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THE *p*-NITROPHENYLETHYL (NPE) GROUP

A VERSATILE NEW BLOCKING GROUP FOR PHOSPHATE AND AGLYCONE PROTECTION IN NUCLEOSIDES AND NUCLEOTIDES

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Abstract—The syntheses of new monomeric building blocks for oligonucleotide synthesis via the phosphotriester approach containing the *p*-nitrophenylethyl group for phosphate and aglycone protection are described. Blocking of the amide function in guanosines at O^6 can be achieved by the Mitsunobu reaction forming the corresponding O^6-*p*-nitrophenylethyl derivatives (**4, 5, 10**). Sugar-protected thymidine (**16**) and uridine (**17**) have been alkylated at O^4 in an S_N1-type reaction by *p*-nitrophenylethyl iodide-silver carbonate in benzene to form the O^4-*p*-nitrophenylethyl derivatives (**18, 19**). Protection of the amino group in 2′-deoxycytidine (**25**) and cytidine (**26**) can be performed directly by 1-(*p*-nitrophenylethoxycarbonyl)-benzotriazole in DMF to obtain the corresponding carbamates (**27, 28**) as a new type of N^4-acylated cytidine derivative. *p*-Nitrophenylethoxycarbonylation of the amino group in 2′-deoxyadenosine (**33**) and adenosine (**34**) requires previous sugar protection by acyl or silyl groups and can then be achieved by *p*-nitrophenylethyl chloroformate or better by 1-methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride to form N^6-*p*-nitrophenylethoxycarbonyladenosines (**38, 39, 40, 42**). The various *p*-nitrophenylethyl blocking groups are stable under mild hydrolytic conditions (e.g. ammonia and triethylamine) but can be cleaved selectively by DBU or DBN in aprotic solvents. 5′-O-Monomethoxytritylation (**12, 29, 43**) as well as phosphorylations at the 3′-OH group can be effected to give the corresponding 3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphotriesters (**13, 22, 30, 44**) also in high yields. Oximate cleavage of the latter compounds to the phosphodiesters (**14, 24, 32, 46**) and detritylation to the 5′-unblocked phosphotriesters (**15, 23, 31, 45**) do not affect the aglycone protecting groups, thereby demonstrating their general versatility. The newly synthesized compounds have been characterized on the basis of their elementary analyses (C, H, N), and UV- and ^{1}H-NMR-spectra.

Recent developments of new methodologies in the chemical synthesis of oligonucleotides[1-4] have concentrated mainly on the phosphotriester approach[5,6] as the most versatile method for the assembly of defined oligonucleotide sequences. The acceptance of the Catlin–Cramer-Principle[7] for stepwise and block condensations created a great variety of special phosphate blocking groups and led to the recommendation of a large number of universally applicable phosphotriester combinations suiting the synthetic purposes. The main trend is hereby directed towards combinations of 3′-terminal phosphotriesters bearing preferentially the *o*-chloro[8] and *p*-chlorophenyl group,[9] respectively, as the basic stable protecting group, whereas the structural modifications are predominantly achieved by the second phosphate blocking function which can be regarded as the flexible part of this approach. In the nucleoside-3′-(*o*-chlorophenyl, 2-cyanoethyl)-phosphates,[10,11] the cyanoethyl groups has been substituted by the trichloroethyl,[12-17] tribromoethyl,[18-20] methylthio,[21] 4-nitrophenyl,[22,23] 2-phenylsulfonylethyl,[24,25] 9-fluorenylmethyl,[26,27] 2-anthraquinonylmethyl,[28] 5-benzisoxazolylmethyl,[29] cyclohexylamido, and morpholido group[30] as well as by the *p*-nitrophenylethyl group,[31-33] whereas the *p*-chlorophenyl function has mainly been combined with the 2-cyanoethyl[9,34-37] and occasionally with the 5-chloro-8-quinolinyl group.[38,39] The latter blocking group is proving to function as an interesting alternative to the chlorophenyl groups in internucleotide phosphate protection.[38-41] Our own contribution to the methodology of oligonucleotide synthesis illustrates clearly that especially from the points of view of stability of phosphotriesters and their deprotection, the *p*-nitrophenylethyl group appears to be, so far, the most advanced phosphate protecting group. This is due to its chemical inertness in the condensation step, its stability towards mild acid and base hydrolyses, and its easy cleavage by DBU or DBN in a β-elimination reaction[42-47] which does not affect the P–O bonds at all. These outstanding properties of the *p*-nitrophenylethyl group suggest a more general application of this interesting group for the protection of various other functions present in the aglycones of the nucleosides and nucleotides. We have therefore investigated the use of the *p*-nitrophenylethyl group for O-protection of the amide functions of guanine, uracil, and thymine residues as well as in form of the *p*-nitrophenylethoxycarbonyl group for the blocking of the amino groups of adenine and cytosine residues. The advantage of the uniformity of the blocking group pattern over the multiplicity of the previously used combinations appears obvious at least during the deprotection steps which are considerably simplified by our new ap-

proach requiring, in general, only a DBU treatment and an acid cleavage of the monomethoxytrityl group[47] to release the free oligonucleotide from its fully protected synthetic precursor.

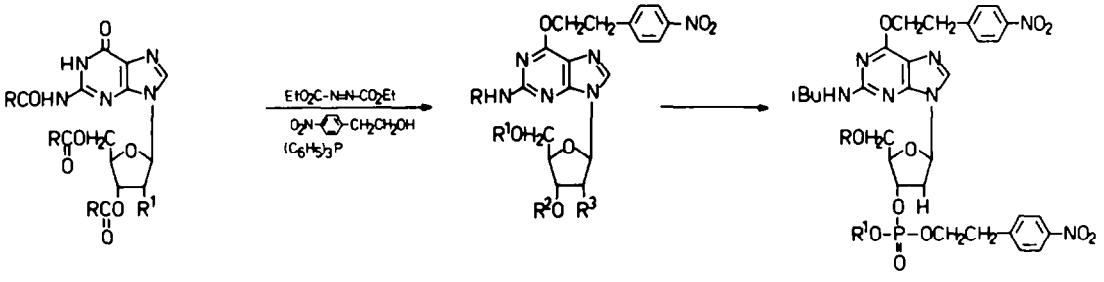
2'-Deoxyguanosine and guanosine protection

A long-standing problem in oligonucleotide synthesis is dealing with the side-reactions of the guanine residue[5,10,35,48-50] which is susceptible preferentially to sulfonylation at O^6 by condensing agents[51-53] and to phosphorylation by activated nucleotides.[54] Therefore, protection of the amide function in guanine residues seems to be an obvious necessity if such types of side reactions are to be eliminated. Various efforts have recently been directed towards the solution of this problem including the introduction of 6-O-(2-nitrophenyl) group,[55] a number of 6-O-substituted silyl, sulfonyl, phosphoryl, and phosphinothioyl derivatives[56] as well as substituted ethyl groups which are prone to β-elimination cleavage reactions.[57] The last investigation involves the introduction of the *p*-nitrophenylethyl group into the O^6-position by a complicated procedure, starting from 2-isobutyrylamino-6-(2,4,6-triisopropylbenzenesulfonyoxy)-3',5'-bis-O-t-butyldimethylsilyl-9-β-D-2'-deoxyribofuranosylpurine, first by reaction with triethylamine and subsequently by substitution of the resulting 6-trimethylammonium group by the *p*-nitrophenylethoxy residue. Careful deprotection of the silyl groups led finally to N^2-isobutyryl-O^6-*p*-nitrophenylethyl-2'-deoxyguanosine (**6**).

After a series of unsuccessful attempts to displace the 6-substituent in 6-chloro- and 6-methanesulfonyl-2-amino-9-β-D-ribofuranosylpurine nucleophilically by *p*-nitrophenylethanol under various reaction conditions, we found[58] that the "Mitsunobu reaction"[59] offered a good chance of direct O^6-alkylation if the 2-amino group is acylated, and hinders in this way a possible reaction at the adjacent N-1 ring atom.

Treatment of N^2,3',5'-tri-O-isobutyryl-2'-deoxyguanosine (**1**) and N^2,2',3',5'-tetra-isobutyrylguanosine (**2**) respectively with 1.5 molecular equivalents each of diethyl azodicarboxylate, triphenylphosphine and 2-(*p*-nitrophenyl)-ethanol in dioxan at room temp led after 24 hr directly to the corresponding O^6-*p*-nitrophenylethyl derivatives **4** and **5** in 85 and 86% yields, respectively. The *p*-nitrophenylethyl group can be cleaved selectively in 40 min by 0.5 M DBU in pyridine to regenerate the starting materials **1** and **2**. Reaction with conc ammonia in dioxan cleaves the sugar acyl groups to give, after 3 days and 20 hr, respectively, N^2-isobutyryl-O^6-*p*-nitrophenylethyl-2'-deoxyguanosine (**6**) and -guanosine (**7**) as crystalline compounds, whereas treatment with conc ammonia in methanol effects the same transformation after 3 hr. Prolonged treatment under the latter conditions finally also cleaves the N^2-acyl group without harming the O^6-blocking group and yields O^6-*p*-nitrophenylethyl-2'-deoxyguanosine (**8**) and -guanosine (**9**) respectively. A similar reaction sequence starting from N^2,2',3',5'-tetra-benzoylguanosine (**3**) proceeds analogously with the formation of the guanosine derivative **11**, via the intermediate **10**.

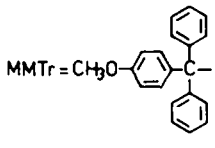
The preparation of the appropriate starting materials for oligodeoxynucleotide synthesis was then achieved from N^2-isobutyryl-O^6-*p*-nitrophenylethyl-2'-deoxyguanosine (**6**) which, on monomethoxytritylation, first yielded the corresponding 5'-O-monomethoxytrityl derivative **12** in 86% yield. Secondly, phosphorylation was carried out in the usual manner by treatment of **12** with 2,5-dichlorophenyl phosphorodichloridate in presence of 1,2,4-triazole and subsequent addition of



	R	R^1
1	$(CH_3)_2CH$	H
2	$(CH_3)_2CH$	O-iBu
3	C_6H_5	OBz

iBu = $(CH_3)_2CHCO$

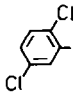
Bz = C_6H_5CO



	R	R^1	R^2	R^3
4	iBu	iBu	iBu	H
5	iBu	iBu	iBu	iBu
6	iBu	H	H	H
7	iBu	H	H	OH
8	H	H	H	H
9	H	H	H	OH
10	Bz	Bz	Bz	OBz
11	Bz	H	H	OH
12	iBu	MMTr	H	H

	R	R^1
13	MMTr	2,5-$Cl_2C_6H_3$
14	MMTr	$Et_3\overset{+}{N}H$
15	H	2,5-$Cl_2C_6H_3$

2,5-$Cl_2C_6H_3$ =



2-(*p*-nitrophenyl)-ethanol to form the fully blocked phosphotriester N^2-isobutyryl-5′-O-monomethoxytrityl-O^6-*p*-nitrophenylethyl-2′-deoxyguanosine-3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (**13**) again in 86% yield. Detritylation of **13** with 2% *p*-toluenesulfonic acid in methylene chloride–methanol (4:1) proceeded in 86% yield to give the corresponding 5′-unprotected phosphotriester **15**, whereas the oximate-cleavage[60] of the 2,5-dichlorophenyl group to the triethylammonium N^2-isobutyryl-5′-O-monomethoxytrityl-O^6-*p*-nitro-phenylethyl-2′-deoxyguanosine-3′-*p*-nitrophenylethyl-phosphate (**16**) could be performed with *p*-nitrobenzaldoxime in dioxan/water/triethylamine in 90% isolated yield.

