NUCLEOTIDES, XIII*): PHOSPHORYLATIONS OF ADENOSINE AND 2'-DEOXY-ADENOSINE BY PHOSPHOROCHLORIDATES

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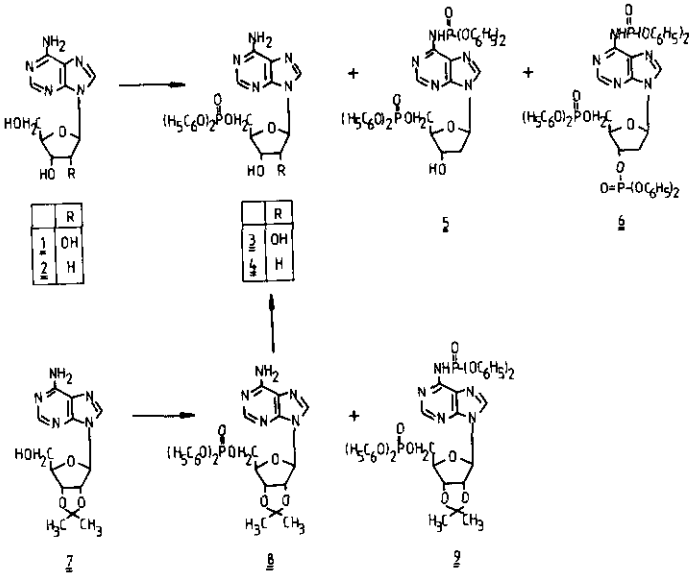
ABSTRACT - Phosphorylations of adenosine and 2'-deoxyadenosine by various phosphorylating agents such as diphenylphosphorochloridate have been achieved at positions 3', 5' and N^6 using appropriate starting materials for unambiguous synthesis. There is a gradual reactivity of the different functions in these molecules with a preference of the 5'-OH-group followed by the 6-amino group and the 3'-OH-function in the third place. The various phosphotriesters and phosphoramidate diesters have been characterized by physical means and their properties are discussed.

INTRODUCTION - Since phosphomono- and diesters are ionic species they do in general not penetrate the cell wall[1] and have therefore little or no effect on intracellular biological systems. Moreover such compounds are subject to degradation by phosphorylitic enzymes converting them back to nucleosides. The transport barrier of the cell wall might however be overcome by the use of electroneutral phosphotriesters which may be expected to be also stable to various phosphorylitic enzymes. Since there is a great interest in biologically active phosphates from several points of views we decided to synthesize and investigate various phosphotriesters of adenosine (1) and 2'-deoxyadenosine (2) with the phosphoester function located at position 3',5' and N^6.

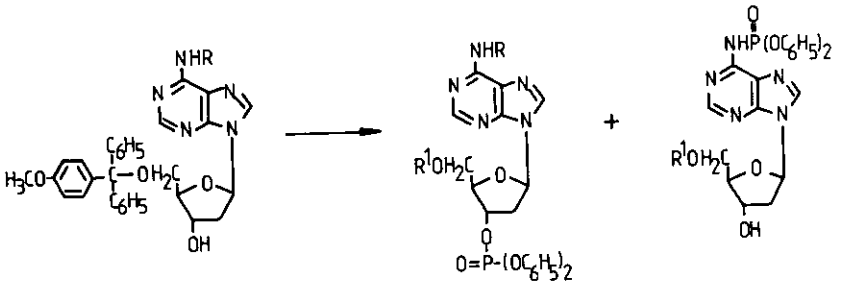
RESULTS - These investigations have been initiated by direct phosphorylations of adenosine (1) and 2'-deoxyadenosine (2) with diphenylphosphoromonochloridate in pyridine at room temperature giving the 5'-phosphotriesters 3 and 4 in 32 % and 57 % yield respectively as main reaction products. Small amounts of N^6,5'-di-(5) and N^6,3',5'-triphosphorylated products (6) are also formed and could be separated by preparative thin layer chromatography. In order to decrease the

formation of unwanted side products and to improve the yield of 3 2',3'-O-isopropylidene-adenosine (7) was phosphorylated in a similar fashion giving 85 % of 2',3'-O-isopropylidene-adenosine-5'-O-diphenyl phosphate (8) as well as a small amount of the corresponding N^6,5'-diphosphotriester 9. Acid treatment of 8 cleaved the isopropylidene group but also led to adenine formation with hydrolysis of the glycosidic linkage. The reaction was therefore stopped after appearing of adenine in the chromatogram yielding after isolation 60 % of 3 and 23 % of unchanged starting material 8.



The synthesis of 2'-deoxyadenosine-3'-O-diphenyl phosphate (13) was initially approached by phosphorylation of 5'-O-monomethoxytrityl-2'-deoxyadenosine (10)[2] by diphenylphosphorochloridate in pyridine leading to two closely running products in a ratio of 2/3 and traces of a third faster moving component (14). Tedious chromatographical separation of the former mixture indicated isomeric monophosphate triesters (12 and 17) which on removal of the monomethoxytrityl group by 80 % acetic acid gave crystalline substances (13, 18) of correct elementary analysis.

