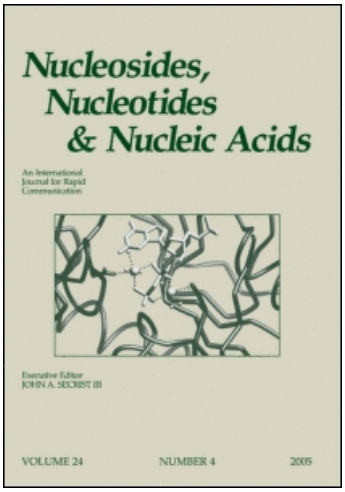

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Synthesis of the Nucleotide Analogue: (R,S)-9-[1-(2-Hydroxyethylthio)-2-phosphonylethyl] Adenine

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SYNTHESIS OF THE NUCLEOTIDE ANALOGUE :(R,S)-9-[1-(2-HYDROXYETHYLTHIO)-2-PHOSPHONYLETHYL] ADENINE.

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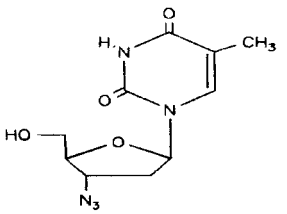
Abstract : The acyclic nucleotide analogue (R,S)-9-[1-(2-hydroxyethylthio)-2-phosphonylethyl] adenine [HETPEA, **4**] was prepared by coupling the adenine potassium salt with diethyl ethynylphosphonate followed by condensation of the product with 2-mercaptoethanol.

Acquired immunodeficiency syndrome (AIDS) is caused by a retrovirus, human immunodeficiency virus (HIV) [1,2]. Because an urgent need exists to find effective chemotherapeutic drugs against AIDS, many substances have been evaluated for antiretroviral activity *in vitro*. Some compounds reported to be effective against HIV replication *in vitro*, are currently undergoing clinically trials for AIDS treatment [3,4]. But to date, few nucleoside derivatives have proven as active as 3'-azido-2',3'-dideoxythymidine (AZT, Zidovudine, **1**) which has been unambiguously demonstrated to provide clinical benefit [5]. In general, these substances have mechanisms of action that involve initial phosphorylation in cells to nucleotide analogues and then inhibition of essential viral enzymes. As nucleotides are negatively charged, they have little potential for efficacy since cellular membranes have low permeability to charged molecules.

Dedicated to the memory of Dr. Roland K. Robins.



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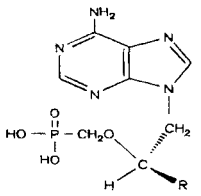

2, R = H
3, R = CH_2OH

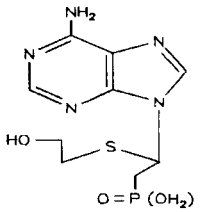

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Fig. 1

However, several negatively charged phosphonate analogues have been found to exert potent and selective activity against adeno-, herpes-, pox- and retroviruses[6]. Among them, the acyclic adenosine derivative 9-(2-phosphonylmethoxyethyl)adenine, (PMEA) 2 (Fig.1) inhibits HIV-induced cytopathogenicity in MT-4 cells and HIV antigen expression in H-9 cells [7]. (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine , (S)-HPMPA 3 (Fig. 1) is also active against various other viruses [8]. This paper deals with the preparation of the novel acyclonucleotide 4 containing a phosphonate group at C(2') position and a sulfur atom in the nucleoside sugar moiety with excission of C(3').

The key step of our strategy involves N-9-alkylation of adenine with the phosphonate derivative 9 through a Michael-type addition. *Trans*-dichloroethylene 5 was reacted with phenyllithium to afford lithium ethynylchloride 6 [9] (Fig. 2). This compound was then silylated by treatment with chlorotrimethylsilane to give the expected chloroethynyltrimethylsilane 7 (41%) which was further converted to the phosphonate derivative 8 through an Arbuzov reaction with triethyl phosphite [10].

