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# Synthesis and biological evaluation of dinucleoside methylphosphonates of 3′-azido-3′-deoxythymidine and 2′, 3′-dideoxycytidine

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## Summary

The 5′ → 5′ dinucleoside methylphosphonates of 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxycytidine (DDC) were prepared and evaluated for their inhibitory properties against different viruses, including human immunodeficiency virus (HIV). The synthesis of the compounds was achieved by reaction of AZT or *N*$^4$-(4-monomethoxytrityl)-2′,3′-dideoxycytidine with in situ prepared methylphosphonic bis(triazolide), followed in the latter case by an acidic treatment. The two title compounds showed in vitro anti-HIV activity, that was 200- to 450-fold less pronounced than that shown by the corresponding monomeric nucleosides AZT and DDC. The decreased antiviral activity may be ascribed to nuclease resistance of the methylphosphonate linkage.

*O,O′-bis*(3′-azido-3′deoxythymidin-5′-yl)methylphosphonate; *O,O′-bis*(2′,3′-dideoxycytidin-5′-yl)methylphosphonate; Human immunodeficiency virus

## Introduction

Among the current strategies for treating acquired immunodeficiency syndrome (AIDS), the most promising one focuses on the search of drugs which interfere with human immunodeficiency virus (HIV, the etiologic agent of AIDS) repli-

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cation (De Clercq, 1989a,b). Among the antiretroviral compounds which have so far proven effective both in vitro and in vivo are the 2′,3′-dideoxynucleoside analogues, in particular 3′-azido-3′-deoxythymidine (AZT, also called Retrovir® and zidovudine), 2′,3′-dideoxycytidine (DDC) and 2′,3′-dideoxyinosine (DDI), the former being at this time the only drug licensed by the Food and Drug Administration for the treatment of AIDS patients (Yarchoan and Broder, 1989). These compounds enter cells and are converted by cellular enzymes to their triphosphates which on the one hand are potent inhibitors of the virus-associated reverse transcriptase and on the other hand result in chain termination when they are incorporated in the growing DNA chain.

Previously, we developed a mutual-prodrug approach based on the design of nucleotide dimers and we demonstrated that the symmetrical 5′ → 5′ dinucleoside monophosphate of 9(β-D-arabinofuranosyl)adenine (araA, vidarabine, VIRA-A) retains the antiproliferative and antiherpetic in vitro activities of its monomeric units, but only so at higher concentrations (Puech et al., 1988). In partial accordance with our results, it has been shown recently that various nucleotide homo- and heterodimers of AZT linked via a 5′ → 5′ phosphodiester bridge have enhanced in vitro anti-HIV potency in comparison with their monomer components (Busso et al., 1988; Hahn et al., 1989). It was assumed that the favorable therapeutic index obtained with these dimers may result either from intracellular liberation of one mole each of mononucleotide and nucleoside if they intactly cross the cell membrane, or from a slow extracellular production of two moles of nucleosides if they are hydrolyzed before their cellular uptake.

As a part of our pursuit of a prodrug approach based on the development of new dimeric derivatives as potential antiviral agents, we report in this communication the unambiguous synthesis of the homodinucleoside methylphosphonates of AZT and DDC and their biological (antiviral and cytotoxic) effects.

## Materials and Methods

### *Starting materials*

AZT was obtained from Burroughs-Wellcome Co. (Research Triangle Park, NC). 2′-Deoxycytidine hydrochloride was purchased from Fluka. General procedures and instrumentation used have been described previously (Puech et al., 1988; Debart et al., 1989). 3′-Amino-3′-deoxythymidine (AMT) was prepared by L. Beacham at Burroughs Wellcome Co. 3′-Azido-3′-deoxy-5′-*O*-(β-D-glucopyranuronosyl)thymine (GAZT, the 5′-*O*-glucuronide of AZT) was purified from the urine of cynomolgus monkeys treated with AZT and fully characterized (Good et al., in press).

### *Anti-human immunodeficiency virus (HIV) assays*

The method for determination of the cytopathic effect of HIV in MT-4 cells has previously been described (Balzarini et al., 1988). Briefly, MT-4 cells were seeded at $10^6$ cells/ml and infected with HIV at 1000 $CCID_{50}$/ml. Then, $10^5$ cells were brought into wells of a microtiter tray containing 100 μl of various dilutions of the test compounds. After 5 days incubation at 37°C, the number of viable cells was determined microscopically in a hematocytometer by trypan blue exclusion.

