-2-O-[(diisopropylphosphonomethyl)-1-0-(methylsulfonyl)-1,2-propanediol (1.82 g, 5.47 mmol) prepared according to the process described by K.-L. Yu et al. (J. Med. Chem. 1992, 35, 2958-2969) in anhydrous dimethyl sulfoxide (30 ml) is added to a stirred mixture of 8-azaguanine (1 g, 6.57 mmol) in anhydrous dimethyl sulfoxide (30 ml) and cesium carbonate (2.14 g, 6.57 mmol) under nitrogen atmosphere and the reaction mixture is warmed at 100 °C for 7 hours.

After cooling at room temperature, the mixture is filtered and the filtrate is evaporated to dryness in vacuo.

2b) Separation of the isomers N8 and N9 substituted.

As it is described in the Example 1, the N8 substituted derivative (0.55 g, 20%) (IVb) has been separated as first fraction as an oily
product by chromatography on a silica gel column eluting with a mixture of CHCl₃-MeOH-NH₄OH (90:9:1).

TLC (CHCl₃-MeOH 80:20); Rf 0.46.

¹H NMR (Me₂SO-d₆): 6 1.10-1.20 (m, 15 H, CH₃, POCHCH₃); 3.60-3.98 (m, 2H, OCH₂P); 4.15 (dd, J=6.0, 9.0 Hz, 1H, H-2'); 4.40-4.55 (m, 4H, H-1', POCH); 6.50 (s, 2H, NH₂); 11.00 (s, 1H, NH).

Analysis: calc. for C₁₄H₂₅N₆O₅P: C 43.3; H 6.49; N 21.64;
Found: C 43.42; H 6.35; N 21.70.

The compound (S)-5-Amino-3-[2-(diisopropylphosphono)methoxy]propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Vb) (0.70 g, 27%) has been separated as second eluted as an oil from the same column chromatography of the derivative N8 substituted.

TLC (CHCl₃-MeOH 80:20); Rf 0.42.

¹H NMR (Me₂SO-d₆): 6 1.12-1.21 (m, 15 H, CH₃, POCHCH₃); 3.60-3.92 (m, 2H, OCH₂P); 4.12 (m, 1H, H-2'); 4.35 (d, J=6.0 Hz, H-1'); 4.40-4.58 (m, 2H, H-1', POCH); 4.65 (m, 1H, POCH); 6.48-6.92 (2s, 2H, NH₂); 10.90-11.25 (2s, 1H, NH).

Analysis: calc. for C₁₄H₂₅N₆O₅P: C 43.3; H 6.49; N 21.64;
Found: C 43.22; H 6.55; N 21.60.

2c) Preparation of (S)-5-amino-3-[2-(phosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Ib).

The derivative (S)-5-amino-3-[2-(phosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Ib) has been prepared as described for the isomer (R) (compound Ia) (Example 1), as a white solid.

M.p. 250-252 °C. TLC (H₂O-MeCN 80:20); Rf 0.77.

UV (pH 12) λ max 252 nm (ε 5900); 278 (ε 8900).
[a]^{20} D$^* +4.82$ (c 0.44, H$_2$O).

$^{31}$P NMR (Me$_2$SO-d$_6$): 16.3.

$^1$H NMR (Me$_2$SO-d$_6$): 6 1.10 (d, J=5.8 Hz, 3H, CH$_3$); 3.40 (d, J=9.3 Hz, 2H, OCH$_2$P); 4.05 (m, 1H, H-2'); 4.40-4.60 (m, 2H, 2H-1'); 6.50 (s, 2H, NH$_2$); 11.25 (s, 1H, NH).

$^{13}$C NMR (Me$_2$SO-d$_6$): 6 155.9; 155.6; 151.8; 124.3, 74.9 (d, $^3$J$_{CP}$ = 9.1 Hz, C-2'); 65.2 (d, $^1$J$_{CP}$=159.5 Hz, OCH$_2$P), 49.9 (C-1'), 17.9 (C-3').

Analysis: calc. for C$_6$H$_{13}$N$_6$O$_5$P: C 31.59; H 4.31; N 27.63; Found: C 31.47; H 4.44; N 27.71.

**EXAMPLE 3**

3a) Preparation of (R)-5-amino-7-chloro-2-[2-(diisopropylphosphonomethoxy)propyl]-2H-1,2,3-triazolo[4,5-d]pyrimidine (IVc) and of the isomer (R)-5-amino-7-chloro-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazole[4,5-d]-pyrimidine (Vc).

