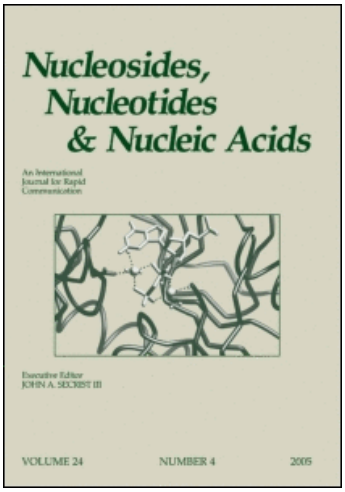

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Franchetti, P. , Cappellacci, L. , Cristalli, G. , Grifantini, M. , Pani, A. , La Colla, P. and Nocentini, G.(1991) 'Synthesis and Evaluation of Anti-HIV-1 and Antitumor Activity of 2',3'-didehydro-2',3'-dideoxy-3-deazaadenosine, 2',3'-dideoxy-3-Deazaadenosine and Some 2',3'-dideoxy-3-deaza-adenosine 5'-dialkyl Phosphates[1]', Nucleosides, Nucleotides and Nucleic Acids, 10: 7, 1551 — 1562
To link to this Article: DOI: 10.1080/07328319108046681
URL: http://dx.doi.org/10.1080/07328319108046681

NUCLEOSIDES & NUCLEOTIDES, 10(7), 1551-1562 (1991)

SYNTHESIS AND EVALUATION OF ANTI-HIV-1 AND ANTITUMOR ACTIVITY OF 2',3'-DIDEHYDRO-2',3'-DIDEOXY-3-DEAZAADENOSINE, 2',3'-DIDEOXY-3-DEAZAADENOSINE AND SOME 2',3'-DIDEOXY-3-DEAZAADENOSINE 5'-DIALKYL PHOSPHATES[1]

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Abstract- The 4-amino-1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-1*H*-imidazo[4,5-c]pyridine (**1**) and 4-amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-imidazo[4,5-c]pyridine (**2**), 3-deaza analogues of the anti-HIV agents 2',3'-didehydro-2',3'-dideoxyadenosine (d4A) and 2',3'-dideoxyadenosine (ddA), have been synthesized. The reaction of 3-deazaadenosine (**3**) with 2-acetoxyisobutyryl bromide yielded a mixture of *cis* and *trans* 2',3'-halo acetates which was converted into olefinic nucleoside (**1**) on treatment with a Zn/Cu couple and then with methanolic ammonia. The 2',3'-dideoxy-3-deazaadenosine (**2**) was obtained by catalytic reduction of **1**. A number of phosphate triester derivatives of **2** have also been prepared. The diethyl-, dipropyl- and dibutyl-phosphates **7a-c** and 3-deazaadenosine have shown anti-HIV activity at non-cytotoxic doses. Compounds **7a-c** have also shown significant cytostatic activity against murine colon adenocarcinoma cells.

Numerous purine and pyrimidine derivatives belonging to the classes of 2',3'-dideoxy- and 2',3'-didehydro-2',3'-dideoxynucleosides have emerged as potent *in vitro* inhibitors of HIV.[2] From this group, several compounds are presently undergoing clinical trials as anti-AIDS drugs, and one of the most promising appears to be 2',3'-dideoxyinosine (ddI),[3] the deamination product of 2',3'-dideoxyadenosine (ddA).