### *Transformation of murine C3H/3T3 embryo fibroblast cells by Moloney murine sarcoma virus (MSV)*

C3H/3T3 cells were seeded into 48-well Nunc Tissue Culture Cluster plates at 20000 cells per ml and grown for 24 h. Cell cultures were then infected by 75 foci-forming units of MSV during 60 to 90 min, whereafter medium was replaced by 1 ml fresh culture medium containing different concentrations of the test compound. After 6 days, the transformation of the cell cultures was examined microscopically.

### *Inhibition of tumor cell growth*

The cytostatic assays were performed in Nunc 96-well microtiter plates. To each well were added 7.5 × $10^4$ Raji or MOLT/4F cells and a given amount of the test compound. The cells were allowed to proliferate for 72 h at 37°C in a humidified, $CO_2$-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter.

### *Inhibition of [methyl-$^3$H]dThd and [5-$^3$H]dCyd incorporation into MOLT/4F and L1210 cell DNA*

The incorporation of [methyl-$^3$H]dThd and [5-$^3$H]dCyd into cellular DNA was also measured in Nunc 96-well microtiter plates. To each well were added $10^5$ cells, 0.25 μCi of [methyl-$^3$H]dThd or 0.25 μCi of [5-$^3$H]dCyd, and a given amount of the test compound. The cells were allowed to proliferate for 20 h at 37°C. At the end of this incubation period, the contents of the wells (200 μl) were brought onto 25-mm glass fiber filters and washed twice with cold phosphate-buffered saline (PBS), twice with cold 10% trichloroacetic acid, twice with cold 5% TCA and once with cold ethanol. The filters were then allowed to dry for 10 min at 60°C and assayed for radioactivity in a toluene-based scintillant.

### *Enzyme hydrolysis studies*

The procedures to determine hydrolysis products of dinucleoside methylphosphonates were performed as recently described (Puech et al., 1988; Balzarini et al., 1988).

### *Disposition of O,O′-bis(3′-azido-3′-deoxythymidin-5′-yl)methylphosphonate [AZTp(CH$_3$)AZT]* (**10**) in rats

Groups of two male CD rats (Charles River Breeding Laboratories) were housed in metabolic cages and were given single, 10 mg/kg, i.v. or oral doses (4 animals/2 cages each) of AZTp(CH$_3$)AZT (**10**). Urine and fecal excretions were individually collected. Urines were collected during the 0–8, 8–24 and 24–48 h periods post dose; feces were collected during the 0–24 and 24–48 h periods post administration. Entire fecal collections were homogenized in 9 volumes (9 ml/g) deionized water and centrifuged (12000 × *g*, 30 min). A sample of each fecal homogenate supernatant and urine collection were filtered (Millipore Millex filter, 0.45 μm) and analyzed by high-performance liquid chromatography (HPLC) for determination of the concentrations of AZTp(CH$_3$)AZT (**10**) and its potential metabolites, AZT, 3′-azido-3′-deoxy-5′-*O*-(β-D-glucopyranuronosyl)thymidine (GAZT), 3′-amino-3′-deoxythymidine (AMT), 3′-azido-3′-deoxythymidine 5′-*O*-(methylphosphonyl) [MP-AZT, prepared by alkaline hydrolysis of AZTp(CH$_3$)AZT (**10**)], thymidine (Thd) and thymine (Th). Urines and fecal homogenate supernatants were analyzed by HPLC as follows: samples (100 μl) were injected using a WISP 710B (Waters Assoc.) onto an Adsorbosphere™ C$_{18}$ column (250 × 4.6 mm I.D., 5 μm spherical particles, Alltech Assoc./Applied Science) which was preceded by an in-line filter (0.5 μm, Scientific Systems, Inc.) and an Adsorbosphere™ C$_{18}$ guard column (10 × 4.6 mm I.D., 5 μm spherical particles, Alltech Assoc./Applied Science). The column was eluted at a flow rate of 1 ml/min using a mobile phase buffered to pH 7.2 with 25 mM ammonium phosphate. A gradient was generated by two Constametric III pumps and a Dynamic Mixer (LDC/Milton Roy) and consisted of two contiguous 20-min linear increases in acetonitrile content, the first from 0 to 15% and the second from 15 to 60% acetonitrile in buffer, followed by a 5-min isocratic segment at 60% acetonitrile in buffer. The u.v. absorbance of the column effluent was monitored at 267 and 254 nm using a Spectromonitor III and a u.v. Monitor D (LDC/Milton Roy). The pumps were controlled and the absorbance data was digitized and integrated using a DS-80Z microcomputer (Digital Specialties). Retention times for Th, AMT, Thd, GAZT, MP-AZT, AZT and AZTp(CH$_3$)AZT (**10**) were 14, 17.5, 19, 21, 23, 31 and 38 min, respectively. Peak areas and area ratios (267 nm/254 nm) were compared to those of authentic standards to determine concentrations of parent compound and metabolites.