A structural assignment of the monophosphate triesters 4, 13 and 18 could be derived from comparisons of their UV-spectra with 2'-deoxyadenosine (2) since O-phosphorylation of the sugar moiety does not change in contrary to N^6-substitution the shape of the UV-spectrum (Fig.1).



	R
10	H
11	Bz

	R	R^1
12	H	MMTr
13	H	H
14	$O=P(OC_6H_5)_2$	MMTr
15	Bz	MMTr
16	Bz	H

	R^1
17	MMTr
18	H

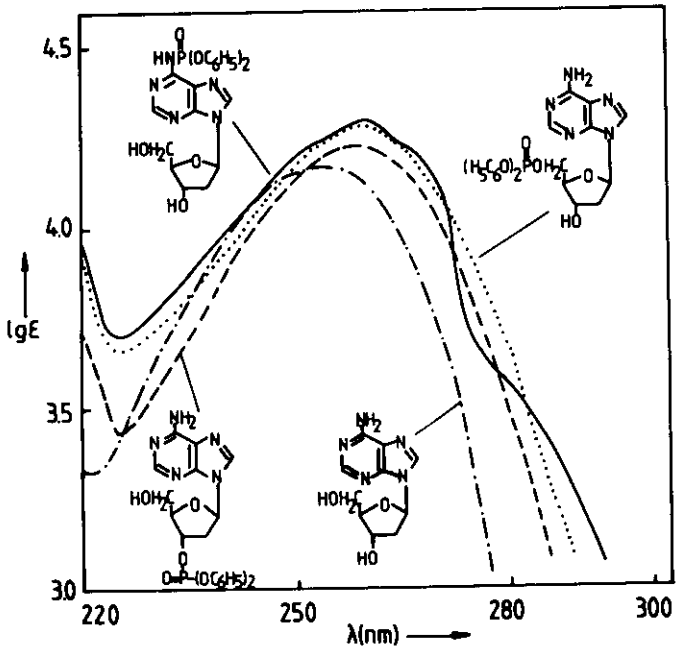
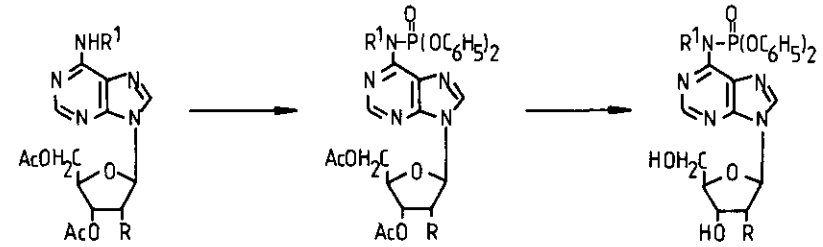


Fig. 1 - UV-Spectra of 2'-deoxyadenosine and its O- and N-diphenylphosphorylated derivatives in methanol.

These results have been confirmed by unambiguous syntheses of compound 13 and 18 starting from N^6-benzoyl-5'-O-monomethoxytrityl-2'-deoxyadenosine (11)[3] and 3',5'-di-O-acetyl-2'-deoxyadenosine (19)[4] respectively. 11 reacted smoothly with diphenylphosphorochloridate to give 15 in 88 % yield and subsequent treatment with 80 % acetic acid followed by conc. ammonia in dioxane (2/1) led to an isolated yield of 72 % of 13. On the other hand phosphorylation of 19 took place in 88 % yield to 22 which could be deblocked by conc. ammonia/dioxane (3/1) quantitatively at room temperature to 18. The identity of the products from the different procedures has then been proven by chromatographical and spectrophotometrical means.



	R	R^1
19	H	H
20	OAc	H
21	OAc	CH_3

	R	R^1
22	H	H
23	OAc	H
24	OAc	CH_3

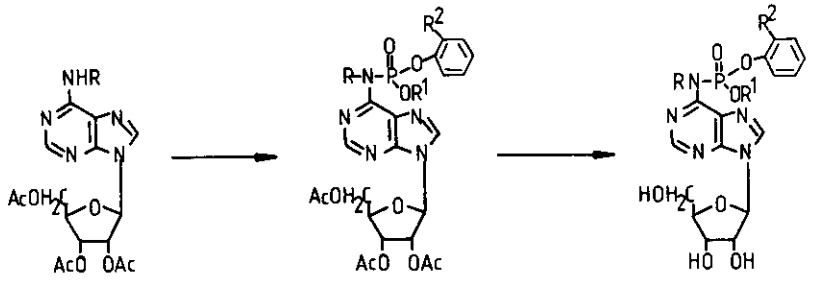
	R	R^1
18	H	H
25	OH	H
26	OH	CH_3

Analogously 2',3',5'-tri-O-acetyladenosine (20)[5] could be phosphorylated to 23 and deprotected to 25 in appreciable yields.

