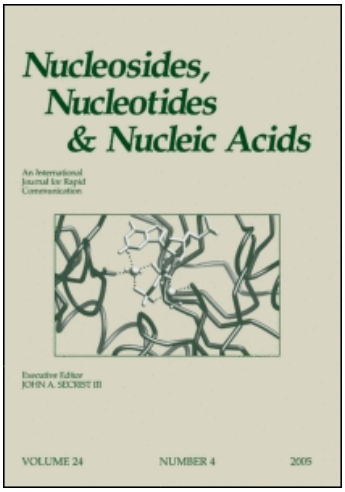

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The p-Nitrophenylethyl Group - An Universal Blocking Group in Nucleoside and Nucleotide Chemistry

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THE p-NITROPHENYLETHYL GROUP - AN UNIVERSAL BLOCKING GROUP IN NUCLEOSIDE AND NUCLEOTIDE CHEMISTRY

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Summary. The development of the p-nitrophenylethyl (NPE) group for phosphate, phosphite and amide protection as well as the p-nitrophenylethoxycarbonyl (NPEOC) group for amino and hydroxy protection offers the first universally applicable blocking groups in nucleoside, nucleotide and oligonucleotide chemistry.

Oligo- and polynucleotide syntheses have accomplished recently a fast development due to the very effective phosphotriester approach[1,2], which leads with suitably protected building blocks by direct condensations or via appropriate phosphites[3,4] followed by oxidation to fully blocked phosphotriesters as the key intermediates. The various blocking groups used for aglycon, sugar and phosphate protection[5,6] are legion and more new types are still developed and recommended for general application in this field. The vast number of protecting groups implicates unlimited combinations which quite often prove their effectiveness or insufficiency only in the last step of the synthesis - the deprotection step.

Our recent contributions to the methodology of the chemical synthesis of oligonucleotides have been concentrated to the search of a more general and universally applicable blocking group to substitute the variable protecting group strategy by a more simple and unified approach. We have shown[7] that the p-nitrophenylethyl (NPE) and p-nitrophenylethoxycarbonyl (NPEOC) groups respectively is so far the most versatile blocking group for protection of all functions of nucleosides and nucleotides. This new type of protecting group has first been

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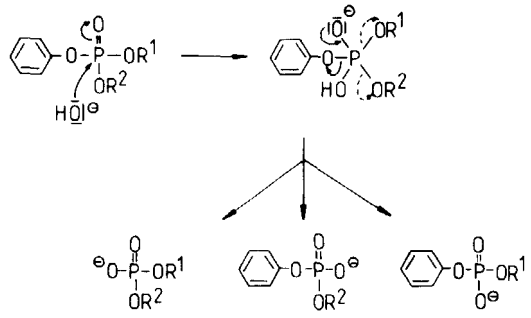
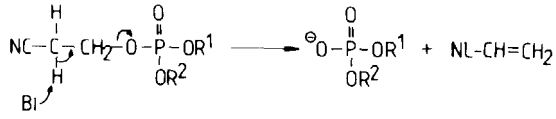
ADDITION-ELIMINATION MECHANISM

NaOH , NEt_4OH , NH_3/H_2O , Aldoximate



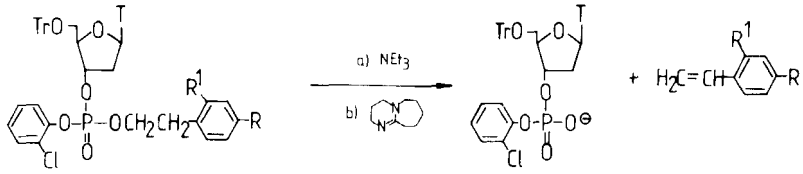
β-ELIMINATION MECHANISM



developed for phosphate blocking[8] taking into account that from a mechanistical point of view any substituent, which is removed by a β-elimination process and leading to a C-O cleavage, is superior to those which afford a nucleophilic attack on phosphorus and may lead this way to the scission of the wrong P-O bond.