Thymidine and uridine protection

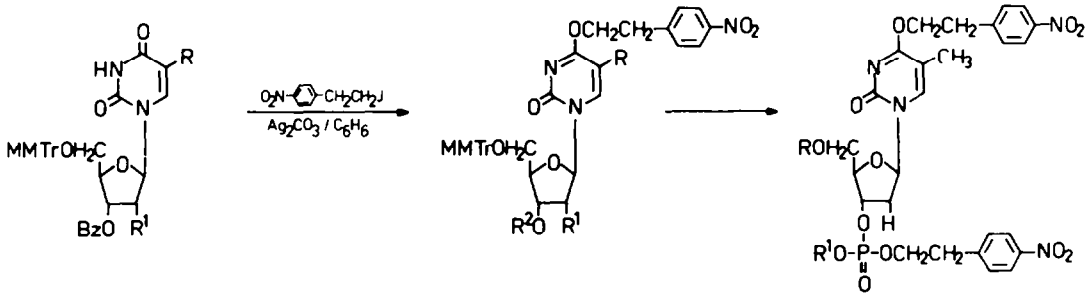
More extended studies of the phosphotriester approach recently also revealed some so far unknown side reactions at the aglycone residues of thymidine and uridine.[52] Condensing agents of the type of arenesulphonyl 1,2,4-triazolide, nitrotriazolide and tetrazolide respectively as well as phosphorylating agents activate the amide function and lead to 4-azol-1-ylpyrimidin-2-one ribonucleosides and ribonucleotides. 2′,3′,5′-Tri-O-acetyluridine has been converted by 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT) into 2′,3′,5′-tri-O-acetyl-4-(3-nitro-1,2,4-triazol-1-yl)-1-β-D-ribofuranosylpyrimidin-2-one[52,55] and protected uridines and the less reactive thymidines have behaved similarly on treatment with *p*-chlorophenyl phosphorochloridate and 1,2,4-triazole.[61–63] It is noteworthy that this base-modification introduces the possibility of nucleophilic displacement reactions leading to cytidine derivatives with ammonia and amines, respectively, as well as to the corresponding 4-hydrazino- and 4-methoxypyrimidin-2-one nucleosides. A first successful amide protection at O^4 in uridine has recently been achieved by phenol and 2,4-dimethylphenol,[55] two blocking groups which can be cleaved simultaneously with the substituted aryl phosphotriester functions by the oximate procedure.

Our own efforts in this connection have been directed towards the introduction of the *p*-nitrophenylethyl group onto O.[4] Since the Mitsunobu reaction[59] does not afford O^4 but N-3 substitution, the silver-catalysed S_N1-type alkylation with *p*-nitrophenylethyl iodide in benzene was investigated. 3′-O-Benzoyl-5′-O-monomethoxytritylthymidine (**16**) and 2′,3′-di-O-benzoyl-5′-O-monomethoxytrityluridine (**17**), respectively, reacted in 65 and 66% yields to give the corresponding O^4-*p*-nitrophenylethyl derivatives **18** and **19**, which can be debenzoylated with ammonia in methanol or dioxan to give O^4-*p*-nitrophenylethyl-5′-O-monomethoxytrityl-thymidine (**20**) and -uridine (**21**), respectively.

Phosphorylation of **20** with 2,5-dichlorophenyl phosphorodichloridate in the presence of 1,2,4-triazole and subsequent treatment with 2-(*p*-nitrophenyl)-ethanol gave 5′-O-monomethoxytrityl-O^4-*p*-nitrophenylethylthymidine-3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (**22**) in 82% yield. Deblocking of the monomethoxytrityl group by *p*-toluenesulfonic acid in methylene chloride/methanol led to the 5′-unblocked phosphotriester **23** in 81% yield, and oximate treatment of **22** resulted in the formation of the corresponding 3′-*p*-nitrophenylethylphosphodiester **24**, which was isolated as its triethylammonium salt in 97% yield.

2′-Deoxycytidine and cytidine protection

Amino protection in the 2′-deoxycytidine and cytidine series is usually effected by N^4-acylation involving for example the acetyl, pivaloyl, benzoyl,[64,65] and substituted benzoyl groups. Comparative studies concerned with the stability of N^4-acylated 2′-deoxycytidines[66] indicate a distinct increase in the stability of the amide bond towards base-promoted deacylation in substituting aliphatic for aromatic acyl groups. As expected, electron-donating substituents, especially in *p*-position, further enhanced stability as is exemplified by the anisoyl and *p*-dimethylaminobenzoyl groups. However, an even stronger effect can be achieved by the steric shielding of an *o*-methylphenyl group on the carbonyl function. Thus, the 2-methyl- and 2,4-dimethylbenzoyl groups are, so far, the most stable 4-amino protecting groups in this series.



	R	R^1
16	CH_3	H
17	H	OBz

	R	R^1	R^2
18	CH_3	H	Bz
19	H	OBz	Bz
20	CH_3	H	H
21	H	OH	H

	R	R^1
22	MMTr	2,5-$Cl_2C_6H_3$
23	H	2,5-$Cl_2C_6H_3$
24	MMTr	$Et_3\overset{+}{N}H$

In order to extend our simplified blocking group pattern to include 2′-deoxycytidine and cytidine derivatives, we investigated the use of the *p*-nitrophenylethoxycarbonyl group for this purpose. Based on observations of Butula,[67,68] that 1-alkoxycarbonyl-benzotriazoles selectively acylate amino groups in presence of OH functions, 1-(*p*-nitrophenylethoxycarbonyl)benzotriazole has been synthesized as the most potentially promising acylating agent. Reactions with unprotected 2′-deoxycytidine (**25**) and cytidine (**26**) proceed smoothly in DMF at 60° and lead in, 90% yields, to the corresponding N^4-*p*-nitrophenylethoxycarbonyl derivatives **27** and **28**. This approach appears to have an advantage over the recently published N-benzoyloxycarbonylation by 1-(benzyloxycarbonyl)-3-ethyl-imidazolium tetrafluoroborate.[69]

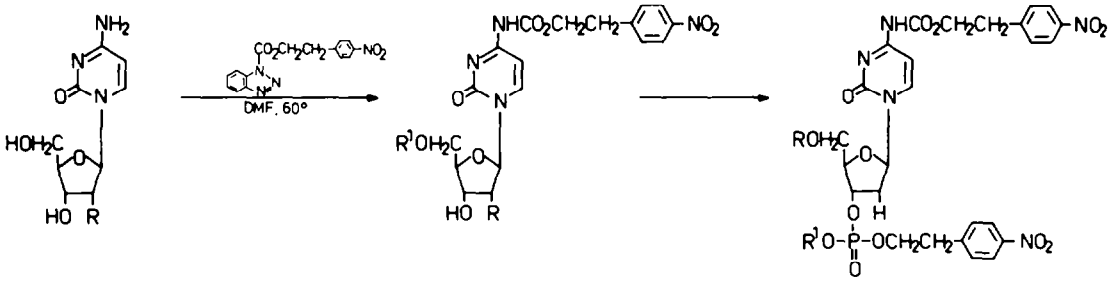
The new blocking group is stable against ammonia and triethylamine in methanol, dioxane, and water but can be cleaved quantitatively to regenerate the starting materials by DBN or DBU in aprotic solvents. Methoxytritylation of **27** proceeds in 88% yield to 5′-O-monomethoxytrityl-N^4-*p*-nitrophenylethoxycarbonyl-2′-deoxycytidine (**29**) and phosphorylation with 2,5-dichlorophenyl phosphorodichloridate, 1,2,4-triazole, and 2-(*p*-nitrophenyl)-ethanol leads in the usual manner to the fully protected 5′-O-monomethoxytrityl-N^4-*p*-nitrophenylethoxycarbonyl-2′-deoxycytidine-3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (**30**) in 86% yield. Detritylation of the phosphotriester **30** with *p*-toluenesulfonic acid in methylene chloride/methanol occurs almost quantitatively to give **31**, and oximate-promoted cleavage of the 2,5-dichlorophenyl groups leads to the protected phosphodiester **32**, which was isolated as its triethylammonium salt in 93% yield.

2′-Deoxyadenosine and adenosine protection

The N^6-benzoyl group has long proved to be satisfactory for the protection of the aglycone moiety of adenosine and 2′-deoxyadenosine derivatives in both the phosphodi- and phosphotri-ester approaches. The latter protecting group is more stable than its 4-chloro, 3,4-dichloro-, and 4-nitro-derivatives; it is even more stable than its aliphatic counterparts such as isobutyryl and phenylacetyl.[71] Some increase in stability can be obtained by the anisoyl group[70] and the more lipophilic 4-t-butylbenzoyl residue[71] reveals some advantages from the point of view of synthesis and isolation of protected oligonucleotides by the phosphotriester method.

p-Nitrophenylethoxycarbonylation of the 6-amino group in 2′-deoxy-(**33**) and adenosine (**34**) could not be achieved directly with 1-*p*-nitrophenylethoxycarbonyl)-benzotriazole in the same way as in the cytosine series. This is due to the lower nucleophilic potential of the amino function in the adenosine series. The more reactive *p*-nitrophenylethyl chloroformate, however, smoothly acylates 3′,5′-di-O-acetyl-2′-deoxyadenosine (**35**) and 2′,3′,5′-tri-O-acetyladenosine (**36**), respectively, in pyridine to give mixtures of the N^6-mono- and di-*p*-nitrophenylethoxycarbonyl derivatives which can be selectively deprotected on treatment with aqueous ammonia in methanol to give N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxy-(**40**) and -adenosine (**41**) in 82 and 85% overall yields, respectively. By analogy with recent reports,[69,73] monoalkoxycarbonylation of **35** and **36** can be effected directly with 1-methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride. The latter reagent was prepared from *p*-nitrophenylethyl chloroformate and N-methylimidazole in methylene chloride, and its use led to crystalline 3′,5′-di-O-acetyl-N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine (**38**) and the 2′,3′,5′-tri-O-acetyl-N^6-*p*-nitrophenylethoxycarbonyladenosine (**39**) in 85% yields. Deacetylation with triethylamine in methanol then proceeded almost quantitatively to give **40** and **41**, respectively, in 95% isolated yields. An even more simple one-pot reaction leading to **40** was developed starting from **33**, which was treated first with 1-trimethylsilylimidazole in methylene chloride to form selectively the 3′,5′-bis-O-trimethylsilyl-2′-deoxyadenosine (**37**). Addition of 1-methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride causes monoacylation to **42** and work-up with ammonia in aqueous methanol leads to N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine (**40**) in 73% overall yield.