## Results

### *Chemical synthesis*

*N$^4$-(4-Monomethoxytrityl)-1-[2-deoxy-5-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]cytosine* (**3**). 5′-*O*-*tert*-butyldimethylsilyl-2′-deoxycytidine (**2**) (3.29 g, 9.64 mmol) (Ogilvie, 1973) was first coevaporated three times with anhydrous pyridine and with toluene until the pyridine had been removed completely and then suspended in anhydrous methylene chloride (60 ml). 4-Monomethoxytrityl chloride (mMTrCl, 6.0 g, 19.43 mmol, 2 eq), 4-dimethylaminopyridine (DMAP, 0.06 g, 0.49 mmol, 0.05 eq) and then triethylamine (2.67 ml, 1.94 g, 19.17 mmol, 2 eq) were added and the reaction mixture was stirred at room temperature for 75 min. Saturated aqueous sodium bicarbonate was added, and the resulting mixture was extracted with methylene chloride. The combined organic layers were washed with water and then dried over sodium sulfate and evaporated to leave crude 3, which was precipitated from hexane. Further purification was accomplished by silica gel column chromatography using as eluent a stepwise gradient of methanol (0–10%) in methylene chloride. Pooling and evaporation of the appropriate fractions as indicated by thin-layer chromatography (TLC) gave pure 3 (4.9 g, 83%) which was precipitated from hexane: UV (methanol) $\lambda_{max}$ 280 nm (15100), $\lambda_{min}$ 250 nm (11,200); $^1$H NMR ($Me_2SO$-$d_6$) δ ppm 8.41 (s, 1H, NH), 7.70 (d, 1H, H-6; $J_{5,6}$ = 7.5 Hz), 7.4–6.7 (m, 14H, trityl), 6.26 (d, 1H, H-5), 6.03 (t, 1H, H-1′; $J_{1',2'} = J_{1',2''}$ = 6.3 Hz), 5.24 (d, 1H, OH-3′; J = 8.5 Hz), 4.1 (br, 1H, H-3′), 3.8–3.6 (m, 3H, H-4′,5′,5″), 3.72 (s, 3H, $OCH_3$), 2.2–2.0 and 1.9–1.8 (2m, 2 × 1H, H-2′ and 2″), 0.88 [s, 9H, $(CH_3)_3CSi$], 0.07 (s, 6H, 2 $CH_3Si$); mass spectrum (FAB < 0, matrix = glycerol-thioglycerol, 50:50, v/v): 704 [M + glycerol − H]$^-$, 612 [M − H]$^-$, 382 [B]$^-$.

### N$^4$-*(4-Monomethoxytrityl)-1-[2,3-dideoxy-5-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]cytosine (5)*

A solution of 3 (3.0 g, 4.89 mmol) and 1,1′-thiocarbonyldiimidazole (1.74 g, 9.78 mmol, 2 eq) in anhydrous 1,2-dichloroethane (25 ml) was stirred and gently refluxed for 1 h under argon. The reaction mixture was cooled at room temperature and the solvent was evaporated in vacuo. The residue was chromatographed on a silica gel column using as eluent a stepwise gradient of acetone (0–90%) in *n*-hexane. The intermediate 4 thus obtained was dissolved in anhydrous toluene (20 ml) and to the refluxing solution under argon was dropwise added over 45 min a mixture of tri-*n*-butyltin hydride (2.6 ml, 9.81 mmol) and α,α′-azobis(isobutyronitrile) (AIBN, 0.20 g, 1.22 mmol) in anhydrous toluene (90 ml). The reaction was gently refluxed for 6 additional hours to completion (TLC, $CH_3OH$-$CH_2Cl_2$, 6:94). After cooling, the solvent was evaporated in vacuo and the residue was suspended in hexane and extracted with acetonitrile. The combined acetonitrile layers were evaporated. Sufficiently pure 5 (1.43 g, 49%) was isolated as an oil after silica gel column chromatography of the resulting residue, using as eluent a stepwise gradient of methanol (0–3%) in methylene chloride: UV (methanol) $\lambda_{max}$ 282 nm (11700), $\lambda_{min}$ 252 nm (9000); $^1$H NMR ($Me_2SO$-$d_6$) δ ppm 8.37 (s, 1H, NH), 7.84 (d, 1H, H-6; $J_{5,6}$ = 7.5 Hz), 7.4–6.7 (m, 14H, trityl), 6.23 (d, 1H, H-5), 5.8 (m, 1H, H-1′), 4.0 (m, 1H, H-4′), 3.8–3.6 (m, 2H, H-5′,5″), 3.72 (s, 3H, $OCH_3$), 2.3-2.1 (m, 1H, H-2′), 1.9–1.7 (m, 3H, H-2″,3′,3″), 0.90 [s, 9H, $(CH_3)_3$ CSi], 0.08 (s, 6H, 2 $CH_3Si$); mass spectrum (FAB < 0, matrix = glycerol-thioglycerol, 50:50, v/v): 596 [M-H]$^-$.

