115. Nucleotides

Part XXVII[1])

Bis[2-(*p*-nitrophenyl)ethyl] Phosphorochloridate, a New Versatile Phosphorylating Agent in Nucleotide Chemistry

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A new, versatile phosphorylating agent, bis[2-(*p*-nitrophenyl)ethyl] phosphorochloridate (**3**), has been prepared and is used for 3'- and/or 5'-phosphorylations of nucleosides. The resulting bis[2-(*p*-nitrophenyl)ethyl] phosphotriesters are versatile synthons in oligonucleotide synthesis leading finally to 3'- and/or 5'-terminated monophosphates in excellent yields.

1. Introduction. – Phosphorylations of nucleosides can be regarded as the most crucial point of any oligonucleotide synthesis performed by the phosphodiester and phosphotriester approach [2–5]. Numerous phosphorylating agents have been proposed and are in use for this purpose and work quite satisfactorily depending upon the various substituents and activating functions. The normal procedures, however, are usually devised in this manner that the end products contain one more base residue than phosphate-ester group. Therefore, a more general method which is suitable for the introduction of phosphate groups at the 3'- and 5'-ends of oligonucleotide chains is required. So far, the use of aryl phosphoroamido chloridates [6] and *O*-aryl *S*-methyl phosphorochloridothioates [7] has been recommended in this connection, but this still does not offer a simple solution of the problem due to some difficulties and potential side reactions in the final deprotection steps.

Recently, we have shown [8] that a new class of blocking groups based on substituted 2-phenylethanols offers some advantages over the commonly used protecting groups due to the relatively high stability under hydrolytic conditions and the easy and clean cleavage in aprotic solvents by a β-elimination mechanism. We are in favour especially of the 2-(*p*-nitrophenyl)ethyl (Npe) and 2-(*p*-nitrophenyl)ethoxycarbonyl (NpeOCO) group [9] as universal, very versatile blocking groups in the phosphotriester approach offering a simple and unified protecting pattern.

An extension of this strategy is the development of a new phosphorylating agent, bis[2(*p*-nitrophenyl)ethyl] phosphorochloridate (**3**) [10], which exhibits the same structural features of high stability in the corresponding phosphotriester form and can, therefore, be carried along in the 3'- and/or 5'-position as a terminal function during the

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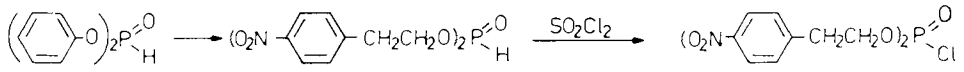
condensations towards the built-up of oligonucleotide chains. The chemical nature of the Npe group guarantees thereby a direct conversion from the phosphotriester stage *via* the intermediary phosphodiesters to the corresponding monophosphates.

2. Syntheses. – The preparation of bis[2-(*p*-nitrophenyl)ethyl] phosphorochloridate (**3**) was achieved from diphenyl phosphonate (**1**), first by transesterification with 2 equiv. of 2-(*p*-nitrophenyl)ethanol to bis[2-(*p*-nitrophenyl)ethyl] phosphonate (**2**) and then by the conversion of **2** to **3** on treatment with 1 equiv. of sulfonyl chloride. Since the new phosphorylating agent could neither by distilled nor recrystallized purely, crystalline **2** had to be used for the last step to get a chromatographically and spectroscopically homogeneous material. The elemental analysis of **3** gives further proof of the correct composition.