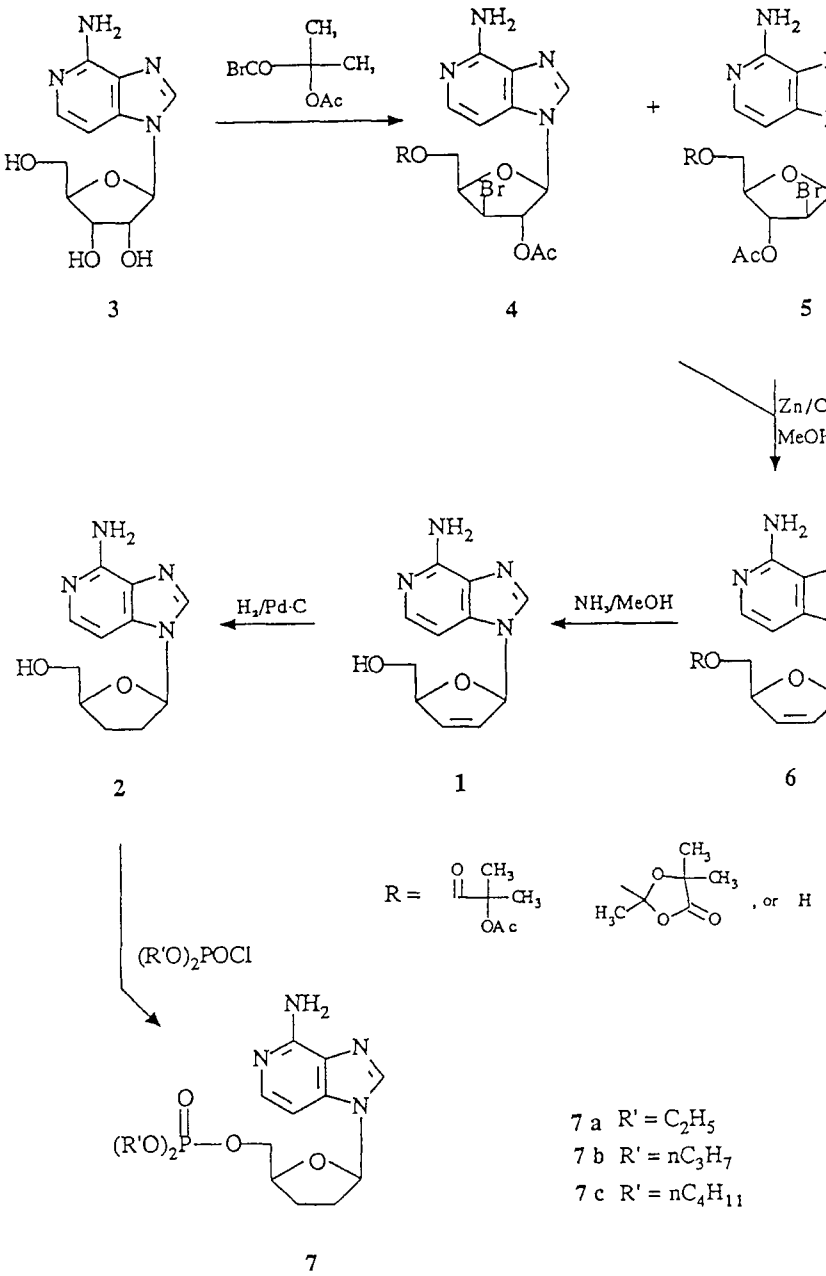
In the search for more active and metabolically stable ddA derivatives, compounds bearing substitutions in either the sugar or the purine moiety have been synthesized and tested. Among the latter are 2-halo-2',3'-dideoxy-

adenosine,[4] 2',3'-dideoxy-derivatives of toyocamycin, sangivamycin, tubercidin, and their 2',3'-unsaturated counterparts.[5] All of them have proved to be poor substrates of adenosine deaminase (ADA), but only 2',3'-dideoxy-2-aminoadenosine has shown an anti-HIV activity comparable to that of ddA.

To obtain compounds endowed with potential anti-HIV activity coupled with resistance towards deamination, we synthesized the 3-deaza analog of ddA, 2',3'-dideoxy-3-deazaadenosine (3-deaza-ddA) (**2**) and its unsaturated derivative, 2',3'-didehydro-2',3'-dideoxy-3-deazaadenosine (3-deaza-d4A) (**1**). The hope was that the new products would retain some of the interesting biochemical and biological activities of 3-deazaadenosine (3-deaza-A). This compound, in fact, in addition to resistance to deamination,[6,7] selectively inhibits the synthesis of the mRNAs for the pr76 gag and pr180 gag-pol proteins of avian retroviruses[8,9] as well as for the M-protein of influenza virus,[10] and induces differentiation of human promyelocytic leukemia HL60 cells *in vitro*.[11] Contrary to previous findings,[12] 3-deazaa-A has been shown to be converted intracellularly into its mono- and triphosphates.[13] Nevertheless, the possibility was taken into account that compounds **1** and **2** could not be quantitatively phosphorylated by cellular kinases. We therefore synthesized the 5'-monophosphate derivatives (**7a-c**) of 3-deaza-ddA in the form of dialkyl phosphate triesters, to facilitate their intracellular uptake.[14]