### $N^4$-*(4-Monomethoxytrityl)-1-(2,3-dideoxy-β-D-ribofuranosyl)-cytosine* (**6**)

Compound **5** (1.3 g, 2.18 mmol) was dissolved in dry tetrahydrofuran (THF, 12 ml), and a 1 M solution of tetra-*n*-butylammonium fluoride in THF (12 ml) was added. The solution was stirred for 1 h at ambient temperature and then poured into saturated aqueous sodium bicarbonate and extracted with methylene chloride. The organic layers were combined, washed with water, dried over sodium sulfate, and evaporated to dryness. The resulting crude material was twice chromatographed, first on a silanized silica gel column [RP-2, Merck No 7719; 4 × 20 cm; eluent, linear gradient of methanol (0–90%) in water] and then on a silica gel column [eluent, stepwise gradient of methanol (0–5%) in methylene chloride] to afford pure **6** (0.93 g, 88%): UV (methanol) $\lambda_{max}$ 280 nm (12400), $\lambda_{min}$ 252 nm (9400); $^1$H NMR ($CDCl_3$ + $D_2O$) δ ppm 7.52 (d, 1H, H-6; $J_{5,6}$ = 7.6 Hz), 7.3–6.8 (m, 14H, trityl), 5.95 (dd, 1H, H-1′; J = 3.6 and 6.6 Hz), 5.03 (d, 1H, H-5), 4.1 (m, 1H, H-4′), 3.8 and 3.6 (2m, 2 × 1H, H-5′ and 5″), 3.79 (s, 3H, $OCH_3$), 2.4 and 2.1 (2m, 2 × 1H, H-2′ and 2″), 2.0–1.8 (m, 2H, H-3′,3″); mass spectrum (FAB $< 0$, matrix = glycerol-thioglycerol, 50:50, v/v): 482 $[M\text{-}H]^-$, 382 $[B]^-$.

### O,O′-*Bis(2′,3′-dideoxycytidin-5′-yl)methylphosphonate [ddCp($CH_3$)ddC]* (**8**)

Methylphosphonic dichloride (0.089 g, 0.67 mmol) was added to a solution of triazole (0.21 g, 3.04 mmol) and collidine (0.81 ml, 6.11 mmol) in dry pyridine (2.5 ml), and the resulting mixture was stirred at 0°C for 1 h under argon. Nucleoside **6** (0.62 g, 1.28 mmol) and tetrazole (0.428 g, 6.11 mmol) were first coevaporated three times with anhydrous pyridine, dissolved in pyridine (6 ml), then added to the above mixture, and the resulting reaction mixture was stirred at room temperature for 14 h. Solvent was evaporated under vacuum and the residue was coevaporated several times with a mixture of toluene-ethanol (4:1, v/v). The residue was poured into saturated aqueous sodium bicarbonate and repeatedly extracted with chloroform. The combined chloroform extracts were washed with water, dried over sodium sulfate, filtered and evaporated to dryness. The residue was chromatographed on a silica gel column with a stepwise gradient of methanol (0–15%) in methylene chloride as eluent. The fractions containing pure compound were pooled and evaporated to give the protected dimer **7** (0.57 g, 87%), which was dissolved in a solution of 4% trifluoroacetic acid in methylene chloride (30 ml). The reaction mixture was stirred at room temperature for 10 h, neutralized by addition of saturated aqueous sodium bicarbonate, diluted with methylene chloride, and extracted with water. The aqueous layer was evaporated to dryness, and the residue was chromatographed on a RP-2 silanized silica gel column (1.5 × 15 cm) with a linear gradient of ethanol (0–15%) in water as eluent. The fractions containing the pure compound were pooled, evaporated, dissolved in water, filtered (HV-4 Millipore), and lyophilized to give **8** (0.24 g, 78% from **6**) as a powder: UV (Methanol) $\lambda_{max}$ 271 nm (14300), $\lambda_{min}$ 252 nm (11000); $^1$H NMR ($Me_2SO\text{-}d_6$) δ ppm 7.67 (d, 2H, 2H-6; $J_{5,6}$ = 7.4 Hz), 7.1 (br s, 4H, $2NH_2$), 6.0 (m, 2H, 2H-1′), 5.72 (d, 2H, 2H-5), 4.3–4.0 (m, 6H, 2H-4′,5′,5″), 2.3 (m, 2H, 2H-2′), 2.1–1.9 (m, 2H, 2H-3′), 1.9–1.8 (m, 2H, 2H-