Scheme 1



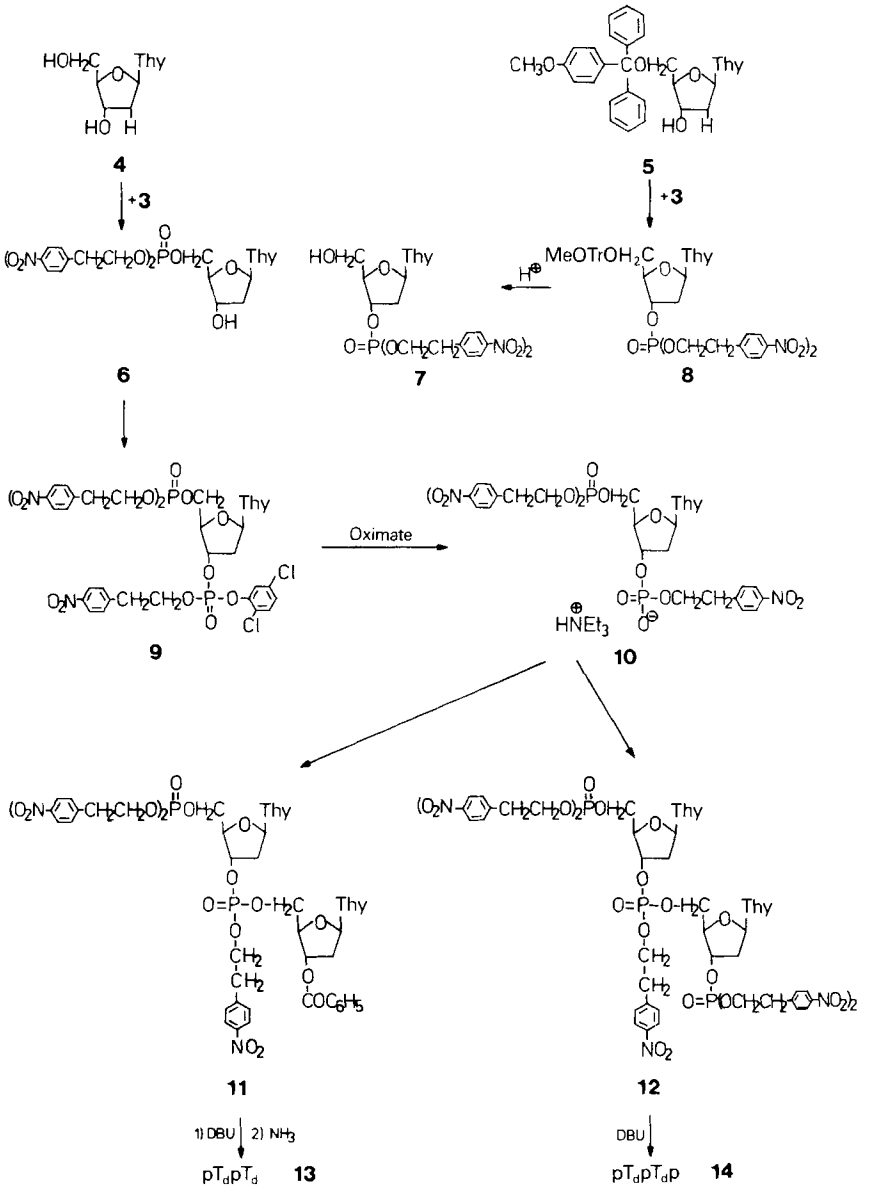
1 **2** **3**

The phosphorylating properties of **3** have first been studied with thymidine (**4**) which reacted in pyridine without any further activation relatively selectively at 0° in 73% yield to thymidine 5'-{bis[2-(*p*-nitrophenyl)ethyl] phosphate} (**6**) as the main product, besides two more components in small amounts which can be regarded as the isomeric 3'-mono- (**7**) and the 3',5'-bis(phosphotriester). The 5'-*O*-(monomethoxytrityl)thymidine (**5**) reacted with **3** in presence of *N*-methylimidazole at r.t. in 81% yield to the corresponding 3'-{bis[2-(*p*-nitrophenyl)ethyl] phosphate} **8**. Subsequent demonomethoxytritylation took place with 2% *p*-toluenesulfonic acid in ethylenechloride/MeOH in 92% yield to give thymidine 3'-{bis[2-(*p*-nitrophenyl)ethyl] phosphate} (**7**).

The chemical stability of the bis[2-(*p*-nitrophenyl)ethyl]-phosphotriester function became obvious in further phosphorylation and condensation reactions. Treatment of **6** with 2,5-dichlorophenyl phosphorodichloridate and 1,2,4-triazole and subsequent addition of 2-(*p*-nitrophenyl)ethanol led in high yield to 5'-*O*-{bis[2-(*p*-nitrophenyl)ethyl]-phosphoryl}thymidine 3'-{bis[2-(*p*-nitrophenyl)ethyl]phosphate} (**9**), and its cleavage by oximate/Et_3N afforded the corresponding phosphodiester **10**. This intermediate was then condensed with 3'-*O*-benzoylthymidine or **7** using *Efimov*'s activating couple 2,4,6-triisopropylbenzenesulfonyl chloride/*N*-methylimidazole [11] to form the dinucleoside bis-(phosphotriester) **11** and tris(phosphotriester) **12**, respectively. Their isolation was achieved by chromatography on preparative silica-gel plates.

Deprotonation of these two fully blocked dimers turned out to be very simple, since **11** contains only two types of protecting groups and **12** only one type. Treatment of **12** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine at r.t. for 24 h afforded in almost quantitative yield 5'-*O*-phosphorylthymidylyl(3'→5')thymidine 3'-phosphate (**14**), and analogously well proceeded the deblocking of **11** giving **13** by subsequent treatment first with DBU and then with conc. NH_3. Isolation and purification of **13** and **14** were achieved by *DEAE-Sephadex-A-25* chromatography with $Et_3NH^+HCO_3^-$ buffer (pH 7.5) using a linear gradient (0.001–0.6M), yielding chromatographically pure material in 94 and 95% yield, respectively. Enzymatic degradations with spleen and snake-venom

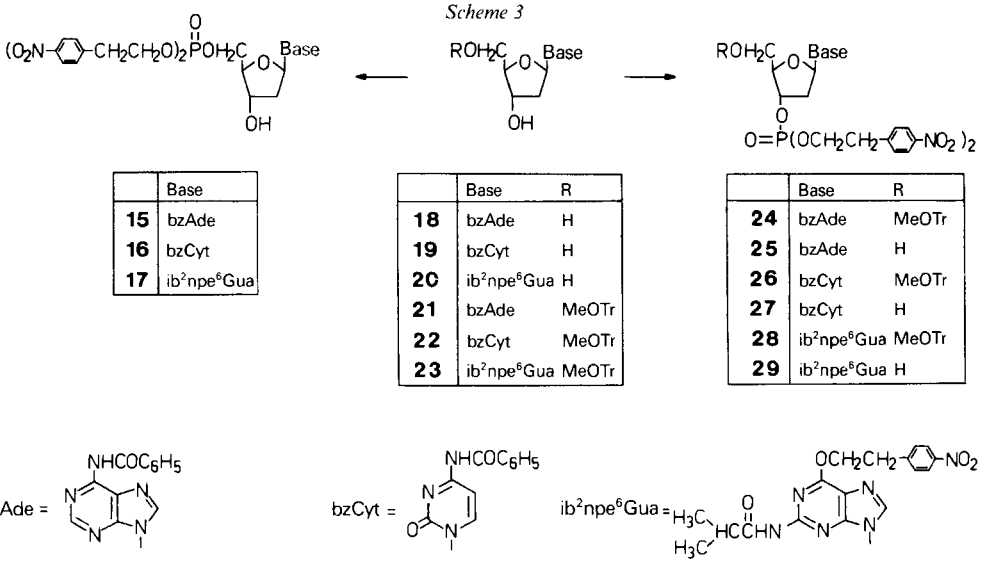
Scheme 2



phosphodiesterases worked, as expected, only with the latter enzyme and **13** to give thymidine 5′-monophosphate, whereas in **14** cleavage of the internucleotidic linkage was prevented by the 3′- and 5′-terminal phosphate groups, providing thus an additional proof of the anticipated structure.