It could be demonstrated by the synthesis of various di- and monosubstituted phenylethylphosphotriesters[8] that the p-nitrophenylethyl group offers so far the best features due to its chemical inertness in the condensation step, its stability towards mild acid and base hydrolyses and its easy cleavage by DBU and DBN in aprotic media.

Another advantage of the NPE-group can be seen from the combination with the 2.5-dichlorophenyl residue in mixed phosphotriesters (1) since primary cleavage of the latter function by the oxime method[9] allows either formation of a new internucleotidic linkage or a further degradation of the p-nitrophenylethylphosphodiester (2) to the corresponding terminal monophosphate (4). It is obvious that in the presence of both an NPE phosphotri- and phosphodiester (2) cleavages proceed

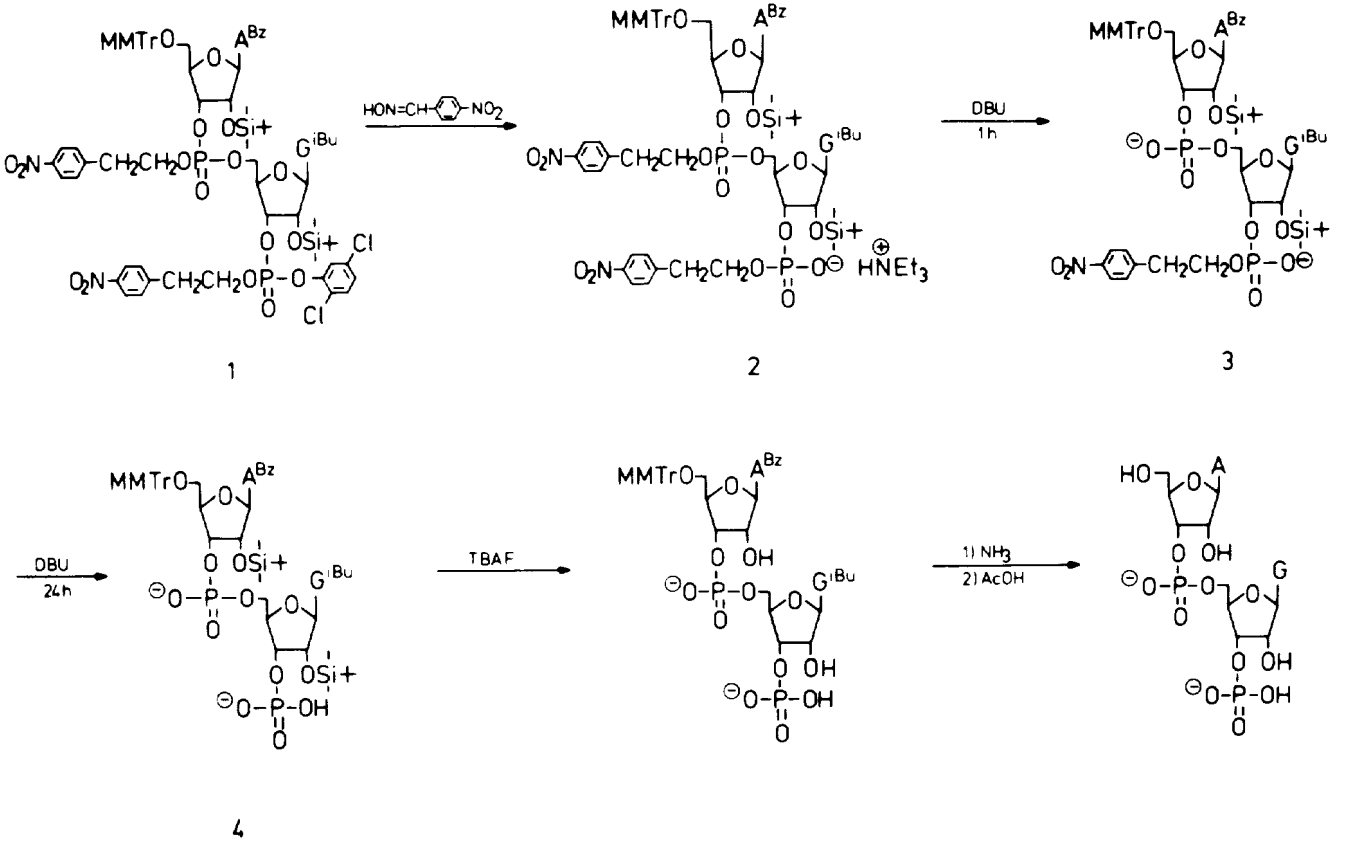


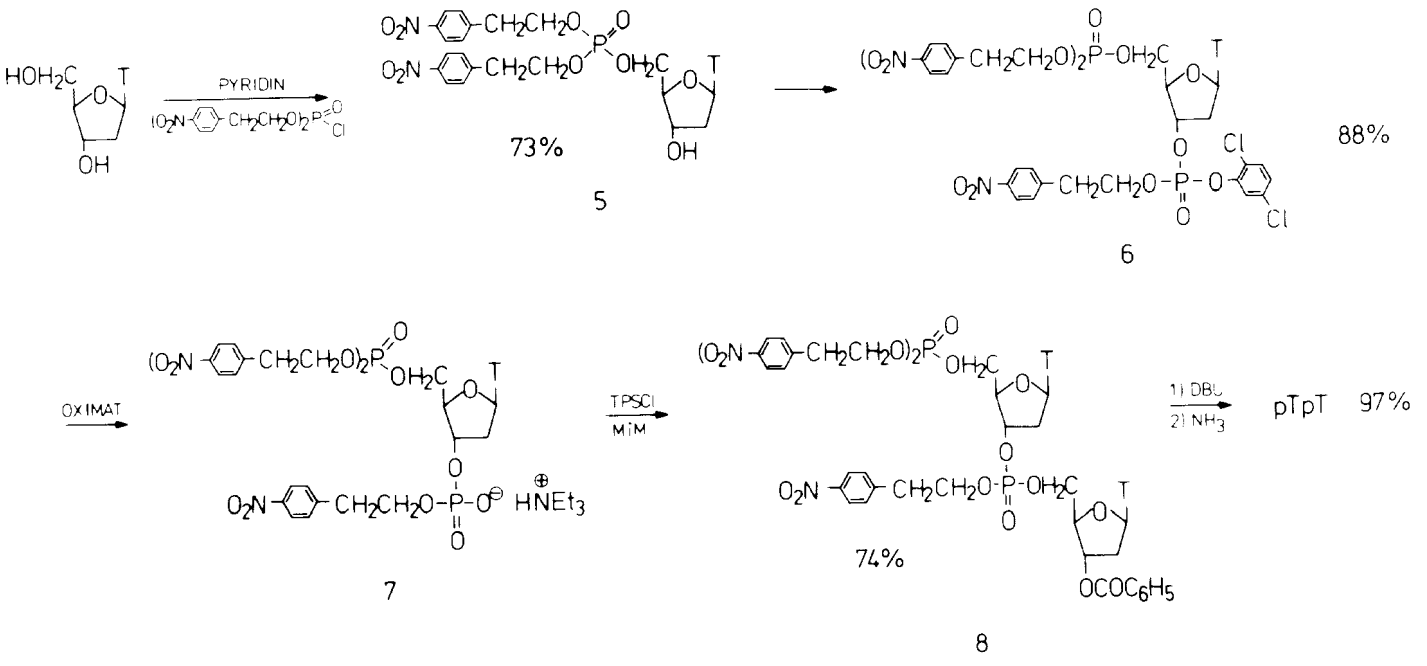
O_2N NO_2 < O_2N CN < $NC\,CH_2CH_2$ < NC NO_2 < NC CN < O_2N Cl	Et_3N
< O_2N < NO_2 < NC	DBU DBN
<	stabile