2″), 1.8–1.7 (m, 2H, 2H-3″), 1.52 (d, 3H, $CH_3P$; $J_{P,CH_3}$ = 17.6 Hz); $^{31}P$ NMR ($Me_2SO$-$d_6$) 31.90 ppm; mass spectrum (FAB > 0, matrix = glycerol): 483 $[M+H]^+$, 112 $[BH_2]^+$; (FAB < 0, matrix = glycerol): 481 $[M\text{-}H]^-$.

*O,O′-Bis(3′-azido-3′-deoxythymidin-5′-yl)methylphosphonate [AZTp(CH₃)AZT] (**10**)*.

This compound was prepared as described above for the synthesis of **7**. AZT (**9**) (0.75 g, 2.81 mmol) in solution in pyridine (3 ml) with tetrazole (0.95 g, 13.56 mmol) was reacted at room temperature with methylphosphonic bis(triazolide) [prepared from methylphosphonic dichloride (0.20 g, 1.49 mmol) and triazole (0.47 g, 6.81 mmol) in dry pyridine (2 ml) in the presence of collidine (1.8 ml, 13.58 mmol)]. After 14 h, 0.5 M aqueous sodium bicarbonate (20 ml) was added and the reaction mixture evaporated to dryness. The residue was reevaporated three times in toluene solvent and then chromatographed on a RP-2 silanized silica gel column (2.0 × 18 cm) with a linear gradient of methanol (0–70%) in water. The fractions containing the pure compound were pooled, evaporated, dissolved in dioxane, filtered (HV-4 Millipore), and lyophilized to give **10** (0.62 g, 74%) as a powder: UV (Methanol) $\lambda_{max}$ 264 nm (20100), $\lambda_{min}$ 233 nm (4900); $^1H$ NMR ($Me_2SO$-$d_6$) δ ppm 11.4 (br s, 2H, 2NH-3), 7.51 (s, 2H, 2H-6), 6.13 (t, 2H, 2H-1′; $J_{1',2'}$ = $J_{1',2''}$ = 6.5 Hz), 4.5 (m, 2H, 2H-3′), 4.2 (m, 4H, 2H-5′,5″), 4.0 (m, 2H, 2H-4′), 2.5–2.2 (m, 4H, 2H-2′,2″), 1.79 [s, 6H, 2($CH_3$)-5], 1.56 (d, 3H, $CH_3P$; $J_{P,CH_3}$ = 17.5 Hz); $^{31}P$ NMR ($Me_2SO$-$d_6$) 32.87 ppm; mass spectrum (FAB < 0, matrix = glycerol): 593 $[M\text{-}H]^-$; (FAB > 0, matrix = glycerol): 595 $[M+H]^+$.

## Antiviral activity

The dinucleoside methylphosphonates **8** and **10** of DDC and AZT were evaluated for their in vitro inhibitory effects on the replication of a number of DNA viruses [i.e. herpes simplex viruses type 1 (KOS) and type 2 (G), vaccinia virus] and RNA viruses (vesicular stomatitis virus, coxsackie virus B4, poliovirus-1, parainfluenza-3 virus, reovirus-1, Sindbis virus and Semliki forest virus) in primary rabbit kidney, HeLa and VERO B cell cultures. Neither **8** or **10** showed a marked antiviral effect at doses up to 400 μg/ml. At this concentration, no detectable alteration of host cell morphology was noted.