To a stirred mixture of 5-amino-7-chloro-1,2,3-triazolo[4,5-d]pyrimidine prepared according to the process described by Shealy Y.F. et al. (J. Org. Chem. 1962, 27, 4518-4523) (1g, 5.86 mmol) in anhydrous dimethyl sulfoxide (30 ml) and cesium carbonate (2.37 g, 7.29 mmol) under nitrogen atmosphere, is added the compound (R)-2-0-[(diisopropylphosphono)methyl]-1-0-(methylsulfonyl)-1,2-propanediol (IIa) (1.95 g, 5.86 mmol) in anhydrous dimethyl sulfoxide (30 ml) and the reaction mixture is warmed at 95 °C for 6 hours. After cooling to room temperature, the mixture is filtered and the filtrate is evaporated to dryness in vacuo. The obtained residue is purified by flash chromatography on silica gel eluting with a mixture of CHCl$_3$-MeOH (95:5). The compound (R)-5-amino-7-chloro-2-[2-
(diisopropylphosphonomethoxy)propyl]-2H-1,2,3-triazolo[4,5-d]
pyrimidin-7-one (IVc) as a yellow oil (0.59 g, 25%) is separated as
first fraction.

TLC (CHCl₃-MeOH 80:20).

5 ¹H NMR (CDCl₃): 6 1.21-1.27 (m, 15H, POCH₃ and CH₃); 3.48 (dd, J=9.6,
13.7 Hz, 1H, OCH₂P); 3.56 (dd, J=7.2, 14.3 Hz, 1H, OCH₂P); 4.20 (m,
1H, H-2'); 4.57-4.73(m, 4H, 2H-1', 2POCH); 5.25 (br s, 2H, NH₂).

Analysis: calc. for C₁₄H₂₄ClN₆O₄P: C 41.34; H 5.95; N 20.66;
Found: C 41.40; H 5.87; N 20.69.

The compound (R)-5-amino-7-chloro-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidine
(Vc) has been obtained as second fraction. from the chromatography of
the former compound N8 substituted as a yellow foam (1.57 g, 66%).

TLC (CHCl₃-MeOH 80:20).

10 ¹H NMR (CDCl₃): 1.26-1.41 (m, 15H, POCH₃ and CH₃); 3.39 (dd, J=9.7,
13.8 Hz, 1H, OCH₂P); 3.54 (dd, J=7.2, 14.2 Hz, 1H, OCH₂P); 4.15 (m,
1H, H-2'); 4.58-4.73(m, 4H, 2H-1', 2POCH); 5.15 (br s, 2H, NH₂).

Analysis: calc. for C₁₄H₂₄ClN₆O₄P: C 41.34; H 5.95; N 20.66;
Found: C 41.29; H 5.91; N 20.73.

3b)(R)-5-Amino-7-chloro-3-[2-(phosphonomethoxy)propyl]-3H-1,2,3-
triazolo[4,5-d]pyrimidine (Ic).

A solution of (R)-5-amino-7-chloro-3-[2-(diisopropylphosphonomethoxy) propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidine (Vc) (1.45 g, 3.56 mmol) is
treated with bromotrimethylsilane (5.45 g, 35.6 mmol) in anhydrous
acetonitrile (20 ml) at 28 °C for 18 hours. The mixture is evaporated
to dryness in vacuo and the residual oil treated with a mixture of
H₂O-acetone (5:30 ml) and stirred for 30 min. at 28°C. The mixture is
cooled at -20°C for 12 hours. The obtained precipitate is filtered and washed with acetone obtaining (R)-5-amin o-7-chloro-3-[2-(phosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidine as a white crystalline solid (0.97 g, 85%).

M.p. 200-202 °C; TLC (H₂O-CH₃CN 80:20).

3¹P NMR (Me₂SO-d₆): 16.1.

¹H NMR (Me₂SO-d₆): δ 1.09 (d, J=5, 7 Hz, 3H, CH₃); 3.38 (d, J=9, 2 Hz, 2H, OCH₂P); 4.01 (m, 1H, H-2'); 4.38-4.57 (m, 2H, H-1'); 6.20 (s, 2H, NH₂).