SYNTHESIS

The synthesis of 3-deaza-ddA (**2**) was carried out starting from 3-deaza-A[15](**3**) as reported in Scheme 1. Reaction of **3** with 2-acetoxyisobutyryl bromide (Mattocks's bromide)[16] provided a mixture of *cis* and *trans* bromo acetates **4** and **5**, in which the 5'-position was protected as a mixture of either the 2-acetoxyisobutyrate or the dioxolane.[17] The mixture of bromo acetates **4** and **5** was treated with the Zn/Cu couple at room temperature in MeOH to afford protected olefins **6**. Treatment of the crude mixture of olefins with a saturated solution of ammonia in methanol at 20 °C overnight gave 3-deaza-d4A (**1**) in 47% yield. Catalytic hydrogenation of olefin **1** gave 3-deaza-ddA (**2**) in greater than 56% isolated yield. After we had communicated this synthesis,[1] Seela et al.[18] and Serafinowski[19] reported two different methods of synthesis of 3-deaza-ddA. The 5'-O-dialkyl phosphate triester derivatives of **2** (**7a-c**) have been synthesized in moderate yield by reacting 3-deaza-ddA with the appropriate dialkyl phosphorochloridate in dry pyridine (Scheme 1). The compounds are characterized by microanalysis and ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.



Scheme 1

TABLE 1- *Cytotoxicity and anti-HIV activity of 1, 2 and 7a-c, in the absence or in the presence of Coformycin (CF).*

	alone		+CF 25 μM	
Compd	MNTD[a]	ED_{90}[b]	MNTD	ED_{90}
3-deaza-A	30	30	30	30
d4A	54	10	54	10
1	100	>100	100	>100
ddA	>1000	10	>1000	1.1
2	200	>200	200	>200
7a	25	25	25	25
7b	20	20	20	20
7c	12	12	12	12

[a]MNTD (Maximum Non Toxic Dose): compound concentration allowing exponential MT4 growth for three cell cycles. [b]ED_{90} (Effective Dose 90): compound concentration required to reduce by 90% the HIV-1 yield in MT4 cells. Virus yield in untreated controls was 2.3×10^5 $CCID_{50}$ per mL.

BIOLOGICAL EVALUATION

Antiviral activity. Compounds **1**, **2**, and **7a-c** were evaluated for their inhibitory effects on the yield of infectious HIV-1 in MT4 cells (Table 1). As reference compounds, 3-deaza-A, ddA, and d4A were used.

In agreement with the antiviral activity shown against other retroviruses,[8,9] 3-deaza-A inhibited the multiplication of HIV at concentrations non toxic for uninfected cells. Among the 3-deaza derivatives of ddA, compound **2** was inactive whereas **7a-c** inhibited by 90% the HIV multiplication at non cytotoxic concentrations. Although the potency of these compounds was comparable to that of ddA, their selectivity indices (s.i. = $MNTD/ED_{90}$) were considerably lower. Nevertheless, the anti-HIV activity of **7a-c** is quite remarkable in view of the fact that dialkyl esters of AZT 5'-monophosphate are inactive.[20] Contrary to d4A, which showed a s.i. of 5, compound **1** was totally inactive.

In the presence of Coformycin (CF), an inhibitor of both ADA and adenosin-monophosphate deaminase, none of the compounds tested showed an

TABLE 2- *Antitumor activity of 1, 2, 7a-c against P388, HL60, B16, C26, and CHO cell lines.*

	ID_{50}[a] (μM)				
Compd	P388	HL60	B16	C26	CHO
3-Deaza-A	193.1	208.8	362.3	27.7	208.2
d4A	63.3	139	69.44	18.02	45.5
1	235.8	331.6	187.1	59.2	218.1
ddA	197.5	b	320.5	279.7	307.3
2	804.8	>1000	729.4	153.4	>1000
7a	343.8	>1000	998.1	83.5	530.3
7b	90.7	>1000	82.5	11.8	375.6
7c	69.9	143.0	57.5	8.1	76.2

