Crystal Structure and Conformation of the Phosphotriester Adenosine 5'-*O*-(Diethyl phosphate). Possible Steric and Conformational Mechanisms for the Biochemical and Biological Effects Arising from Phosphate Alkylation

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Abstract: Phosphotriesterified oligonucleotides are often the major products resulting from the attack of mutagenic and carcinogenic alkylating agents on DNA and RNA. In order to elucidate the electronic and conformational perturbations arising from phosphate esterification, which may be the basis of its biochemical and biological effects, the X-ray structure of the triesterified nucleotide adenosine 5'-*O*-(diethyl phosphate) ($C_{14}H_{22}N_5O_7P$) was undertaken. The compound (FW = 403.33) crystallizes in the triclinic space group $P1$ ($Z = 1$) with unit cell parameters of $a = 6.799$ (1), $b = 7.923$ (1), $c = 9.003$ (1) Å, $\alpha = 86.86$ (1), $\beta = 77.98$ (1), $\gamma = 77.85$ (1)°, $V = 463$ Å^3, $D_c = 1.444$ g cm^{-3}, and $D_M = 1.45$ g cm^{-3}. The structure was solved by heavy-atom methods and refined by the full-matrix least-squares technique to an R index of 0.039 ($R_w = 0.051$) using 1917 intensities. The D-ribofuranosyl ring is in a symmetrical twist conformation, C(2')-endo-C(1')-exo ($^2_1$T), with pseudorotational parameters $P = 145.0$ (2)° and $\tau_m = 39.4$ (2)°. The adenine base is anti (69.0 (3)°) about the C(1')-N(9) glycosyl bond, and the conformation about the exocyclic bond C(4')-C(5') is the preferred gauche$^+$ (50.8 (3)°). Hydrogen bonding is centered about the ribosyl hydroxyls and the N(6) amino group of the base. The molecular packing is dominated by intermolecular base-alkyl stacking and alkyl-alkyl van der Waals interactions. The methyl group of one of the ethoxy groups is two-site disordered. Diethylation of the phosphate results in neutralization of the charge and geometric and conformational perturbations of the phosphodiester. All four P-O bonds are significantly shorter than those of the nonalkylated nucleotides. The three combinations of phosphodiester linkages display the (g^-,t), (g^+,g^-), and (t,t) conformations which are different from the familiar (g^-,g^-) conformation of right-handed polynucleotide helices. The lack of preference of the alkylated sugar phosphate backbone for the (g^-,g^-) phosphodiester conformation would tend to destack the bases and promote alkyl-base stacking. This will lead to sugar phosphate backbone configurations which would provide a mechanism for the biochemical and biological effects induced by phosphate alkylation.

Alkylation by many mutagenic and carcinogenic agents is not confined alone to the purine and pyrimidine bases of the nucleic acids. Phosphate alkylation can also be affected by methyl methanesulfonate and ethyl methanesulfonate at neutral pH.[1] The phosphodiester backbone is particularly susceptible to ethylation by the potent carcinogen ethylnitrosourea. The resulting phosphotriester product represents 60% of the total ethylation in TMV-RNA.[2] Similar results are observed both in vitro[3] and in vivo[4] when DNA is treated with ethylnitrosourea. RNA can be readily alkylated, but its sugar phosphate backbone forms unstable ethoxy linkages and undergoes chain scission.[5] DNA, on the other hand, is able to form stable alkyl phosphotriesters[6] which remain nearly unchanged after 72 h in cell culture.[7] Although these alkyl groups are persistent, there is no evidence that mutations result.

The biological and biochemical effects resulting from the alkylation of the phosphodiester backbone are not well understood but appear not to involve base mispairing.[8] Conformational perturbations in the sugar phosphate backbone, neutralization of the phosphate group, and the interference of the alkyl group with protein binding have often been invoked to explain these effects. To gain a detailed understanding of the conformational, electronic, and geometric perturbations resulting from alkylation of the phosphodiester backbone, we have determined the crystal and molecular structure of the first free (noncyclic) triesterified nucleotide adenosine 5'-*O*-(diethyl phosphate). On the basis of the conformational analysis of this compound and the observed crystal packing we suggest possible mechanisms which may explain the biochemical and biological effects resulting from phosphotriesterification.

Experimental Section

A crystal of adenosine 5'-*O*-(diethyl phosphate), crystallized from water and air-dried, with dimensions 0.3 × 0.15 × 0.15 mm, was selected for intensity data collected on an Enraf-Nonius CAD4 diffractometer using Ni-filtered Cu Kα radiation (λ 1.5418 Å). The unit cell parameters were refined by a least-square algorithm using 25 automatically centered reflections. A total of 2142 reflections were measured to a 2θ value of 150°, of which 1917 unique reflections with intensities greater than $1.5\sigma(I)$ were used in the structural analysis. Three reflections monitored throughout data collection revealed negligible crystal decay. An absorption correction employing an empirical ϕ curve as well as Lorentz and polarization corrections was applied to the intensities.

Structure Determination and Refinement

The structure was solved by the heavy-atom method, which revealed the five atoms of the phosphate group in the initial Patterson map. The remaining non-hydrogen atoms were located by subsequent Fourier syntheses. One of the methyl groups displayed a two-site disorder with each site having a 50% occupancy factor. The atomic positions for the non-hydrogen atoms were refined by full-matrix least-squares technique using anisotropic thermal parameters. Difference Fourier syntheses revealed 18 of the 22 hydrogen atoms which were submitted to full-matrix least-squares refinement using isotropic thermal parameters. The remaining hydrogen atoms were fixed from geometric considerations. The full-matrix least-squares refinement converged at $R = \sum||F_o| - |F_c||/\sum|F_o| = 0.039$ ($R_w = 0.051$).[9] The function minimized was $\sum w(|F_o| - |F_c|)^2$, where a modified counting statistics weighting scheme[10] was used with the weight of each reflection proportional to $1/[\sigma^2(F) + (0.04F_o)^2]$.