Now the question arose where exactly the phosphorylation took place, at the aglycon at N-1 or at the exocyclic amino group N^6. Since no assignment could be made on the basis of ^{1}H-NMR-spectroscopy due to the fact that the N-H signal is embedded in the aryl protons we decided to phosphorylate 2',3',5'-tri-O-acetyl-N^6-methyladenosine (21) to 24, deblock it to 26 and study their ^{1}H-NMR-spectra. They show both the N-methyl signal as a doublet indicating a

coupling with phosphorous of 10 Hz and confirming thereby the position of the phosphoramidate bond at N^6.

The N^6-phosphorylation reaction was furthermore extended to other phosphorylating agents. Bis-o-chlorophenyl phosphoromonochloridate reacted with 20 and 21 to the N^6-diphenyl phosphoroamidate diesters 27 and 28 noticing a much slower reaction with 21 from steric reasons. Deblocking afforded the N^6-phosphorylated nucleosides 31 and 32. The synthesis of N^6-phosphoramidate diesters with two different ester functions was also undertaken starting from 20 by reaction with phenyl and o-chlorophenyl phosphorodichloridate respectively and subsequent treatment of the resulting intermediates with cyanoethanol to give 29 and 30 in 59 % and 76 % yield.



	R
20	H
21	CH_3

	R	R^1	R^2
27	H	o-ClC_6H_4	Cl
28	CH_3	o-ClC_6H_4	Cl
29	H	CH_2CH_2CN	H
30	H	CH_2CH_2CN	Cl

	R	R^1	R^2
31	H	o-ClC_6H_4	Cl
32	CH_3	o-ClC_6H_4	Cl
33	H	CH_2CH_2CN	H
34	H	CH_2CH_2CN	Cl

Treatment of these compounds with conc. aqueous ammonia/dioxane (3/1) removed the acetyl groups in the normal manner but left unexpectedly the phosphoramidate diester function in tact giving 33 and 34 respectively. We believe that the high acidity of the phosphoramidate group, which could be seen from the acidic pK_as of about 7, will counteract the normally easily achievable ß-elimination of the cyanoethyl group by anion formation. If the reaction was carried out for a longer time traces of adenosine and other side products

could be detected by tlc.

Not only the different shape of the UV-spectra of the N^6-phosphorylated adenosines in comparison to 1 and 2 (Fig. 1) could be taken for characterization but much better indications are obtained from the acidic properties of the molecules and the spectral changes going from the neutral form to the monoanion (Fig. 2).

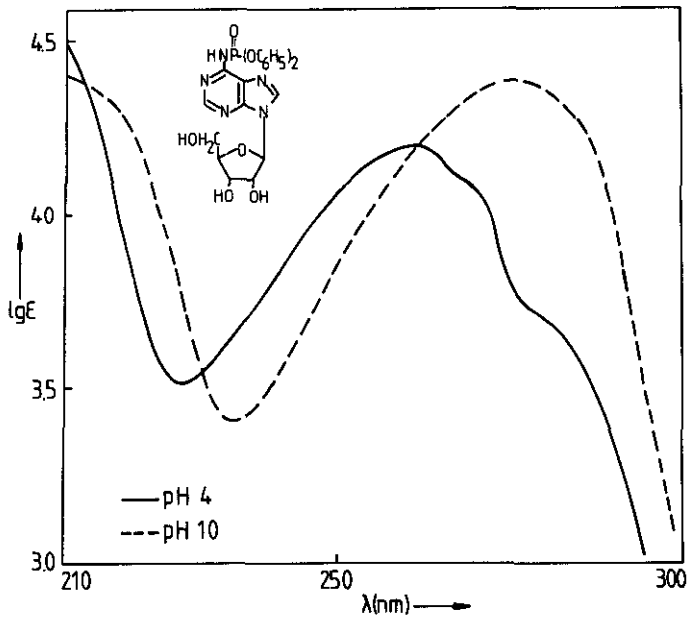


Fig. 2 - UV-Spectra of the neutral and monoanion form of N^6-diphenylphosphoryl-adenosine (25).

NMR-spectra in general offer an excellent tool for localizing the phosphoester functions at the sugar part of the molecules through the characteristic shifts of the adjacent protons in comparison to the starting material.

It is also interesting to note that the CD-spectra of the 3'-O- and N^6-phosphorylated products show negative cotton effects around 265 nm similar to adenosine, whereas 5'-O-phosphotriesters exhibit a positive effect like 8-bromo-

Table 1 - Physical data of adenosine and 2'-deoxyadenosine phosphotriesters.