[a]ID_{50} (Inhibitory Dose 50): compound concentration required to reduce by 50% radioactive UdR incorporation. Values are the mean of at least three separate experiments. [b]Inactive at maximum concentration tested (1000 μm)

increased antiviral activity or toxicity. The sole exception was ddA, whose anti-HIV activity (but not cytotoxicity) was potentiated by 10 fold. Identical results were obtained in experiments performed with other ADA inhibitors.[21] A likely explanation is that 3-deaza derivatives are less susceptible than ddA to deamination. This hypothesis was confirmed by cell-free experiments with purified ADA from human erythrocytes. In fact, the results revealed that the 3-deaza derivatives **1**, **2** and **7a-c** are resistent to deamination. It is noteworthy that, in addition to a lack of anti-HIV activity, compound **2** is also less cytotoxic than its dialkyl monophosphate esters. This could be explained by postulating that **2** is inefficiently phosphorylated by cellular kinases.

Cytostatic activity. Compounds **1**, **2**, and **7a-c** were also evaluated *in vitro* for cytostatic activity against murine leukemia (P388), human promyelocytic leukemia (HL60), murine melanoma (B16), murine colon adenocarcinoma (C26), and chinese hamster ovary (CHO) cell lines (Table 2). The 3-deaza-A, ddA, and d4A as reference compounds were used.

All the compounds, showed little or moderate cytostatic activity against the majority of cell lines. Some inhibitory effect was obtained against murine colon

adenocarcinoma cells, against which the most active compounds were **7c** and **7b**, while the least active were **7a** and **2**. As far as the phosphate triesters of **2** were concerned, it could be concluded that a correlation exists between their lipophilicity and efficacy as cytostatic agents.

EXPERIMENTAL SECTION

The melting points (uncorrected) were taken on a Buchi apparatus. Elemental analyses were determined on a Carlo Erba Model 1106 analyzer. Ultraviolet spectra were recorded with an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin layer chromatography (TLC) was run on silica gel 60 F-254 plates (Merck); silica gel 60 (Merck) for column chromatography was used. Nuclear magnetic resonance ^{1}H, ^{13}C and ^{31}P NMR spectra were determined at 300, 75 and 121 MHz respectively, with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-1*H*-imidazo[4,5-c]pyridine (1) (2',3'-didehydro-2',3'-dideoxy-3-deazaadenosine). To a slurry of 3-deaza-A[15] (**3**) (1 g, 3.75 mmol) in acetonitrile (15 mL), a mixture of acetonitrile (4 mL), H_2O (0.075 mL, 4.16 mmol) and 2-acetoxyisobutyryl bromide[16] (2.25 mL, 15 mmol) was added. The reaction mixture was stirred for 1.5 h at room temperature, then quenched with saturated $NaHCO_3$ (40 mL) and solid $NaHCO_3$ to adjust to pH 8, and stirred for a further 5 min. The resulting yellow solution was extracted with methylene chloride (5 x 20 mL) and then concentrated to 10 mL. Methanol (20 mL) was added and then concentrated to 10 mL. This organic phase containing compounds **4** and **5** was used directly in the next step; the solution was added to the freshly prepared Zn/Cu couple[17] and the heterogeneous reaction mixture stirred for 5 min. The reaction mixture was filtered through Celite and the Celite pad was washed further with methanol (10 mL). To the filtrate collected, Amberlyst 27 exchange resin (OH form) was added portionwise until a gradual increase in the pH was observed (ca. pH 6-7). The mixture was filtered through Celite and the pad was washed with methanol (10 mL). The filtrate, which contained **6**, was concentrated to 10 mL and then added, at 0 °C, to a saturated solution of ammonia in methanol (10 mL). The solution was allowed to warm to 20 °C and stirred for 24 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on a silica gel

column using $CHCl_3$-MeOH-NH_4OH (85:14:1). Compound **1** was collected as a white solid (0.42 g, 47%); mp 198-200 °C; UV λ_{max} (MeOH) 210 nm (ϵ 8800), 266 nm (ϵ 5200). ^1H NMR (Me_2SO-d_6) δ 3.52 (m, 2H, H-5', H-5"), 4.83 (m, 1H, H-4'), 4.90 (t, 1H, OH), 6.18 (s, 2H, NH_2), 6.20 (m ,1H, H-3'), 6.51 (m, 1H, H-2'), 6.88 (d, 1H, J = 5.8 Hz, H-7), 6.90 (m, 1H, H-1'), 7.67 (d, 1H, J = 5.8 Hz, H-6), 8.11 (s, 1H, H-2). ^{13}C NMR (Me_2SO-d_6) δ 140.1 (C-2), 126.7 (C-3a), 152.3 (C-4), 140.3 (C-6), 96.9 (C-7), 137.7 (C-7a), 89.5 (C-1'), 134.4 (C-2'), 125.4 (C-3'), 87.8 (C-4'), 62.8 (C-5'). *Anal.* Calcd. for $C_{11}H_{12}N_4O_2$: C 56.89; H 5.21; N 24.13. Found: C 56.75; H 5.43; N 24.25.

