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Synthesis of New Acyclic Nucleoside Phosphonic Acids by Michael Addition

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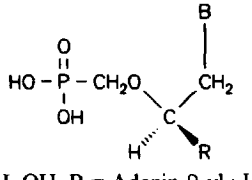
Abstract : New acyclic nucleoside phosphonic acids in the purine and pyrimidine series were prepared *via* one step by Michael addition. These compounds are the first reported acyclonucleosides enamines which incorporate the α, β-unsaturated phosphonic acid as a phosphate mimic.

Acyclic nucleoside phosphonic acids, which contain a phosphonate group linked to the *N-1* or *N-9* alkyl side-chain of pyrimidines or purines, have been identified as a new class of antiviral agents with a broad spectrum of activity against retroviruses and DNA viruses[1-3]. The lead compounds of the nucleoside phosphonate analogue series are 9-(*S*)-(3-hydroxy-2-phosphonyl methoxypropyl)adenine (HPMPA) **1** and 9-(2-phosphonylmethoxyethyl) adenine (PMEA) **2**, both of which exhibit potent antiviral effects[4-6]. The mechanism of action of the antiviral activity of these compounds is believed to involve binding of the viral DNA polymerases by the diphosphate derivatives of the nucleoside phosphonates and subsequent incorporation of the analogues into viral DNA[7].



1 R = CH_2OH, B = Adenin-9-yl : HPMPA

2 R = H, B = Adenin-9-yl : PMEA

All of these antiviral compounds contain a characteristic phosphonylmethoxy group linked in the β-position of an ethyl or hydroxypropyl group. It is well known that conjugate additions of organometallic reagents to α, β-unsaturated carbonyl compounds constitue a powerful method which is extensively used in stereospecific synthesis[8]. In contrast, analogous *1,4*-addition reactions to α, β-unsaturated phosphoryl compounds have received little attention[9,10]. Recently, new unsaturated acyclonucleotide analogues of purines and pyrimidines possessing the α, β-unsaturated phosphoryl function at carbon 3 position regarding to the heterocycle, have been described[11,12].

We report herein the synthesis of a novel class of acyclonucleoside precursors in which the side-chain contains an ethylenylphosphonyl group.

The alkylation of the heterocyclic bases with diethyl ethynylphosphonate **3**[13] was performed by Michael type addition using Solid-Liquid Phase Transfer Catalysis conditions (tBuOK/18-crown-6/CH_3CN). The reaction of synthon **3** with adenine or uracil at room temperature led exclusively to *N*-9 and *N*-1 adducts, respectively. The *E* and *Z* isomers were formed in the ratios of 2:8 (adenine) and 5:5 (uracil).

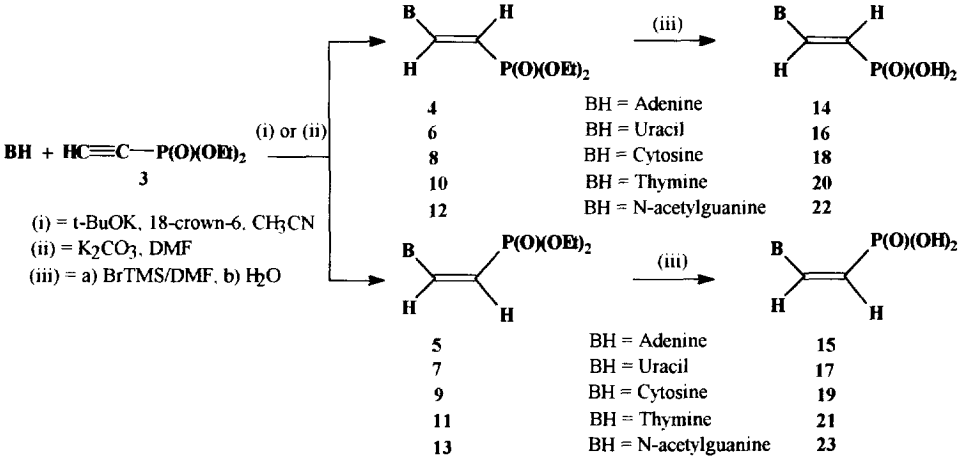


Table1 : Experimental results of the condensation of nucleobases with the sython **3**.

Bases	Reaction conditions	T (°C)	Time (h)	E/Z ratio	Yields (%)	Dialkylated products
Adenine	tBuOK, 18-crown-6, CH_3CN	RT	40	2/8	35	-
	K_2CO_3 /DMF	RT	22	2/8	70	-
Uracil	tBuOK, 18-crown-6, CH_3CN	RT	60	5/5	36	-
	K_2CO_3 /DMF	RT	18	8/2	76	
Cytosine	tBuOK, 18-crown-6, CH_3CN	RT	2	4/6	31	N1-N4 (20%)
	tBuOK, 18-crown-6, CH_3CN	0°C	2	1.5/8.5	41	-
	K_2CO_3 /DMF	RT	4	8/2	72	-
Thymine	tBuOK, 18-crown-6, CH_3CN	RT	24	4/6	10	N1-N3 (44%)
	tBuOK, 18-crown-6, CH_3CN	0°C	30	4/6	32	-
	K_2CO_3 /DMF	RT	24	8/2	81	-
N-acetylguanine	tBuOK, 18-crown-6, CH_3CN	RT	12	4/6	35	-
	K_2CO_3 /DMF	RT	6	2/8	67	-