	pK_a	UV - Absorption Spectra		pH	Species	CD - Spectra in Methanol							
		λ_{max} (nm)	lgε			λ_{max} (nm)				Ⓗ			
1		260	4.19	MeOH	o	225			265	+1731			-2990
2		260	4.18	MeOH	o	220			267,5	+1652			-2911
3		259	4.18	MeOH	o	215	[224]	250	264	-415	[± 0]	+415	+996
4		258	4.27	MeOH	o	215	[230 - 245]		263	-4800	[± 0	]	+1400
13		258	4.21	MeOH	o	214	[235]		258	+3850	[+500]		-1850
18	7.09	260 [268][285] 261 [269][285] [214] 276	4.27 [4.13][3.40] 4.24 [4.18][3.68] [4.42] 4.45	MeOH 4.0 10.0	o o -	216	237	245	268	+5250	+300	+700	-1970
25	6.96	259 [267][285] 261 [269][285] [215] 276	4.25 [4.14][3.29] 4.20 [4.14][3.57] [4.38] 4.39	MeOH 4.0 10.0	o o -	222	[235]		265	+6270	[+700]		-3620
31	6.48	262 [270][285] 263 [271][284] [211] 274	4.28 [4.22][3.77] 4.22 [4.16][3.83] [4.23] 4.35	MeOH 4.0 10.0	o o -	222	[235]		262	+4330	[+1900]		-3660
33	7.14	259 [269][280] 261 [268][285] [208] 275	4.21 [4.09][3.56] 4.23 [4.15][3.43] [4.39] 4.38	MeOH 4.0 10.0	o o -	220	232	235	262	+5600	+1550	+1900	-2050
34	6.92	261 [268][283] 257 [264][278] [212] 269	4.20 [4.12][3.44] 4.26 [4.14][3.58] [4.39] 4.35	MeOH 4.0 10.0	o o -		[243]		270		[+350]		-2880
26		264 [274] 266 [276]	4.31 [4.17] 4.24 [4.09]	MeOH 7.0	o o	225	[240]		272	+5670	[+550]		-3940
32		265 [273] 266 [276]	4.23 [4.14] 4.17 [4.03]	MeOH 7.0	o o	225	[250]		270	+6140	[±0]		-3150

adenosine. Whether these findings should be explained on the basis of the syn-anti concept or might be due to other special conformational arrangements have to await further detailed investigations in this field (Fig. 3).

It should further be mentioned that the diarylphosphoryl group is a strong activator of amino groups acidifying the N-H function to such a large extent that easy deprotonation can take place followed by substitution with various electrophiles. Diarylphosphoramidates of this type can be regarded as the phosphorus analogs of sulfonamides offering the advantages of much milder deblocking of this functional group. We will shortly report on this synthetic approach in more detail.

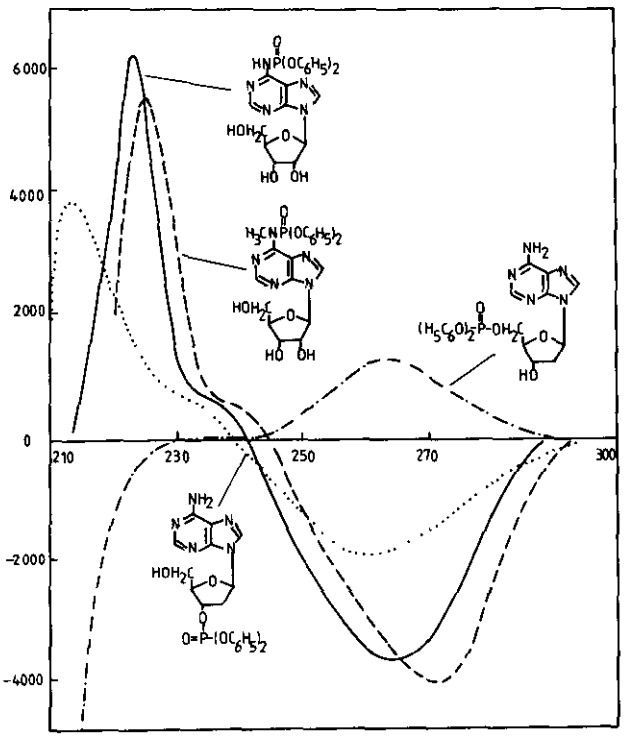


Fig. 3 - CD-Spectra of adenosine and 2'-deoxy-adenosine derivatives in methanol.

EXPERIMENTAL

UV- and CD-Spectra were taken with Cary-Recording Spectrometers, Model 118 and 1115/15 of Applied Physics. - ^{1}H-NMR-Spectra were measured with a Jeol JNM-MH-100 and a Bruker HFX-90. - Chromatographical studies were performed on thin layer sheets of Schleicher & Schüll, silica gel F 1500 LS 254 and cellulose F 1440 LS 254. - Preparative thick layer chromatography was done on silica gel PF_{254} and column chromatography on silica gel (0.05-0.2 mm particals) both from Fa. Merck, Darmstadt. - All substances were dried in a vacuum desiccator over P_4O_{10} or in the oven at 100°C. - The melting points have not been corrected and are only accurate for crystalline substances.

Adenosine-5'-O-diphenyl phosphate (3). - a) 0.133 g (0.5 mmole) of adenosine (1) were dissolved in 4 ml of absol. pyridine and then added 0.11 ml (0.6 mmole) of diphenylphosphoromonochloridate dropwise with stirring. After 40 min. the mixture was evaporated to dryness in vacuum and then three times coevaporated

with 5 ml benzene each. The residue was dissolved in little chloroform and applied to a preparative silica gel plate (40x20x0.2 cm). On chromatography with chloroform/methanol (9/1) two main bands were obtained which were eluted with chloroform. The lower moving material gave after evaporation and recrystallization from methanol/ether 0.081 g (32 %) colourless crystals from mp. 115-117°C.