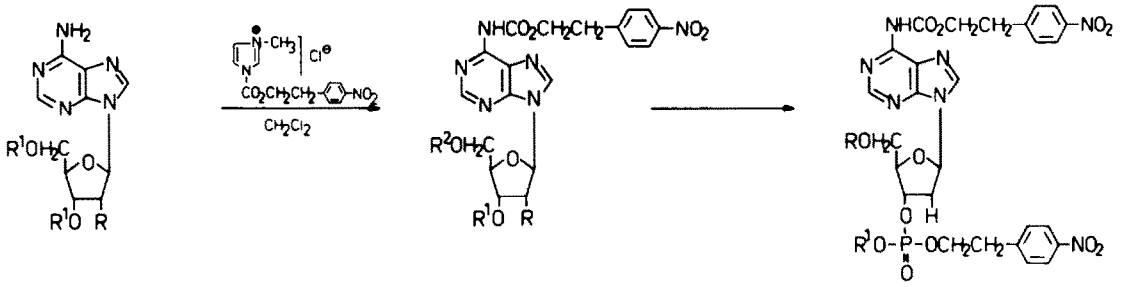
Deprotection of the amino blocking group to regenerate the starting materials **33** and **34** may again



	R
25	H
26	OH

	R	R^1
27	H	H
28	OH	H
29	H	MMTr

	R	R^1
30	MMTr	2,5-$Cl_2C_6H_3$
31	H	2,5-$Cl_2C_6H_3$
32	MMTr	$Et_3\overset{+}{N}H$



	R	R^1
33	H	H
34	OH	H
35	H	Ac
36	OAc	Ac
37	H	$Si(CH_3)_3$

	R	R^1	R^2
38	H	Ac	Ac
39	OAc	Ac	Ac
40	H	H	H
41	OH	H	H
42	H	$Si(CH_3)_3$	$Si(CH_3)_3$
43	H	H	MMTr

	R	R^1
44	MMTr	2,5-$Cl_2C_6H_3$
45	H	2,5-$Cl_2C_6H_3$
46	MMTr	$Et_3\overset{+}{N}H$

best be effected with DBN or DBU in pyridine. Monomethoxytritylation of **40** could be performed in the usual manner with monomethoxytrityl chloride in pyridine to 5′-O-monomethoxytrityl-N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine (**43**) in high yield and further phosphorylation with 2,5-dichlorophenyl phosphorodichloridate, 1,2,4-triazole, and 2-(*p*-nitrophenyl)-ethanol in pyridine afforded the fully protected monomeric nucleotide building block, 5′-O-monomethoxytrityl-N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine-3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (**44**). During the detritylation experiments it was noticed that the carbamate function in the aglycone moiety seems to be more stable towards acid cleavage than the N^6-benzoyl group since the phosphotriester intermediate **45** was isolated in 93% yield, whereas, due to particle debenzoylation, the yield of the corresponding benzoyl derivative was always only 85%. Furthermore, preliminary qualitative chromatographic studies also indicate a somewhat enhanced stability of the glycosidic bond in **44** and **45** compared to the N^6-benzoyl analogs. Quantitative measurements relating to these observations are under investigation and these results will soon be published elsewhere. Finally oximate cleavage of the 2,5-dichlorophenyl group in **44** led to the phosphodiester **46** in high yield. The amino blocking group was completely stable to the conditions of *p*-nitrobenzaldoxime/triethylamine treatment in dioxan/water.

Structure and characterization

The structural assignments of the newly synthesized compounds are based on the well-defined constitutions of the starting materials whereby C, H, N-elementary analysis prove the empirical formulae and high resolution ^{1}H-NMR-spectra ensure in addition the correctness of the assigned structures (Table 1). In the *p*-nitrophenylethyl substituted nucleosides and nucleotides, the AA′BB′-pattern of the *p*-nitrophenyl group attracts very special attention due to its characteristic signals which are listed in Table 1 together with the chemical shifts of the O–CH_2-triplet and the anomeric protons. The 250 MHz-spectra, in general, show clearly separated signals as may be seen for example, from the spectrum of N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine (**40**).

The purity of the compounds has been checked by chromatographic means and further structural support is provided by the UV-absorption spectra, measured in methanol solution.

EXPERIMENTAL

UV-absorption spectra were measured with a Cary 118 spectrometer. ^{1}H-NMR-spectra are taken at 250 MHz with a Bruker WM-250 spectrometer; as standards have the $CHCl_3$ and DMSO signals been used. Schleicher and Schüll precoated silica gel sheets F 1500 LS 254 were used for TLC, which was performed in the following solvent systems: A [$CHCl_3$–MeOH (19:1)]; B [$CHCl_3$–MeOH (9:1)]; C [$CHCl_3$–MeOH–Et_3N (90:5:5)];D [toluene–EtOAc–MeOH (5:4:1)]; E [toluene–EtOAc (7:3)]. Merck silica gel PF_{254} was used for separations on preparative plates (40 × 20 × 0.2 cm) and Merck particle size 0.05–0.2 mm for preparative column chromatography. Drying of the substances was achieved in a Büchi oven T0-50 under high vacuum over silica gel blue (Merck). Absol. pyridine was obtained by refluxing with *p*-toluenesulfonyl chloride, distillation, treatment with CaH_2, distillation and storage over molecular sieve. The other solvents have been dried and purified in the usual manner. M.ps are not corrected. Several compounds have not been obtained in crystalline form but as amorphous solids on evaporation and drying in high vacuum.

N^2,3′,5′-Triisobutyryl-2′-deoxyguanosine (**1**)[65]

Compound **4** (0.156 g, 0.25 mmol) was treated with a 0.5 molar soln of DBU in abs pyridine (5 ml) for 1 hr at room temp. It was then neutralized with 1 M AcOH (2.5 ml) and evaporated to dryness. The residue was purified by preparative TLC on a silica gel plate (40 × 20 × 0.2 cm) using $CHCl_3$–MeOH (19:1). The main band was eluted by $CHCl_3$–MeOH (1:1) and yielded on evaporation and drying in high vacuum N^2,3′,5′-triisobutyryl-2′-deoxyguanosine as

Table 1. Physical data of *p*-nitrophenylethyl substituted nucleosides and nucleotides

Compound	UV-Spectra in Methanol λ_{max} (nm)	UV-Spectra in Methanol lg ε	^{1}H-NMR-Spectra (δ-values in ppm) o-H-phenyl	m-H-phenyl	1'-H	-O-CH_2-CH_2-	Solvent
4	218 269	4.42 4.40	7.49d	8.13d	6.33t	4.81t	$CDCl_3$
5	217 268	4.46 4.46	7.50d	8.14d	6.02d	4.82t	$CDCl_3$
6	218 269	4.44 4.42	7.64d	8.16d	6.31t	4.77t	D_6-DMSO
7	228 268 [282]	4.43 4.40 [4.25]	7.65d	8.17d	5.88d	4.78t	D_6-DMSO
8	250 279	4.16 4.25	7.62d	8.17d	6.19t	4.65t	D_6-DMSO
9	251 278	4.17 4.26	7.62d	8.17d	5.76d	4.66t	D_6-DMSO
10	230 270	4.77 4.55	*	8.16d	6.25d	4.89m[a]	$CDCl_3$
11	230 271	4.31 4.49	7.62d	8.16d	5.92d	4.75t	D_6-DMSO
12	[236] 269	[4.32] 4.45	7.64d	8.16d	6.34t	4.77t	D_6-DMSO
13	[222] 270 [282]	[4.71] 4.56 [4.43]	7.48d 7.35d	8.07d 8.13d	6.33t	4.83t	$CDCl_3$
14	[236] 270 [282]	[4.35] 4.58 [4.47]	7.46d 7.49d	8.01d 8.04d	6.34t	4.79t	$CDCl_3$
15	217 270 [282]	4.62 4.54 [4.40]	7.46d 7.36d	8.08d 8.08d	6.21t	4.77t	$CDCl_3$
18	[227] 275 [280]	[4.54] 4.25 [4.25]	*	8.15d	6.53t	4.63t	$CDCl_3$
19	273 [280]	4.31 [4.28]	*	8.15d	5.92	4.61t	$CDCl_3$
20	275 [280]	4.18 [4.17]	7.40d	8.14d	6.37	4.60t	$CDCl_3$
21	274 [280]	4.25 [4.23]	7.41d	8.16d	5.80d	4.65t	$CDCl_3$
22	273 [280]	4.35 [4.32]	* *	8.10 8.17	6.45m[a]	4.65t	$CDCl_3$
23	273 [280]	4.38 [4.36]	7.30d 7.36d	8.12d 8.16d	6.04	4.63t 4.46dt	$CDCl_3$
24	275 [280]	4.38 [4.37]	* *	8.01d 8.13d	6.33	4.60t 4.04t	$CDCl_3$
27	242 281	4.24 4.16	7.59d	8.15d	6.08t	4.35t	D_6-DMSO
28	243 280	4.23 4.14	7.60d	8.17d	5.76d	4.35t	D_6-DMSO
29	235 [277] 281	4.41 [4.16] 4.17	7.40d	8.20d	6.28t	4.40t	$CDCl_3$
30	229 273	4.56 4.41	7.38d *	8.07d 8.14d	6.25m[a]	4.40t	$CDCl_3$
31	245 273	4.38 4.40	7.36d 7.38d	8.11d 8.14d	6.17m[a]	4.40t	$CDCl_3$
32	274	4.41	* *	8.02d 8.18d	6.20m[a]	4.37t	$CDCl_3$
38	267	4.45	7.39d	8.11d	6.44d	4.53t	$CDCl_3$
39	267	4.46	7.40d	8.14d	6.18d	4.52t	$CDCl_3$
40	267	4.44	7.60d	8.16d	6.42t	4.30t	D_6-DMSO
41	267	4.45	7.61d	8.17d	5.99d	4.40t	D_6-DMSO
43	268	4.45	7.40d	8.12d	6.46t	4.52t	$CDCl_3$
44	267	4.59	7.40d *	8.06d 8.13d	6.42m[a]	4.52t	$CDCl_3$
45	267	4.58	7.38d 7.42d	8.12d 8.13d	6.28m[a]	4.50t	$CDCl_3$
46	267	4.58	7.40d *	8.02d 8.13d	6.47t	4.50t	$CDCl_3$

s = singlet, d = doublet, t = triplet, m = multiplet;

* = covered by aromatic H, a) = complex splitting due to the presence of diastereomeric mixtures.

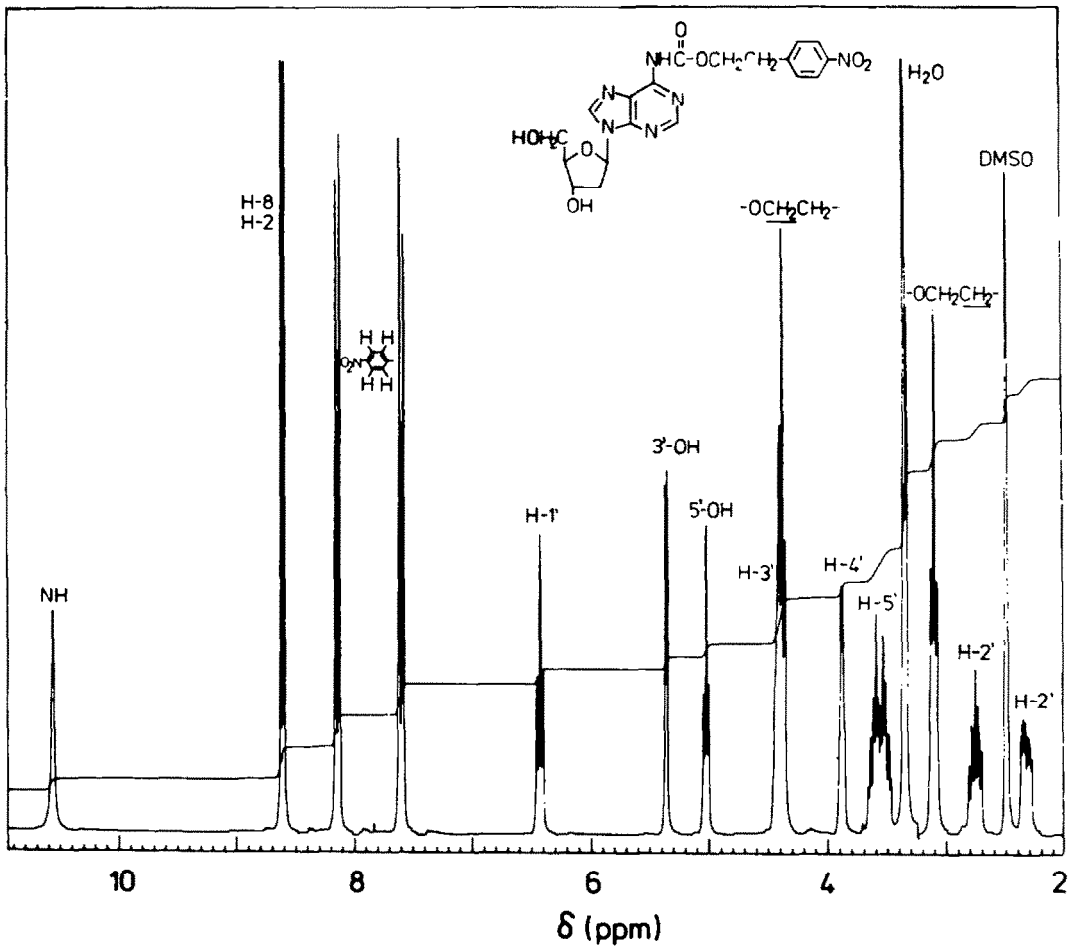


Fig. 1. 250 MHz-^{1}H-NMR spectrum of N^6-*p*-nitrophenylethoxycarbonyl-2'-deoxyadenosine (**40**) in [D$_6$]DMSO.

a chromatographically pure solid foam; yield, 0.1 g (84%). The material was identical with an authentic sample.