The crude intermediate 8 was desilylated using a 10% aqueous sodium carbonate solution. The resulting diethylethynyl phosphonate 9 was characterized by ^1H NMR and

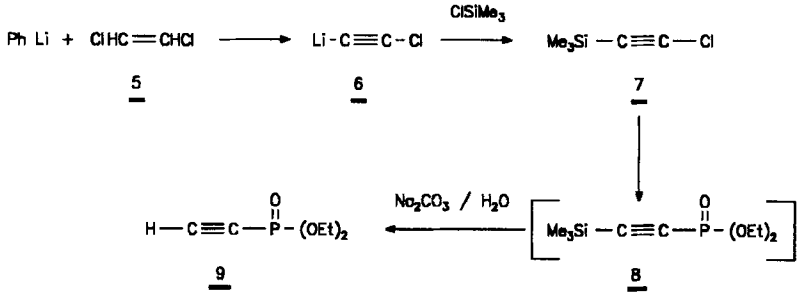

Fig. 2

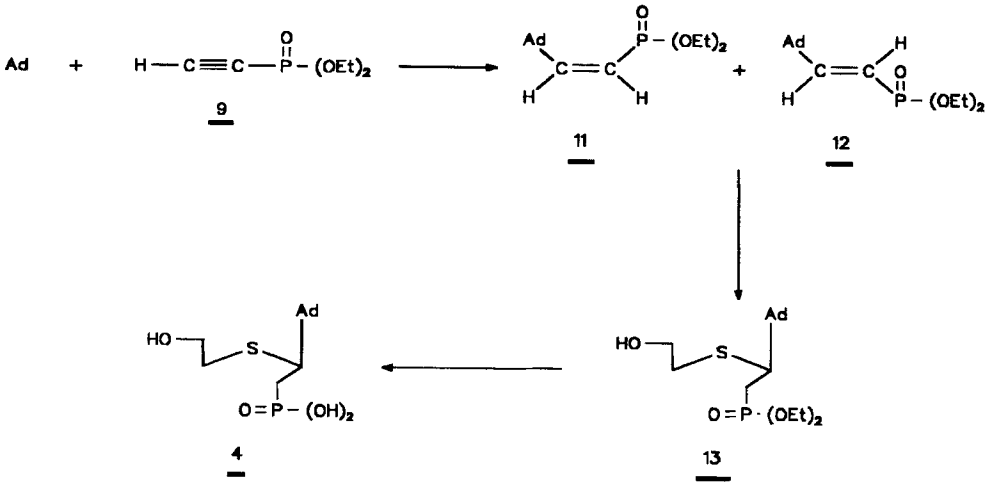

Fig. 3

IR spectroscopy methods [10]. Alkylation of the *in situ* generated adenine potassium salt with **9** was accomplished by Michael-type addition using solid-liquid phase transfer catalysis (SLPTC) [11] (Fig. 3). The usefulness of the phase transfer process for the selective preparation of N-9 adenine derivative has been demonstrated in our laboratory, as well as its application to the preparation of N-1 pyrimidine and other N-9 purine derivatives[12]. When 18-crown-6 was used as catalyst, the Z-isomer **11** and E-isomer **12** were obtained in 35% overall yield in an approximate 8:2 ratio, respectively.

As the yield was moderate under solid-liquid PTC conditions, the reaction was also carried out with potassium carbonate as base and DMF as solvent without catalyst. This gave the two isomers in 70% overall yield. Isomers **11** and **12** were separated by

chromatography on silica gel and fully characterized by the usual physical methods. The mixture of **11** and **12** was then treated with 2-mercaptoethanol in THF under the SLPTC conditions described above to provide compound **13** in 87% yield. This compound was converted into the phosphonic acid **4** by treatment with bromotrimethylsilane and subsequent hydrolysis [13]. After HPLC purification, **4** was recovered in 30% yield. The compounds were tested for *in vitro* for antiherpetic and anti-HIV-1 activity. None of the compounds (**11**-**13**,**4**) were active.

Experimental :

^{1}H-NMR spectra were recorded in a Bruker AM 300 spectrometer in DMSO-d_6 or in $CDCl_3$. Chemical shifts (δ) are quoted in parts per million relative to tetramethylsilane set at 0.0 ppm as internal reference. The signals are described as : s, singulet; d, doublet; t, triplet; dd, double doublet; m, multiplet.UV spectra were recorded with CARY 219 spectrophotometer. Thin layer chromatography used silica gel plates (Ek-F_{254}) and Merck silica gel (230-400 mesh) was employed for column chromatography. High performance liquid chromatographic (HPLC) purifications were carried out on a SFCC C_{18} XLODS (3µm) column. The HPLC system (Waters Millipore) included a Model U6K injector, two Models 6000 pumps, a Model 680 gradient controller and a Model 990 photodiode array detector interfaced with a NEC APC IV computer. Mass spectra were performed by JEOL JMS DX 300. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison France.