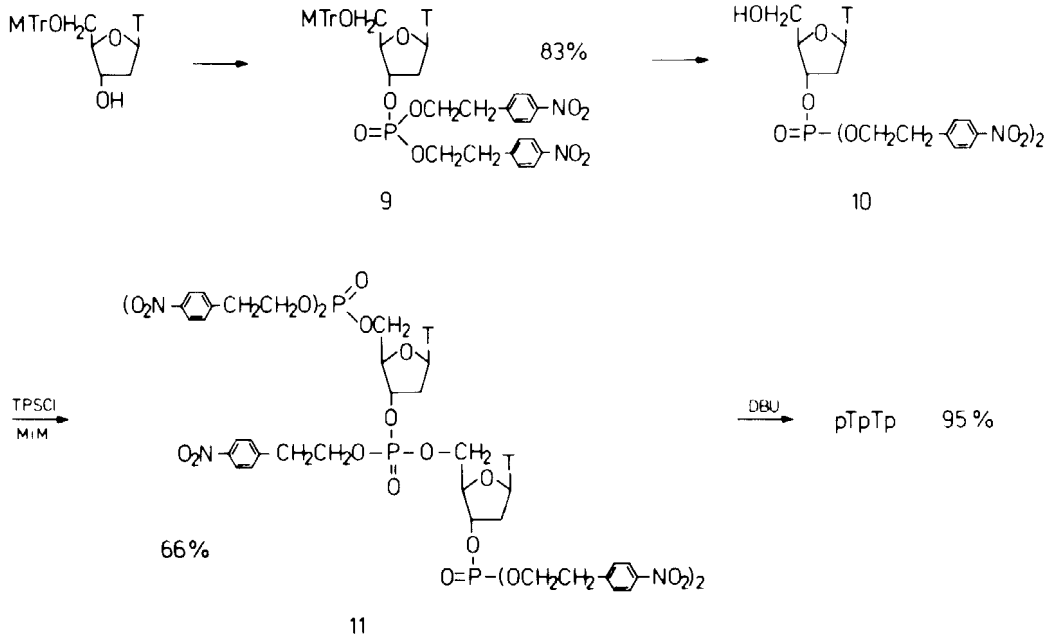
with different rates according to electronic reasons and giving rise even to the isolation of the phosphodiester species (3).

This interesting behaviour prompted us to develop di-(p-nitrophebylethyl)-phosphorochloridate[10] as a new phosphorylating agent. Nucleosides are phosphorylated preferentially in 5'-position to the corresponding phosphotriester (5) which is chromatographically separated from minor quantities of the 3'-isomer and the disubstituted analog. Conversion of 5 into the mixed diphosphotriester 6 works in high yield as does the oximate cleavage to the corresponding 3'-phosphodiester 7. Condensation with 3'-O-benzoylthymidine leads to the fully protected dinucleosidediphosphotriester 8, which needs for deprotection only DBU and subsequent ammonia treatment to give pTpT in 97 % isolated yield.