The transformation of the commonly used 2′-deoxyribonucleosides such as the N^6-benzoyl-2′-deoxyadenosine (**18**), N^4-benzoyl-2′-deoxycytidine (**19**), and N^2-isobutyryl-

Scheme 3



	Base
15	bzAde
16	bzCyt
17	ib²npe⁶Gua

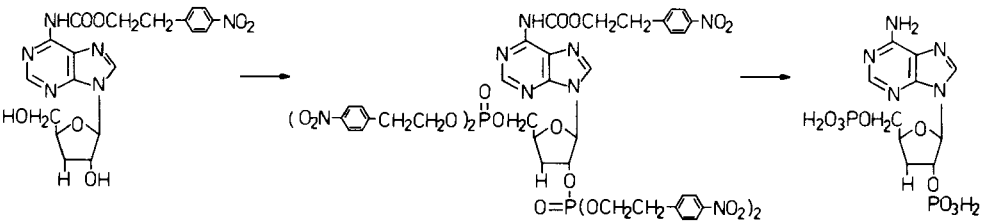
	Base	R
18	bzAde	H
19	bzCyt	H
20	ib²npe⁶Gua	H
21	bzAde	MeOTr
22	bzCyt	MeOTr
23	ib²npe⁶Gua	MeOTr

	Base	R
24	bzAde	MeOTr
25	bzAde	H
26	bzCyt	MeOTr
27	bzCyt	H
28	ib²npe⁶Gua	MeOTr
29	ib²npe⁶Gua	H

O^6-[2-(*p*-nitrophenyl)ethyl]-2′-deoxyguanosine (**20**) into their 3′- and 5′-bis[2-(*p*-nitrophenyl)ethyl] phosphotriesters was performed on analogous routes. Direct phosphorylation of **18**–**20** with **3** again led to the 5′-phosphotriesters **15**–**17** as the predominant products, whereas the corresponding 3′-isomers **25**, **27**, and **29** were obtained from the protected 5′-*O*-monomethoxytrityl derivatives **21**, **22**, and **23**, respectively, by the sequence phosphorylation to **24**, **26**, and **28** and final detritylation. High yields at all steps may be regarded as a characteristic feature in this series.

The synthetic value of the new phosphorylating agent **3** has further been demonstrated by the conversion of 3′-deoxyadenosine (cordycepin) into its 2′,5′-diphosphate **32**. For this purpose, cordycepin was first protected at the amino group using the procedure of transient protection by silylation [12] and subsequent acylation by 1-methyl-3-[2-(*p*-nitrophenyl)ethoxycarbonyl]imidazolium chloride [9] to form N^6-[2-(*p*-nitrophenyl)-ethoxycarbonyl]-3′-deoxyadenosine (**30**) in 94% yield. Phosphorylation with **3** worked very well giving N^6-[2-(*p*-nitrophenyl)ethoxycarbonyl]-5′-*O*-{bis[2-(*p*-nitrophenyl)-

Scheme 4



30 **31** **32**

ethyl]phosphoryl}-3'-deoxyadenosine 2'-{bis[2-(*p*-nitrophenyl)ethyl] phosphate} (**31**) which was isolated in 87% yield after silica-gel chromatography. The uniform protection in **31** reveals another example of a one-step deblocking procedure leading, by a β-elimination process, in high yield to the final product **32**.

3. Physical Data. – The newly synthesized compounds have been characterized by their UV spectra based upon the C, H, N-elemental analysis to prove the correct composition *(Table)*. The molecular features of structurally related compounds such as **6**, **7**, and **8**, **9** and **10**, **16**, **26**, and **27** as well as **17** and **31** are nicely reflected by the similarities of their UV absorption maxima and the extinction coefficients.

The ^{1}H-NMR spectra (high resolution) are complex due to the various blocking groups and overlapping signals. The fully protected phosphotriesters **9**, **11**, and **12**, furthermore, consist of diastereoisomeric mixtures which limit the NMR method significantly for structural proof. Some characteristic signals of the more simple compounds are listed in the *Table*.

Comparisons of the NMR data reveal some peculiarities as seen in the upfield shift of the CH_3–C(5) signal to 1.40 ppm in **8** or the downfield shift of H–C(2) in **30**.

4. Conclusion. – Bis[2-(*p*-nitrophenyl)ethyl] phosphorochloridate (**3**) can be regarded as a new versatile phosphorylating agent which yields, on reaction with primary and secondary OH groups, stable aliphatic phosphotriesters. The relatively high hydrolytic stability towards weak acids and bases allows their use in oligonucleotide synthesis leading to 3'- and/or 5'-terminal phosphates. Cleavage of the 2-(*p*-nitrophenyl)ethyl protecting groups can be achieved in aprotic solvents with DBU in a β-elimination process which guarantees also a clean interconversion of the intermediary, more stable phosphodiester into the monophosphate. A further advantage of this methodology is seen by the introduction of a free phosphate group into molecules carrying other base-labile functions.

Experimental Part

General. TLC: precoated silica-gel thin layer sheets *F 1500 LS 254* and cellulose thin layer sheets *F 1440* from *Schleicher & Schüll*. Prep. TLC: silica gel *60 PF_{254} (Merck)*. Column chromatography: silica gel *Merck 60* (0.063–0.2 mesh). Ion-exchange chromatography: *DEAE Sephadex A 25 (Pharmacia)*. M.p.: *Büchi* apparatus, model Dr. *Tottoli;* no corrections. UV/VIS: *Cary* recording spectrometer, model *118, Applied Phys. Corp.*, and *Uvikon 820, Kontron;* λ_{max} in nm (1 g ε). ^{1}H-NMR: *Bruker WM 250* in δ (ppm) with respect to TMS.