From the faster moving band 0.066 g (18 %) amorphes powder with mp. 75-77°C was obtained which is designated as N^6-diphenylphosphoryl-adenosine-5'-O-diphenyl phosphate according to the UV- and NMR-spectra.

b) 2.1 g (4 mmole) 2',3'-O-isopropylidene-adenosine-5'-O-diphenyl phosphate (8) were stirred with 4.5 ml 98 % formic acid in the dark at room temp. for 4 days. The reaction was stopped by addition of 5 ml of ethanol and evaporation. This process was repeated three times, then the residue dissolved in chloroform and applied to preparative silica gel plates (40x20x0.2 cm). After development in chloroform/methanol (9/1) three bands were cut out and eluted with chloroform/methanol (1/1). The upper fraction gave 0.5 g (23 %) starting material, the middle band consisted of traces of adenine and the slowest moving band yielded after evaporation and recrystallization from methanol/ether 1.2 g (60 %) colourless crystals with mp. 115-117°C. UV: λ_{max}^{MeOH} 259 nm, lgε 4.18.

$C_{22}H_{22}N_5O_7P$ x 1.5 H_2O (526.5) Calc. C 50.19 H 4.79 N 13.30

Found C 50.05 H 4.39 N 13.09.

2'-Deoxyadenosine-5'-O-diphenyl phosphate (4). - 0.5 g (2 mmole) 2'-deoxyadenosine (2) are dissolved in 8 ml absol. pyridine and then 1.43 ml (8 mmole) of diphenylphosphoromonochloridate added dropwise with stirring at room temp. After 2 h the reaction mixture is evaporated to dryness, coevaporated three times with 3 ml of benzene each and then the residue taken up in chloroform. This solution is washed with water, dried over Na_2SO_4 and after evaporation to a small volume applied to preparative silica gel plates for chromatography with chloroform/methanol (9/1). Three bands are cut out and eluted with chloroform/methanol (1/1). The main band with the lowest R_f-value gave on recrystallization from chloroform/n-hexane 0.56 g (58 %) colourless crystals of mp. 135-137°C.

UV: λ_{max}^{MeOH} 258 nm, lgε 4.27.

$C_{22}H_{22}N_5O_6P$ (483.4) Calc. C 54.67 H 4.55 N 14.50
Found C 54.55. H 4.71 N 14.49.

From the second band 0.17 g (12 %) N^6-diphenyl phosphoryl-2'-deoxyadenosine-5'-O-diphenyl phosphate (5) were isolated on evaporation as an amorphous solid of mp. 75-78°C.

The third band gave 0.246 g (13 %) N^6-diphenylphosphoryl-2'-deoxyadenosine-3',5'-di-O-diphenyl phosphate (6) on evaporation in form of an amorphous solid of mp. 78-81°C.

2',3'-O-Isopropylidene-adenosine-5'-O-diphenyl phosphate (8). - 0.307 g (1 mmole) of 2',3'-O-isopropylidene-adenosine (7) were treated in 2.5 ml of absol. pyridine with 0.27 ml of diphenylphosphoromonochloridate at room temp. with stirring for 1 h and then evaporated to dryness. Evaporation was repeated three times with 3 ml of benzene each. The residue was dissolved in chloroform, washed with water, dried over Na_2SO_4 and then applied to a silica gel plate (40x20x0.2 cm) for chromatography in ethyl acetate. The lower moving main band was eluted with chloroform/methanol (1/1) and yielded after evaporation and recrystallization from benzene/n-hexane 0.47 g (85 %) colourless crystals of mp. 95-97°C. UV: λ_{max}^{MeOH} 258 nm, lgε 4.11.

$C_{25}H_{26}N_5O_7P \times H_2O$ (557.5) Calc. C 53.86 H 5.06 N 12.56 P 5.21
Found C 53.98 H 4.95 N 12.56 P 5.55.

From the second band 0.077 g (10 %) 2',3'-O-isopropylidene-N^6-diphenylphosphoryl-adenosine-5'-O-diphenyl phosphat (9) was obtained on evaporation as an amorphous solid.

Diphenyl phosphorylation of 5'-O-monomethoxytrityl-2'-deoxyadenosine (10). - 3.7 g (7 mmole) of 10[2] were treated in 40 ml of absol. pyridine by dropwise addition of 3.75 g (14 mmole) of diphenylphosphoromonochloridate at room temp. with stirring. After 1.5 h the reaction mixture was evaporated to dryness, the residue dissolved in chloroform, washed with water and after drying over Na_2SO_4 and evaporation to a small volume applied to a silica gel column (34x3.5 cm)

for chromatography first with chloroform and second with chloroform/methanol (99/1). The first fraction gave on evaporation 2.93 g (35 %) 5'-O-monomethoxytrityl-N^6-diphenylphosphoryl-2'-deoxyadenosine-3'-O-diphenylphosphate (14) as a solid foam.