Chloroethynyltrimethylsilane 7:

A suspension of bromobenzene (156 g, 0.89 mol) and lithium (13.92g, 1.98 mol) in ether (2 liter) was refluxed overnight. *Trans*-dichloroethylene (42.68g, 0.44 mol) was added and the reflux continued for 2h, followed by addition of chlorotrimethylsilane (56ml, 0.44 mol). The mixture was refluxed for 12 h. The solution was filtered, the solvents were evaporated and the residue was distilled to afford 23.9 g (41%) of compound **7**

bp : 88 - 93°C.

I.R (CCl_4) : 2160 cm^{-1}.

^{1}H NMR (CCl_4) : δ 0.16 ppm ($(Me)_3Si$).

Diethyl ethynylphosphonate 9 :

A mixture of compound **7** (11g, 0.082 mol) and triethyl phosphite (16.4 ml, 0.096 mol) was refluxed for 5h. The reaction mixture was then treated with (61 ml) an 10% aqueous sodium carbonate solution. The solution was kept for 2h at room temperature, extracted

with ether (3x25ml) and dried ($MgSO_4$). The solvent was evaporated and product **9** was distilled (bp : 78°C/10 ^{3} Pa) to afford 6.64g (50%).
I.R (CCl_4) : 2140 cm^{-1}, 3180 cm^{-1}.
^1H NMR (CCl_4) δ : 4.10 (qd, 4H, 2 x O-CH_2, J_{H-P} = 9Hz, $J_{CH2 - CH3}$ = 7Hz); 3.63 (d, 1H, acetylenic H, J_{H-P} = 14Hz), 1.36 (t,6H, 2 x CH_3, $J_{CH2 - CH3}$ = 7Hz).

(Z)-diethyl 2-(adenin-9-yl)-1ethylenephosphonate 11 and **(E)-diethyl 2-(adenin-9-yl)-1ethylenephosphonate 12**:
Method A :
A mixture of adenine (1g, 7.40 mmol), potassium *tert*-butoxide (720 mg, 6.43 mmol) and 18-crown-6 (326 mg, 1.23 mmol) in acetonitrile (150 ml) was stirred at room temperature for 15 min. Diethyl ethynylphosphonate **9** (1.44 g, 8.89 mmol) was then added and the solution was kept for 40 h at room temperature. The solid was filtered off and the filtrate was evaporated. The residue was chromatographed on a silica gel column with the eluent system CH_2Cl_2/MeOH (95/5) to afford 640 mg (29%) of product **11** and 134 mg (6%) of product **12**.

Method B:
A solution of adenine (1g, 7.40 mmol), K_2CO_3 (511 mg, 37 mmol) in dimethyl formamide (100 ml) was stirred at room temperature for 15 mn. Diethyl ethynylphosphonate **9** (1.44 g, 8.89 mmol) was then added and the solution was kept for 22h at room temperature. Work-up of the mixture was the same as described under method A. 1.23 g(56%) of product **11** and 307 mg (14%) of product **12** were obtained.

Compound **11** :
-mp : 174 -176°C ($CHCl_3$/Ether).
-R_f : 0.46 (CH_2Cl_2/MeOH : 90/10).
-UV (MeOH) : λ_{max}, nm : 201 (ε : 8932), 245 (ε : 10760).
- Mass spectrum (FAB) : 298 $[M + H]^+$.
^1H NHR ($CDCl_3$) δ : 9,15 (s, 1H, H_8) ; 8.32 (s, 1H, H_2); 7.7 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 12.09 Hz , $J_{1',P}$ = 42.39 Hz); 6.3 (brs, 2H, NH_2) ; 5.03 (dd, 1H, $H_{2'}$, $J_{1',2'}$ = 12.09 Hz, $J_{2',P}$ = 7.17 Hz); 4.10 (qd, 4H, 2xOCH_2, J_{CH2-P} = 7.88 Hz, $J_{CH2-CH3}$ = 7.15 Hz); 1.30 (t, 6H, 2xCH_3, $J_{CH2-CH3}$ = 7.15 Hz).
-Anal. Calc.for $C_{11}H_{16}N_5O_3P$ 1/9 H_2O : C, 44.11; H, 5.46; N, 23.38. Found : C, 44.12; H, 5.60; N, 23.20.