¹³C NMR (Me₂SO-d₆): δ 157.2; 155.8; 151.4; 125.1, 74.8 (d, 3JCp=10.5 Hz, C-2'), 65.5 (d, 1JCp=160 Hz, OCH₂P), 47.9 (C-1'), 16.3 (C-3').

Analysis: calc. for C₈H₁₂CIN₆O₄P: C 29.78; H 3.75; N 26.05.
Found: C 29.69; H 3.80; N 26.12.

The derivatives of formula (V) of the present invention are most conveniently prepared by the process reported in scheme 2 (method B).

This procedure is carried out through the following steps:

a) the mesylate (III) is reacted with sodium azide in an organic solvent to give the azido derivative (VI);

b) the azido derivative (VI) is converted into the amino derivative (VII) by catalytic hydrogenation;

c) the amino derivative (VII) is reacted with 2-amino-6-chloro-5-nitro-4-(3H)-pyrimidinone (VIII) [prepared as described by Davoll, J. and Evans, D.D. (J. Chem. Soc. 1960. 5041-5049) to give the derivative of formula (IX);

d) compound (IX) is catalytically hydrogenated and then converted into compound (V) by nitrosation in acidic medium.

The reaction of step a) is carried out in dimethylformamide, under
nitrogen atmosphere at 80 °C. The molar ratio between compound (III) and sodium azide is 1:10. The solvent is then removed by evaporation. The residue is treated with water and extracted with chloroform. Compound (VI) is obtained pure after evaporation of the organic solvent.

The reaction of step b) is carried out in methanol over palladium on carbon under hydrogen atmosphere at 40 psi. The molar ratio between compound (VI) and catalyst is 30:1. After filtration, compound VII is obtained enough pure by evaporation of the organic solvent.

The reaction of step c) is executed by reaction of (VII) with compound (VIII) in a dipolar aprotic solvent, such as dimethylformamide in the presence of triethylamine at room temperature. The derivative (IX) is purified by flash chromatography on silica gel eluting with a mixture of solvents such as CHCl₃-MeOH (95:5).

The first reaction of step d) is executed in methanol by catalytic hydrogenation (Raney nickel at a hydrogen pressure of 50 psi). The molar ratio between (IX) and catalyst ranges from 8:1 to 2:1 and preferentially is 4:1. To the reaction mixture is then added acetic acid in deoxygenated water (40:10). To this mixture stirred and cooled at 0°C sodium nitrite in deoxygenated water is added. Compound (V) is obtained by extraction of the mixture with chloroform.

The following example serves to illustrate the process reported in Scheme 2.
Example 4

Preparation of (R)-5-Amino-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]-pyrimidin-7-one (Va) (Method B).

Preparation of (R)-1-Azido-2-0-[(diisopropylphosphonomethoxy)methoxy]propene (VIa)

A stirred mixture of (R)-2-0-[(diisopropylphosphonomethyl)-1-0-(methylsulfonyl)-1,2-propanediol (III) (5 g, 15.04 mmol) in anhydrous dimethylformamide (100 mL) and sodium azide (9 g, 138.4 mmol) is heated to 80°C for 12 h under nitrogen atmosphere. At this time the
reaction is completed by TLC. The resulting mixture is allowed to
cool to room temperature and then concentrated in vacuo. The residue
is diluted with 50 mL of water, and extracted with chloroform (3 x 50
mL). The combined organic extracts are dried over Na₂SO₄, filtered
and evaporated to dryness in vacuo to give (R)-1-azido-2-0-
[(diisopropylphosphono)methoxy]propane (VIIa) as an oil (3.6 g, 84%).
TLC (CHCl₃-MeOH 95:5): Rf 0.76
IR (cm⁻¹): 2100 (azide).
¹H NMR (CDCl₃): δ 1.18 (d, J = 6.2 Hz, 3H); 1.30 (d, J = 6.2 Hz, 12
H); 3.22 (d, J = 5.7 Hz, 2H); 3.65 (d, J = 5.7 Hz, 2H); 3.75 (m, 1H);
4.73 (m, 2H).
Analysis: Calc. for C₁₀H₂₂N₃O₄P: C 43.01, H 7.94, N 15.05; Found C
42.98, H 7.89, N 15.09.
Preparation of (R)-2-0-[(Diisopropylphosphonomethoxy)propyl]amine
VIIa).
The title compound is obtained by reaction of (R)-1-azido-
2-0(diisopropylphosphono)methoxy]propane (VIIa) (3 g, 10.74 mmol) in 50
mL of MeOH with palladium on carbon (10%, 0.50 g) under hydrogen
atmosphere at 40 psi for 1.5 h. The catalyst is removed by filtration
and the filtrate is evaporated in vacuo to give (R)-2-0-
[(diisopropylphosphonomethoxy)propyl]amine (VIIa) as a colorless oil
(2.5 g, 92.3%).
TLC (CHCl₃-MeOH 80:20): Rf 0.2.
¹H NMR (CDCl₃): δ 1.02 (d, J = 6.2 Hz, 3H); 1.23 (d, J = 6.2 Hz, 12
H); 1.38 (br s, 2H); 2.62 (m, 2H); 3.40 (m, 1H); 3.55, 3.72 (2dd, J =
4.7, 9.5 Hz, 2H); 4.58-4.70 (m, 2H).
Analysis: Calc. for C₁₀H₂₄N₃O₄P: C 47.42, H 9.55, N 5.53; Found C
47.48, H 9.50, N 5.60.