N^2,2',3',5'-*Tetraisobutyrylguanosine* (**2**)

Compound **5** (71 mg, 0.1 mmol) was treated with 2 ml of 0.5 molar soln of DBU in abs pyridine for 3 hr at room temp. The soln was then neutralized with 1 ml of 1 molar AcOH, evaporated to dryness and the residue was chromatographed on a preparative silica gel plate (20 × 20 × 0.2 cm) with $CHCl_3$–MeOH (19:1). The main band was eluted and yielded on evaporation and drying N^2,2',3',5-tetraisobutyrylguanosine identical chromatographically and spectrophotometrically with an authentic sample; yield, 46 mg (81%).

N^2,2',3',5'-*Tetrabenzoylguanosine* (**3**)

Compound **10** (85 mg, 0.1 mmol) was treated analogously to the preceding procedure and gave on isolation pure N^2,2',3',5'-tetrabenzoylguanosine; yield, 61 mg (87%). The material was chromatographically and spectrophotometrically identical with an authentic sample.

N^2,3',5' - *Triisobutyryl* - O^6 - p - *nitrophenylethyl* - 2' - *deoxy* - *guanosine* (**4**)

Compound **1**[65] (0.477 g, 1 mmol), triphenylphosphine (0.393 g, 1.5 mmol), diethyl azodicarboxylate (0.261 g, 1.5 mmol), and *p*-nitrophenylethanol (0.25 g, 1.5 mmol) were stirred in 25 ml abs dioxan for 24 hr at room temp. After evaporation to a smaller volume and dilution in 500 ml $CHCl_3$, the soln was washed with water (2 × 100 ml). The $CHCl_3$ layer was separated, dried over Na_2SO_4 and then concentrated to a small volume which was separated on a silica gel column (16 × 2.5 cm). Elution was done first with 300 ml $CHCl_3$, then with a mixture of $CHCl_3$–MeOH (99:1), which eluted the product after a prefraction of 100 ml in the following 600 ml. This fraction was evaporated to dryness and the residue recrystallized from $CHCl_3$–petroleum ether to give N^2,3',5'-triisobutyryl-O^6-*p*-nitrophenylethyl-2'-deoxyguanosine (Found: C, 57.10; H, 5.91; N, 13.11. $C_{30}H_{37}N_6O_9$ (625.7) requires: C, 57.59; H, 5.96; N, 13.43%) as colourless crystals; m.p. 123–126°; yield, 0.529 g (85%); R_f 0.49 (system A).

N^2-2',3',5'-*Tetraisobutyryl*-O^6-p-*nitrophenylethylguanosine* (**5**)

Compound **2**[65] (1.127 g, 2 mmol), diethyl azodicarboxylate (0.52 g, 3 mmol), triphenylphosphine (0.79 g, 3 mmol), and *p*-nitrophenylethanol (0.5 g, 3 mmol) were stirred in 40 ml abs dioxan for 3 hr at room temp. On evaporation to a smaller volume diethyl hydrazinedicarboxylate separated and was filtered off. The filtrate was evaporated to dryness and the residue dissolved in $CHCl_3$ and separated on a silica gel column (20 × 5 cm). Elution with $CHCl_3$ yielded a main fraction which was still contaminated with some triphenylphosphine. Column chromatography was therefore repeated in the same manner. Evaporation to dryness of the main fraction and drying in high vacuum gave pure N^2,2',3',5'-tetraisobutyryl-O^6-*p*-nitrophenylethylguanosine (Found: C, 57.18; H, 6.12; N, 12.01. $C_{34}H_{44}N_6O_{11}$ (712.7) requires: C, 57.29; H, 6.22; N, 11.79%) as colourless amorphous solid; yield, 1.01 g (70%); R_f 0.87 (system A).

N²-Isobutyryl-O⁶-p-nitrophenylethyl-2'-deoxyguanosine (**6**)

(a) Compound **1**[65] (0.477 g, 1 mmol), triphenylphosphine (0.393 g, 1.5 mmol), diethyl azodicarboxylate (0.261 g, 1.5 mmol), and *p*-nitrophenylethanol (0.25 g, 1.5 mmol) were stirred in 25 ml abs dioxan for 24 hr at room temp. After evaporation to 1:4 of the volume, 500 ml $CHCl_3$ was added and then washed with water (2 × 100 ml). The organic layer was dried over Na_2SO_4, evaporated to dryness and then the residue dissolved in 15 ml dioxan. Conc ammonia (20 ml) was added and then stirred for 3 days. Evaporation to dryness and recrystallization of the residue from MeOH gave N²-isobutyryl-O⁶-*p*-nitrophenylethyl-2'-deoxyguanosine (Found: C, 54.09; H, 5.31; N, 17.18. $C_{22}H_{26}N_6O_7$ (486.5) requires: C, 54.23; H, 5.39; N, 17.28%) as colourless crystals; m.p. 210–211°; yield, 0.365 g (75%); R_f 0.13 (system B).

(b) Compound **4** (0.16 g, 0.26 mmol) was dissolved in dioxan (5 ml), conc ammonia (5 ml) added and then stirred at room temp for 3 days. After evaporation to dryness, the residue was purified by preparative TLC on a silica gel plate (20 × 20 × 0.2 cm) in $CHCl_3$–MeOH (4:1). The main band was eluted with $CHCl_3$–MeOH (1:1) and gave on evaporation N²-isobutyryl-O⁶-*p*-nitrophenylethyl-2'-deoxyguanosine as an amorphous, chromatographically pure solid; yield, 0.105 g (85%).

N²-Isobutyryl-O⁶-p-nitrophenylethylguanosine (**7**)

(a) Compound **5** (0.071 g, 0.1 mmol) was treated with a mixture of 1.5 ml MeOH and 1.5 ml 25% aqueous ammonia for 4 hr at room temp. After evaporation to dryness, the residue was purified by preparative TLC on a silica gel plate (20 × 20 × 0.2 cm) $CHCl_3$–MeOH (4:1). The main band was eluted and gave on evaporation and drying N²-isobutyryl-O⁶-*p*-nitrophenylethylguanosine as a colourless solid; yield, 38 mg (76%).

(b) Compound **2**[65] (11.27 g, 20 mmol) was dissolved in 400 ml abs dioxan. After addition of triphenylphosphine (7.88 g, 30 mmol), diethyl azodicarboxylate (5.24 g, 30 mmol), and *p*-nitrophenylethanol (5.0 g, 30 mmol) and stirring for 3 hr at room temp the reaction was evaporated to dryness. The residue was dissolved in 250 ml MeOH and after addition of 250 ml 25% aqueous ammonia stirred for 3 hr at room temp. The soln was neutralized with glacial AcOH, evaporated to dryness and the residue chromatographed on a silica gel column (20 × 9 cm) with $CHCl_3$–MeOH (19:1). The main fraction was collected and gave on evaporation N²-isobutyryl-O⁶-*p*-nitrophenylethylguanosine (Found: C, 52.64; H, 5.14; N, 16.60. $C_{22}H_{26}N_6O_8$ (502.5) requires: C, 52.59; H, 5.22; N, 16.73%) as an amorphous, chromatographically pure solid; yield, 8.7 g (86%). Recrystallization from dioxan or ethanol gave colourless crystals; m.p. 199–201°; R_f 0.39 (system B).

O⁶-p-Nitrophenylethyl-2'-deoxyguanosine (**8**)

Compound **4** (0.16 g, 0.26 mmol) was dissolved in MeOH (50 ml) and after addition of conc aqueous ammonia (50 ml) was stirred for 5 days at room temp. The soln was evaporated and the residue fractionated on silica gel (20 × 20 × 0.2 cm) in the system toluene–EtOAc–MeOH (10:8:3). The main band was eluted with $CHCl_3$–MeOH (1:1) and gave on evaporation and drying in high vacuum, O⁶-*p*-nitrophenylethyl-2'-deoxyguanosine (Found: C, 50.12; H, 4.65; N, 19.15. $C_{18}H_{20}N_6O_6 \times H_2O$ (434.4) requires: C, 49.76; H, 5.10; N, 19.34%) as an amorphous solid; yield, 0.081 g (76%); R_f 0.28 (system B).

O⁶-p-Nitrophenylethylguanosine (**9**)

Compound **7** (1.0 g, 2 mmol) was suspended in MeOH (75 ml) and conc aqueous ammonia (75 ml) and then stirred for 6 days at room temp. After evaporation of the soln to dryness, the residue was recrystallized from water to give O⁶-*p*-nitrophenylethylguanosine (Found: C, 50.18; H, 4.55; N, 19.72. $C_{18}H_{20}N_6O_7$ (432.4) requires: C, 50.00; H, 4.66; N, 19.44%) as yellowish crystal powder; m.p. 108°; yield, 0.51 g (59%); R_f 0.48 (system B).

N²,2',3',5'-Tetrabenzoyl-O⁶-nitrophenylethylguanosine (**10**)

Compound **3** (3.5 g, 5 mmol), triphenylphosphine (2.62 g, 10 mmol), *p*-nitrophenylethanol (1.67 g, 10 mmol), and diethyl azodicarboxylate (1.74 g, 10 mmol) were stirred for 18 hr at room temp leading to a complete soln of all components. The soln was evaporated to dryness, the residue dissolved in $CHCl_3$ and then chromatographed on a silica gel column (20 × 5 cm) with $CHCl_3$. The main fraction was collected, evaporated and the residue recrystallized from CCl_4. The second fraction, which contained some triphenylphosphine, was also evaporated and the residue recrystallized twice from CCl_4 to give pure N²,2',3',5'-tetrabenzoyl-O⁶-nitrophenylethylguanosine (Found: C, 63.52; H, 4.12; N, 9.83. $C_{46}H_{36}N_6O_{11} \times H_2O$ (866.8) requires: C, 63.74; H, 4.42; N, 9.70%) as colourless crystals; m.p. 168°; yield, 3.09 g (71%); R_f 0.91 (system A).