1. *Bis[2-(*p*-nitrophenyl)ethyl] Phosphonate* (**2**). A mixture of 2.34 g (10 mmol) of diphenyl phosphonate and 3.35 g (20 mmol) of 2-(*p*-nitrophenyl)ethanol is heated to 100° for 4 h with stirring. The formed phenol is then distilled off at 100°/0.2 Torr. An excess of 2-(*p*-nitrophenyl)ethanol is removed by treatment of the remaining oil with Et_2O. The residue is then crystallized from 8 ml of toluene: 3.19 g (84%) of yellowish crystals. M.p. 66°. UV (MeOH): 268 (4.30). ^{1}H-NMR ($CDCl_3$): 3.10 (*t*, 4 H); 4.27 (*m*, 4 H); 6.75 (*d*, 1 H); 7.40 (*d*, 4 H); 8.15 (*d*, 4 H). Anal. calc. for $C_{16}H_{17}N_2O_7P$ (381.9): C 50.53, H 4.50; found: C 50.56, H 4.26.

2. *Bis[2-(*p*-nitrophenyl)ethyl] Phosphorochloridate* (**3**). To a soln. of 0.3 g (2.2 mmol) of SO_2Cl_2 in 5 ml of abs. CCl_4 is dropped, with stirring under anh. conditions, a soln. of 0.76 g (2 mmol) of **1** in 4 ml of abs. CH_2Cl_2. The mixture is stirred for 15 min. Then, the solvents and gaseous products are evaporated at r.t. under high vacuum to yield 0.84 g (100%) of a thick yellowish oil. This material is pure enough for the phosphorylations. It cannot be distilled without decomposition. UV (CH_3CN): 270 (4.26). ^{1}H-NMR ($CDCl_3$): 3.10 (*t*, 4 H); 4.37 (*m*, 4 H); 7.35 (*d*, 4 H); 8.15 (*d*, 4 H). Anal. calc. for $C_{16}H_{16}ClN_2O_7P$ (416.4): C 46.34, H 3.89; found: C 46.15, H 4.05.

Table. *Physical Data of Nucleoside Bis[2-(p-nitrophenyl)ethyl] Phosphotriester in $CDCl_3$*

	UV				^{1}H-NMR (δ [ppm])[a]									
	λ_{max} [nm]		lg ε		NH		$C_6H_4NO_2$			H–C(6)	H–C(1')	CH_3–C(5)/ H–C(5)	H–C(8)	H–C(2)
6		267		4.45	9.60 (*s*)		8.15 (*m*);	7.35 (*m*)		7.30 (*s*)	6.25 (*m*)	1.85 (*s*)		
7		267		4.47	9.55 (*s*)		8.15 (*m*);	7.40 (*m*)		7.45 (*s*)	6.15 (*m*)	1.85 (*s*)		
8	233	267	4.31	4.46	8.00 (*s*)		8.12 (*m*);	7.30 (*m*)		7.30[b])	6.35 (*m*)	1.40 (*s*)		
9		267		4.57	8.42 (*s*)	8.47 (*s*)	8.12 (*m*);	7.30 (*m*)		7.10 (*ds*)	6.25 (*m*)	1.88 (*s*)		
10		268		4.57	9.20 (*s*)		8.10 (*m*);	7.40 (*m*)		7.30[b])	6.33 (*dd*)	1.88 (*s*)		
11		266		4.67	8.87 (*s*)	8.95 (*s*)	8.13 (*m*);	7.40 (*m*)		[b])	6.24 (*m*)	1.85 (*s*)		
					9.03 (*s*)	9.26 (*s*)					6.29 (*m*)	1.90 (*s*)		
12		267		4.80	9.09 (*s*)	9.17 (*s*)	8.10 (*m*);	7.34 (*m*)		7.15	5.99 ('*t*')	1.82 (*s*)		
					9.23 (*s*)	9.51 (*s*)				7.21	6.13 (*m*)	1.85 (*s*)		
15		267		4.56	9.05 (*s*)		8.02 (*m*);	7.28 (*m*)			6.43 ('*t*')		8.69 (*s*)	8.13 (*s*)
16	262	310 (sh)	4.60	4.06 (sh)	9.00 (br. *s*)		8.11 (*m*);	7.33 (*m*)		8.05 (*d*)	6.24 ('*t*')	7.50[b])		
17	217	269	4.63	4.67	8.27 (*s*)		8.10 (*d*);	8.04 (*d*);	7.99 (*d*);		6.63 ('*t*')		8.00 (*s*)	
							7.48 (*d*);	7.29 (*d*);	7.20 (*d*)					
24	232	276	4.53	4.59	8.92 (*s*)		8.09 (*m*);	7.30 (*m*)			6.40 (*dd*)		8.67 (*s*)	8.07 (*s*)
25		276		4.58	9.03 (*s*)		8.14 (*m*);	7.35 (*m*)			6.25 (*dd*)		8.72 (*s*)	8.04 (*s*)
26	262	310 (sh)	4.61	4.06 (sh)	8.57 (*s*)		8.12 (*m*);	7.30 (*m*);	8.04 (*d*)		6.24 ('*t*')	7.55 (*d*)[b])		
27	262	310 (sh)	4.63	4.08 (sh)	8.88 (*s*)		8.14 (*m*);	7.33 (*m*);	8.18 (*d*)		6.16 ('*t*')	7.55[b])		
28	236	279	4.40	4.67	7.78 (*s*)		8.10 (*m*);	7.48 (*d*);	7.35 (*m*)		6.27 (*dd*)		7.87 (*s*)	
29	218	270	4.32	4.66	7.83 (br. *s*)		8.14 (*m*);	7.48 (*d*);	7.37 (*m*)		6.10 (*dd*)		7.83 (*s*)	
30		267		4.33	10.54 (br. *s*)		8.16 (*d*);	7.62 (*d*)			5.99 (*d*)		8.68 (*s*)	8.60 (*s*)
31	214 (sh)	267	4.76 (sh)	4.72			8.07 (*m*);	7.37 (*m*)			5.99 (*d*)		8.63 (*s*)	8.28 (*s*)

[a]) '*t*' = pseudo-*t*.