The elution of the column was continued with chloroform/methanol (98/2) yielding the main fraction (2.84 g) which consisted of the closely running isomers 12 and 17. Their separation was achieved by preparative thin layer chromatography on silica gel plates (40x20x0.2 cm) in the system ethylacetate/methanol (95/5). The upper band gave on evaporation 1.7 g (32 %) of 5'-O-monomethoxytrityl-N^6-diphenylphosphoryl-2'-deoxyadenosine (17) of mp. 75-77°C according to elementary analysis and UV-spectrum: λ_{max}^{MeOH} 231, 259, [269], [283] nm, lg ε 4.31, 4.29, [4.19], [3.69].

$C_{42}H_{38}N_5O_7P$ (755.7) Calc. C 66,75 H 5.07 N 9.27
Found C 66.49 H 5.36 N 9.41.

The slower moving band yielded 1.13 g (22 %) amorphous powder on evaporation and was assigned as 5'-O- monomethoxytrityl-2'-deoxyadenosine-3'-O-diphenyl phosphate (12) with mp. 82.84°C. UV: λ_{max}^{MeOH} 231, 260 nm, lgε 4.26, 4.25. The structures of 12 and 17 have furthermore been proven by detritylation with 80 % acetic acid to 13 and 18 respectively.

2'-Deoxyadenosine-3'-O-diphenyl phosphate (13). - 0.59 g (1 mmole) N^6-Benzoyl-2'-deoxyadenosine-3'-O-diphenyl phosphate (16) were dissolved in 1 ml of dioxane and then 5 ml of conc. ammonia added. After 1 day stirring at room temp. the mixture was evaporated to dryness, dissolved in chloroform and applied to silica gel plates (40x20x0.2 cm) for chromatography in chloroform/methanol (4/1). The main band was eluted and yielded after evaporation and recrystallization from chloroform/n-hexane 0.347 g (72 %) of colourless crystals of mp. 126-127°C.

$C_{22}H_{22}N_5O_6P$ (483.4) Calc. C 54.92 H 4.79 N 14.63 P 6.20
Found C 54.67 H 4.55 N 14.50 P 6.41.

N^6-Benzoyl-5'-O-monomethoxytrityl-2'-deoxyadenosine-3'-O-diphenyl phosphate (15). - 0.313 g (0.5 mmole) of N^6-benzoyl-5'-O-monomethoxytrityl-2'-deoxy-

adenosine (11)[3] were dissolved in 4 ml absol. pyridine and then 0.37 ml (2 mmole) of diphenylphosphoromonochloridate dropwise added. After 1 h stirring at room temp. was evaporated the residue taken up in chloroform, washed with water, dried over Na_2SO_4 and then concentrated to a small volume. Purification was achieved by chromatography on a silica gel plate (40x20x0.2 cm) with chloroform/methanol (95/5) and elution of the main band yielding 0.38 g (88 %) amorphous solid of mp. 58-60°C. UV: λ_{max}^{MeOH} 230, [259], 278 nm, lgε 4.44, [4.17], 4.33.

$C_{49}H_{42}N_5O_8P \times 3\ H_2O$ (913.9) Calc. C 64.40 H 5.29 N 7.66
Found C 64.86 H 5.38 N 7.23.

N^6-Benzoyl-2'-deoxyadenosine-3'-O-diphenyl phosphate (16). - 0.24 g (0.27 mmole) of 15 were stirred in 1 ml 80 % acetic acid for 5 h at room temp. and then the reaction mixture evaporated to dryness and finally coevaporated several times with ethanol. Purification was done by chromatography on a silica gel plate (40x20x0.2 cm) with chloroform/methanol (95/5). Elution of the main band and evaporation gave 0.12 g (76 %) colourless solid with mp. 68-70°C. UV: λ_{max}^{MeOH} = [227], [260], 278 nm, lgε [4.19], [4.19], 4.37.

$C_{29}H_{26}N_5O_7 \times 2\ H_2O$ (609.6) Calc. C 57.43 H 4.96 N 11.48
Found C 57.53 H 4.51 N 11.20.

N^6-Diphenylphosphoryl-2'-deoxyadenosine (18). - 2.84 g (5 mmole) of 22 were dissolved in 12.5 ml of dioxane, 20 ml of conc. ammonia added and stirred at room temp. for 16 h. Evaporation to dryness. The residue was dissolved in chloroform, washed with water, dried over Na_2SO_4 and again evaporated to a solid. Recrystallization from ethyl acetate/n-hexane yielded 1.53 g (63 %) colourless crystals of mp. 144-145°C.

$C_{22}H_{22}N_5O_6P$ (483.4) Calc. C 54.67 H 4.55 N 14.50 P 6.41
Found C 54.93 H 4.41 N 14.38 P 6.26.