**Preparation of (R)-2-Amino-5-nitro-6-[2-(diisopropylphosphonomethoxy)-propylamino]-3H-pyrimidin-4-one (IXa).**

A mixture of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (VIII) (1 g, 4.79 mmol), (R)-2-O-[(diisopropylphosphonomethoxy)propyl]amine (VIIa) (1.22 g, 4.81 mmol) and triethylamine (1 mL, 7.2 mmol) in anhydrous dimethylformamide (20 mL) is stirred under nitrogen atmosphere for 2 h at room temperature. The reaction mixture is filtered and the filtrate is evaporated to dryness in vacuo. The residue is purified by flash chromatography on silica gel eluting with CHCl₃-MeOH (95:5) to give (R)-2-amino-5-nitro-6-[2-(diisopropylphosphonomethoxy)propylamino]-3H-pyrimidin-4-one (IXa) as an oil which is crystallized from diethyl ether/petroleum ether (1.65 g, 84%, yellow crystals).

M.p. 158-160°C.

TLC (CHCl₃-MeOH 85:15); Rf 0.58.

°H NMR (CDCl₃); δ 1.23 (d, J = 6.2 Hz, 3H); 1.35 (m, 12 H); 3.48 (m, 1 H); 3.83 (m, 4H); 4.77 (m, 2 H); 5.90, 8.31 (2 br s, 2 H); 9.82 (t, 1H); 10.72 (br s, 1 H).

Analysis: calc. for C₁₄H₂₆N₅O₇P: C 41.28, H 6.43, N 17.19; Found: C 41.32, H 6.45, N 17.21.

**Preparation of (R)-5-Amino-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]-pyrimidin-7-one (Va)**

A mixture of (R)-2-Amino-5-nitro-6-[2-(diisopropylphosphonomethoxy)propylamino]-3H-pyrimidin-4-one (IXa) (0.8 g, 1.96 mmol) in 40 mL of anhydrous MeOH and Raney nickel (0.4 g) is reacted at a hydrogen pressure of 50 psi. After 1 h the reaction is completed by TLC. The stirred crude mixture is cooled at 0°C; a mixture of acetic
acid/deoxygenated water (40:10) and a solution of sodium nitrite (0.55 g, 8.0 mmol) in 30 mL of deoxygenated water are added. The reaction mixture is stirred at room temperature for 3 h, filtered and the filtrate is evaporated to dryness. Water (40 mL) and CHCl₃ (50 mL) are added to the residue. The aqueous layer is extracted with CHCl₃ (3 x 50 mL). The combined organic extracts are dried over Na₂SO₄ and filtered. The solvent is evaporated in vacuo to yield 0.7 g (91.7%) of (R)-5-amino-3-[2-(diisopropylphosphonomethoxy)propyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (Va) as a yellow foam.

10 CYTOTOXICITY AND ANTI-HIV ACTIVITY OF THE DERIVATIVES OF THE PRESENT INVENTION

The cytotoxicity and the anti-HIV activity of derivatives (Ia) and (Ib) prepared as described in the Examples 1 and 2 have been tested in comparison with some known antiviral compounds. The relative experimentation has been carried out on MT-4 cells which have the characteristic to allow HIV replication.