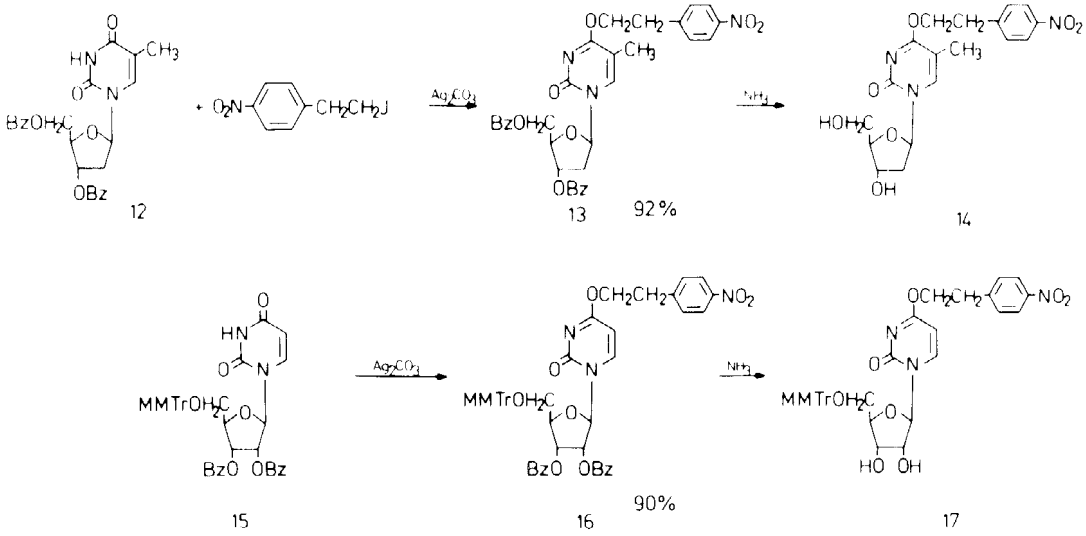
A terminal 3',5'-diphosphate can also be achieved by this approach in condensing for example 7 with a 3'-di-(p-nitrophenylethyl)-phosphotriester (10) derived from an sppropriately blocked precursor (9) to give 11. Deprotection is then a one step procedure working in extremely high yield.

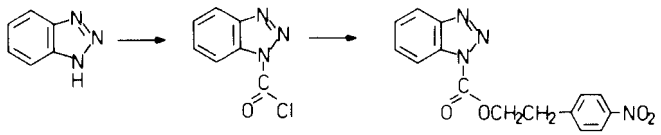






The outstanding features of the NPE group for phosphate protection indicated a more general use of this function to block temporarily also other substituents at the aglycon and sugar moieties of nucleosides and nucleotides. Since several side reactions of even the amide groups have been detected recently it is recommended to protect also O^4 and O^6 in pyrimidine and purine nucleosides respectively. O^4-Alkylation of sugar-blocked thymidines (12) and uridines (15) proceed in high yields





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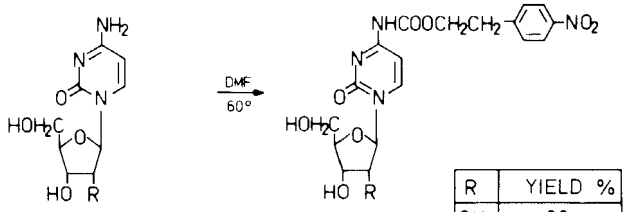