N²-Benzoyl-O⁶-p-nitrophenylethylguanosine (**11**)

Compound **10** (0.17 g, 0.2 mmol) was stirred in saturated methanolic ammonia (3 ml) and dioxan (1 ml) at room temp for 8 days. It was then evaporated and the residue chromatographed on a preparative silica gel plate (40 × 20 × 0.2 cm) with $CHCl_3$–MeOH (9:1). The main band was eluted, the eluate evaporated to an amorphous gel, which crystallized on treatment with MeOH to give N²-benzoyl-O⁶-*p*-nitrophenylethylguanosine (Found: C, 54.99; H, 4.49; N, 15.24. $C_{25}H_{24}N_6O_8 \times 1/2\ H_2O$ (545.5) requires: C, 55.04; H, 4.62; N, 15.41%) as colourless crystals; m.p. 194; yield, 61 mg (57%); R_f 0.41 (system B).

N² - Isobutyryl - 5'-O - monomethoxytrityl-O⁶ - p - nitrophenylethyl - 2' - deoxyguanosine (**12**)

Compound **6** (1.06 g, 2.2 mmol) was evaporated in abs pyridine (3 × 5 ml) and then monomethoxytrityl chloride (1.39 g, 4.5 mmol) and 20 ml abs pyridine was added. After stirring for 24 hr at room temp MeOH (5 ml) was added and the soln evaporated to a smaller volume and then extracted with 800 ml $CHCl_3$. The $CHCl_3$ layer was washed with water (3 × 300 ml), dried over Na_2SO_4, evaporated and finally coevaporated with toluene (3 × 10 ml). The residue was chromatographed on a silica gel column (14 × 2.5 cm) and eluted first with 500 ml $CHCl_3$ and then with 900 ml $CHCl_3$–MeOH (99:1) which contained the product. This fraction was evaporated and the residue reprecipitated from $CHCl_3$ into *n*-hexane with stirring to give N²-isobutyryl-5'-O-monomethoxytrityl-O⁶-*p*-nitrophenylethyl-2'-deoxyguanosine (Found: C, 64.78; H, 5.40; N, 10.40. $C_{42}H_{42}N_6O_8 \times H_2O$ (776.8) requires: C, 64.93; H, 5.71; N, 10.87%) as colourless amorphous powder; yield, 1.437 g (84%); R_f 0.28 (system A).

N² - Isobutyryl - 5' - O - monomethoxytrityl - O⁶ - p - nitrophenylethyl-2'-deoxyguanosine-3'-(2,5-dichlorophenyl, p-nitrophenylethyl)-phosphate (**13**)

Compound **12** (1.137 g, 1.5 mmol) was coevaporated with abs pyridine (3 × 4 ml) and then dissolved in 4 ml abs pyridine. This soln was added dropwise to a mixture of 1,2,4-triazole (0.34 g, 5 mmol) and 2,5-dichlorophenylphosphorodichloridate in 4 ml abs pyridine and stirred in an ice-bath. After 30 min, when the starting material had disappeared (checked by chromatography), *p*-nitrophenylethanol (0.473 g, 2.8 mmol) was added and the mixture stirred at room temp for another 6 hr. Phosphate buffer (5 ml, pH 7) was added to stop the reaction. The mixture was extracted with 800 ml $CHCl_3$, washed with buffer (2 × 200 ml), the organic layer dried over Na_2SO_4, evaporated to dryness and then coevaporated with toluene (2 × 50 ml). The residue obtained was dissolved in a little $CHCl_3$, chromatographed on a silica gel column (25 × 2.5 cm) first with $CHCl_3$ (800 ml) and then $CHCl_3$–MeOH(99:1; 1000 ml) to elute the product. The main fraction was evaporated, the residue dissolved in 10 ml $CHCl_3$ and this soln added slowly dropwise into *n*-hexane (400 ml) with stirring to give N²-isobutyryl-5'-O-monomethoxytrityl-O⁶-*p*-nitrophenylethyl-2'-deoxy-

guanosine-3′-(2,5-dichlorophenyl,*p*-nitrophenylethyl)-phosphate (Found: C, 59.48; H, 4.83; N, 8.53. $C_{56}H_{52}Cl_2N_7O_{13}P$ (1132.9) requires: C, 59.37; H, 4.62; N, 8.65%) as a colourless powder; yield, 1.461 g (86%); R_f 0.33 (system A), R_f 0.53 (system D).

Triethylammonium N²-*isobutyryl*-5′-O-*monomethoxytrityl*-O⁶-p-*nitrophenylethyl*-2′-*deoxyguanosine*-3′-p-*nitrophenylethylphosphate* (**14**)

A soln of *p*-nitrobenzaldoxime (1.66 g, 10 mmol) in dioxan (20 ml), water (20 ml), and Et_3N (20 ml) was stirred for 10 min at room temp and then **13** (1.132 g, 1 mmol) was added. The mixture was stirred for 3 hr, then evaporated to dryness and coevaporated with toluene (2 × 20 ml). The material obtained was dissolved in $CHCl_3$ and chromatographed on a silica gel column (12 × 2.5 cm) first with $CHCl_3$ (400 ml) and then $CHCl_3$–MeOH mixtures (99:1, 300 ml; 98:2, 300 ml; 97:3, 300 ml; 95:5, 300 ml) and finally with $CHCl_3$–MeOH–Et_3N (90:5:5; 500 ml) to elute the product. This fraction was evaporated, then coevaporated with toluene (2 × 20 ml) and the residue dissolved in $CHCl_3$ (8 ml). This soln was added slowly dropwise to *n*-hexane (300 ml) with intensive stirring to give triethylammonium N²-isobutyryl-5′-O-monomethoxytrityl-O⁶-*p*-nitrophenylethyl-2′-deoxyguanosine-3′-*p*-nitrophenylethylphosphate (Found: C, 61.71; H, 6.12; N, 10.29. $C_{56}H_{65}N_8O_{13}P$ (1089.7) requires: C, 61.72; H, 6.01; N, 10.82%) as a colourless, amorphous powder; yield, 0.98 g (90%); R_f 0.39 (system C).

N²-*Isobutyryl*-O⁶-p-*nitrophenylethyl*-2′-*deoxyguanosine*-3′-(2,5-*dichlorphenyl*, p-*nitrophenylethyl*)-*phosphate* (**15**)

Compound **13** (1.132 g, 1 mmol) was added to a 2% soln of *p*-toluene-sulfonic acid in CH_2Cl_2–MeOH (4:1; 20 ml) and stirred for 30 min at room temp. The reaction was stopped by addition of phosphate buffer (20 ml; pH 7) and then extracted several times with $CHCl_3$ (600 ml). The organic layer was washed with buffer (2 × 100 ml), water (100 ml) and then dried over Na_2SO_4. The extract was evaporated to a small volume, chromatographed on a silica gel column (20 × 2.5 cm) and eluted first with $CHCl_3$ (700 ml) and then $CHCl_3$–MeOH (99:1; 800 ml). The latter fraction was again evaporated, the residue dissolved in $CHCl_3$ (10 ml) and this soln added dropwise into *n*-hexane (400 ml) with intensive stirring to give N²-isobutyryl-O⁶-*p*-nitrophenylethyl-2′-deoxyguanosine-3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (Found: C, 50.42; H, 4.32; N, 11.21. $C_{36}H_{32}Cl_2N_7O_{12}P$ (860.6) requires: C, 50.24; H, 4.21; N, 11.39%) as a colourless amorphous powder; yield, 0.741 g (86%); R_f 0.11 (system A), R_f 0.33 (system D).

3′-O-*Benzoyl*-5′-O-*monomethoxytritylthymidine* (**16**)[74]

Compound **18** (0.2 g, 0.26 mmol) and diazabicyclo [5.4.0]undecen (DBU) (0.4 g, 0.26 mmol) in abs pyridine (5 ml) was stirred for 3 hr at room temp. It was neutralized with 1N AcOH (2.6 ml) and evaporated to dryness. The residue was dissolved in $CHCl_3$ (40 ml), washed with phosphate buffer (30 ml; pH 7) and then the $CHCl_3$ layer dried over Na_2SO_4. The soln was chromatographed on a silica gel plate (40 × 20 × 0.2 cm) after evaporation to a small volume and developed in $CHCl_3$–MeOH(19:1). The main band was eluted with the same solvent mixture and gave on evaporation 3′-O-benzoyl-5′-O-monomethoxytritylthymidine as an amorphous solid; yield, 0.13 g (81%). The substance was chromatographically and spectrophotometrically identical with an authentic material.

2′,3′-*Di*-O-*benzoyl*-5′-O-*monomethoxytrityluridine* (**17**)

Compound **19** (0.227 g, 0.26 mmol) was treated analogously and gave after preparative TLC, 2′,3′-di-O-benzoyl-5′-O-monomethoxytrityluridine as a colourless solid foam; yield, 0.166 g (88%). The identity with an authentic sample was checked chromatographically and spectrophotometrically.

p-*Nitrophenylethyliodide*

NaI (8.8 g, 59 mmol) was dissolved in abs ethyl methyl ketone (70 ml) and then *p*-nitrophenylethyl chloride (10.0 g, 54 mmol) in ethyl methyl ketone (30 ml) was added. After boiling under reflux for 24 hr, the ppt was filtered off, washed with acetone and the united filtrates evaporated. The residue was dissolved in $CHCl_3$ (150 ml), washed with a 1% $Na_2S_2O_3$ aq (2 × 50 ml), dried over Na_2SO_4 and then again evaporated. The residue was recrystallized from diethyl ether and little $CHCl_3$ to give *p*-nitrophenylethyliodide (Found: C, 34.73; H, 2.88; N, 4.97. $C_8H_8INO_2$ (277.0) requires: C, 34.68; H, 2.91; N, 5.06%) as yellowish crystals; m.p. 100–101°; yield, 13.9 g (93%).

3′-O-*Benzoyl*-5′-O-*monomethoxytrityl*-O⁴-p-*nitrophenylethylthymidine* (**18**)

Compound **16**[74] (21.0 g, 33.9 mmol) and silver carbonate (14.0 g, 51 mmol) were heated under reflux in abs benzene (190 ml) for 1 hr. After cooling to 50° *p*-nitrophenylethyliodide (19.4 g, 70 mmol) was added and the mixture stirred for 3 days at this temp. The ppt was filtered off and the filtrate evaporated to dryness. The residue was dissolved in CH_2Cl_2 and chromatographed on a silica gel column (400 g) first with CH_2Cl_2 and then with $CHCl_3$. The main fraction was collected and evaporated in vacuum to a solid foam; yield, 17.0 g (65%). A small sample was further purified by preparative TLC on a silica gel plate with $CHCl_3$ and gave after elution, evaporation, and reprecipitation from a little $CHCl_3$ into *n*-hexane 3′-O-benzoyl-5′-O-monomethoxytrityl-O⁴-*p*-nitrophenylethylthymidine (Found: C, 70.15; H, 5.37; N, 5.30. $C_{45}H_{41}N_3O_9$ (767.8) requires: C, 70.39; H, 5.38; N, 5.47%) as an amorphous powder; R_f 0.9 (system A).