4-Amino-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)-1*H*-imidazo[4,5-c]pyridine (2) (2',3'-dideoxy-3-deazaadenosine). To a slurry of **1** (0.5 g, 2.13 mmol) in 90% aqueous ethanol (70 mL), palladium on carbon (5%) (0.15 g) was added and the reaction mixture was exposed to hydrogen at 20 psi of pressure for 4 h. The mixture was filtered and washed with ethanol (30 mL). The filtrate was evaporated to dryness in vacuo, and the residue was purified by silica gel column chromatography using $CHCl_3$-MeOH-NH_4OH (85:14:1) as eluent. Compound **2** was collected as a white solid (0.294 g, 56%); mp 164-166 °C [lit.18: colorless foam; 19: colorless amorphous powder 119-126°C]; UV (MeOH) λ_{max} 210 nm (ϵ 8700), 266 nm (ϵ 5400) [lit.18: 267 nm; 19: 235 and 268 nm]. The ^1H, and ^{13}C NMR spectra are identical to those reported in literature.[18]

Further elution of the column with the same mixture of eluents gave 13 mg (6%) of 3-deazaadenine.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-imidazo[4,5-c]pyridine 5'-diethyl phosphate (7a) (2',3'-dideoxy-3-deazaadenosine 5'-diethyl phosphate). Compound **2** (0.2 g, 0.86 mmol) was dissolved in pyridine (15 mL), and diethyl phosphorochloridate (0.296 g, 0.248 mL, 1.72 mmol) was added dropwise with vigorous stirring at 4 °C. After stirring for 6 h at room temperature, the reaction was quenched with H_2O (0.031 mL, 1.72 mmol), and the solvent was removed under reduced pressure. The resulting oil was purified on a silica gel column using $CHCl_3$-MeOH (85-15) as eluent. Pooling and evaporation of appropriate fractions of the first eluate gave **7a** as a white solid (0.087 g, 29%); mp 84-86 °C; UV (MeOH) λ_{max} 210 nm (ϵ 9900), 268 nm (ϵ 5500). ^1H NMR (Me_2SO-d_6) δ 1.18 (dt, 6H, CH_3), 2.04-2.18 (2m, 2H, H-3', H-3"), 2.45 (m, 2H, H-2', H-2"), 3.92 (m, 5H, H-5' and CH_2OP), 4.12 (m, 1H, H-5"), 4.33 (m, 1H, H-4'), 6.22 (s, 2H, NH_2), 6.18 (dd, 1H, J = 4.4, 6.5 Hz, H-1'), 6.83 (d, 1H, J = 5.8 Hz, H-7), 7.68 (d, 1H, J = 5.8 Hz, H-6), 8.25 (s, 1H, H-2); ^{31}P NMR (Me_2SO-d_6) δ 0.076; ^{13}C NMR (Me_2SO-d_6) δ 138.1 (C-2), 126.6 (C-3a), 151.5 (C-4), 139.5 (C-6), 97.3 (C-7), 137.7 (C-7a), 85.3 (C-1'), 30.7 (C-2'), 25.4 (C-3'), 78.6 (C-4'), 67.9 (CH_2OP), 63.2 (C-