[b]) Covered by other signals.

3. *Thymidine 5'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**6**). A soln. of 0.242 g (1 mmol) of thymidine (**4**) in 4 ml of abs. pyridine is evaporated. The residue is again dissolved in 3 ml of abs. pyridine and cooled to 0°, then, 0.62 g (1.5 mmol) of **3** in 3 ml of abs. pyridine are dropwise added with stirring. After 45 min, the soln. is distributed 3 times in 20 ml of $CHCl_3$ and 15 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4), evaporated, and coevaporated 3 times with 15 ml of toluene each. The residue is chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 92:8. The main band is eluted with 250 ml of $CHCl_3$/MeOH 7:1 to yield, after drying at 40°/0.2 Torr, 0.452 g (73%) of a colourless foam. Anal. calc. for $C_{26}H_{29}N_4O_{12}P$ (620.5): C 50.33, H 4.71, N 9.03; found: C 50.13, H 4.67, N 9.13.

4. *Thymidine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**7**). To a 2% soln. of TsOH in CH_2Cl_2/MeOH 7:3 are added 0.893 g (1 mmol) of **8**. After stirring for 1 h at r.t., 100 ml of $CHCl_3$ are added. Then, the mixture is treated twice with 100 ml of phosphate buffer (pH 7), the org. layer dried (Na_2SO_4) and evaporated, the residue in little $CHCl_3$ chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with CH_2Cl_2/MeOH 97:3, and the main band eluted with 300 ml of CH_2Cl_2:MeOH 6:1. On evaporation and drying at 40°/0.2 Torr, 0.565 g (92%) of a colourless foam is obtained. Anal. calc. for $C_{26}H_{29}N_4O_{12}P$ (620.5): C 50.33, H 4.71, N 9.03; found: C 50.45, H 4.66, N 9.00.

5. *5'-O-(Monomethoxytrityl)thymidine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**8**). To a soln. of 0.624 g (1.5 mmol) of **3** in 4 ml of abs. pyridine are added 0.514 g (1 mmol) of 5'-*O*-(monomethoxytrityl)thymidine (**5**), followed by 0.8 ml (10 mmol) of *N*-methylimidazole. After stirring for 2 h at r.t., 5 ml of phosphate buffer (pH 7) are added, and stirring is continued for 10 min. Then, the mixture is distributed 3 times between 30 ml of $CHCl_3$ and 25 ml of phosphate buffer, the org. layer dried (Na_2SO_4), evaporated, and coevaporated 3 times with 15 ml of toluene. The residue in little $CHCl_3$ is chromatographed on a silica-gel column (25 × 3 cm) with 300 ml of $CHCl_3$ followed by $CHCl_3$/MeOH 97:3. The main fraction is evaporated and the residue in 6 ml of $CHCl_3$ added slowly with stirring to 100 ml of hexane. The precipitate is dried at 40° under vacuum: 0.725 g (81%) of a colourless amorphous powder.

6. *5'-O-{Bis[2-(p-nitrophenyl)ethyl]phosphoryl}thymidine 3'-[2,5-Dichlorphenyl 2-(p-Nitrophenyl)ethyl Phosphate]* (**9**). A soln. of 0.11 g (1.6 mmol) of 1,2,4-triazole and 0.2 g (0.75 mmol) of 2,5-dichlorophenyl phosphorodichloridate in 2 ml of abs. pyridine is stirred for 30 min at r.t. The mixture is cooled to 0°, and then a soln. of 0.31 g (0.5 mmol) of **6** in 3 ml of abs. pyridine is added dropwise. After stirring for 1 h, 0.167 g (1 mmol) of 2-(*p*-nitrophenyl)ethanol is added and stirring continued for 24 h at r.t. The mixture is distributed 3 times between 15 ml of $CHCl_3$ and 10 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4) and evaporated and the remaining oil coevaporated 3 times with 10 ml of toluene. The residue in little $CHCl_3$ is chromatographed on a silica-gel column (15 × 2.5 cm) with CH_2Cl_2 followed by CH_2Cl_2/MeOH 96:4. The main fraction is evaporated and the residue dried at 40°/0.2 Torr to give 0.44 g (88%) of a colourless foam. Anal. calc. for $C_{40}H_{39}Cl_2N_5O_{17}P_2$ (994.6): C 48.30, H 3.95, N 7.04; found: C 48.54, H 4.03, N 7.43.

7. *5'-O-{Bis[2-(p-nitrophenyl)ethyl]phosphoryl}thymidine 3'-[2-(p-Nitrophenyl)ethyl Phosphate]* (**10**). A mixture of 0.61 g (5 mmol) of pyridine-4-carbaldehyde oxime, 20 ml of dioxane, 20 ml of Et_3N and 20 ml of H_2O is stirred for 30 min. Then, 0.44 g (0.44 mmol) of **9** are added and stirred for 1 h at r.t. The solvents are evaporated at 30°, and the residue is coevaporated 3 times with 15 ml of abs. pyridine and 3 times with 15 ml of toluene. The residue is chromatographed on a silica-gel column (10 × 3 cm) with CH_2Cl_2/MeOH 95:5 (removal of excess of reagents) and $CHCl_3$/MeOH/NEt_3 9:1:1. The product fraction is evaporated and coevaporated 3 times with 10 ml of toluene and the resulting foam dried at 40°/0.2 Torr: 0.33 g (80%) of a yellowish amorphous solid. Crystals are obtained by dissolving 0.1 g in 2 ml of MeOH and slow dilution with AcOEt by diffusion on standing in a desiccator. An oil is formed which crystallizes after *ca.* 2 weeks: 0.08 g. Anal. calc. for $C_{40}H_{52}N_6O_{17}P_2$ (950.8): C 50.53, H 5.51, N 8.84; found: C 50.47, H 5.48, N 8.93.