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Table I. Atomic Parameters for Adenosine 5′-*O*-(Diethyl phosphate). Fractional Positional Parameters Are Multiplied by 10^4 for Non-Hydrogen Atoms and 10^3 for Hydrogen Atoms

$$B_{eq} = \frac{4}{3}\sum_i\sum_j B_{ij}a_ia_j$$

atom	x	y	z	B_{eq} or B	multiplicity
P	−7 (0)	39 (0)	4 (0)	2.65 (2)	
O(1)	13 (3)	−1769 (3)	−271 (2)	3.54 (4)	
O(2)	453 (4)	376 (3)	1560 (2)	4.27 (5)	
C(9)	233 (9)	−767 (6)	2848 (4)	6.17 (12)	
C(10-1)	−1023 (22)	43 (16)	4169 (10)	7.45 (34)	0.50
C(10-2)	787 (23)	−209 (15)	4062 (11)	12.18 (33)	0.50
O(3″)	1638 (4)	879 (4)	−1078 (3)	4.25 (5)	
C(12)	1397 (8)	1630 (7)	−2516 (5)	7.03 (12)	
C(13)	3371 (8)	1829 (7)	−3442 (5)	6.38 (11)	
O(5′)	−2067 (3)	1302 (3)	−156 (3)	3.97 (5)	
C(5′)	−4034 (4)	842 (3)	496 (3)	3.06 (5)	
C(4′)	−5498 (3)	2426 (3)	1218 (3)	2.39 (4)	
O(4′)	−4831 (3)	2851 (2)	2545 (2)	2.85 (4)	
C(3′)	−5654 (3)	4037 (3)	195 (2)	2.17 (4)	
O(3′)	−7761 (3)	4803 (2)	305 (2)	3.17 (3)	
C(2′)	−4510 (3)	5176 (3)	887 (2)	2.06 (4)	
O(2′)	−5186 (3)	6954 (2)	691 (2)	2.85 (4)	
C(1′)	−4958 (3)	4658 (3)	2554 (2)	2.07 (4)	
N(9)	−3488 (3)	4982 (3)	3411 (2)	2.42 (4)	
C(8)	−1369 (4)	4577 (4)	2999 (3)	3.02 (5)	
N(7)	−451 (3)	4960 (3)	4044 (3)	3.23 (5)	
C(5)	−2044 (3)	5669 (3)	5190 (3)	2.37 (4)	
C(4)	−3930 (3)	5681 (3)	4830 (2)	2.08 (4)	
N(3)	−5785 (3)	6283 (3)	5703 (2)	2.76 (4)	
C(2)	−5598 (3)	6890 (4)	6999 (3)	2.85 (5)	
N(1)	−3886 (3)	6965 (3)	7507 (2)	2.60 (4)	
C(6)	−2054 (3)	6352 (3)	6600 (2)	2.35 (4)	
N(6)	−334 (3)	6418 (3)	7079 (2)	3.38 (5)	
H1(C-5′)	−379 (4)	−6 (3)	109 (3)	1.6 (5)	
H2(C-5′)	−437 (6)	47 (5)	−43 (4)	3.6 (7)	
H(C-4′)	−686 (5)	209 (4)	155 (4)	2.9 (6)	
H(C-3′)	−503 (4)	386 (3)	−76 (3)	1.2 (4)	
H(O-3′)	−796 (6)	580 (5)	21 (4)	3.7 (7)	
H(C-2′)	−288 (5)	476 (4)	44 (3)	2.6 (6)	
H(O-2′)	−472 (5)	721 (4)	−18 (3)	2.4 (6)	
H(C-1′)	−614 (5)	525 (4)	311 (3)	2.2 (5)	
H(C-8)	−79 (5)	408 (4)	202 (4)	2.6 (6)	
H1(N-6)	−47 (6)	694 (5)	794 (4)	3.5 (7)	
H2(N-6)	69 (6)	617 (5)	654 (4)	3.9 (8)	
H(C-2)	−682 (6)	726 (4)	775 (4)	3.1 (6)	
H1(C-9)	−4 (7)	−164 (6)	253 (5)	5.4 (10)	
H2(C-9)[a]	151 (0)	−104 (0)	319 (0)	4.0 (0)	
H1(C-12)	30 (8)	229 (7)	−271 (6)	6.3 (12)	
H2(C-12)	85 (15)	62 (14)	−283 (13)	14.4 (29)	
H1(C-13)	328 (13)	238 (10)	−440 (10)	11.3 (22)	
H2(C-13)	428 (16)	262 (12)	−307 (12)	14.6 (31)	
H3(C-13)[a]	437 (0)	63 (0)	−342 (0)	4.0 (0)	

[a] The atomic coordinates of these hydrogen atoms were fixed from geometric considerations. No hydrogen atoms were assigned to the C(10-1) or C(10-2) methyl carbon atoms.

The maximum values of the shift over error ratios were <0.01 and <0.023 for the non-hydrogen and hydrogen atoms, respectively.

