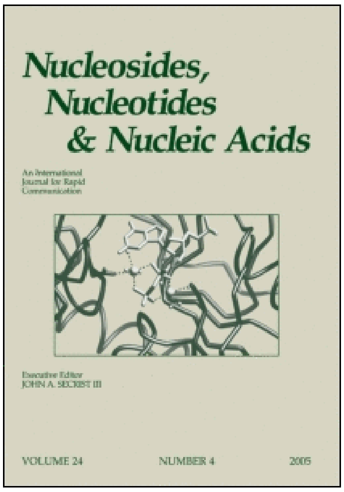

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New Phosphonate Analogues of 3'-Thia-2',3'-dideoxycytidine(BCH-189) Synthesis and Anti-HIV Evaluation

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New phosphonate analogues of 3'-thia-2',3'-dideoxycytidine (BCH-189)

Synthesis and anti-HIV evaluation.

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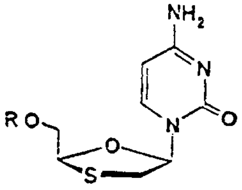
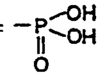
Abstract: New phosphonate analogues of 3'-thia-2',3'-dideoxycytidine (BCH-189) were synthesized in 5 steps via cyclocondensation of 2-mercaptoacetaldehyde di[2-methoxyethyl]acetal with 3-diethylphosphonopropionaldehyde as a key step, followed by a Lewis acid catalyst addition of the appropriate nucleic base. Anti-HIV evaluation of these analogues showed that the α-form 3b of 3'-thia-2',3'-dideoxycytidine-4'-ethylene phosphonic acid was inactive while the β-form 3a was found to be less potent than the parent compound (BCH-189), showing that the replacement of the oxygen in position 5' by a methylene group in the monophosphorylated intermediate diminishes the antiviral activity.

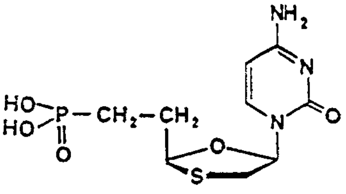
Although antiretroviral therapy with AZT, or DDI seems to have beneficial effects in AIDS patients, the emergence of AZT resistant HIV variants (1,2) or the hematologic toxicity of AZT (3) have stimulated considerable efforts for the discovery of superior therapeutic agents. In this respect, the synthesis of numerous nucleosides inhibitors of the viral reverse transcriptase have been achieved. The discovery by Dr. Belleau et al. (4) of 3'-thia-2',3'-dideoxycytidine (BCH-189), which has entered clinical trials (5,6,7), has raised up considerable interest (8,9). This agent suppresses HIV replication showing a better therapeutic index than AZT. Because of the specific antiretroviral activity of the oxathiolane nucleoside we report in this paper the synthesis of a 2',3'-dideoxynucleoside prototype, phosphono oxathiolane-cytosine, in which the 3'methylene carbon is replaced by a sulfur atom, and the 5'position is substituted by a methylene phosphonate group. Indeed several nucleoside analogues are known to be converted into their 5'-phosphate 2 by cellular kinases and then acting as reverse-transcriptase inhibitors (7,10a). We wanted to proof whether a phosphonate analogue such as 3 which mimics the key monophosphorylated intermediate 2 would retain or even improves the antiviral activity of the parent compound BCH-189 1. One has to recall that this isosteric replacement has been carried out with dideoxynucleosides such as AZT (10b) but no antiviral activity was reported for these new analogues.