8. *5'-O-{Bis[2-(p-nitrophenyl)ethyl]phosphoryl}thymidylyl{3'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-3'-O-benzoylthymidine* (**11**). A mixture of 0.3 g (0.315 mmol) of **10** and 0.084 g (0.242 mmol) of 3'-*O*-benzoylthymidine in 5 ml of abs. pyridine is evaporated twice. To the residue in 2.6 ml of abs. pyridine are added 0.22 g (0.73 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride and 0.174 ml (2.18 mmol) of *N*-methylimidazole. The mixture is stirred at r.t. for 4 h and then treated 3 times each with 15 ml of $CHCl_3$ and 10 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4), evaporated, and coevaporated 3 times with toluene. The residue in little $CHCl_3$ is chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with toluene/AcOEt/MeOH 3:5:2. The slowest moving band is eluted with 200 ml of CH_2Cl_2/MeOH 6:1, evaporated, and dried at 40°/0.2 Torr: 0.212 g (74%) of a solid foam. Anal. calc. for $C_{51}H_{53}N_7O_{22}P_2$ (1177.9): C 52.00, H 4.54, N 8.32; found: C 52.29, H 4.57, N 8.06.

9. *5'-O-{Bis[2-(p-nitrophenyl)ethyl]phosphoryl}thymidylyl {3'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}thymidine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**12**). A mixture of 0.45 g (0.473 mmol) of **10** and 0.225 g (0.363 mmol) of **7** in 4 ml of abs. pyridine is evaporated and the residue dissolved in 3.6 ml of abs. pyridine. Then, 0.33 g (1.09 mmol) of 1,4,6-triisopropylbenzenesulfonyl chloride are added. After cooling to 0°, 0.26 ml (3.26 mmol) of *N*-methylimidazole are added and stirred at r.t. for 24 h. The mixture is treated 3 times each with 20 ml of $CHCl_3$ and 15 ml of phosphate buffer (pH 7), the org. phase washed with 20 ml of sat. NaCl soln., dried (Na_2SO_4), and evaporated, and the residue coevaporated 3 times with 15 ml of toluene. The residue in little $CHCl_3$ is chromatographed on 3 prep. silica-gel plates (40 × 20 × 0.2 cm) with toluene/AcOEt/MeOH 6:10:3. The main band is eluted with 150 ml of $CHCl_3$/MeOH 6:1 and the residue in little $CHCl_3$ rechromatographed on a small silica-gel column (20 × 2 cm) with $CHCl_3$/MeOH 95:5. The main fraction yielded, after drying at 40°/0.2 Torr, 0.35 g (66%) of a colourless foam. Anal. calc. for $C_{60}H_{64}N_9O_{28}P_3$ (1452.1): C 49.63, H 4.44, N 8.68; found: C 49.39, H 4.74, N 8.57.

10. *5'-O-Phosphorylthymidylyl (3'→5')thymidine* (**13**). To 6.6 ml of 0.5M DBU in abs. pyridine are added 11.2 mg (9.5 μmol) of **11** and stirred for 24 h at r.t. The mixture is neutralized by addition of 3.5 ml of 1M AcOH in abs. pyridine and evaporated. The residue in 3 ml of conc. NH_3 is stirred for 18 h at r.t., the solvent evaporated, and the residue coevaporated several times with bidistilled H_2O. The residue in 8 ml of H_2O is chromatographed on a *DEAE-Sephadex* column (50 × 1 cm) with a gradient of 0.001–0.6M $Et_3NH^+HCO_3^-$ buffer (fractions of 12.5 ml; **13** in *Fractions 38–58*). Evaporation at 30° followed by coevaporation 8 times each with 50 ml of bidistilled H_2O (removal of $Et_3NH^+HCO_3^-$) give 94% of **13** (determined by UV at 267 nm and referring to an $\varepsilon = 19000$ for thymidine).

11. *5'-O-Phosphorylthymidylyl (3'→5')thymidine 3'-Phosphate* (**14**). To 10 ml of 0.5M DBU in abs. pyridine are added 15.4 mg (10.6 μmol) of **12** and stirred at r.t. for 24 h. Neutralization is done by addition of 5 ml of 1M AcOH in abs. pyridine. The mixture is evaporated, the residue in 8 ml of bidistilled H_2O chromatographed on a *DEAE-Sephadex* column (50 × 1 cm) with a gradient of 0.001–0.6M $Et_3NH^+HCO_3^-$ buffer (fractions of 12.5 ml; **14** in *Fractions 68–83*). Evaporation at 30° and 10-fold coevaporation with each 50 ml of bidistilled H_2O give 95% of **14** (determined by UV at 267 nm and based on $\varepsilon = 19000$ for thymidine).

12. N^6-*Benzoyl-2'-deoxyadenosine 5'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**15**). A soln. of 0.177 g (0.5 mmol) of *N*^{6}-benzoyl-2'-deoxyadenosine [13] in 4 ml of abs. pyridine is evaporated. To the residue in 5 ml of abs. pyridine, 0.332 g (0.8 mmol) of **3** are added and stirred at r.t. for 45 min. The mixture is distributed 3 times between 20 ml of $CHCl_3$ and 20 ml of phosphate buffer (pH 7) and the org. layer dried (Na_2SO_4), evaporated, and coevaporated 3 times each with 15 ml of toluene. The oily residue in little $CHCl_3$ is chromatographed on 2 silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 92:8. The main band is eluted with 200 ml of $CHCl_3$/MeOH 4:1 and the residue dried at 40°/0.2 Torr: 0.264 g (72%) of an amorphous solid. Anal. calc. for $C_{33}H_{32}N_7O_{11}P$ (733.6): C 54.03, H 4.40, N 13.36; found: C 54.12, H 4.54, N 13.25.