Scattering factors for the non-hydrogen atoms were taken from Cromer and Waber[11] and those for the hydrogen atoms were from Stewart et al.[12]

Results and Discussion

An ORTEP[13] drawing indicating the atom numbering and overall molecular conformation is given in Figure 1. The fractional positional and isotropic thermal parameters for all atoms are presented in Table I.[14] The bond lengths and angles for the non-hydrogen atoms are listed in Table II. The ribofuranosyl, glycosyl, and sugar phosphate backbone torsion angles are given in Table III.

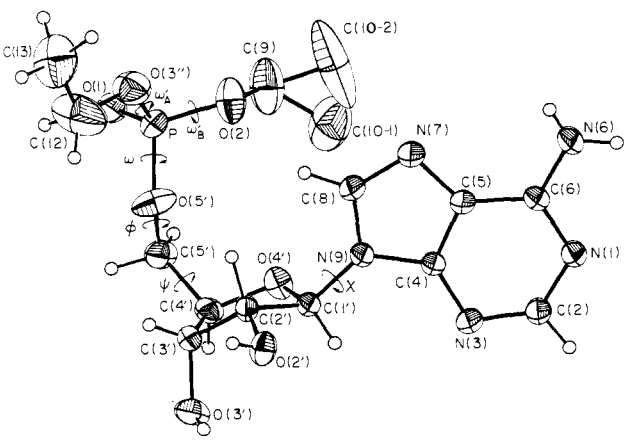

Figure 1. ORTEP drawing of adenosine 5′-*O*-(diethyl phosphate). The non-hydrogens are represented by 50% probability ellipsoids, while the hydrogen atoms are drawn as spheres of arbitrary size. Note both carbon atoms C(10-1) and C(10-2) of the disordered methyl group bonded to C(9) are included.

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Table II. Non-Hydrogen Bond Lenghts (Å) and Angles (deg) for Adenosine 5′-*O*-(Diethyl phosphate)

N(1)-C(2)	1.350 (3)	C(2)-N(3)	1.326 (3)	N(3)-C(4)	1.346 (3)
C(4)-C(5)	1.385 (3)	C(5)-C(6)	1.405 (3)	C(6)-N(1)	1.349 (3)
C(6)-N(6)	1.340 (3)	C(5)-N(7)	1.377 (3)	N(7)-C(8)	1.312 (4)
C(8)-N(9)	1.383 (4)	N(9)-C(4)	1.370 (3)	N(9)-C(1′)	1.453 (3)
C(1′)-C(2′)	1.519 (3)	C(2′)-C(3′)	1.538 (3)	C(2′)-O(2′)	1.400 (3)
C(3′)-C(4′)	1.531 (3)	C(3′)-O(3′)	1.418 (3)	C(4′)-C(5′)	1.511 (3)
C(4′)-O(4′)	1.442 (3)	O(4′)-C(1′)	1.416 (3)	C(5′)-O(5′)	1.456 (4)
O(5′)-P	1.569 (3)	P-O(1)	1.464 (2)	P-O(2)	1.546 (2)
O(2)-C(9)	1.434 (4)	C(9)-C(10-1)*	1.409 (11)	C(9)-C(10-2)*	1.351 (13)
P-O(3″)	1.559 (3)	O(3″)-C(12)	1.421 (5)	C(12)-C(13)	1.459 (7)

C(2)-N(1)-C(6)	117.8 (2)	N(1)-C(2)-N(3)	129.6 (3)
C(2)-N(3)-C(4)	110.9 (2)	N(3)-C(4)-C(5)	126.3 (2)
N(3)-C(4)-N(9)	128.3 (2)	C(5)-C(4)-N(9)	105.4 (2)
C(4)-C(5)-C(6)	117.3 (2)	C(4)-C(5)-N(7)	111.4 (3)
C(6)-C(5)-N(7)	131.4 (2)	C(5)-C(6)-N(6)	123.0 (2)
C(5)-C(6)-N(1)	118.1 (2)	C(8)-N(9)-C(4)	106.0 (2)
N(1)-C(6)-N(6)	118.9 (2)	C(5)-N(7)-C(8)	104.0 (3)
N(7)-C(8)-N(9)	113.2 (3)	C(4)-N(9)-C(1′)	126.6 (2)
C(8)-N(9)-C(1′)	127.3 (2)	N(9)-C(1′)-O(4′)	107.9 (2)
N(9)-C(1′)-C(2′)	114.4 (2)	C(1′)-C(2′)-C(3′)	101.5 (2)
C(2′)-C(1′)-O(4′)	104.6 (2)	C(3′)-C(2′)-O(2′)	114.6 (2)
C(1′)-C(2′)-O(2′)	111.1 (2)	C(2′)-C(3′)-O(3′)	112.1 (2)
C(2′)-C(3′)-C(4′)	102.7 (2)	C(3′)-C(4′)-O(4′)	107.2 (2)
C(4′)-C(3′)-O(3′)	108.4 (2)	O(4′)-C(4′)-C(5′)	108.9 (2)
C(3′)-C(4′)-C(5′)	114.8 (2)	C(5′)-O(5′)-P	120.3 (2)
C(4′)-O(4′)-C(1′)	108.7 (2)	O(5′)-P-O(2)	107.5 (2)
C(4′)-C(5′)-O(5′)	108.6 (2)	O(5′)-P-O(1)	113.7 (2)
O(5′)-P-O(3″)	102.5 (2)	O(2)-P-O(3″)	100.3 (2)
O(1)-P-O(2)	114.7 (2)	P-O(2)-C(9)	124.2 (3)
O(1)-P-O(3″)	116.7 (2)	O(2)-C(9)-C(10-1)[a]	113.5 (7)
P-O(3″)-C(12)	124.1 (3)	O(2)-C(9)-C(10-2)[a]	113.0 (8)
O(3″)-C(12)-C(13)	111.0 (5)		

[a] C(10) and C(11) represent the disordered methyl carbon atoms bonded to carbon atom C(9).