5'), 15.7 (CH_3). *Anal.* Calcd. for $C_{15}H_{23}N_4O_5P$: C 48.64, H 6.25, N 15.12. Found: C 48.55, H 6.38, N 14.98.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-imidazo[4,5-c]pyridine 5'-dipropyl phosphate (7b) (2',3'-Dideoxy-3-deaza-adenosine 5'-dipropyl phosphate). This compound was prepared in an analogous manner to **7a** above, except that the dipropyl phosphorochloridate was produced in situ by the reaction of dipropyl phosphite and CCl_4 anhydrous (molar ratio 1:2) in triethylamine;[14] furthermore, the reaction mixture was stirred for 48 h at room temperature. Separation by chromatographic silica gel column gave the product **7b** as a white foam. Thus, from 0.2 g of **2**, (0.095 g, 28 %) was isolated. UV (MeOH) λ_{max} 210 nm (ε 10100), 266 nm (ε 4900). ^1H NMR (Me_2SO-d_6) δ 0.87 (m, 6H, CH_3), 1.58 (m, 4H, CH_2CH_2OP), 2.05-2.18 (2m, 2H, H-3', H-3"), 2.47 (m, 2H, H-2', H-2"), 3.90 (m, 4H, CH_2OP), 3.98 and 4.12 (2m, 2H, H-5', H-5"), 4.34 (m, 1H, H-4'), 6.18 (dd, 1H, J = 4.4, 6.5 Hz, H-1'), 6.32 (s, 2H, NH_2), 6.85 (d, 1H, J = 5.8 Hz, H-7), 7.68 (d, 1H, J = 5.8 Hz, H-6), 8.22 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6) δ 0.251. ^{13}C NMR (Me_2SO-d_6) δ 138.9 (C-2), 126.6 (C-3a), 151.6 (C-4), 139.4 (C-6), 97.2 (C-7), 137.8 (C-7a), 85.3 (C-1'), 31.5 (C-2'), 25.8 (C-3'), 78.5 (C-4'), 67.9 (CH_2OP), 66.8 (C-5'), 25.0 (CH_3CH_2), 13.7 (CH_3). *Anal.* Calcd. for $C_{17}H_{27}N_4O_5P$: C 55.57; H 7.40; N 15.24. Found: C 55.73; H 7.38; N 15.46.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-imidazo[4,5-c]pyridine 5'-dibuthyl phosphate (7c) (2',3'-dideoxy-3-deaza-adenosine 5'-dibuthyl phosphate). This compound was prepared in an entirely analogous manner to **7b** above, except that a lower reaction time was employed (24 h) at room temperature. Chromatographic separation gave compound **7c** as a white foam. Thus, from 0.2 g of **2**, (0.13 g, 36%) was isolated. UV (MeOH) λ_{max} 212 nm (ε 11200), 266 nm (ε 5400). ^1H NMR (Me_2SO-d_6) δ 0.84 (m, 6H, CH_3), 1.27 (m, 4H, CH_3CH_2), 1.51 (m, 4H, CH_2CH_2OP), 2.05-2.18 (2 m, 2H, H-3', H-3"), 2.48 (m, 2H, H-2', H-2"), 3.88 (m, 4H, CH_2OP), 3.97 and 4.11 (2m, 2H, H-5', H-5"), 4.31 (m, 1H, H-4'), 6.18 (dd, 1H, J = 4.39, 6.36 Hz, H-1'), 6.55 (s, 2H, NH_2), 6.87 (d, 1H, J = 5.8 Hz, H-7), 7.67 (d, 1H, J = 5.8 Hz, H-6), 8.22 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6) δ 0.266. ^{13}C NMR (Me_2SO-d_6) δ 138.6 (C-2), 126.7 (C-3a), 151.7 (C-4), 139.4 (C-6), 97.2 (C-7), 137.7 (C-7a), 85.3 (C-1'), 30.7 (C-2'), 25.4 (C-3'), 78.5 (C-4'), 68.0 (CH_2OP), 66.7 (C-5'), 31.5 (CH_2CH_2OP), 18.0 (CH_3CH_2), 13.2 (CH_3). *Anal.* Calcd. for $C_{19}H_{31}N_4O_5P$: C 53.51, H 7.32, N 13.13. Found: C 53.68, H 7.54, N 13.29.

Biological determination

Cells. In anti-HIV assay, the following cells were used: H9/IIIB cells, a H9 subline which is persistently infected with HTLV-IIIB; C8166 and MT4 cells, a

CD4+ T-cell line containing a genome of HTLV-I and expressing only the tat gene, in which HIV induces an easily detectable, syncytium-forming cytopathic effect (CPE). These cell lines were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 U per mL penicillin and 100 μg per mL streptomycin, at 37 °C in a CO_2 incubator.