13. N^4-*Benzoyl-2'-deoxycytidine 5'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**16**). A soln. of 0.166 g (0.5 mmol) of *N*^{4}-benzoyl-2'-deoxycytidine [13] in 4 ml of abs. pyridine is evaporated. To the residue in 5 ml of abs. pyridine, 0.332 g (0.8 mmol) of **3** are added and stirred at r.t. for 30 min. The mixture is distributed 3 times between 20 ml of $CHCl_3$ and 20 ml of phosphate buffer (pH 7) and the org. layer dried (Na_2SO_4), evaporated, and coevaporated 3 times with 15 ml of toluene. The oily residue in little $CHCl_3$ is chromatographed on 2 silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 9:1. The main band is eluted with 200 ml of $CHCl_3$/MeOH 4:1 to give, after drying of the residue at 40°/0.2 Torr, 0.241 g (68%) of an amorphous solid. Anal. calc. for $C_{32}H_{32}N_5O_{12}P$ (709.6): C 54.16, H 4.55, N 9.87; found: C 53.93, H 4.72, N 9.91.

14. N^2-*Isobutyryl-O*^{6}-*[2-(p-nitrophenyl)ethyl]-2'-deoxyguanosine 5'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**17**). As in *Exper. 13*, 0.245 g (0.5 mmol) of *N*^{2}-isobutyryl-*O*^{6}-2-[(*p*-nitrophenyl)ethyl]-2'-deoxyguanosine [9] are treated with 0.33 g (0.8 mmol) of **3**. Prep. silica-gel chromatography on plates (40 × 20 × 0.2 cm) in $CHCl_3$/MeOH 95:5 yields 0.32 g (74%) of a solid foam. Anal. calc. for $C_{38}H_{41}N_8O_{14}P$ (864.8): C 52.78, H 4.78, N 12.96; found: C 52.69, H 4.88, N 12.93.

15. N^6-*Benzoyl-5'-O-monomethoxytrityl-2'-deoxyadenosine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**24**). To a soln. of 0.314 g (0.5 mmol) of *N*^{6}-benzoyl-5'-*O*-monomethoxytrityl-2'-deoxyadenosine (**21**) [13] in 2 ml of abs. pyridine are added 0.2 ml of *N*-methylimidazole and 0.415 g (1 mmol) of **3**. The mixture is stirred for 12 h at r.t. and then distributed 3 times between 20 ml of $CHCl_3$ and 20 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4), evaporated, and coevaporated 3 times with 20 ml of toluene. The residue in little $CHCl_3$ is chromatographed on a silica-gel column (25 × 2 cm) with $CHCl_3$ and then with $CHCl_3$/MeOH 100:1. The product fraction yields, after drying of the residue at 40°/0.2 Torr, 0.452 g (90%) of an amorphous solid. Anal. calc. for $C_{53}H_{48}N_7O_{12}P$ (1005.9): C 63.28, H 4.81, N 9.75; found: C 63.44, H 4.98, N 9.58.

16. N^6-*Benzoyl-2'-deoxyadenosine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**25**). In 33 ml of a 1.5% soln. of TsOH in CH_2Cl_2/MeOH 7:3 are stirred, for 20 min, 0.68 g (0.675 mmol) of **24**. The soln. is diluted with 70 ml of $CHCl_3$ and shaken 3 times each with 100 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4) and evaporated and the residue chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 95:5. The main band is eluted with 300 ml of $CHCl_3$/MeOH 4:1 to give, after drying of the residue at 40°/0.2 Torr, 0.431 g (87%) of a colourless amorphous solid. Anal. calc. for $C_{33}H_{32}N_7O_{11}P$ (733.6): C 54.03, H 4.40, N 13.36; found: C 53.99, H 4.48, N 13.09.

17. N^4-*Benzoyl-5'-O-monomethoxytrityl-2'-deoxycytidine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**26**). To a soln. of 0.302 g (0.5 mmol) of N^4-benzoyl-5'-*O*-monomethoxytrityl-2'-deoxycytidine (**22**) [13] in 2 ml of abs. pyridine and 0.2 ml of *N*-methylimidazole are added 0.415 g (1 mmol) of **3** and stirred at r.t. for 4 h. The soln. is distributed 3 times between 20 ml of $CHCl_3$ and 20 ml of phosphate buffer (pH 7) and the org. layer dried (Na_2SO_4), evaporated, and 3 times coevaporated with 20 ml of toluene. The residue in little $CHCl_3$ is chromatographed on 2 silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 92:8. The main band is eluted with $CHCl_3$/MeOH 4:1 to give, after drying of the residue at 40°/0.2 Torr, 0.412 g (84%) of an amorphous solid. Anal. calc. for $C_{52}H_{48}N_5O_{13}P$ (981.9): C 63.61, H 4.93, N 7.13; found: C 62.90, H 4.95, N 7.11.

18. N^4-*Benzoyl-2'-deoxycytidine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**27**). To a soln. of 50 ml of 1% TsOH in CH_2Cl_2/MeOH 7:3 are added 0.49 g (0.5 mmol) of **26** and stirred for 40 min at r.t. The soln. is diluted with 50 ml of $CHCl_3$ and shaken 3 times each with 100 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4) and evaporated and the residue chromatographed on 2 silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 92:8. The main band is eluted with 200 ml of $CHCl_3$/MeOH 4:1 to yield, after drying of the residue at 40°/0.2 Torr, 0.32 g (90%) of an amorphous solid. Anal. calc. for $C_{32}H_{32}N_5O_{12}P$ (709.6): C 54.16, H 4.55, N 9.87; found: C 53.93, H 4.72, N 9.91.