3',5'-Di-O-acetyl-N^6-diphenylphosphoryl-2'-deoxyadenosine (22). - 0.67 g (2 mmole) of 3',5'-Di-O-acetyl-2'-deoxyadenosine (19)[4] were stirred in 10 ml of absol. pyridine and then 1.1 ml (5 mmole) of diphenylphosphoromonochloridate added dropwise at room temp. After 3 h stirring the mixture was evaporated four times with addition of 3 ml of benzene each and then the residue taken up

in chloroform. Washing with water, drying over Na_2SO_4 and evaporation to a small volume, which was applied to a silica gel column. On chromatography in the system chloroform/methanol (99/1) the main fraction was collected, evaporated and the residue reprecipitated from chloroform/n-hexane (5/500) to yield 1.06 g (88 %) colourless amorphous powder of mp. 62-66°C. UV: λ_{max}^{MeOH} 259, [268], [280] nm, lgε 4.29, [4.18], [3.59].

$C_{26}H_{26}N_5O_8P$ (567.5) Calc. C 55.32 H 4.90 N 11.86
Found C 55.03 H 4.62 N 12.34.

2',3',5'-Tri-O-acetyl-N^6-diphenylphosphoryladenosine (23). - 0.786 g (2 mmole) 2',3',5'-Tri-O-acetyladenosine (20)[5] were stirred in 10 ml of absol. pyridine and then 0.88 ml (4 mmole) diphenylphosphoromonochloridate slowly added. After 4 h stirring at room temp. was the reaction mixture evaporated to dryness and then coevaporated three times with 3 ml benzene each. The residue was treated with 5 ml of water and then extracted five times with each 5 ml of chloroform. The organic layer was dried over Na_2SO_4, evaporated to a small volume and then applied to a silica gel column for chromatography in chloroform/methanol (99/1). The main fraction was collected and gave on evaporation 1.1 g (91 %) amorphous solid of mp. 78-80°C. UV: λ_{max}^{MeOH} 259, [268], [280], lgε 4.31, [4.21], [3.58].

$C_{28}H_{28}N_5O_{10}P$ (625.5) Calc. C 53.76 H 4.51 N 11.19
Found C 53.68 H 4.69 N 11.28.

2',3',5'-Tri-O-acetyl-N^6-methyl-N^6-diphenylphosphoryladenosine (24). - 0.404 g (1 mmole) 2',3',5'-Tri-O-acetyl-N^6-methyladenosine (21)[6] were treated in 4 ml absol. pyridine with 1.7 ml (8 mmole) of diphenylphosphoromonochloridate by dropwise addition and stirring at room temp. After 3 h ice water was added and again stirred for 30 min. The reaction mixture was then extracted with chloroform, the organic layer dried and then evaporated to a small volume which is applied to preparative silica gel plates (40x20x0.2 cm) for separation in chloroform/methanol (95/5). The main band was eluted and gave on evaporation 0.534 g (84 %) amorphous powder of mp. 78-80°C. UV: λ_{max}^{MeOH} 264, [272], [290] nm, lgε 4.34, [4.26], [3.71].

$C_{29}H_{30}N_5O_{10}P$ (629.6) Calc. C 54.46 H 4.73 N 10.95
Found C 54.03 H 4.90 N 10.80.

N^6-Diphenylphosphoryl-2'-deoxyadenosine (25). - 0.625 g (1 mmole) 23 were stirred with 1.5 ml dioxane and 7 ml of conc. ammonia for 14 h in the dark at room temp. The reaction mixture was coevaporated with methanol to dryness and the residue purified by silica gel chromatography with chloroform/methanol (9/1) to yield 0.425 g (88 %) amorphous solid of mp. 86-89°C.

$C_{22}H_{22}N_5O_6P$ (483.4) Calc. C 52.91 H 4.44 N 14.02 P 6.20
Found C 53.15 H 4.53 N 14.01 P 6.04.

N^6-Methyl-N^6-diphenylphosphoryl-adenosine (26). - From 0.32 g (0.5 mmole) of 24 analogously to the preceeding procedure were obtained 0.231 g (90 %) amorphous solid on evaporation of the main fraction of mp. 68-70°C.

$C_{23}H_{24}N_5O_7P$ (513.5) Calc. C 53.80 H 4.71 N 13.64
Found C 53.84 H 5.29 N 13.21.

2',3',5'-Tri-O-acetyl-N^6-bis-o-chlorophenylphosphoryl-adenosine (27). - 0.393 g (1 mmole) of 20 were dissolved in 3 ml of absol. pyridine and then treated with 1.01g (3 mmole) of bis-o-chlorophenylphosphoromonochloridate with stirring at room temp. for 3.5 h. The reaction mixture was then cooled with ice, ice water was added and stirred for another 30 min. before extraction with chloroform. The organic layer was dried over Na_2SO_4, then evaporated to dryness and coevaporated three times with benzene. The residue was applied to preparative silica gel plates (40x20x0.2 cm) and developed in ethyl acetate. The main band gave after elution and evaporation 0.682 g (98 %) of a solid foam with mp. 76-79°C.
UV: λ_{max}^{MeOH} 259, [266], [280] nm, lgε 4.29, [4.27], [3.89].

$C_{28}H_{26}Cl_2N_5O_{10}P \times 0.5\ H_2O$ (703.4) Calc. C 47.80 H 3.87 N 9.85
Found C 47.67 H 3.83 N 9.89.