2′,3′-*Di*-O-*benzoyl*-5′-O-*monomethoxytrityl*-O⁴-p-*nitrophenylethyluridine* (**19**)

Compound **17** (8.0 g, 11 mmol) and silver carbonate (4.7 g, 17 mmol) were boiled under reflux in abs benzene (90 ml) for 1 hr. After cooling to 50° *p*-nitrophenylethyliodide (6.11 g, 22 mmol) was added and stirred at this temp for another 24 hr. The ppt was filtered off, the filtrate evaporated, the residue dissolved in CH_2Cl_2 and this soln chromatographed on a silica gel column (100 g) in the same solvent. The main fraction was collected, evaporated in vacuum to give a colourless solid foam; yield, 6.37 g (66%). A small sample was further purified by preparative TLC on a silica gel plate and gave after elution, evaporation and reprecipitation from a little $CHCl_3$ into *n*-hexane 2′,3′-di-O-benzoyl-5′-O-monomethoxytrityl-O⁴-*p*-nitrophenylethyluridine (Found: C, 70.33; H, 5.03; N, 4.60. $C_{51}H_{43}N_3O_{11}$ (873.9) requires: C, 70.09; H, 4.96; N, 4.81%) as a colourless amorphous powder; R_f 0.94 (system A).

5′-O-*Monomethoxytrityl*-O⁴-p-*nitrophenylethylthymidine* (**20**)

Compound **18** (1.0 g, 1.3 mmol) was dissolved in dioxan (5 ml) and after addition of a satd soln of ammonia in abs MeOH (10 ml) stirred for 18 hr at room temp. The soln was evaporated to dryness, the residue dissolved in $CHCl_3$ and this soln chromatographed on a silica gel column (40 g) first in $CHCl_3$ and then with gradient $CHCl_3$–MeOH-mixtures (95:5). The main fraction was collected and gave on evaporation in vacuum a colourless solid foam; yield, 0.7 g (80%). A small sample was further purified by preparative TLC and reprecipitation from a little $CHCl_3$ into *n*-hexane to give 5′-O-monomethoxytrityl-O⁴-*p*-nitrophenylethylthymidine (Found: C, 68.96; H, 5.70; N, 6.08. $C_{38}H_{37}N_3O_8$ (663.7) requires: C, 68.76; H, 5.62; N, 6.33%) as a colourless amorphous powder; R_f 0.50 (system A).

5′-O-*Monomethoxytrityl*-O⁴-p-*nitrophenylethyluridine* (**21**)

Compound **19** (1.0 g, 1.14 mmol) was dissolved in dioxan (5 ml) and then a satd soln of ammonia in abs MeOH (10 ml) was added. It was stirred for 24 hr at room temp and

then the soln evaporated to dryness. The residue was dissolved in $CHCl_3$, and chromatographed on a silica gel column (40 g) first with $CHCl_3$ and then with $CHCl_3$–MeOH (98:2). The main fraction was collected and gave on evaporation in vacuum 5'-O-monomethoxytrityl-O⁴-*p*-nitrophenylethyluridine (Found: C, 66.86; H, 5.39; N, 6.25. $C_{37}H_{35}N_3O_9$ (666.0) requires: C, 66.75; H, 5.29; N, 6.31%) as a colourless amorphous foam; yield, 0.628 g (82%); R_f 0.55 (system A).

5'-O-Monomethoxytrityl-O⁴-p-nitrophenylethylthymidine-3'-(2,5-dichlorophenyl, p-nitrophenylethyl)-phosphate (**22**)

Compound **20** (3.0 g, 4.5 mmol) was dissolved in abs pyridine (10 ml) and then added dropwise to a soln of 1,2,4-triazole (0.94 g, 13.6 mmol) and 2,5-dichlorophenylphosphorodichloridate (1.77 g, 6.33 mmol) in abs pyridine (5 ml) which had previously been stirred for 15 min in an ice-bath. The mixture was then stirred for 30 min at room temp, *p*-nitrophenylethanol (1.37 g, 8.2 mmol) added and stirring continued for another 3.5 hr. The soln was then evaporated to a small volume, extracted with $CHCl_3$ (150 ml) and washed with phosphate buffer pH 7 (50 ml). The $CHCl_3$ layer was dried over Na_2SO_4, evaporated to dryness and the residue dissolved again in CH_2Cl_2. This soln was chromatographed on a silica gel column (80 g) first with CH_2Cl_2 and then with $CHCl_3$. The main fraction was collected and gave on evaporation in vacuum a colourless solid foam; yield, 3.87 g (82%). A small sample was further purified by preparative TLC on silica gel with $CHCl_3$ and repricipitated from a little $CHCl_3$ into *n*-hexane to give 5'-O-monomethoxytrityl-O⁴-*p*-nitrophenylethylthymidine - 3' - (2,5 - dichlorophenyl, *p* - nitrophenylethyl)-phosphate (Found: C, 60.25; H, 5.05; N, 5.24. $C_{52}H_{47}Cl_2N_4O_{13}P$ (1037.8) requires: C, 60.18; H, 4.56; N, 5.39%) as a colourless amorphous powder; R_f 0.90 (system A).

O⁴-p-Nitrophenylethylthymidine-3'-(2,5-dichlorophenyl, p-nitrophenylethyl)phosphate (**23**)

Compound **22** (1.0 g, 1.09 mmol) was treated with a 2% soln of *p*-toluenesulfonic acid in CH_2Cl_2–MeOH (4:1) (20 ml) for 30 min at room temp. The mixture was diluted with $CHCl_3$ (100 ml), washed with phosphate buffer pH 7 (2 × 50 ml) and then the organic layer dried over Na_2SO_4. The extract was evaporated to dryness, the residue dissolved in a little $CHCl_3$ and chromatographed on a silica gel column (40 g) with $CHCl_3$. The main fraction gave on evaporation and drying in vacuum a colourless solid foam; yield, 0.68 g (81%). A small sample was further purified by preparative TLC on a silica gel plate in $CHCl_3$. The main band gave on elution, evaporation, and reprecipitation from $CHCl_3$ into *n*-hexane O⁴-*p*-nitrophenylethylthymidine-3'-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (Found: C, 50.38; H, 404; N, 7.08. $C_{32}H_{31}Cl_2N_4O_{12}P$ (765.5) requires: C, 50.20; H, 4.08; N, 7.32%) as a colourless amorphous powder; R_f 0.6 (system A).

Triethylammonium 5' - O - monomethoxytrityl - O⁴ - p - nitrophenylethylthymidine-3'-p-nitrophenylethylphosphate (**24**)

p-Nitrobenzaldoxime (1.66 g, 10 mmol) was dissolved in a mixture of dioxan (20 ml), water (20 ml), and Et_3N (20 ml) and stirred for 10 min. Then **22** (1.037 g, 1 mmol) was added and the soln stirred for 30 min. The mixture was evaporated to dryness, coevaporated with abs pyridine (2 × 10 ml) and abs toluene (2 × 10 ml). The residue was dissolved in a little $CHCl_3$ and chromatographed on a silica gel column (20 g) for separation first with $CHCl_3$–MeOH (19:1) to elute the reagents and then with $CHCl_3$–MeOH–Et_3N (90:5:5) to give the product. The main fraction was collected and formed on evaporation in vacuum a solid foam; yield, 0.964 g (97%). A small sample was further purified by chromatography on a preparative silica gel plate with the system $CHCl_3$–MeOH–Et_3N (90:5:5) to yield triethylammonium 5'-O-monomethoxytrityl-O⁴-*p*-nitrophenylethylthymidine-3'-*p*-nitrophenylethyl phosphate (Found: C, 60.61; H, 5.95; N, 6.74. $C_{52}H_{60}N_5O_{13}P \times 2H_2O$ (1030.0) requires: C, 60.63; H, 6.26; N, 6.79%) as a colourless amorphous powder; R_f 0.42 (system C).

1-(p-Nitrophenylethoxycarbonyl)-benzotriazole

2-(*p*-Nitrophenylethyl)ethanol (8.35 g, 50 mmol) and 1-(chlorocarbonyl)-benzotriazole (9.05 g, 50 mmol) were stirred in cold abs CH_2Cl_2 (70 ml) at 0–5° and then a soln of Et_3N (10 ml) in CH_2Cl (10 ml) was added dropwise. After 1 hr stirring the soln was warmed to room temp and kept for another 30 min. Then CH_2Cl_2 (250 ml) was added and the soln shaken with ice-water (3 × 200 ml). The organic layer was dried over Na_2SO_4 and then evaporated to dryness. The residue was recrystallized from abs benzene (120 ml) to give 1-(*p*-nitrophenylethoxycarbonyl)-benzotriazole (Found: C, 57.59; H, 3.79; N, 17.95. $C_{15}H_{12}N_4O_4$ (312.3) requires: C, 57.69; H, 3.87; N, 17.94%) as yellowish crystals; m.p. 135–136°; yield, 14.4 g (92%); ^{1}H-NMR[$CDCl_3$, 250 MHz]: δ = 3.37 (2H, t), 4.88 (2H, t), 7.52 (1H, m), 7.54 (2H, d), 17.65 (1H, m), 8.05 (1H, d), 8.22 (2H, d); R_f 0.65 (system E).

N⁴-p-Nitrophenylethoxycarbonyl-2'-deoxycytidine (**27**)

Compound **25** (3.2 g, 14 mmol) and 1-(p-nitrophenylethoxycarbonyl)-benzotriazole (4.7 g, 15 mmol) were heated in abs DMF (42 ml) to 60° for 15 hr. The DMF was distilled off in vacuum and the residue treated with a mixture of 1,2-dichloroethane (30 ml) and water (30 ml) with vigorous shaking. The ppt was filtered off, washed with water and dried at 50° to give N⁴-*p*-nitrophenylethoxycarbonyl-2'-deoxycytidine (Found: C, 50.63; B, 4.67; N, 13.03. $C_{18}H_{20}N_4O_8 \times 0.5\ H_2O$ (429.4) requires: C, 50.35; H, 4.93; N, 13.05%) as colourless crystals; yield, 5.35 g (91%). The compound could be recrystallized from MeOH; m.p. 114–120°; R_f 0.28 (system A).

N⁴-p-Nitrophenylethoxycarbonyl cytidine (**28**)

Cytidine **26** (1.21 g, 5 mmol) and 1-(p-nitrophenylethoxycarbonyl)-benzotriazole (1.56 g, 5 mmol) were heated in abs DMF (20 ml) to 60° for 20 hr with stirring. The DMF was evaporated under high vacuum at a bath temp of 30°. The remaining oil was shaken with a mixture of water (20 ml) and 1,2-dichloroethane, whereby a solid was formed. This material was filtered off by suction, washed with water and dichloroethane and then dried at 60° in an oven to give N⁴-*p*-nitrophenylethoxycarbonylcytidine (Found: C, 47.36; H, 4.51; N, 12.29. $C_{18}H_{20}O_9 \times H_2O$ (454.4) requires: C, 47.58; H, 4.88; N, 12.33%) as a colourless powder, m.p. 85–89°; yield, 1.96 g (90%). The compound can be recrystallized from MeOH; R_f 0.30 (system B).