19. N^2-*Isobutyryl-5'-O-monomethoxytrityl-*O^6-*[2-(p-nitrophenyl)ethyl]-2'-deoxyguanosine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**28**). A soln. of 0.38 g (0.5 mmol) of N^2-isobutyryl-5'-*O*-monomethoxytrityl-O^6-2-[(*p*-nitrophenyl)ethyl]-2'-deoxyguanosine (**23**) [9] in 5 ml of abs. pyridine is evaporated. To the residue in 2 ml of abs. pyridine, 0.2 ml of *N*-methylimidazole and 0.415 g (1 mmol) of **3** are added and stirred at r.t. for 4 h. The soln. is then distributed 3 times between 30 ml of $CHCl_3$ and phosphate buffer (pH 7) and the org. layer dried (Na_2SO_4), evaporated, and coevaporated 3 times with 20 ml of toluene. The residue is chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 95:5. The main band is eluted with $CHCl_3$/MeOH 5:1 to yield, after drying at 40°/0.2 Torr, 0.52 g (91%) of a solid foam. Anal. calc. for $C_{58}H_{57}N_8O_{15}P$ (1137.1): C 61.26, H 5.05, N 9.85; found: C 61.01, H 4.98, N 9.72.

20. N^2-*Isobutyryl-*O^6-*[2-(p-nitrophenyl)ethyl]-2'-deoxyguanosine 3'-{Bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**29**). To 50 ml of a soln. of 1% TsOH in CH_2Cl_2/MeOH 7:3 are added 0.568 g (0.5 mmol) of **28** and stirred for 30 min at r.t. The soln. is diluted with 100 ml of $CHCl_3$ and shaken 3 times each with 100 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4) and evaporated and the residue chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 20:1. The main band is eluted with 100 ml of $CHCl_3$/MeOH 4:1 to yield, after drying of the residue at 40°/0.2 Torr, 0.358 g (82%) of an amorphous solid. Anal. calc. for $C_{38}H_{41}N_8O_{14}P$ (864.8): C 52.78, H 4.78, N 12.96; found: C 52.58, H 4.84, N 13.02.

21. N^6-*[2-(p-Nitrophenyl)ethoxycarbonyl]-3'-deoxyadenosine* (**30**). A mixture of 30 ml of abs. dioxane, 30 ml of hexamethyldisilazane, a few crystals of $(NH_4)_2SO_4$, and 5.38 g (20 mmol) of 3'-deoxyadenosine (cordycepin) [14] is boiled under reflux for 3 h under exclusion of moisture. The mixture is evaporated and the residue taken up in 120 ml of abs. toluene. Insoluble material is filtered off, the filtrate evaporated, and the residue dissolved in 400 ml of abs. CH_2Cl_2. To this soln. are added 12.5 g (40 mmol) of 1-methyl-3-[2-(*p*-nitrophenyl)ethoxycarbonyl]-imidazolium chloride and stirred for 18 h at r.t. A precipitate is filtered off, the filtrate evaporated, and the residue treated with 200 ml of MeOH and 50 ml of Et_3N with vigorous stirring over night. The precipitate is filtered off, washed with cold MeOH, and dried at 60° to give 8.33 g (94%) of colourless crystals. M.p. 124°. Anal. calc. for $C_{19}H_{20}N_6O_7 \cdot H_2O$ (462.4): C 49.35, H 4.80, N 18.17; found: C 49.15, H 4.45, N 17.85.

22. N^6-*[2-(p-Nitrophenyl)ethoxycarbonyl]-3'-deoxyadenosine 2',5'-Bis{bis[2-(p-nitrophenyl)ethyl] Phosphate}* (**31**). To a soln. of 0.111 g (0.25 mmol) of **30** in 1.5 ml of abs. pyridine, 0.15 ml of *N*-methylimidazole and 0.415 g (1 mmol) of **3** are added. The mixture is stirred at r.t. for 18 h, evaporated, the residue dissolved in 100 ml of $CHCl_3$, and washed twice with 50 ml of H_2O. The org. layer is dried (Na_2SO_4), evaporated, and coevaporated 3 times with 10 ml of toluene. The residue is chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 95:5. The main band is eluted with 300 ml of $CHCl_3$/MeOH 4:1 to yield, after drying of the residue

at 40°/0.2 Torr, 0.26 g (87%) of an amorphous solid. Anal. calc. for $C_{51}H_{50}N_{10}O_{21}P_2$ (1201.0): C 51.00, H 4.19, N 11.66; found: C 50.95, H 4.46, N 11.32.

23. *3'-Deoxyadenosine 2',5'-Bis{diammonium Phosphate}* (**32**). To 30 ml of 0.5M DBU in abs. pyridine are added 36 mg (30 μmol) of **31**. After stirring at r.t. for 48 h, the mixture is neutralized with 15 ml of 1M AcOH in pyridine and evaporated. The residue is chromatographed on a *DEAE-Sephadex* column (60 × 1 cm) with a linear gradient of 0.001–0.6M $Et_3NH^+HCO_3^-$ buffer (pH 7). The product is eluted at a buffer concentration of 0.35–0.45M, and this fraction is then evaporated and coevaporated many times with H_2O to remove the buffer. The residue is chromatographed on a large cellulose sheet (58 × 60 cm) with i-PrOH/conc. NH_3/H_2O 55:10:35 to give, after elution of the main band, 97% of **32** (350 *OD*).

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