2',3',5'-Tri-O-acetyl-N^6-methyl-N^6-bis-o-chlorophenylphosphoryladenosine (28). - 0.101 g (0.25 mmole) of 21[8] were treated with 0.34 g (1 mmole) of bis-o-chlorophenylphosphoromonochloridate for 28 h analogously to the preceeding procedure and yielded 0.155 g (88 %) of an amorphous solid of mp. 56-58°C. UV: λ_{max}^{MeOH} = 263, [271], lgε 4.28, [4.16].

$C_{29}H_{28}Cl_2N_5O_{10}P$ (708.5) Calc. C 49.16 H 3.98 N 9.88
Found C 48.97 H 4.20 N 9.60.

2',3',5'-Tri-O-acetyl-N^6-cyanoethyl-phenyl-phosphoryladenosine (29). - 0.393 g (1 mmole) of 20 were dissolved in 2.5 ml of absol. pyridine and then 0.422 g (2 mmole) of phenylphosphorodichloridate added and stirred for 3 h at room temp. 0.35 ml (4.9 mmole) of cyanoethanol were added to the reaction mixture and stirring continued for another 15 h. Further work-up was analogous to the preceeding procedure and gave 0.354 g (59 %) of an amorphous powder on evaporation with a mp. of 62-65°C. UV: λ_{max}^{MeOH} 259, [268], $1g\varepsilon$ 4.24, [4.10].

$C_{25}H_{27}N_6O_{10}P \times 1\ H_2O$ (620.5) Calc. C 48.39 H 4.71 N 13.54

Found C 48.53 H 4.49 N 13.10.

2',3',5'-Tri-O-acetyl-N^6-o-chlorophenyl-cyanoethylphosphoryl-adenosine (30). - 0.426 g (2.02 mmole) of o-Chlorophenylphosphorodichloridate were dissolved in 25 ml of absol. pyridine, then 0.276 g (0.405 mmole) 1,2,4-triazole added and stirred at room temp. for 45 min. After addition of 0.393 g (1 mmole) of 20 stirring was continued for 30 min. till all starting material has disappeared. 0.35 ml (5 mmole) of cyanoethanol was then added to the reaction mixture which was stirred at room temp. for 24 h to complete the reaction. After cooling with ice, ice water was added, the mixture stirred for 30 min. and then extracted with chloroform. The organic layer was dried, evaporated to a small volume and applied to a silica gel column for chromatography in chloroform/methanol (99/1). The main fraction was collected, evaporated, and the residue reprecipitated from chloroform (5 ml) / n-hexane (500 ml) yielding 0.486 g (76 %) amorphous powder with mp. 68-70°C. UV: λ_{max}^{MeOH} 258, [265], [285], $1g\varepsilon$ 4.23, [4.18], [3.53].

$C_{25}H_{26}Cl\ N_6O_{10}P$ (636.9) Calc. C 47.14 H 4.11 N 13.19 P 4.86

Found C 47.12 H 4.47 N 12.52 P 5.04.

N^6-Bis-o-chlorophenylphosphoryl-adenosine (31). - 0.347 g (0.5 mmole) 27 were treated in 0.75 ml of dioxane with 3 ml of conc. ammonia by stirring at room temp. in the dark for 24 h. The solution was evaporated to dryness and the residue purified by chromatography on preparative silica gel plates (40x20x0.2 cm) in chloroform/methanol (85/15). The main band was eluted with chloroform/methanol (3/7) yielding 0.228 g (80 %) amorphous powder of mp. 91-93°C.

$C_{22}H_{22}Cl_2N_5O_7P$ (570.3) Calc. C 46.33 H 3.88 N 12.28

Found C 46.41 H 3.87 N 11.84.

N^6-Methyl-N^6-bis-o-chlorophenylphosphoryl-adenosine (32). - Analogously to the preceeding procedure from 0.142 g (0.2 mmole) of 28 yielding 0.095 g (82 %) amorphous solid of mp. 84-88°C.

$C_{23}H_{22}Cl_2N_5O_7P$ (582.4) Calc. C 47.44 H 3.81 N 12.02
Found C 47.82 H 4.04 N 11.60.

N^6-Cyanoethyl-phenylphosphoryl-adenosine (33). - Analogously to procedure 31 from 0.12 g (0.2 mmole) of 29 yielding 0.064 g (68 %) of amorphous solid of mp. 89-91°C.

$C_{19}H_{21}N_6O_7P \times 1\ H_2O$ (476.4) Calc. C 46.16 H 4.69 N 16.99
Found C 45.94 H 5.11 N 16.83.

N^6-o-Chlorophenyl-cyanoethylphosphoryl-adenosine (34). - Analogously to procedure 31 from 0.313 g (0.5 mmole) of 30 yielding after recrystallization from ethyl acetate/methanol (8/1) 0.38 g (74 %) colourless crystals of mp. 58-60°C.

$C_{19}H_{20}Cl\ N_6O_7P \times 1.5\ H_2O$ (537.8) Calc. C 42.43 H 4.31 N 15.62
Found C 42.59 H 4.10 N 14.95.

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