The cytotoxicity of the compounds is expressed as the concentration necessary to reduce of the viability of normal MT-4 cells by 50% (CC50). The activity is expressed as the concentration required to protect 50% of the MT-4 cells from the virus-induced cytopathogenic effect (EC50). The MT-4 cells have been cultured in RPMI 1640 additioned of fetal calf serum (FCS) at 10%, penicillin 100 U/ml and streptomycin 100 µg/ml. The cultures have been incubated at 37 °C in a 5% CO₂ atmosphere and have been periodically checked for the absence of mycoplasmas contamination.

The experimental protocols used for the evaluation of cytotoxicity and antiviral activity are based on the MTT colorimetric method, owing to
its easy execution and to the quickness of the answer. The MTT is a tetrazolium salt which is transformed by the mitochondrial succinate dehydrogenase enzyme to formazane which is a product having blue colour. The amount of formazane produced is directly proportional to the number of living cells.

As far as the cytotoxicity of the compounds is concerned, the various experiments have been carried out according to the following procedure: 50 µl of culture medium containing 1 x 10^4 MT-4 cells have been added, in microplates wells, to 50 µl of culture medium containing different concentrations of the compounds under examination, and to culture medium not containing said compounds. After 4 days of incubation at 37 °C 20 µl of MTT (2.5 mg/ml) have been added to each well.

After 4 hours of incubation at 37 °C the formazane produced has been solubilized by adding 150 µl/well of a mixture formed by isopropanol, 0.34% of HCl and 5% of the nonionic detergent Nonidet P80 (np-40).

The produced amount of formazane has been then measured at the spectrophotometer by optical density reading at 570 nm.

For the estimation of the anti-HIV activity of the compounds, the MT-4 cells, seeded at a density equal to 1 x 10^6 cells/ml have been acutely infected with type 1 HIV (strain IIIB) and with type 2 HIV (strain CBL20) at an infection multiplicity (m.o.i.) of 0.01. After 1 hour of incubation at 20 °C and subsequent removal of the inoculum, the cells have been washed for three times and then suspended again at a density equal to 1 x 10^5 cells/ml, in absence or presence of the compounds under examination. After 4 days of incubation at 37 °C, the cellular viability has been determined by the above described MTT method.

The results of the evaluation of the cytotoxicity and of the anti-HIV
activity of the derivatives (Ia) and (Ib) of the present invention and of some comparison compounds are shown in the Table 1. The derivatives of the present invention, either in the (R) form or in the (S) form, turn out to be not cytotoxic at the maximum tested concentration 5 (329 μM).

As far as the anti-HIV activity is concerned, only the isomer (R) turned out to be active with regard to HIV-1 and HIV-2, showing a behaviour and a particularly high potency.

Analogous results have been obtained with several other derivatives having formula (I) according to the present invention. Moreover a synergistic effect has been found by association of the compounds of the present invention with AZT and ddI.

**TABLE 1 - Comparative evaluation of cytotoxicity and anti-HIV activity of the compounds of the present invention and reference compounds.**

<table>
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<tr>
<td>50</td>
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<tr>
<td>(Ia)</td>
<td>&gt;329</td>
<td>12</td>
<td>12.5</td>
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<tr>
<td>(Ib)</td>
<td>&gt;329</td>
<td>93</td>
<td>200</td>
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<td>PMEA</td>
<td>229</td>
<td>5.3</td>
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<tr>
<td>ddC</td>
<td>&gt;24</td>
<td>1.9</td>
<td>ND</td>
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\(^a\) Concentration of compound (μM) necessary to reduce the viability of the not infected MT-4 cells by 50%.

\(^b\) Concentration of compound (μM) necessary to protect 50% of MT-4...
cells from the cytopathic effect induced by HIV-1 and HIV-2. Due to their characteristics the derivatives having formula (I) according to the present invention and their pharmacologically acceptable salts may be used with success, alone or in associations with other antivirals or in pharmaceutical compositions, in the therapy of the HIV virus infections.

Said combinations comprise at least a derivative having formula (I) together with AZT and/or ddI and/or ddC.

Said pharmaceutical compositions comprise a pharmaceutically effective dose of at least a derivative having formula (I) or of one of its pharmaceutically acceptable salts in mixture with pharmaceutically acceptable diluents and/or excipients.