Compound **12**

- mp : 168 - 170°C ($CHCl_3$/Ether).
-R_f : 0.42 (CH_2Cl_2/MeOH : 90/10).
-UV (MeOH) : λ_{max}, nm : 201 (ε : 6846) ; 239 (ε : 12996).
- Mass spectrum (FAB) : 298 $[M + H]^+$.
-^1H NHR ($CDCl_3$) δ : 8.40 (s, 1H, H_8) ; 7.92 (s, 1H, H_2); 7.83 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 15.54 Hz, $J_{1',P}$ = 17.43 Hz), 6.93 (dd, 1H, $H_{2'}$, $J_{2',1'}$ = 15.42 Hz, $J_{2',P}$ = 11.97 Hz), 5.87 (brs, 2H, NH_2), 4.15 (qd, 4H, 2 x OCH_2, $J_{CH2,CH3}$ = 7.27 Hz, $J_{CH2,P}$ = 7.90 Hz); 1.33 (t, 6H, 2 x CH_3, $J_{CH2,CH3}$ = 7.27 Hz).
-Anal. Calc.for $C_{11}H_{16}N_5O_3P$: C, 44.45; H, 5.42; N, 23.56. Found : C, 44.49; H, 5.54; N, 23.66.

(R,S)-diethyl 2-(adenin-9-yl)-2-(2-hydroxyethylthio)ethylphosphonate] 13:

A mixture of 2-mercaptoethanol (968 mg, 12.39 mmol), potassium *tert*-butoxide (8 mg, 0.07 mmol) and 18-crown-6 (8 mg) in THF was stirred at room temperature for 1/2 h and then added to a solution of **11** and **12** (526 mg, 1.77 mmol) in THF (2ml) at room temperature. The reaction mixture was stirred overnight. After addition of glacial acetic acid (3 drops) the solvent was removed under reduced pressure. The residue was chromatographed,. elution with CH_2Cl_2/MeOH (90/10) gave 577 mg (87 %) of **13**.

- mp : 139 - 141° C ($CHCl_3$ / ether).
-Rf : 0.52 (CH_2Cl_2 / MeOH 80 / 20).
-UV (MeOH) λ_{max}, nm: 202 nm (ε : 22 737); 260 nm (ε : 13980).
- Mass spectrum (FAB) : 376 $[M + H]^+$.
- ^1H NMR ($DMSOd_6$), δ : 8.40 (s, 1H, H_8); 8.13 (s, 1H, H_2); 7.24 (brs, 2H, NH_2) ; 5.98 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 10.30 Hz); 4.82 (t, 1H, OH, J_{OH-CH2} = 5.39 Hz); 3.80 (m, 4H, $CH_2O(O)P$); 3.39 (td, 2H, CH_2OH, J_{CH2-OH} = 5.39 Hz, $J_{CH2-CH2}$ = 6.73 Hz); 3.27-2.65 (m, 1H, $H_{2'}$); 2.5 (t, 2M, CH_2S, $J_{CH2-CH2}$ = 6.73 Hz); 1,24 (t, 2xCH_3, $J_{CH2-CH3}$ = 7.01 Hz)
-Anal. Calc.for $C_{13}H_{22}N_5O_4PS$: C, 41.59; H, 5.91; N, 18.65. Found : C, 41.66; H, 5.94; N, 18.59.

(R,S)-9-[1-(2-hydroxyethylthio) -2-phosphonylethyl] adenine 4 :

The diethyl ester **13** (100 mg, 0.26 mmol) was dissolved in CH_3CN (6ml) and treated with bromotrimethylsilane (323 mg, 2.11mmol) at room temperature. The reaction mixture was stirred for 20 h. Purification of compound **4** was done by HPLC to afford 25 mg (30%) of product **4**.

- mp : 179 -182°C (MeOH).
- R_f : 0.45 (isopropanol)/NH_4OH/H_2O : 3/1/1).

- UV (H2O) : λ_{max}, nm : 204 (ε : 17982), 260 (ε : 14100).
- Mass spectrum (FAB) : 320 $[M + H]^+$.
^1H NMR (D_2O) δ: 8.53 (s, 1H, H_8); 8.21 (s, 1H, H_2), 5.93 (m, 1H, $H_{1'}$) ; 3.50 (m , 2H, CH_2); 2.72 (m,1H, $H_{2'}$) 2.57 (m, 2H, CH_2); 2.42 (m, 1H, $H_{2'}$).
-Anal. Calc.for $C_9H_{14}N_5O_4PS$: C, 33.86; H, 4.42; N, 21.93 Found : C, 34.06; H, 4.11; N, 22.33.

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We dedicate this article to the memory of Professor R.K.ROBINS.

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