## Inhibitory effects of the dinucleoside methylphosphonate derivatives on the replication of Moloney murine sarcoma virus (MSV) and human immunodeficiency virus (HIV)

The dinucleoside methylphosphonate of AZT **10** proved markedly active against MSV-induced transformation of murine C3H/3T3 cells and HIV-induced cytopa-

TABLE 1

Inhibitory effects of the DDC and AZT dinucleoside methylphonates on the replication of MSV and HIV

| Compound | 50% Effective dose (μg/ml) | |
|---|---|---|
| | Anti-MSV activity | Anti-HIV activity |
| DDC | 11.9 ± 4.8 | 0.06 ± 0.02 |
| 8[a] | > 200 | 12.7 ± 2.0 |
| AZT | 0.005 ± 0.003 | 0.001 ± 0.0003 |
| 10[a] | 1.0 ± 0.11 | 0.45 ± 0.5 |

[a] It was ascertained by TLC and HPLC that these synthesized compounds did not contain any detectable contamination by DDC and AZT, respectively.

TABLE 2

Inhibitory effects of the DDC and AZT dinucleoside methylphosphonates on human tumor cell proliferation

| Compound | 50% Inhibitory dose (μg/ml) | | |
|---|---|---|---|
| | Raji | MOLT/4F | MT-4[a] |
| DDC | 3.67 ± 0.35 | 1.15 ± 0.12 | 46.1 ± 6.7 |
| 8 | > 200 | > 200 | > 200 |
| AZT | 17.6 ± 5.1 | 30.5 ± 1.50 | 6.91 ± 2.0 |
| 10 | > 200 | > 200 | > 200 |

[a] The 50% inhibitory dose was measured by determining the number of viable cells after 5 days of incubation.

thogenicity in human MT-4 cells. The dinucleoside methylphosphonate of DDC 8 showed only a marked antiviral activity in the HIV-infected MT4 cells. However, both **8** and **10** proved at least 200- to 450-fold less effective as antiviral agents than their corresponding monomers DDC and AZT (Table 1).

## Cytostatic activity

The dinucleoside methylphosphonates **8** and **10** were also evaluated for their inhibitory effects on the proliferation of human Raji, MOLT/4F and MT-4 cells (Table 2). Unlike DDC and AZT, the dinucleosides **8** and **10** were devoid of any cytostatic activity at a concentration as high as 200 μg/ml.

## [Methyl-$^3$H]dThd and [5-$^3$H]dCyd incorporation into MOLT/4 cell DNA

The DDC and AZT dinucleoside methylphosphonate derivatives were evaluated for their inhibitory effects on the incorporation of [methyl-$^3$H]dThd and [5-$^3$H]dCyd into MOLT/4 cell DNA. Only the AZT derivative **10** slightly affected [methyl-$^3$H]dThd incorporation at a dose that was 75-fold higher than AZT.

TABLE 3

Inhibitory effects of the DDC and AZT dinucleoside methylphosphonates on the incorporation of [methyl-$^3$H]dThd and [5-$^3$H]dCyd into MOLT/4 cell DNA

| Compound | 50% Effective dose (μg/ml) | |
|---|---|---|
| | Incorporation of [methyl-$^3$H]dThd | Incorporation of [5-$^3$H]dCyd |
| DDC | > 500 | > 100 |
| 8 | > 500 | > 100 |
| AZT | 3.1 ± 1.0 | > 100 |
| 10 | 224 ± 184 | > 100 |

Neither DDC nor its derivative 8 interfered with [methyl-$^3$H]dThd incorporation at 500 μg/ml. [5-$^3$H]dCyd incorporation into Molt/4 DNA was not inhibited at 100 μg/ml by any of the compounds listed in Table 3.

### Enzymatic hydrolysis of 8 and 10

The dinucleoside methylphosphonates **8** and **10** were subject to treatment with phosphodiesterase I, phosphodiesterase II and human serum. Incubations were done for 0, 1, 4, 24 and 48 h at 37°C and the reactions were examined by thin layer chromatography (TLC). None of the enzyme preparations were able to hydrolyse **8** and **10** to a significant extent within an incubation period as long as 48 h.

### Disposition of AZTp(CH$_3$)AZT (10) in rats

No dinucleoside methylphosphonate **10** or any of its predicted metabolites (including AZT, MP-AZT, GAZT, AMT, Thd and Th) were detected in the feces of rats after i.v. or oral administration. Following i.v. administration, 2% and 41% of the dose was recovered in urine as AZT and parent compound **10**, respectively. No other potential metabolites could be detected, although small amounts of Th could have been obscured by other compounds endogenous to rat urine. After oral dosing, no AZTp(CH$_3$)AZT (**10**) or any of the predicted metabolites were detected in the urine. The incomplete recoveries of the administered doses might be explained by an unexpected metabolism of **10** or by a whole body or tissue specific retention.