Table III. Selected Torsion Angles (deg) of Adenosine 5′-*O*-(Diethyl phosphate)

τ_0	C(4′)-O(4′)-C(1′)-C(2′)	-32.1 (3)
τ_1	O(4′)-C(1′)-C(2′)-C(3′)	39.4 (3)
τ_2	C(1′)-C(2′)-C(3′)-C(4′)	-31.4 (2)
τ_3	C(2′)-C(3′)-C(4′)-O(4′)	13.7 (3)
τ_4	C(3′)-C(4′)-O(4′)-C(1′)	11.3 (3)
ψ'_A	O(3′)-C(3′)-C(4′)-C(5′)	133.8 (3)
ψ_A	C(3′)-C(4′)-C(5′)-O(5′)	50.8 (3)
ϕ_A	C(4′)-C(5′)-O(5′)-P	138.9 (3)
ω_A	C(5′)-O(5′)-P-O(3″)	170.3 (3)
ω'_A	O(5′)-P-O(3″)-C(12)	-40.1 (4)
ϕ'_A	P-O(3″)-C(12)-C(13)	-162.8 (6)
ω_B	C(5′)-O(5′)-P-O(2)	-84.5 (2)
ω'_B	O(5′)-P-O(2)-C(9)	106.7 (4)
ϕ'_B	P-O(2)-C(9)-C(10-1)	-126.0 (9)
ϕ''_B	P-O(2)-C(9)-C(10-2)	178.2 (9)
χ_{CN}	O(4′)-C(1′)-N(9)-C(8)	69.0 (3)
ω_C	C(9)-O(2)-P-O(3″)	-146.6 (4)
ω'_C	O(2)-P-O(3″)-C(12)	-150.7 (4)

Sugar Geometry and Conformation. The ribofuranose ring is found in the C(2′)-endo-C(1′)-exo, ${}^{2}_{1}\mathrm{T}$ symmetrical twist conformation,[15] where the phase angle and amplitude of pseudorotation are 145.0 (2)° and 39.4 (2)°, respectively.[16] This distortion in the sugar pucker from the standard ${}^{2}\mathrm{T}_{3}$ pucker increases the purine ring to ethyl group distance, thus avoiding unfavorable intramolecular interactions between the two groups. An additional consequence of this puckering is an increase in the C(2′)-C(3′) (1.538 (3) Å) and C(3′)-C(4′) (1.531 (3) Å) bond lengths. A similar result is obtained for guanosine dihydrate molecule **B**, which assumes the closely related ${}_{1}\mathrm{T}^{2}$ twist conformation (P = 139.2°), where the C(2′)-C(3′) and C(3′)-C(4′) bond distances are 1.536 (4) and 1.530 (4) Å, respectively.[17] The conformation about the C(4′)-C(5′) bond is the preferred gauche$^+$ (50.8 (3)°).

The glycosyl bond length, N(1)-C(1′), and bond angle N(9)-C(1′)-O(4′), are 1.453 (3) Å and 107.9 (2)°, respectively. The conformation about the glycosyl bond is anti with a value of 69.0 (3)° for χ (O(4′)-C(1′)-N(9)-C(8)).

Base Geometry. The values of the bond lengths and angles for the purine base are similar to those reported for the standard adenine ring[18] where the largest deviations in bond length and angle are 0.016 Å for the C(8)-N(9) bond and 1.0° for the C(2)-N(1)-C(6) bond angle. The least-squares plane through the nine atoms of the purine ring, which is flat, is $0.105X + 0.902Y - 0.419Z = 2.151$ where X, Y, and Z are measured in angstroms along the crystallographic a, b, and c axes, respectively. The exocyclic nitrogen atom N(6) is within the plane (0.011 (3) Å) while the substituent atom C(1′) deviates -0.038 (3) Å from the plane.

Sugar Phosphate Geometry. The triesterification of adenosine 5′-monophosphate by addition of the two ethyl groups neutralizes the sugar phosphate backbone and significantly changes the geometry. The three phosphoester bonds, P-O(2), P-O(3″), and P-O(5′), are 1.546 (2), 1.559 (3), and 1.569 (3) Å, respectively, and are shorter than the average value (~1.59 Å) observed for nucleotide (monophosphates) structures. The P-O(1) bond length (1.464 (2) Å) is similar to the nonesterified P-O bond distances of the 3′,5′ cyclic nucleotide phosphotriesters, ethyl cAMP (1.44 (1) Å)[19] and 2′-acetyluridine-3′,5′-cyclophosphate benzyl triester (1.466 (2) Å).[20] However, this bond length is significantly shorter than the average nonesterified P-O bond length reported for the O-alkylated mononucleotide diesters uridine 5′-*O*-(methyl phosphate) methanol[21] and adenosine 5′-*O*-(methyl phosphate)[22] (av

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Table IV. Hydrogen Bond Lengths (Å) and Angles (deg) in Adenosine 5′-*O*-(Diethyl phosphate)