5'-O-Monomethoxytrityl-N⁴-p-nitrophenylethoxycarbonyl-2'-deoxycytidine (**29**)

Compound **27** (1.68 g, 4 mmol) was evaporated with abs pyridine (2 × 20 ml) and then dissolved in abs pyridine (15 ml). *p*-Monomethoxytrityl chloride (1.48 g, 4.8 mmol) was added and the mixture stirred for 16 hr at room temp. The soln was then diluted with phosphate buffer pH 7 (100 ml) and extracted with $CHCl_3$ (1 × 100 ml, 2 × 50 ml). The extracts were washed with phosphate buffer and then dried over Na_2SO_4. The filtrate was evaporated, then coevaporated with abs toluene (3 × 20 ml) and finally applied for short column chromatography[75] (30 g) first with 1,2-dichloroethane and with $CHCl_3$ to remove the reagents and impurities. The product was eluted by a gradient $CHCl_3$–MeOH (100:1 till 100:4). The main fraction was evaporated and gave on coevaporation with CH_2Cl_2 in vacuum 5'-O-monomethoxytrityl-N⁴-*p*-nitrophenylethoxycarbonyl-2'-deoxycytidine (Found: C, 65.67; H, 5.17; N, 7.85. $C_{38}H_{36}N_4O_9$ (692.7) requires: C, 65.89; H, 5.24; 8.09%) as an amorphous solid foam; yield, 2.44 g (88%); R_f 0.34 (system A).

5'-O-Monomethoxytrityl-N⁴-p-nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-(2,5-dichlorophenyl, p-nitrophenylethyl)-phosphate (**30**)

1,2,4-Triazole (0.33 g, 4.8 mmol) was evaporated with abs pyridine (10 mmol) and then the residue dissolved in abs pyridine (6 ml). 2,5-Dichlorophenylphosphorodichloridate was added and the mixture stirred for 20 min at room temp. It was then cooled with ice and a soln of **29** (1.04 g, 1.5 mmol) in abs pyridine (10 ml) added dropwise with stirring. After 1 hr, the starting material had disappeared completely (checked chromatographically). As a second component 2(p-nitrophenyl)-ethanol (0.5 g, 3 mmol) was added and the mixture stirred for 24 hr at room temp. The soln was diluted with phosphate buffer pH 7 (100 ml) and then extracted with $CHCl_3$ (3 × 50 ml). The organic layer was dried over Na_2SO_4, filtered, the filtrate evaporated to dryness and then coevaporated with toluene (3 × 20 ml). The residue was applied for short column chromatography[75] (30 g) eluting subsequently with toluene and toluene–EtOAc (9:1 till 6:4) to remove the reagents and finally with a gradient toluene–EtOAc (1:1 till 1:2) to isolate the product. The main fraction was collected, evaporated and co-evaporated with $CHCl_3$ (50 ml) and CH_2Cl_2 (2 × 50 ml) to give 5'-O-monomethoxytrityl-N⁴-*p*-nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (Found: C, 58.73; H, 4.26;N, 6.45; $C_{52}H_{46}Cl_2N_5O_{14}P$ (1066.8) requires: C, 58.54; H, 4.35; N, 6.56%) as a colourless amorphous solid foam; yield, 1.38 g (86%). Reprecipitation from $CHCl_3$ into *n*-hexane formed a colourless amorphous powder; R_f 0.62 (system A).

N⁴-p-Nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-(2,5-dichlorophenyl, p-nitrophenylethyl)-phosphate (**31**)

Compound **30** (0.32 g, 0.3 mmol) was treated with a 2% soln of *p*-toluenesulfonic acid in CH_2Cl_2–MeOH (4:1) for 20 min at room temp. After dilution with $CHCl_3$ (40 ml), the soln was shaken with phosphate buffer pH 7 (3 × 40 ml), the organic layer then dried over Na_2SO_4 and evaporated to dryness. The residue was fractionated by column chromatography on silica gel (12 × 2 cm) first with $CHCl_3$ and then with $CHCl_3$–MeOH (98:2). The main fraction was collected and gave on evaporation and drying at 50° in high vacuum N⁴-*p*-nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (Found: C, 48.57; H, 3.91; N, 8.68. $C_{32}H_{30}Cl_2N_5O_{13}P$ (794.5) requires: C, 48.38; H, 3.81; N, 8.82%) as a colourless solid foam; yield, 0.23 g (95%). Reprecipitation from little $CHCl_3$ into *n*-hexane yielded an amorphous powder; R_f 0.40 (system A).

Triethylammonium 5'-O-monomethoxytrityl-N⁴-p-nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-p-nitrophenylethyl-phosphate (**32**)

4-Nitrobenzaldoxime (0.5 g, 3 mmol) was dissolved in a mixture of dioxan (6 ml), water (6 ml), and Et_3N (6 ml) and stirred for 20 min at room temp. Then 5'-O-monomethoxytrityl-N⁴-*p*-nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (0.32 g, 0.3 mmol) was added and stirring continued for another 40 min. The mixture was then evaporated in vacuum and coevaporated with abs pyridine (10 ml) and toluene (3 × 10 ml) before chromatography of the residue on a silica gel column (10 × 2 cm) with $CHCl_3$ and $CHCl_3$–MeOH (95:5) to remove the reagents and finally with $CHCl_3$–MeOH–Et_3N (95:5:5) to elute the product. The main fraction was collected, evaporated in vacuum and coevaporated with toluene (10 ml), $CHCl_3$ (20 ml), and CH_2Cl_2 (2 × 20 ml) to give triethylammonium 5'-O-monomethoxytrityl-N⁴-*p*-nitrophenylethoxycarbonyl-2'-deoxycytidine-3'-*p*-nitrophenylethyl-phosphate (Found: C, 60.80; H, 5.96; N, 8.14. $C_{52}H_{59}N_6O_{14}P$ (1023.1) requires: C, 61.05; H, 5.81; N, 8.21%) as a colourless amorphous foam; yield, 0.285 g (93%); R_f 0.25 (system C).

2-(p-Nitrophenyl)ethyl chloroformate

Phosgene (70 g, 0.7 mol) was dissolved in abs toluene (200 ml) with ice-cooling. A soln of 2-(p-nitrophenyl)-ethanol (33.4 g, 0.2 mol) in a mixture of abs CH_2Cl_2 (100 ml) and abs toluene (50 ml) was added dropwise with stirring. After stirring at room temp for 1 hr the temp was raised slowly to 50° within 5 hr. After cooling, the mixture was evaporated in vacuum. The residue started to crystallize, it was then dried in high vacuum at room temp to give pure 2-(p-nitrophenyl-ethyl chloroformate (Found: C, 47.31; H, 3.46; N, 6.21. $C_9H_8ClNO_4$ (229.6) requires: C, 47.08; H, 3.51; N, 6.10%) as yellowish crystals which could be used in this form for further reactions; yield, 46.0 g (100%). The material could be recrystallized from abs toluene to yield yellowish crystals, m.p. 42°; ^{1}H-NMR[$CDCl_3$, 250 MHz]: δ = 3.14 (2H, t), 4.53 (2H, t), 7.38 (2H, d), 8.15 (2H, d).

1-Methyl-3-p-nitrophenylethoxycarbonylimidazolium chloride

2-(p-Nitrophenyl)ethyl chloroformate (4.6 g, 20 mmol) was dissolved in abs CH_2Cl_2 (40 ml) and after ice-cooling 1-methylimidazole (1.7 ml, 20 mmol) was added under exclusion of moisture. The mixture was stirred for 15 min at 0–5° and then another 30 min at room temp. The ppt was collected by suction under dry N_2, washed with abs CH_2Cl_2 and dried in high vacuum to give 1-methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride (Found: C, 49.98; H, 4.44; N, 13.38. $C_{13}H_{14}ClN_3O_4$ (311.7) requires: C, 50.09; H, 4.53; N, 13.48%) as a colourless crystal powder; m.p. 92–95°; yield, 5.0 g (80%). ^{1}H-NMR[$(CD_3)_2SO$, 250 MHz]: δ = 3.27 (2H, t), 4.00 (3H, s), 4.75 (2H, t), 7.70 (2H, d), 7.98 (1H, d), 8.09 (1H, d), 8.17 (2H, d), 10.15 (1H, s).

3',5'-Di-O-acetyl-2'-deoxyadenosine (**35**)[76]

Compound **38** (0.132 g, 0.25 mmol) and DBU (0.38 g, 2.5 mmol) were stirred in abs MeCN (5 ml) at room temp for 18 hr. The mixture was neutralized by addition of 0.16 ml glacial AcOH in abs MeCN (2.5 ml). It was diluted with water (25 ml) and extracted with $CHCl_3$ (3 × 15 ml). The organic layer was dried over Na_2SO_4 and then chromatographed on a silica gel plate (20 × 20 × 0.2 cm) for separation with $CHCl_3$–MeOH (98:2). The main band was eluted with $CHCl_3$–MeOH (2:1), evaporated, dissolved in $CHCl_3$, filtered, and then again evaporated to give 3',5'-di-O-acetyl-2'-deoxyadenosine as a solid foam; yield, 77 mg (92%). Recrystallization from EtOAc gave colourless crystals; yield, 68 mg (81%), which were identical with authentic material.

2',3'5'-Tri-O-acetyladenosine (**36**)[77]

Compound **39** (0.147 g, 0.25 mmol) was treated with DBU (0.38 g, 2.5 mmol) in abs MeCN (5 ml) as in the preceding procedure. After chromatographical separation and evaporation 2',3',5'-tri-O-acetyladenosine was obtained as a colourless glass; yield, 93 mg (92%). Recrystallization from EtOAc gave colourless crystals; yield, 85 mg (84%). The identity with an authentic material was proved by chromatographical and spectrophotometrical comparison.

3',5'-Di-O-acetyl-N⁶-p-nitrophenylethoxycarbonyl-2'-deoxyadenosine (**38**)

Compound **35**[76] (2.35 g, 10 mmol) was dissolved in abs CH_2Cl_2 (60 ml) and then 1-methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride (4.36 g, 14 mmol) was added. After 16 hr stirring at room temp the ppt was filtered off and the filtrate evaporated in vacuum. The residue was dissolved in 1,2-dichloroethane for short column chromatography[75] on silica gel (50 g) and subsequently with 1,2-dichloroethane, $CHCl_3$ and $CHCl_3$–MeOH (100:2). The main fraction was evaporated and gave on treatment with EtOAc a crystalline material. Work-up of the mother liquor yielded another crop of 3',5'-di-O-acetyl-N⁶-*p*-nitrophenylethoxycarbonyl-2'-deoxyadenosine (Found: C, 52.51; H,

4.59; N, 15.93. $C_{23}H_{24}N_6O_9$ (528.5) requires: C, 52.27; H, 4.58; N, 15.90%) as colourless crystals; m.p. 134–136°; yield, 3.15 g (85%). The material was recrystallized from EtOAc acetate, R_f 0.63 (system A).

2',3',5' - Tri - O - acetyl - N⁶ - p - nitrophenylethoxycarbonyladenosine (**39**)

Compound 36[77] (2.02 g, 5 mmol) was dissolved in abs CH_2Cl_2 (50 ml). 1-Methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride (3.12 g, 10 mmol) was added and then the soln stirred for 18 hr at room temp. The suspension was filtered, the residue washed with CH_2Cl_2 (20 ml) and the filtrates evaporated. The residue obtained was dissolved in 1,2-dichloroethane and fractionated by short column chromatography[75] on silica gel (40 g) subsequently with 1,2-dichloroethane, $CHCl_3$ and $CHCl_3$–MeOH (50:1). The product was collected, evaporated and the residue recrystallized from EtOAc to give 2',3',5'-tri-O-acetyl-N⁶-*p*-nitrophenylethoxycarbonyladenosine (Found: C, 51.19; H, 4.36; N, 14.58. $C_{25}H_{26}N_6O_{11}$ (586.5) requires: C, 51.20; H, 4.47; N, 14.33%) as colourless crystals; m.p. 149–151°; yield, 2.55 g (87%); R_f 0.69 (system A).