## Discussion

### *Chemistry*

A successful scheme for the synthesis of the title compounds **8** and **10** requires, in the case of **8**, prior preparation of a protected derivative of DDC, in which the

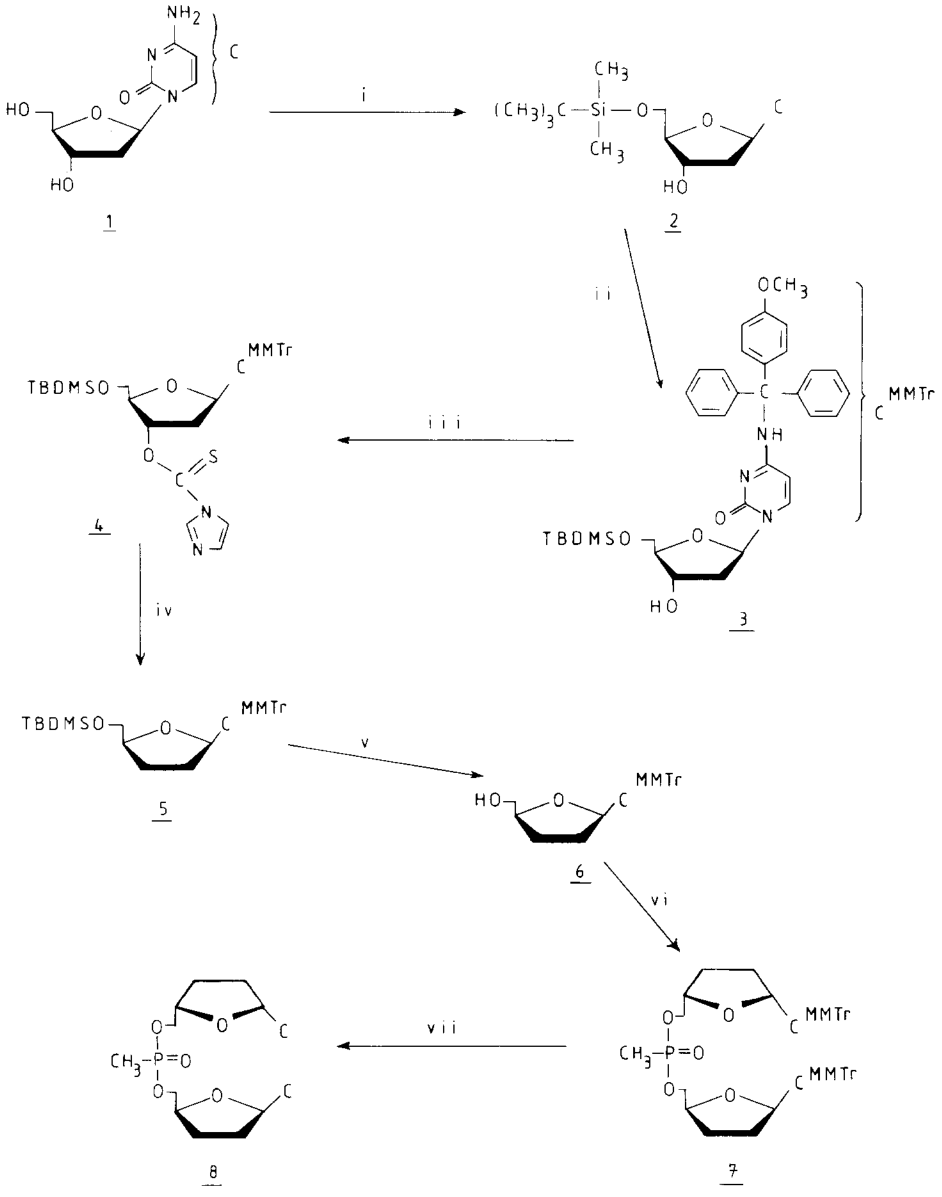

Fig. 1. Synthesis of O,O′-*bis*(2′,3′-dideoxycytidin-5′-yl)methylphosphonate. Reagents and conditions: i, $(CH_3)_3CSi(CH_3)_2Cl/C_5H_5N$; ii, $CH_3OC_6H_4C(Cl)(C_6H_5)_2$, DMAP, $(C_2H_5)_3N/CH_2Cl_2$; iii, thiocarbonyldiimidazole/$ClCH_2CH_2Cl$; iv, $(C_4H_9)_3SnH$, AIBN/$C_6H_5CH_3$; v, $(C_4H_9)_4NF$/THF; vi, $CH_3$ $P(O)Cl_2$, triazole, collidine/$C_5H_5N$ then tetrazole/$C_5H_5N$; vii, $CF_3COOH/CH_2Cl_2$.