A—H···B	translation for B	A—H	H···B	A···B	A—H···B
O(2′)-H(2′)···N(1)	$x,y,z-1$	0.81 (3)	2.05 (3)	2.818 (2)	157 (3)
O(3′)-H(3′)···O(2′)	x,y,z	0.78 (4)	2.38 (4)	2.768 (3)	112 (4)
O(3′)-H(3′)···O(1)	$x-1, y+1,z$	0.78 (4)	2.20 (4)	2.895 (3)	149 (4)
N(6)-H(1)···O(1)	$x,y+1,z+1$	0.76 (4)	2.07 (4)	2.926 (3)	165 (4)
N(6)-H(2)···N(3)	$x+1,y,z$	0.88 (4)	2.38 (4)	3.064 (3)	152 (4)

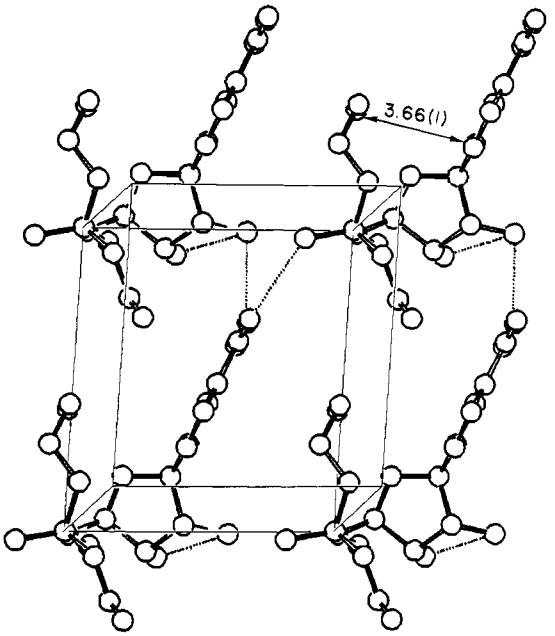


Figure 2. A crystal packing diagram displaying four molecules viewed edge on the adenine rings. The dashed lines show some of the hydrogen-bonding scheme present in the crystal and the intramolecular stacking between C(10-1) and C(8) is indicated by an arrow. The hydrogen atoms have been removed for clarity. Note that the ethoxy carbon atoms C(12) and C(13) make van der Waals contacts with neighboring bases, where the C(12) to N(6) and C(13) to N(3) distances are 3.749 (6) Å and 3.707 (6) Å, respectively.

= 1.486 (13) Å) and the monoclinic[23] and orthorhombic[24] forms of adenosine 5′-monophosphate (av = 1.502 (8) Å). When the average P–O bond length of adenosine 5′-*O*-(diethyl phosphate) (1.534 Å) is compared to the average P–O bond distances of the cyclic 3′,5′-triesters (1.538 Å), the O-akylated mononucleotide diesters (1.537 Å) and adenosine 5′-monophosphate (1.545 Å), only slight differences are observed. Therefore, as ethylation (esterification) of the phosphate group increases, there is a concomitant decrease in the individual P–O bond lengths though the overall bond order about the phosphate group remains nearly constant.

The bond angles, O(3″)–P–O(1), O(5′)–P–O(1), and O(2)–P–O(1), are 116.7 (2)°, 113.7 (2)°, and 114.7 (2)°, respectively, and reflect the double bond character of the P–O(1) bond. The remaining bond angles, involving the diester fragments, O(5′)–P–O(2), O(2)–P–O(3″), and O(5′)–P–O(3″) are 107.5 (2)°, 100.3 (2)°, and 102.5 (2)°, respectively, which are in the range of values observed for diesters themselves.

Hydrogen Bonding and Base–Alkyl Stacking. A packing diagram, illustrating some of the hydrogen bonding and the base-alkyl intramolecular and intermolecular stacking, is given in Figure 2. Of the five hydrogen bonds (Table IV), four are intermolecular and are centered around the ribosyl hydroxyls and the N(6) amino group of the adenine ring. The H(O3′) proton is involved in a bifurcated hydrogen bond intramolecularly with O(2′) and intermolecularly with the phosphate oxygen O(1), where the O-(3′)–H(O3′)–O(1) and O(3′)–H(O3′)–O(2′) angles are 149 (4)° and 112 (4)°, respectively. The sugar puckering is probably intimately related to this intramolecular hydrogen bond. The closest nonbonded intermolecular contact is between the O(3′) hydroxyl oxygen atom and a neighboring C(8) imidazole carbon atom (3.097 (3) Å).

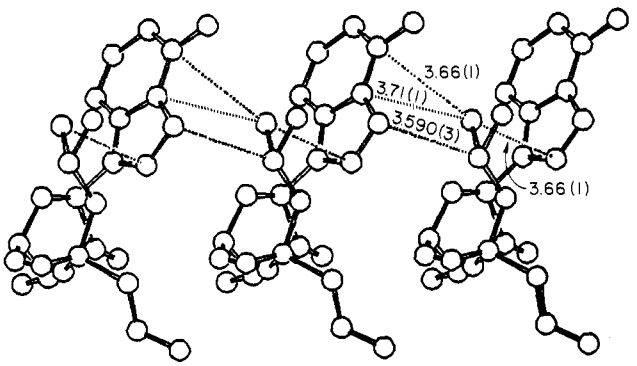


Figure 3. Projection displaying the inter- and intramolecular stacking between the C(9)–C(10-1) ethoxy group and the adenine ring. The dashed lines represent contacts between the two in which the distances range from 3.5 to 3.8 Å (see text).