In antiproliferative assay, the following cells were used: P388 murine lymphocytic leukemia, HL60 human promyelocytic leukemia, B16 murine melanoma, C26 murine colon adenocarcinoma, CHO chinese hamster ovary. CHO were grown in HAM'S F12 supplemented with 3 mM glutamine, 1% vitamins and 10% heat-inactivated FCS. C26 were grown in MEM supplemented with 3 mM glutamine and 5% FCS. The other cell lines were grown in RPMI-1640 supplemented with 100 U per mL penicillin, 100 μg per mL streptomycin, 50 U per mL gentamicin, 3 mM glutamine, 10 mM HEPES buffer, and 15% heat-inactivated new-born calf serum (P388), 10% FCS (B16) or 15% FCS (HL60). All cell lines were checked periodically for mycoplasma contamination and were found negative.

Virus. The HIV-1 used in the assay was obtained from culture supernatants of H9/IIIB cells collected at the end of an exponential growth phase. The titer of virus stock solutions varied from 2 to $4x10^5$ cell culture infectious doses fifty ($CCID_{50}$) per mL.

Toxicity test. MT4 cells were seeded at a density of $1x10^5$ cells per mL in growth medium and cultured with various concentrations of the compounds. Cell numbers were determined with a Coulter counter after 96 h of incubation at 37 °C.

Anti-HIV assay. Exponentially growing MT4 cells were seeded at a density of $1x10^6$ cells per mL and then infected with $1x10^5$ $CCID_{50}$ of HIV-1. After a 2 h incubation at 37 °C the inoculum was removed, the cells were washed three times and then resuspended at $1x10^5$ per mL in RPMI-1640 containing 10% FCS, in the absence or in the presence of the test compounds. After a 4 days incubation at 37 °C, the number of syncytia was evaluated at the inverted microscope and the amount of infectious virus produced was determined by end point titration.

HIV titration. Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in 96 well plates. The infectious virus titer was measured by light microscope scoring of syncytia after 4 days of incubation, and virus titer was calculated as $CCID_{50}$ by the Reed and Muench method.

In Vitro antitumor evaluation.

Compounds solubilization. Immediately before testing, compound **2** was solubilized in water, compounds **1** and **7a-c** were solubilized in DMSO and water. The higher DMSO concentration used (0.5%) did not have any cytotoxic effect in our testing system.

Antiproliferative assay. The assay developed for predictive evaluation of tumor chemosensitivity has been used.[22]

Briefly, various concentrations of each drug were placed with tumor cell suspension (P388, 10^4 cells/well; HL60, $5x10^4$ cells/well; B16, $2.5x10^3$ cells/well; C26, 10^3 cells/well; CHO, $2.5x10^3$ cells/well). Forty eight hours later DNA synthesis was evaluated by adding 0.1 μCi/well of [^{125}]I-5-iodo-2'-deoxyuridine along with 2'-deoxy-5-fluorouridine (0.01 μg/well) to the cultured cells for an additional 18 h. Harvesting was performed by a multiple suction filtration apparatus (Mash II) on a fiberglass filter . Paper disks containing the aspirate cells were read in a gamma-scintillation counter. Results are expressed as percentage ratio of inhibited radioisotope incorporation in the treated cultures vs. untreated controls. Dose resulting in 50% inhibition of radioisotope incorporation (ID_{50}) was determined as suggested by Chou[23]; the mean ID_{50} of at least three experiments was reported.

Adenosine deaminase assay. Compounds **1**, **2** and **7a-c** were tested for their ability to serve as substrates of adenosine deaminase from human erythrocytes as reported in the literature,[24] and found to be resistent to deamination.

Acknowledgment: This investigation was supported by Consiglio Nazionale delle Ricerche (Progetto Strategico "Studio di farmaci per la prevenzione e terapia dell'AIDS) and by grant N 4206 37 from Istituto Superiore di Sanità (Progetto AIDS 1989).

REFERENCES

1) Various aspects of this work were presented at the Third International Conference On Antiviral Research, Brussels, April 22-27, 1990, Franchetti, P.; Cristalli, G.; Grifantini, M.; Cappellacci, L.; Vittori, S.; Marongiu, M. E.; Pani, A. *Antiviral Res.*, **1990**, *1*, 53. Congresso Interdivisionale della Societa' Chimica Italiana CISCI 90, San Benedetto del Tronto, Italy, Sept. 30-

Oct. 5, **1990**, Franchetti, P.; Cappellacci, L.; Cristalli, G.; Grifantini, M; La Colla, P. *Abstract* FP 7.