exocyclic $NH_2$ is blocked with an appropriate group. The synthesis of this building block **6** starts from commercially available 2′-deoxycytidine (**1**) (Fig. 1). The 5′-hydroxyl function of **1** is temporarily protected with a *tert*-butyldimethylsilyl (TBDMS) group to afford **2** (Ogilvie, 1973). Reaction of **2** with monomethoxytrityl (MMT) chloride under 4-dimethylaminopyridine (DMAP) catalysis (Honda et al., 1984) gives the $N^4$-tritylated derivative **3**. Further reaction of **3** with thiocarbonyldi-imidazole (De Bernardo et al., 1985) affords the intermediate thionourethane **4** which is treated with tributyltin hydride in the presence of azo-*bis*(isobutyronitrile) (AIBN) in refluxing toluene (Barton and Subramanian, 1977; Pankiewicz et al., 1982) to afford the 2′,3′-dideoxynucleoside **5**. Desilylation of **5** finally affords the hitherto unknown building block **6**.

The procedure applied for synthesizing the symmetrical (5′ → 5′) linked methylphosphonates **8** and **10** was analogous with slight modifications to the method described previously for the preparation of dissymmetrical (3′ → 5′) dideoxynucleoside methylphosphonates (Dorman et al., 1984). Thus, methylphosphonic dichloride was allowed to react with triazole and collidine in pyridine to form methylphosphonic bis(triazolide) which was used directly in a reaction with 1.9 equivalents of **6** or AZT (**9**) to give, under triazole activation, the dimers **7** and **10**. The former was deprotected by subsequent treatment with trifluoroacetic acid in methylene chloride to afford, after purification, **8** (Figs. 1 and 2).

Purity and structural assignments for the reported compounds are based on their physical properties.

## Biological properties

The dinucleoside methylphosphonate derivatives of AZT and DDC showed in vitro anti-HIV activity at a concentration of 0.45 and 12.7 μg/ml, respectively. They were non-toxic at a concentration of 200 μg/ml. However, the parent compounds DDC and AZT were at least 200- to 450-fold more effective in inhibiting HIV (and MSV) replication than their corresponding dimers. The marked decrease

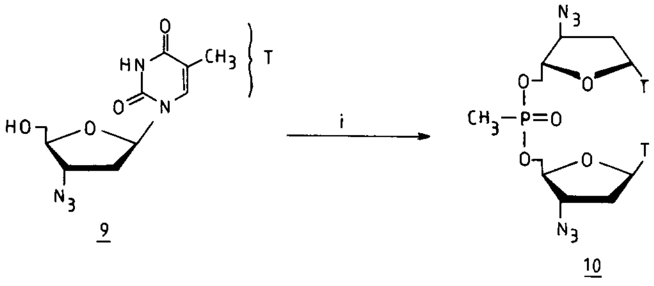

Fig. 2. Synthesis of *O,O′-bis*(3′-azido-3′-deoxythymidin-5′-yl)methylphosphonate. Reagents and conditions: i, $CH_3P(O)Cl_2$, triazole, collidine/$C_5H_5N$, then tetrazole/$C_5H_5N$.

in efficacy of **8** and **10** might be explained by their resistance to enzymatic attack by phosphodiesterases. These observations are in accord with previous reports on the stability of the methylphosphonate internucleosidic bonds (Puech et al., 1988; Agarwal and Riftina, 1979; Miller et al., 1979). A 0.5% conversion of the dinucleotide methylphosphonate derivative to the monomeric compound suffices to account for the biological activity that was observed.

Furthermore, when AZTp($CH_3$)AZT (**10**) was orally administered to rats, no **10**, AZT or any expected derivative of AZT was detected in the feces or urine. When the rats were given intravenous doses of **10**, only 43% of the administered dose (2% AZT and 41% **10**) was recovered in the urine.

These results show that dinucleoside methylphosphonates, although they are nonionic and easily transportable through biological membranes (Miller et al., 1981), are obviously not good prodrugs for the systemic delivery of antiviral nucleosides, owing to their too high stability.

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