Base–base stacking interactions are not present in the crystal structure of adenosine 5′-*O*-(diethyl phosphate). However, stacking interactions are found between the ethoxy carbon atoms C(9) and C(10-1)[25] and a translationally related adenine ring (Figure 3). C(9) is in close contact with N(7) (3.590 (5) Å), while C(10-1) is in contact with C(6) (3.66 (1) Å) and C(5) (3.71 (1) Å). C(10-1) also makes a close intramolecular contact with the base C(8) atom (3.66 (1) Å). The second ethoxy group[25] shows no intramolecular stacking with the adenine ring. The C(10-2) methyl group, however, does exhibit van der Waals contacts (alkyl–alkyl) with a neighboring C(12)–C(13) ethoxy group where the C(10-2) to C(12) and C(13) distances are 3.61 (1) and 3.74 (1) Å, respectively. The two-site disorder displayed by the C(10) methyl group may be explained by the equally attractive alkyl–base and alkyl–alkyl interactions/environs.

The Phosphotriester Backbone Conformation—The ω′,ω Map. There are three sets of phosphodiester linkages: ω'_A[O(5′)–P–O(3″)–C(12)], ω_A[C(5′)–O(5′)–P–O(3″)]; ω'_B[O(5′)–P–O(2)–C(9)], ω_B[C(5′)–O(5′)–P–O(2)]; ω'_C[O(2)–P–O(3″)–C(12)], ω_C[C(9)–O(2)–P–O(3″)] (Table III) and the conformations are depicted in the (ω′,ω) plot (Figure 4). (ω'_A,ω_A) is (g^-,t). (ω'_B,ω_B) is (g^+,g^-), which is close to the conformations found for the cyclic nucleotides.[19,20,26-29] The third combination, (ω'_C,ω_C), is off the (t,t) conformational domain. The (t,t) phosphodiester conformation is not normally observed in phosphodiesters because of the unfavorable lone pair interactions between the ester oxygens.[30] Thus none of the phosphodiester linkages assumes a (g^-,g^-)

(23) Kraut, J.; Jensen, L. H. *Acta Crystallogr.* **1963**, *16*, 79–88.
(24) Neidle, S.; Kühlbrandt, W.; Achari, A. *Acta Crystallogr., Sect. B* **1976**, *B32*, 1850–1855.
(25) C(10-1) and C(10-2) are the two disordered sites of the methyl carbon atom bonded to C(9).
(26) Coulter, C. *Acta Crystallogr., Sect. B* **1969**, *B25*, 2055–2065.
(27) Chwang, A. K.; Sundaralingam, M. *Acta Crystallogr., Sect. B* **1974**, *B30*, 1233–1240.
(28) Sheldrick, W. S.; Rieke, E. *Acta Crystallogr., Sect. B* **1978**, *B34*, 2324–2327.
(29) Sundaralingam, M.; Haromy, T. P.; Prusiner, P. *Acta Crystallogr., Sect. B* **1982**, *B38*, 1536–1540.
(30) Sundaralingam, M. *Biopolymers* **1969**, *7*, 821–869.

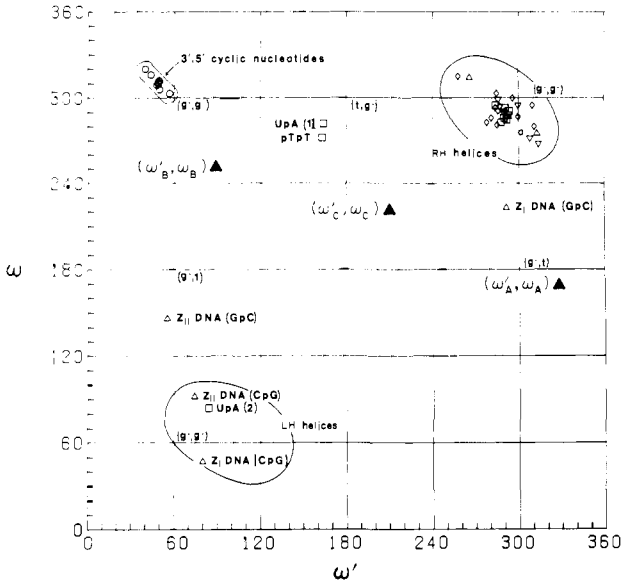


Figure 4. An (ω',ω) plot showing the phosphodiester conformations for the three possible combinations of phosphodiester linkages: (▲) (ω'_A,ω_A), (ω'_B,ω_B), and (ω'_C,ω_C); (▽) RNAs; (△) DNAs; (□) dinucleotides; (◇) drug-bound dinucleotides; (○) cyclic nucleotides. The ellipses enclosing the RH (right-handed) helices and LH (left-handed) helices are of arbitrary size and other right- and left-handed helices may extend beyond their limits.

conformation, favored by right-handed polynucleotide helices,[30] even though this conformation is not sterically prohibited in phosphotriesterified nucleotides or helices.[31]