Said compositions may be prepared in formulations suitable to the administration by oral or by parenteral way.
CLAIMS

1. Purine and 8-azapurine derivatives having the following general formula:

\[
\begin{align*}
&\text{Z} \quad \text{R}_1 \quad \text{R}_2 \\
&\text{N} \quad \text{R}_3 \quad \text{R}_4 \\
&\text{O} \quad \text{P(OH)}_2
\end{align*}
\]

wherein Z is N or CH, R_1 is an amino group or hydrogen, R_2 is an halogen or an OR_5 or NHR_5 group where R_5 is hydrogen or an alkyl-carbonyl group, R_3 is a CH=CH_2 or CH_2X group where X is hydrogen or halogen or N_3 and R_4 is hydrogen or methyl, or wherein R_3 and R_4 together may form also a cyclopropyl or halocyclopropyl group or may be substituted by a =CH_2 group.

2. Process for the preparation of purine and 8-azapurine derivatives having the following general formula:
wherein Z is N or CH. R₁ is an amino group or hydrogen. R₂ is an alogen or an OR₅ or NR₅ group where R₅ is hydrogen or an alkyl carbonyl group. R₃ is a CH=CH₂ or CH₂X group where X is hydrogen or halogen or N₃ and R₄ is hydrogen or methyl, or wherein R₃ and R₄ together may form also a cyclopropyl or halocyclopropyl group. characterized in that it is realized according to the following reaction scheme
wherein in the step a) the compound (II) is reacted with the mesylate (III), in presence of cesium carbonate, in the step b) the two regioisomers (IV) and (V) are separated and in the step c) the compound (V) is treated with bromotrimethylsilane to obtain the derivative (I).

3. Process as claimed in claim 2, characterized in that the reaction of said step a) is carried out with a molar ratio between the compound (III) and the compound (II) in the range 0.8:1 and 1.2:1.

4. Process as claimed in claim 2, characterized in that the reaction of said step a) is carried out with a molar ratio between the compound (III) and the compound (II) equal to 1:1.

5. Process as claimed in claim 2, characterized in that the reaction of said step a) is carried out in anhydrous dimethyl sulfoxide, by stirring and under nitrogen atmosphere at a temperature equal to 95-100 °C.

6. Process as claimed in claim 2, characterized in that said separation of the step b) is carried out submitting the residu obtained by evaporation to dryness in vacuo of the step a) to flash chromatography on a silica gel column.

7. Process as claimed in claim 2, characterized in that said reaction of the step c) is carried out with a molar ratio between bromotrimethylsilane and the compound (V) in the range 15:1 and 8:1.

8. Process as claimed in claim 2, characterized in that said reaction of the step c) is carried out with a molar ratio between bromotrimethylsilane and the compound (V) equal to 10:1.

9. Process as claimed in claim 2, characterized in that the reaction of the step c) is carried out in anhydrous acetonitrile at a
temperature equal to 25-30 °C.

10. Process for the preparation of 8-azapurine derivatives having the following formula:

![Chemical Structure](image)

characterized in that it is realized according to the following reactive scheme:
wherein in the step a) the mesylate (III) is reacted with sodium azide in anhydrous DMF to obtain the compound (VI), in step b) the compound (VI) is reacted with palladium on carbon under hydrogen atmosphere in MeOH to obtain compound (VII), in the step c) the compound (VII) is reacted with 2-amino-6-chloro-5-nitro-4(3H)-pyrimidone (VIII) and triethylamine in anhydrous DMF to obtain the compound (IX) and in the step d) the compound (IX) is reacted with Raney nickel under hydrogen pressure and then with an aqueous solution of sodium nitrite in a mixture of acetic acid and H₂O to obtain the compound (V).

11. Compositions containing as active substance at least a derivative having formula (I) as claimed in claim 1 or one of its salts or esters, in mixture with pharmacologically acceptable diluents and/or excipients.

12. Compositions as claimed in claim 11 in a formulation suitable to the administration per os.

13. Compositions as claimed in claim 11 in a formulation suitable to the administration by parenteral way.

14. Therapeutical method for the AIDS treatment consisting in administering an effective dose of at least a derivative having formula (I) as claimed in claim 1, or of one of its salts or esters, by oral or by parenteral way.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07F9/6561 A61K31/675

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X Patent family members are listed in annex.

Date of the actual completion of the international search

17 January 1996

Date of mailing of the international search report

25.01.96

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Beslier, L
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