1 R= H

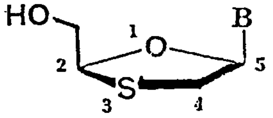
2 R=


$-P(=O)(OH)_2$

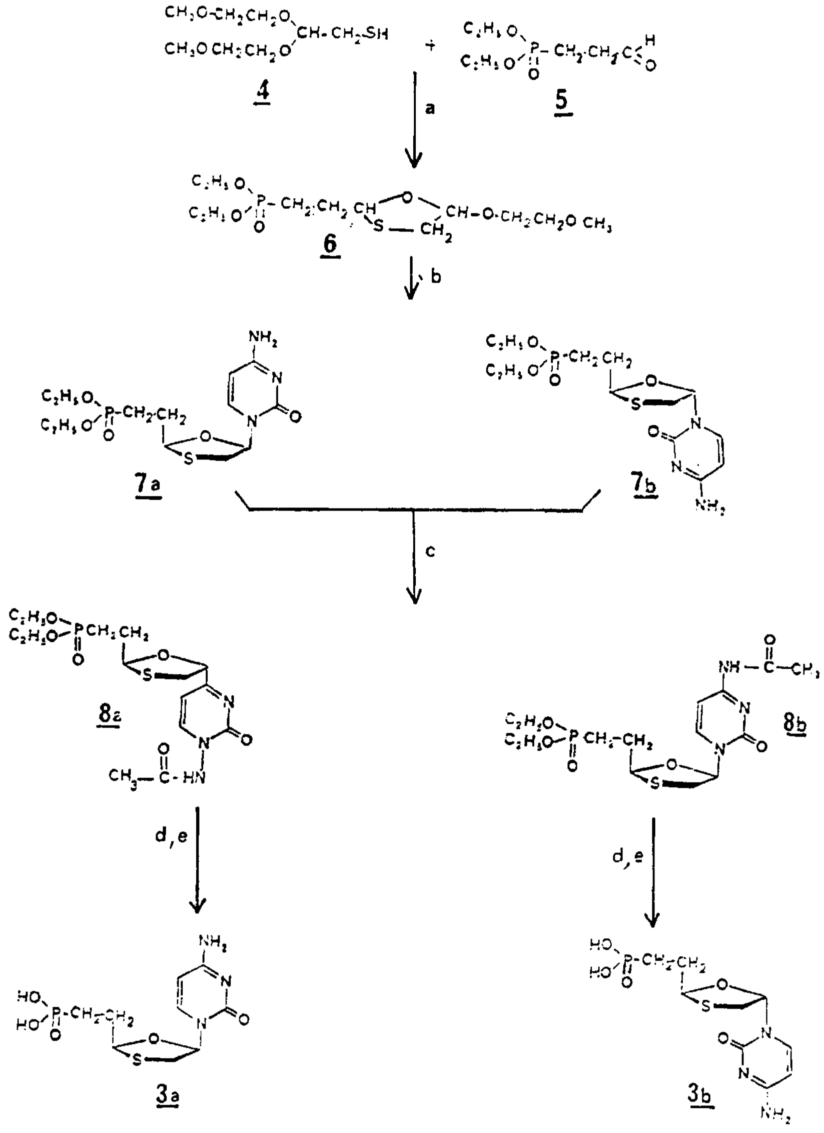


3a

As detailed in scheme 1, condensation of 2-mercaptoacetaldehyde di[2-methoxyethyl]acetal 4 (11,12) with 3-diethylphosphonopropionaldehyde 5 (13) provided a 60% yield of the oxathiolane 6 as a racemic 1:1 mixture of diastereoisomers. Condensation of oxathiolane 6 with silylated cytosine using titanium tetrachloride ($TiCl_4$) as catalyst yield the oxathiolanes 7a and 7b as 1:1 mixture of diastereoisomers. Even if we knew that the use of stannic chloride ($SnCl_4$) led to the exclusive formation of the β adduct (9), $TiCl_4$ was selected for the cytosine catalysis condensation, because we desired to obtain both α and β isomers for biological testing. Indeed in the dioxolane serie, the α -isomer was found more active than the corresponding β -isomer (4). Separation of the mixture of the α and β anomers was carried on in the next step after N-acetylation using acetic anhydride in DMF (14). Indeed the separation of the two N-acetylated anomers 8a and 8b was achieved by flash column chromatography using EtOAc-MeOH [10:0.5] mixture. Pure anomers 8a and 8b were isolated in 80% yield in a 1/1 ratio. The structure of 8a and 8b was assigned through ^1H NMR data. Indeed the chemical shift for the protons H_2 and H_5 of the 1,3-oxathiolane ring are known to be characteristic of the α and β anomers (15,16). Since the structure of the α and β forms of the anti-HIV drug BCH-189 1 have been fully characterized by ^1H NMR spectroscopy, the assignment of the peaks corresponding to the H_2 and H_5 protons in compounds 8a and 8b, was established by direct comparison with the corresponding chemical shift reported in the case of BCH-189. Table 1 summarized the ^1H NMR data.