Possible Mechanisms for the Biochemical and Biological Effects Induced by Phosphotriesterification. Some studies have been carried out on the biochemical and biological effects of phosphotriesterified oligonucleotides. It has been shown that the phosphotriester $G^mP(Et)G^mP(Et)U$[32] was readily taken up by transformed Syrian hamster fibroblasts and reversibly inhibited protein synthesis.[33] Experiments, having a single-site triesterification in decadeoxyoligonucleotides, have shown that the rates and extents of polymerization of these templates are 25–50% less than those of an unmodified control.[34] The triester d-Tp(Et)Tp(Et)Cp(Et)A has been shown to form a hydrogen-bonded complex with the anticodon region (–UpGpApA) of *E. coli* and yeast phenylalanine tRNA.[35] In the same study the triester d-Tp(Et)Gp(Et)G formed a hydrogen-bonded complex with the 3' amino acid accepting 3'-CpCpA terminus of $tRNA^{phe}$. These and previous studies[8,36] show that esterification of the sugar phosphate backbone does not inhibit the formation of Watson–Crick double helical structure. The binding affinity of the operator regions of DNA for their repressor proteins has been shown to drop markedly when the phosphate groups are esterified.[37] Also,

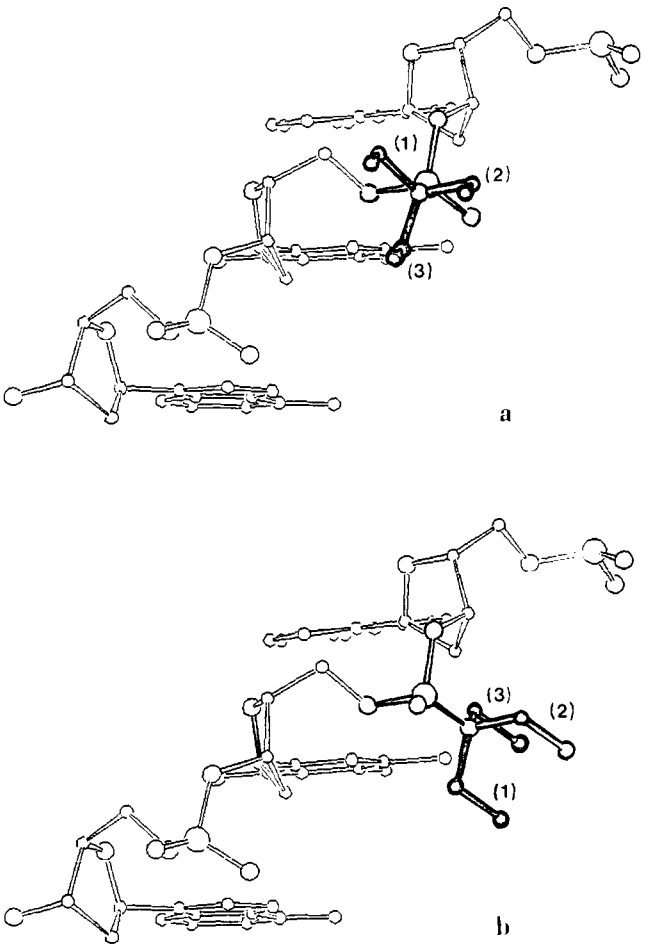


Figure 5. Possible staggered conformations taken by an ethyl group after esterification of phosphate oxygen atom O(1) (a) or O(2) (b) of a duplex DNA. 1, 2, and 3 refer to the gauche⁻ (–60°), trans (180°), and gauche⁺ (60°) conformations, respectively, which the ethyl group takes with respect to the O(5') oxygen atom, in parts a and b. Alkylation of phosphate oxygen atom O(1) results in the *S* configuration about the phosphorus atom, whereas alkylation of phosphate oxygen atom O(2) results in the *R* configuration. Only one strand of the duplex is shown for clarity. It is found that in a duplex DNA only the gauche⁺ conformation in part b is sterically disallowed.

our molecular modeling studies reveal that double helical DNA can sterically accommodate an ethyl group on either of the two anionic oxygen atoms without necessitating conformational change in the sugar phosphate backbone (Figure 5). This lowering of the binding affinity and the lowering of the rates of polymerization mentioned above, therefore, seem to result not from a conformational change of the duplex DNA but rather from the steric interference of the alkyl group in the formation of protein–nucleic acid complex. Additionally, the neutralization of the phosphate groups will also lead to weaker electrostatic interactions between the protein and the sugar phosphate backbone. The loss in the affinity of the protein for the DNA could lead to altered rates of replication and gene expression and possible mutagenicity.[38]

Although the conformation of a double helical DNA is not noticeably affected by phosphate alkylation, the sugar phosphate backbone of *single-stranded* DNA, e.g., during transcription, seems to show greater susceptibility to conformational changes because of the absence of base pairing. The backbone phosphodiester could assume a conformation similar to that found for (ω_B',ω_B), (g^+,g^-), instead of taking the helical (g^-,g^-) conformation.[39] This switch

(31) The structure of the neutral 3'-deoxyadenylthymidine (methyl 5'-phosphonate) dihydrate also displays a non (g^-,g^-) phosphodiester conformation for which the values of (ω',ω) (–91.8°,117.8°) are similar to those of (ω'_B,ω_B) (Chacko, K. K.; Linder, K.; Saenger, W.; Miller, P. S. *Nucleic Acids Res.* **1983**, *11*, 2801–2814). In the monoalkylated nucleotide uridine 5'-*O*-(methyl phosphate) the phosphodiester conformation is (g^-,g^-), while in adenosine 5'-*O*-(methyl phosphate) it is (g^+,g^+) (see ref 21 and 22, respectively).

(32) In this triester the 2' hydroxyl oxygen atom of each guanine had been methylated.

(33) Miller, P. S.; Braiterman, L. T.; T'so, P. O. P. *Biochemistry* **1977**, *16*, 1988–1996.

(34) Miller, P. S.; Chandrasegaran, S.; Dow, D. L.; Pulford, S. M.; Kan, L. S. *Biochemistry* **1982**, *21*, 5468–5474.