R = H, OH

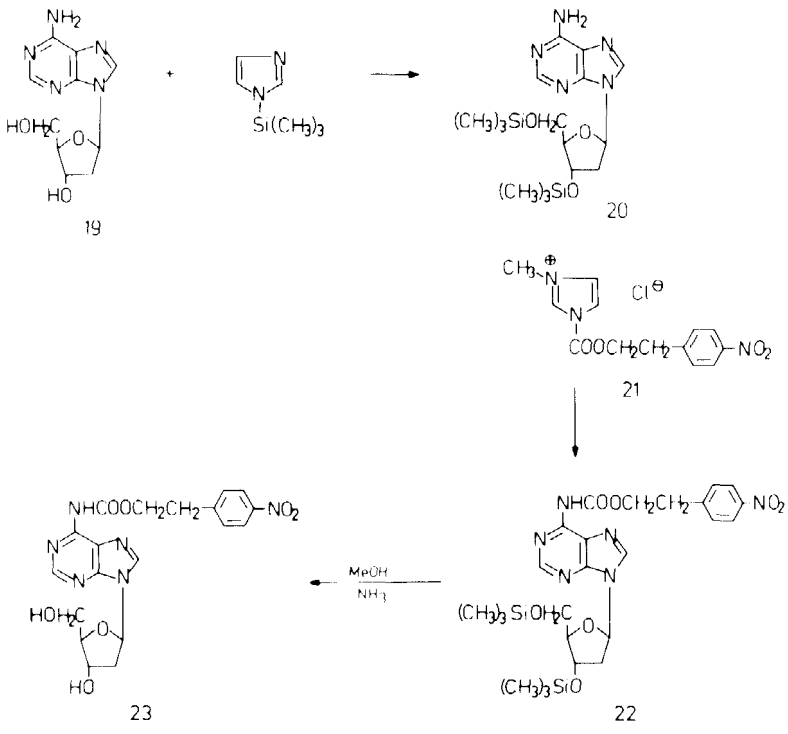
R	YIELD %
OH	90
H	91

with p-nitrophenylethyl iodide in presence of silver carbonate to the corresponding O^4-p-nitrophenylethyl derivatives 13 and 16[10]. Hydrolysis of acyl groups at the sugar moiety can then be performed by ammonia which does not harm the O^4-protecting group (14,17).

Amino protection is usually effected by N-acylation using acetyl, pivaloyl, benzoyl, and substituted benzoyl groups[11]. The structural analog, including the p-nitrophenylethyl residue, has on these grounds to be seen in the p-nitrophenylethoxycarbonyl (NPEOC) group forming the corresponding carbamates[10]. 1-(p-Nitrophenylethoxycarbonyl)-benztriazole (18) turned out to be the perfect reagent to convert unprotected 2'-deoxycytidine and cytidine in DMF at 60°C smoothly into their N^4-p-nitrophenylethoxycarbonyl derivatives.

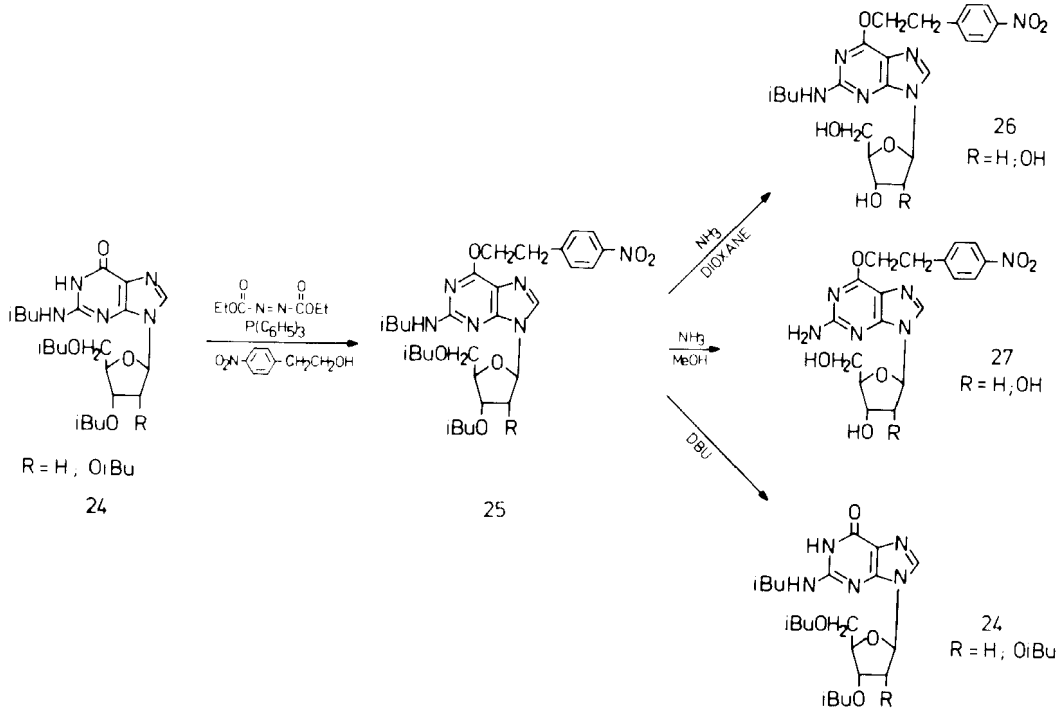
The new blocking group is stable against ammonia and triethylamine in methanol, dioxane, and water but can be cleaved quantitatively by DBU or DBN in aprotic solvents to regenerate the starting materials.