Hydrolysis of the phosphonic ethyl esters 8a and 8b was carried out using trimethylsilylbromide (TMSBr) in DMF (40°C, 5h) leading to the corresponding phosphonic acid derivatives after aqueous work-up. These latter compounds without purification, were directly treated with methanolic ammonia overnight at room temperature, the desired nucleosides 3a and 3b were obtained in 75% yield. NMR data, elemental analyses and high resolution mass spectrometry confirmed the reported structures. It should be pointed out that the synthesized compounds 3a and 3b are racemic mixtures and evaluated for their antiviral properties as racemic mixtures. The antiviral effect of these new nucleosides was based on the cytopathogenic effect of HIV-1 (HIV-1 BRU prototype) on MT4 cells. Syncytium formation was evaluated under an inverted optical microscope, 4 to 7 days after HIV-1 injection. The antiviral testing of 3a and 3b showed that 3b was totally inactive, while 3a was found less potent than the parent analogue BCH-189 1. This result indicates that the phosphorylated modified analogue 3a was not a good substrate for HIV-1 kinases. 3a could probably not be transformed into the phosphorylated intermediate needed for reverse transcriptase activity.



a) p-toluenesulfonic acid catalytic, toluene 3h. reflux (60%)

b) bistrimethylsilyl-cytosine, $TiCl_4$, $(CH_2)_2Cl_2$, 0°C (70%)

c) Ac_2O/DMF, overnight, RT (80%)

d,e) $(CH_3)_3SiBr$/DMF, 5h., 40°C then NH_3/MeOH RT overnight (75%)

Scheme 1

Table 1: 1H NMR data of phosphonate analogues of 3'-thia-2',3'-dideoxycytidine

			BCH-189 1	
	8a	8b	α	β
H_2	5.60	5.21	5.48	5.12
H_5	6.40	6.27	6.30	6.18

Experimental section

Nuclear Magnetic Resonance (^1H, NMR) spectra were obtained with a Varian XL 200 at 200 MHz. The chemical shift values were expressed in δ values (part per million) relative to tetramethylsilane. Elemental analysis was performed at Guelph Laboratories, Guelph, Ontario-Canada. Analysis indicated by the symbols was within ± 0.4% of theoretical values. Analytical thin layer chromatography was carried out on aluminium base sheets precoated with Kieselgel $60F_{254}$ 0.2mm thick (Merck. Darmstadt). Flash column chromatography separations were performed using Merck G60 silicagel (230–400 mesh) under pressure. Mass spectra were determined at the University of Manchester (Dr K.T. Douglas) Manchester-England.

2-mercaptoacetaldehyde di-[2-methoxyethyl]acetal 4 and 3-diethylphosphono propionaldehyde 5 have been synthesized according to known procedures reported respectively in references (11) and (13).

2-diethylphosphonoethyl-5-[2-methoxyethoxy]-1,3-oxathiolane 6

A mixture of 4.9 g (25mM) of 5 and 5.3 g (25mM) of 4 in 80 ml of xylenes in the presence of catalytic amount of p-toluene sulfonic acid is refluxed for one night. Removal of the solvent in vacuo followed by flash column chromatography of the residue on silica gel with EtOAc-Toluene (1:1) as eluent provided 4.73 g of 6 as an oil (yield: 55%). T.L.C. Rf=0.13 (solvent EtOAc-Toluene; 1:1).

^1H NMR ($CDCl_3$): 1.30 (6H,t,$\underline{CH_3}CH_2O$-), 1.9-2.2 (4H,m,$\underline{CH_2}$-$\underline{CH_2}$), 3.1-3.15 and 3.5-3.65(2H,dd,S-$\underline{CH_2}$), 3.30 (3H,s,O$\underline{CH_3}$), 3.5-3.65 and 3.7-3.85 (4H,m,O$\underline{CH_2}$-$\underline{CH_2}$-O),4.05-4.16(4H,m,CH_3-$\underline{CH_2}$-0-P), 5.1-5.14 (1H,t,O-$\underline{CH}$-S), 5.3 and 5.5 (1H,dd,O-$\underline{CH}$-O)
Anal.($C_{12}H_{25}O_6SP$) C,H,P.

4'-diethylphosphonoethyl-3'-thia-2',3'-dideoxycytidine

(mixture of 7a and 7b).

At -10°C, under Argon, to 0.5 g (1.52 mM) of 6 in 25 ml of dry CH_2Cl_2, was added 1.2 equivalent of silylated cytosine, dissolved in 25 ml of CH_2Cl_2. After 30 minutes, 1.22 mM of 1N $TiCl_4$ in CH_2Cl_2 was added. The brown resulting solution is stirred at 0°C for 40 minutes, 1h at room temperature and finally refluxed overnight. After work-up (saturated $NaHCO_3$ solution, EtOAc extraction, solvent evaporation) 503 mg of a brown oily residue was recovered. Purification by flash column chromatography (EtOAc-MeOH, 5:1) provided 250 mg of the mixture 7a,7b, which was directly used for the next step.