Reaction of cytosine with **3** at room temperature led to the *N-1* isomers **8** (*E* form) and **9** (*Z* form) respectively in the ratio 4:6 together with the *N-1,N-4* dialkylated side product in 20% yield. Analogously thymine gave *N-1* isomers **10** (*E* form) and **11** (*Z* form) respectively in a 4:6 ratio with the *N-1,N-3* dialkylated derivatives as side product. In order to avoid the formation of the undesired dialkylated product, the reaction were carried out at 0°C. In these conditions, only monoalkylated *N-1* derivatives have been obtained[14]. The yields were generally moderate 35-41 %. But they were improved to 70-80% using

another condition: K_2CO_3 as base and DMF as solvent at room temperature. The condensation between **3** and nucleobases led exclusively to *N-9* (purine) and *N-1* (pyrimidine) adducts based on their UV spectra [15]. The ratio of the formed isomers (*E* and *Z*) are reported in table 1. All isomers synthesized were separated by chromatography on silica gel and were identified by the usual analytical methods[15]. These isomers were converted into phosphonic acids derivatives **14-23** on treatment with bromotrimethylsilane and no isomerisation occured during the reaction[11]. The deprotection of acetyl group of **23** was achieved using MeOH / HCl[11].

This new procedure offers several advantages for the synthesis of acyclophosphonate nucleoside analogues. It allows introduction of the diethylphosphonyl group at the start of the synthesis by the simple method mentionned above. No prior protection of amino or hydroxyl groups of Uracil, Thymine, Cytosine and Adenine is necessary. Additionally, the compounds prepared here are activated olefins and can lead to numerous other acyclic nucleosides. This work will be reported in the near future.

All these phosphonic acids were tested for their *in vitro* inhibitory effects on the replication of a number of DNA viruses [i.e. (type 1 and type 2) herpex simplex virus] and retroviruses (HIV-1, HIV-2) in various cell culture systems. None of them showed any significant activity at the highest concentration tested (usually 100 μM).

Typical procedure :

1) A solution of heterocyclic base (7.40 mmol), potassium *tert*-butoxide (720 mg, 6.43 mmol), 18-crown-6 (326 mg, 1.23 mmol) and acetonitrile (150 ml) was stirred at room temperature for 15 mn, then diethyl ethynylphosphonate (1.44 g, 8.89 mmol) was added. The mixture was stirred at room temperature for 40 h, filtered and concentrated. The residue was chromatographed on silica gel to yield 35-41 % of the expected products.
2) Solid K_2CO_3 (0.5mmol) was added to a solution of nucleobase (1 mmol) in DMF at room temperature. After 15 mn stirring, the alkylating agent (1.5 mmol) in DMF was added. The reaction evolution was monitored by TLC. The solvent evaporated and the product was purified by column chromatography.

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15. For example:

Product **6**:^{1}H NMR (DMSO-d6) δ (ppm) (Hz): 11.72 (br s, 1H, NH); 7.71 (dd, 1H, H-2', $J_{2',1'}$ = 16.0, $J_{2',P}$ = 18.3), 8.14 (d, 1H, H-6, $J_{6,5}$ = 8.3), 5.83 (d, 1H, H-5, $J_{5,6}$ = 8.3), 6.13 (dd, 1H, H-1', $J_{1',2'}$ = 16.0, $J_{2',P}$ = 10.6), 4.00 (qd, 4H, P-O-CH_2, $J_{CH2,CH3}$ = 7.1, $J_{CH2,P}$ = 8.1), 1.24 (t, 6H, 2 x CH_3, $J_{CH3,CH2}$ = 7.1). MS FAB (positive ions): *m/z* 275 $[M+H]^+$. UV (MeOH): λ_{max} 228 nm (ε 1800); λ_{max} 278 nm (ε 20200).

Product **7**: ^{1}H NMR (DMSO-d6) δ (ppm) J (Hz): 11.66 (br s, 1H, NH); 7.84 (d, 1H, H-6, $J_{6,5'}$ = 8.1); 7.31 (dd, 1H, H-2', $J_{2',1'}$ = 11.2, $J_{1',P}$ = 42.7); 5.77 (d, 1H, H-5, $J_{5,6}$ = 8.1), 5.76 (dd, 1H, H-1', $J_{1',2'}$ = 11.2, $J_{1',P}$ = 9.2), 4.01 (qd, 4H, 2 x CH_2, $J_{CH2,CH3}$ = 7.1, $J_{CH2,P}$ = 8.2), 1.22 (t, 6H, 2 x CH_3, $J_{CH2,CH3}$ = 7.1). MS FAB (positive ions): *m/z* 275 $[M+H]^+$. UV (MeOH): λ_{max} 236 nm (ε 8300); λ_{max} 273 nm (ε 14400).

Product **16**:^{1}H NMR (DMSO-d6) δ (ppm) (Hz): 11.66 (br s, 1H, NH); 8.12 (d, 1H, H-6, $J_{6,5}$ = 7.7); 7.60 (dd, 1H, H-2', $J_{2',1'}$ = 15.9, $J_{2',P}$ = 15.8); 6.06 (dd, 1H, H-1', $J_{1',2'}$ = 15.9, $J_{2',P}$ = 9.3); 5.77 (d, 1H, H-5, $J_{5,6}$ = 7.7); MS FAB (negative ions): *m/z* 217 $[MH]^-$. UV (H_2O): λ_{max} 279 nm (ε 15100).

Product **17**:^{1}H NMR (DMSO-d6) δ (ppm) (Hz): 11.58 (br s, 1H, NH); 7.79 (d, 1H, H-6, $J_{6,5}$ = 7.6); 7.27 (dd, 1H, H-2', $J_{2',1'}$ = 11.4, $J_{2',P}$ = 27.2); 5.69 (dd, 1H, H-1', $J_{1',2'}$ = 11.4, $J_{2',P}$ = 7.7); 5.73 (d, 1H, H-5, $J_{5,6}$ = 7.6); MS FAB (negative ions): *m/z* 217 $[MH]^-$. UV (H_2O): λ_{max} 274 nm (ε 14200).

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