Analogous protection of 2'-deoxy- and adenosine could not be achieved directly in the same manner as in the cytidine series due to the lower nucleophilic potential of the amino group. The more reactive p-nitrophenylethyl chloroformate, however, acylates smoothly 3',5'-di-O-acetyl-2'-deoxy- and 2',3',5'-tri-O-acetyladenosine respectively in pyridine and even more effective for p-nitrophenylethoxycarbonylation is the 1-methyl-3-p-nitrophenylethoxycarbonylimidazolium chloride (21). A simple one-pot reaction is illustrated by the conversion of 2'-deoxy-



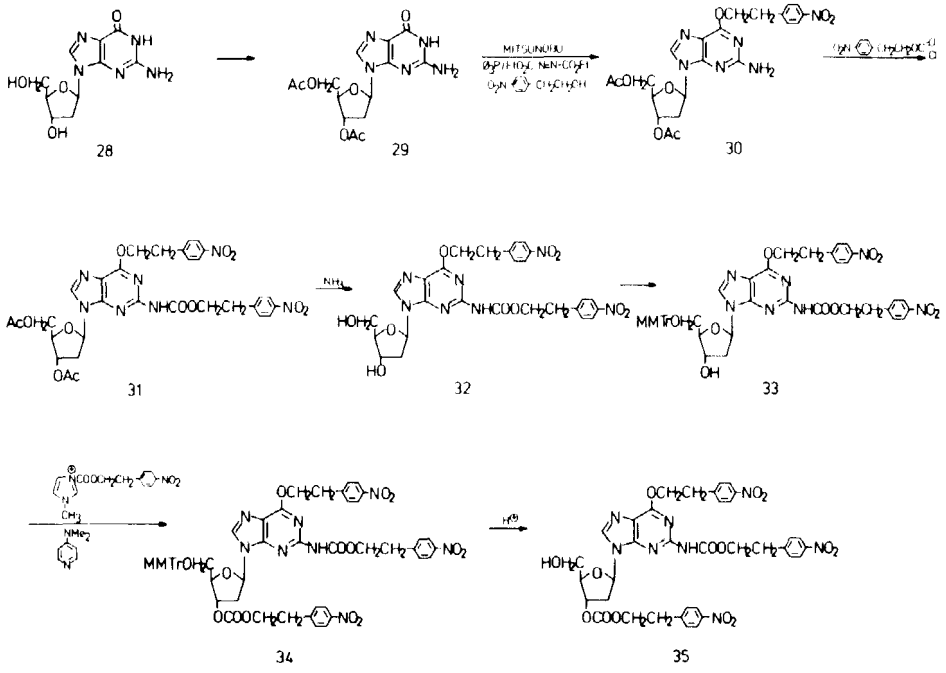
adenosine (19) into its N^6-p-nitrophenylethoxycarbonyl derivative (23). Starting with a trimethylsilylation to protect the sugar hydroxyl groups (20) is followed by acylation to form 22, which on work-up with ammonia in aqueous methanol leads to N^6-p-nitrophenylethoxycarbonyl-2'-deoxyadenosine (23) in 73 % overall yield.