(±)-4'-diethylphosphonoethyl-3'-thia-2',3'-dideoxy-N4-acetyl cytidine: β isomer 8a and α isomer 8b.
Under nitrogen, to 0.135 g (0.375 mM) of mixture 7a,7b, dissolved in 7 ml of dry DMF, was added 1.5 equivalent (0.55 mM) of freshly distilled acetic acid anhydride and 3 equivalents of triethylamine (1.125 mM). The mixture is stirred overnight at room temperature. After solvent evaporation, the residue was purified by flash column chromatography (EtOAc-MeOH, 9:1). The two isomers 8a and 8b were separated (80% yield).

β -isomer 8a : TLC Rf:0.20 (solvent EtOAc-MeOH,3:1)
^{1}H NMR (CDCl$_3$) : 1.31 (6H,t,$\underline{CH_3}CH_2O$-) ,1.8-2.1 (4H,m,$\underline{CH_2}$-$\underline{CH_2}$),2.25(3H,s,CO$\underline{CH_3}$),3.22-3.27 and 3.5-3.6(2H,dd,S-$\underline{CH_2}$), 4.08-4.10(4H,m,CH$_3$-$\underline{CH_2}$O-),5.60(1H,t,O-$\underline{CH}$-S),6.40(1H,dd,O-$\underline{CH}$-N) , 7.4(1H,d,$\underline{CH}$=CH), 7.7(1H,d,CH=$\underline{CH}$).
Anal.($C_{15}H_{24}N_3O_6PS$) C,H,P. FABMS (Thioglycerol) m/z 406 (M+H)+.

α -isomer 8b : TLC Rf:0.31 (solvent EtOAc-MeOH,3:1)
^{1}H NMR(CDCl$_3$): 1.31 (6H,t,$\underline{CH_3}CH_2O$-), 1.9-2.1(4H,m,$\underline{CH_2}$-$\underline{CH_2}$), 2.24 (3H,s,CO$\underline{CH_3}$),3.08-3.12 and 3.5-3.6 (2H,dd,S-$\underline{CH_2}$), 4.08-4.10 (4H,m,CH$_3$$\underline{CH_2}$O-), 5.21 (1H,t,O-$\underline{CH}$-S),6.27(1H,dd,O-$\underline{CH}$-N), 7.45(1H,d,$\underline{CH}$=CH), 7.87(1H,d,CH=$\underline{CH}$)
Anal.($C_{15}H_{24}N_3O_6PS$) C,H,P. FABMS (Thioglycerol) m/z 406 (M+H)+.

(±)-4'-phosphonoethyl-3'-thia-2',3'-dideoxycytidine (free acid). β isomer 3a and α isomer 3b.
To 39 mg (0.096 mM) of compound 8a or 8b dissolved in 2 ml of dry CH_2Cl_2 was added 66.3 mg (0.43 mM) of trimethylsilylbromide. The mixture is stirred at room temperature for 5 h. Directly to this solution was added 2 ml of MeOH, and stirring was continued for 2 h. After solvent evaporation, the crude residue was treated for one night at room temperature with a methanolic solution saturated with ammonia. After solvent removal, the residue was washed with ether, and a white compound was recovered in 75% yield.

β -isomer 3a:
^{1}H NMR (DMSO-d$_6$):1.5-1.65(2H,m,P-$\underline{CH_2}$),1.87-1.98(2H,m,P-CH$_2$-$\underline{CH_2}$), 3.25-3.31 and 3.50-3.55(2H,dd,S-$\underline{CH_2}$), 5.65(1H,t,O-$\underline{CH}$-S), 5.92(1H,d,$\underline{CH}$=CH), 6.3(1H,dd,O-$\underline{CH}$-N), 7.78(1H,d,CH=$\underline{CH}$), 8.58(2H,broad,$\underline{NH_2}$)
Anal. ($C_9H_{14}N_3O_5P$) C,H,P. FABMS (Thioglycerol) m/z 308 (M+H)+.

α -isomer 3b:
^{1}H NMR(CD$_3$OD) : 1.5-1.8 (2H,m,P-$\underline{CH_2}$), 2.10-2.20 (2H,m,P-CH$_2$-$\underline{CH_2}$), 3.20 - 3.25 and 3.42 - 3.50 (2H,dd,S-$\underline{CH_2}$), 5.29 (1H,dd,O-$\underline{CH}$-S), 5.90 (1H,d,$\underline{CH}$=CH), 6.25 (1H,dd,O-$\underline{CH}$-N), 7.85 (1H,d,CH=$\underline{CH}$) , 8.62 (2H,broad,$\underline{NH_2}$)
Anal. ($C_9H_{14}N_3O_5P$) C,H,P. FABMS (Thioglycerol) m/z 308 (M+H)+.

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