(35) Miller, P. S.; Barrett, J. C.; T'so, P. O. P. *Biochemistry* **1974**, *13*, 4887–4896.

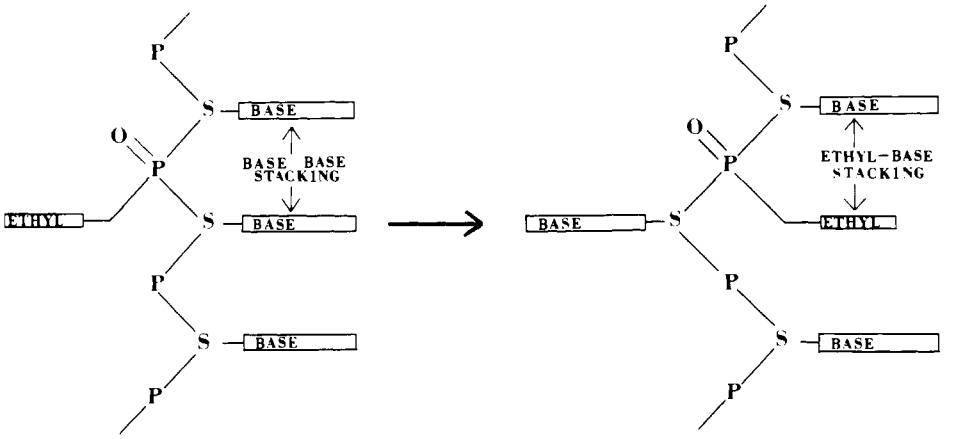
(36) Miller, P. S.; Fang, K. N.; Kondo, N. S.; T'so, P. O. P. *J. Am. Chem. Soc.* **1971**, *93*, 6657–6665.

(37) (a) Ptashne, M.; Jeffrey, A.; Johnson, A. D.; Mauer, R.; Meyer, B. J.; Pabo, C. O.; Roberts, T. M.; Sauer, R. T. *Cell (Cambridge, Mass.)* **1980**, *19*, 1–11. (b) Siebenlist, U.; Simpson, R. B.; Gilbert, W. *Ibid.* **1980**, *20*, 269–281.

(38) (a) Barkley, M. D.; Bourgeois, S. "The Operon"; Cold Spring Harbor Laboratory: 1980; pp 177–220. (b) Reznikoff, W. S.; Abelson, J. N. "The Operon"; Cold Spring Harbor Laboratory: 1980; pp 222–243.

(39) Since the in vivo rate of disappearance of methyl triesters is about an order of magnitude greater than ethyl triesters, it is possible that conformational differences between ethylated and methylated ribopolymers exist.

Scheme I



in the phosphate conformation would lead to the displacement of base stacking interactions by alkyl–base stacking interactions (Scheme I). The possibility of such a conformational change in the sugar phosphate backbone is supported by the spectroscopic finding that dinucleotide phosphotriesters exhibit decreased base–base stacking interactions as compared to the dinucleotides themselves.[8,36] The present crystal structure shows that the environments of the ethyl groups are different in that the C(9)–C(10-1) ethyl group is proximal to the imidazole moiety, while the C(12)–C(13) ethyl group is distal to the base. This difference should be noticeable in solution by NMR studies. Although NMR data are not yet available on our compound, NMR studies on the dideoxyoligonucleoside ethyl phosphotriesters, dIp(Et)I and dAp(Et)A, have shown that the ethyl groups experience shielding by the anisotropic ring current of the purine imidazole moiety.[40] These results indicate the proximity of the alkyl group to the base and suggest alkyl–base stacking interactions similar to those observed in our crystal.

Distortions in the backbone conformation, e.g., (g^-,g^-) to (g^+,g^-), could initiate biochemical events such as a mutation in one of two possible ways. The first would require that RNA polymerase[41] misreads the alkyl group for a nucleotide leading to an altered gene product. The second would require the premature termination of transcription. Here polymerization of the complementary strand is arrested at the alkylated site either by interference of the nonstacked base with polymerase binding (and/or translocation) or by simple dissociation of the polymerase due to the lack of an appropriate base. Esterification of the phosphate anionic oxygen atoms could lead to two enantiomeric triesters. It is possible that one or the other of these enantiomers more readily undergoes the conformational change necessary for alkyl–base stacking.

(40) Kan, L. S.; Barrett, J. C.; Miller, P. S.; T'so, P. O. P. *Biopolymers* **1973**, *12*, 2225–2240.

(41) DNA polymerase may also misread the alkyl group; however, we shall not deal with these effects here.

Summary

The molecular and crystal structure of adenosine 5′-*O*-(diethyl phosphate), the first noncyclic triesterified mononucleotide, is presented. Significant electronic and geometric changes are observed in the phosphodiester linkages. The three sets of phosphodiester linkages are in the (g^-,t), (g^+,g^-), and (t,t) conformations. Two mechanisms for the biochemical and biological effects of phosphate alkylation are discussed. The first is based on the neutralization of the phosphate group by an alkyl group which also sterically interferes with the formation of protein–DNA complexes. The second is based on the conformational changes in the alkylated phosphodiester backbone, where alkyl–base stacking interactions displace base–base stacking interactions. This would be particularly important in single-stranded regions of alkylated DNA, e.g., during transcription.

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Registry No. Adenosine 5′-*O*-(diethyl phosphate), 91190-12-4.

Supplementary Material Available: Tables of anisotropic thermal parameters and observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.