N⁶-p-*Nitrophenylethoxycarbonyl-2'-deoxyadenosine* (**40**)

(a) Compound **38** (3.1 g, 5.86 mmol) was dissolved in MeOH (300 ml) and then Et_3N (60 ml) was added with stirring at room temp. After 16 hr, the ppt was collected by suction and washed with 1,2-dichloroethane. The filtrate was evaporated and the residue treated with a mixture of water (40 ml) and 1,2-dichloroethane (40 ml) to yield a second crop of crystals of N⁶-*p*-nitrophenylethoxycarbonyl-2)-deoxyadenosine (Found: C, 49.29; H, 4.74; N, 18.04. $C_{19}H_{20}N_6O_7 \times H_2O$ (462.4) requires: C, 49.35; H, 4.80; N, 18.17%) in a total yield of 2.46 g (94%). The compound was recrystallized from MeOH to give colourless crystals with m.p. 100–110°; R_f 0.37 (system B).

(b) Compound **35**[76] (1.68 g, 5 mmol) was dissolved in abs pyridine (10 ml) and 2-(p-nitrophenyl)ethyl chloroformate (2.88 g, 12.5 mmol) was added with stirring under ice-cooling for 1 hr. The mixture was then stirred for another 2 hr at room temp and thereafter treated with phosphate buffer pH 7 (100 ml) and extracted with $CHCl_3$ (3 × 50 ml). The united extracts were washed again with phosphate buffer (100 ml), dried over Na_2SO_4 and then evaporated to dryness followed by coevaporation with abs toluene (3 × 20 ml). The residue was fractionated by short column chromatography[75] on silica gel (30 g) first with 1,2-dichloroethane and then with $CHCl_3$ which eluted a mixture of mono- and diacylated adenosine derivatives. This fraction was evaporated to dryness, the residue was dissolved in MeOH (200 ml) and then treated with conc aqueous ammonia (60 ml) at room temp. After 1 hr the mixture was evaporated to dryness and the residue shaken with a mixture of water (20 ml) and 1,2-dichloroethane to form a colourless, chromatographically pure solid of N⁶-*p*-nitrophenylethoxycarbonyl-2'-deoxyadenosine; yield, 1.78 g (80%).

(c) Compound **33** (1.25 g, 5 mmol) and 1-trimethylsilylimidazole (3 ml, 20 mmol) were heated under reflux and exclusion of moisture in abs CH_2Cl_2 (25 ml) for 30 min to achieve trimethylsilylation of the OH-functions to form **37**. The soln was evaporated in vacuum, the residue shaken with abs toluene (50 ml) and after standing for 10 min, the ppt of imidazole was filtered off. It was washed with another 50 ml of abs toluene and then the united filtrates were treated with a 1% soln of potassium biphosphate (150 ml) which had been saturated with NaCl. After separation the organic layer was dried over Na_2SO_4, filtered and on evaporation crystalline **37** was obtained. This material was dissolved in abs CH_2Cl_2 (60 ml), 1-methyl-3-*p*-nitrophenylethoxycarbonylimidazolium chloride (3.11 g, 10 mmol) was added and then the mixture heated under reflux for 3 hr with magnetic stirring. The ppt was filtered off after cooling to room temp and the filtrate was evaporated to dryness. The residue was dissolved in pyridine (25 ml) and then treated for 1 hr with half-conc aqueous ammonia (20 ml) to hydrolyse the trimethylsilyl groups. It was diluted with water (40 ml), then evaporated to a small volume in vacuum and after addition of another 50 ml water concentrated to half of the volume. 1,2-Dichloroethane (25 ml) was added to the suspension and the mixture shaken vigorously. The separated material was collected by suction, washed with water and 1,2-dichloroethane and then dried at 60° in an oven to give **40** as a colourless, chromatographically pure solid; yield, 1.63 g (73%).

N⁶-p-*Nitrophenylethoxycarbonyladenosine* (**41**)

(a) Compound **36** (0.393 g, 1 mmol) and *p*-nitrophenylethyl chloroformate were stirred in abs pyridine (2 ml) for 2 hr at room temp. It was then diluted with $CHCl_3$ (50 ml), shaken with phosphate buffer pH 7 (3 × 50 ml) and the organic layer dried over Na_2SO_4. After filtration it was evaporated and coevaporated with abs toluene (3 × 20 ml). The residue was fractionated on a silica gel column (15 × 3 cm) first with $CHCl_3$ and then with $CHCl_3$–MeOH (92:8) to elute the N⁶-mono- and diacylated mixture. This fraction was evaporated, the residue dissolved in MeOH (40 ml) and then conc aqueous ammonia (15 ml) added. After stirring for 30 min at room temp it was again evaporated and the residue gave on recrystallization from EtOH N⁶-*p*-nitrophenylethoxycarbonyladenosine (Found: C, 49.42; H, 4.26; N, 18.30. $C_{19}H_{20}N_6O_8$ requires: C, 49.57; H, 4.38; N, 18.25%) as colourless needles; m.p. 152–160°; yield, 0.38 g (83%); R_f 0.37 (system B).

(b) Compound **39** (2.1 g, 3.5 mmol) was dissolved in MeOH (200 ml). Et_3N (40 ml) was added and the soln stirred for 18 hr at room temp. The product was filtered off by suction, washed with MeOH and dried at 60° in an oven. The mother liquor was evaporated to dryness, the residue shaken with a mixture of water (20 ml) and 1,2-dichloroethane (20 ml) and the ppt collected and dried to give a second crop of N⁶-*p*-nitrophenylethoxycarbonyladenosine as a crystalline powder; yield, 1.46 g (90%).

5'-O-Monomethoxytrityl-N⁶-p-nitrophenylethoxycarbonyl-2'-deoxyadenosine (**43**)

Compound **40** (1.78 g, 4 mmol) was evaporated with abs pyridine (2 × 20 ml), the residue dissolved in abs pyridine (18 ml) and *p*-monomethoxytrityl chloride (1.49 g, 4.8 mmol) added. After stirring for 16 hr at room temp, the mixture was treated with phosphate buffer pH 7(100 ml) and extracted with $CHCl_3$ (4 × 50 ml). The united extracts were washed with phosphate buffer (100 ml) and after separation and drying the organic layer over Na_2SO_4 the filtrate was evaporated to dryness and finally coevaporated with abs toluene. The residue was applied for short column chromatography[75] on silica gel (30 g) and fractionated with 1,2-dichloroethane followed by $CHCl_3$. The main fraction was collected, evaporated and coevaporated with CH_2Cl_2 (2 × 20 ml) to give 5'-O-monomethoxytrityl-N⁶-*p*-nitrophenylethoxycarbonyl-2'-deoxyadenosine (Found: C, 65.13; H, 4.99; N, 11.54. $C_{39}H_{36}N_6O_8$ (716.8) requires: C, 65.35; H, 5.06; N, 11.73%) as a colourless amorphous solid foam; yield, 2.58 g (90%); R_f 0.46 (system A).

5' - O - Monomethoxytrityl - N⁶ - p - nitrophenylethoxycarbonyl - 2' - deoxyadenosine - 3' - (2,5 - dichlorophenyl, p - nitrophenylethyl) - phosphate (**44**)

1,2,4-Triazole (0.33 g, 4.8 mmol) was first evaporated with abs pyridine (10 ml), then dissolved in abs pyridine (6 ml) and 2,5-dichlorophenylphosphoro dichloridate (0.6 g, 2.25 mmol) was added and the mixture stirred for 20 min. It was then cooled in an ice-bath before dropwise addition of **43** (1.1 g, 1.53 mmol) dissolved in abs pyridine (10 ml). Stirring was continued for 1 hr, 2-(*p*-nitrophenyl)-ethanol (0.5 g, 3 mmol) was added and the mixture kept for 16 hr at room temp with stirring. After dilution with phosphate

buffer pH 7 (100 ml) it was extracted with $CHCl_3$ (3 × 50 ml), washed with phosphate buffer, the organic layer dried over Na_2SO_4 and then the filtrate evaporated and coevaporated with abs toluene (3 × 20 ml). The residue was fractionated by short column chromatography[75] on silica gel (28 g) first with toluene and followed by a gradient toluene–EtOAc (9:1 till 6:4). The product was finally eluted with toluene–EtOAc mixtures (1:1 till 1:3). The main fraction was collected, evaporated in vacuum and coevaporated with $CHCl_3$ (50 ml) and CH_2Cl_2 (2 × 50 ml) to give 5′-O-monomethoxytrityl-N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine-3′-(2,5-dichlorophenyl, *p*-nitrophenylethyl)-phosphate (Found: C, 58.58; H, 4.14; N, 8.77. $C_{53}H_{46}Cl_2N_7O_{13}P$ (1090.9) requires: C, 58.36; H, 4.25; N, 8.99%) as a colourless solid foam; yield, 1.47 g (88%); R_f 0.67 (system A).

N^6-p-*Nitrophenylethoxycarbonyl-2′-deoxyadenosine-3′-(2,5-dichlorophenyl, p-nitrophenylethyl)-phosphate* (**45**)

Compound **44** (0.328 g, 0.3 mmol) was treated with a 2% soln of *p*-toluene-sulfonic acid in CH_2Cl_2–MeOH (4:1) for 10 min with stirring at room temp. The soln was diluted with $CHCl_3$ (40 ml) and then shaken with phosphate buffer pH 7 (3 × 40 ml). The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was fractionated by column chromatography on silica gel (12 × 2 cm) with $CHCl_3$ and $CHCl_3$–MeOH (98:2). The main fraction was collected and evaporated in vacuum to a solid foam. Reprecipitation from a little $CHCl_3$ into *n*-hexane gave N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine-3′-(2,5-dichlorophenyl *p*-nitrophenylethyl)-phosphate (Found: C, 48.66; H, 3.76; N, 11.70. $C_{33}H_{30}Cl_2N_7O_{12}P$ (818.5) requires: C, 48.42; H, 3.69; N, 11.98%) as a colourless amorphous powder; yield, 0.228 g (93%); R_f 0.57 (system A).

Triethylammonium 5′ - O - *monomethoxytrityl* - N^6 - p - *nitrophenylethoxycarbonyl* - 2′ - *deoxyadenosine* - 3′ - p - *nitrophenylethyl* - *phosphate* (**46**)

4-Nitrobenzaldoxime (0.5 g, 3 mmol) was dissolved in a mixture of dioxan (6 ml), water (6 ml), and Et_3N (6 ml) and stirred for 20 min at room temp. Compound **44** (0.328 g, 0.3 mmol) was added and stirring was continued for another 40 min. The mixture was evaporated in vacuum at a bath temp of 30° and then coevaporated with abs pyridine (10 ml) and abs toluene (3 × 10 ml). The residue was fractionated by column chromatography on silica gel (10 × 2 cm) first with $CHCl_3$ and $CHCl_3$–MeOH (95:5) to remove the reagents and impurities. The product was eluted with $CHCl_3$–MeOH–Et_3N (95:5:5). The main fraction was evaporated, coevaporated with abs toluene (2 × 10 ml), $CHCl_3$ (20 ml), and CH_2Cl_2 (2 × 20 ml) to give triethylammonium 5′-O-monomethoxytrityl-N^6-*p*-nitrophenylethoxycarbonyl-2′-deoxyadenosine-3′-*p*-nitrophenylethyl-phosphate (Found: C, 60.59; H, 5.78; N, 10.59. $C_{53}H_{59}N_8O_{13}P$ (1047.0) requires: C, 60.80; H, 5.68; N, 10.70%) as a colourless solid foam; yield, 0.289 g (92%); R_f 0.36 (system C).

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