2) De Clercq, E.; Van Aerschot, A.; Herdewijn, P.; Baba, H.; Pauwels, R.; Balzarini, J. *Nucleosides & Nucleotides,* **1989**, *8*, 659.

3) Yarchoan, R.; Mitsuya, H.; Thomas, R.V.; Pluda, J.M.; Hartman, N.R.; Perno, C.F.; Marczyk, K.S.; Allain, J.P.; Johns, D.G.; Broder, S. *Science,* **1989**, *245*, 412.

4) Heartle, T.; Carrera, C.J.; Wasson, D.B.; Sowers, L.C.; Richman, D.R. ; Carson, D.A. *J. Biol. Chem.,* **1988**, *263*, 5870.

5) Pauwels, R.; Baba, M.; Balzarini, J.; Herdewijn, P.; Desmyter, J.; Robins, M.J.; Zou, R.; Madej, D.; De Clercq, E. *Biochem. Pharmacol.,* **1988**, *37*, 1317.

6) Ikehara, M.; Fukin, T. *Biochem. Biophys. Acta,* **1974**, *338*, 512.

7) Chiang, P.K.; Richards, H.H.; Cantoni, G.L. *Mol. Pharmacol.,* **1977**, *13*, 939.

8) Bader, J.P.; Brown, N.R.; Chiang, P.K.; Cantoni, G.L. *Virology,* **1978**, *89*, 494.

9) Stoltzfus, C.M.; Montgomery, J.A. *J. Virol.,* **1981**, *38*, 173.

10) Fischer, A.A.H.; Mueller, K.; Scholtissek, C. *Virology,* **1990**, *177*, 523.

11) Aarbakke, J.; Miura, G.A.; Prytz, P.S.; Bessesen, A.; Chiang, P.K. *Eur. Paediatr. Haematol. Oncol.,* **1985**, *2*, 189.

12) Miller, R.L.; Adamczyk, D.L.; Miller, W.H.; Kaszalka, G.W.; Rident, J.L.; Beacham, L.M.; Chas, E.Y.; Haggerty, J.J.; Krenitsky, T.A.; Elion, G.B. *J. Biol. Chem,* **1979**, *254*, 2346.

13) Bennett L.L.; Brockman, R.W.; Allan, P.W.; Rose, L.M.; Shaddix, S.C. *Biochem. Pharmacol.,* **1988**, *37*, 1233.

14) Colin, B.; Jones, N. M.; McGuigan, C.; Riley P. A. *Nucleic Acids Res.,* **1989**, *17*, 7195.

15) Montgomery, J.A.; Shortnacy, A.T.; Cayton, S.D. *J. Heterocycl. Chem.,* **1977**, *14*, 195.

16) Mattocks, A.R. *J. Chem. Soc.,* **1964**, 4840.

17) Mansuri, M.M.; Starrett, Jr., J.E.; Wos, J.A.; Tortolani, D.R.; Brodfuehrer, P.R.; Howell, H.G.; Martin, J.C. *J. Org. Chem.,* **1989**, *54*, 4780.

18) Seela, F.; Rosemeyer, H.; Fischer, S. *Helv. Chim. Acta* , **1990**, *73*, 1602.

19) Serafinowski, P. *Synthesis,* **1990**, *9*, 757.

20) McGuinan, C; Nicholls, S.R.; O'Connor, T.J.; Kinchington, D. *Antiviral Chem.,* **1990**, *1*, 25.

21) La Colla, P.; Cristalli, G.; Franchetti, P., Grifantini, M.; Tramontano, E.; Marongiu, M. E.; Pani A. *Abstracts of Papers,* Fourth International Conference on Antiviral Research, New Orleans, **1991**, in press.

22) Menconi, E.; Lepri, E.; Bonmassar, E.; Frati, L.; Barzi, A. *Int. J. Tiss. React.*, **1986**, *8* (6), 485.

23) Chou, T. C. *J. Theor. Biol.*, **1977**, *65*, 345.

24) Lupidi, G.; Cristalli, G.; Marmocchi, F.; Riva, F.; Grifantini, M. *J. Enzyme Inhib.*, **1985**, *1*, 67.

Received 12/19/90

Accepted 3/29/91