The most difficult protections have then been encountered with the various guanosine derivatives. After a series of unsuccessful attempts the O^6-alkylation could be achieved with sugar- and amino-protected 2'-deoxy- and guanosines (24) by the Mitsunobu reaction[12] using 1.5 molecular equivalent each of diethyl azodicarboxylate, triphenylphosphine, and 2-(p-nitrophenyl)-ethanol in dioxane (25)[10]. The p-nitrophenylethyl group can be cleaved selectively by 0.5 M DBU in pyridine to regenerate the starting material 24. Reaction with conc. ammonia in dioxane hydrolyses the sugar acyl groups (26) and prolonged treatment with conc. ammonia in methanol cleaves also the N^2-acyl group (27) without harming the O^6-blocking group.



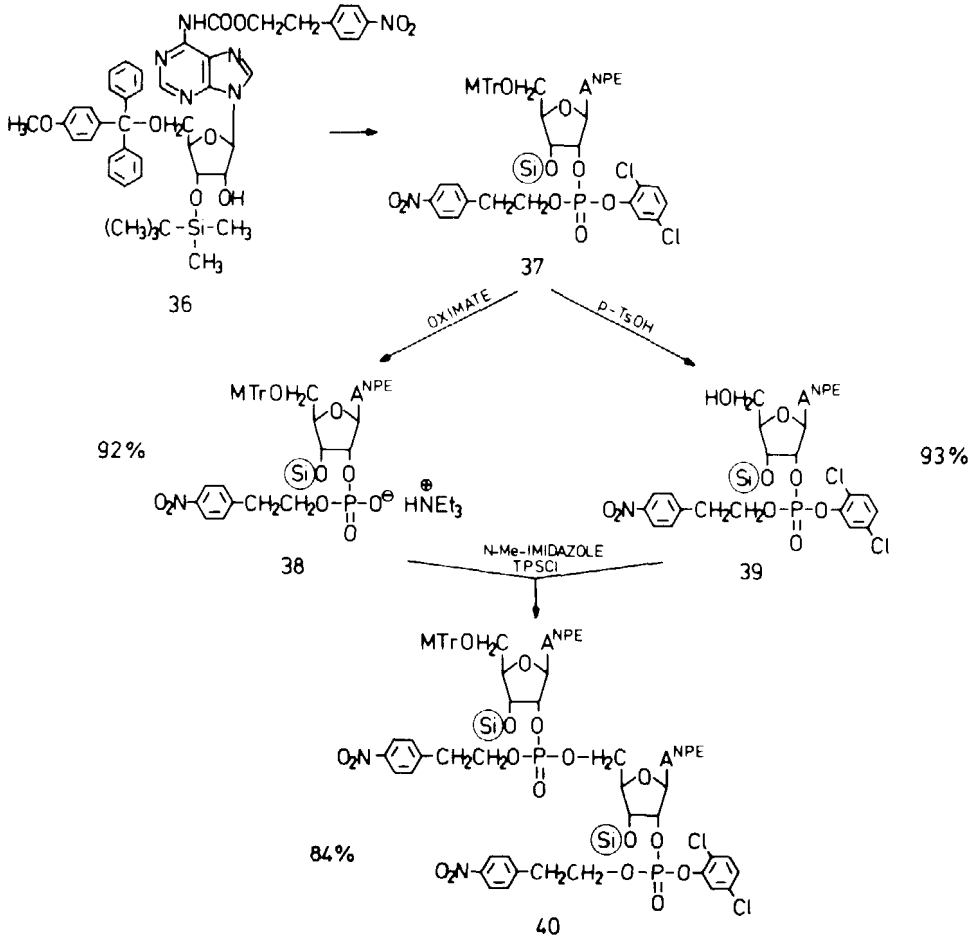
In a cascade of reactions not only the amide function of the guanine moiety but also the amino as well as the hydroxy groups have been protected uniformly as demonstrated by the synthesis of N^2,3'-di-p-nitrophenylethoxycarbonyl-O^6-p-nitrophenylethyl-2'-deoxyguanosine (35). 2'-Deoxyguanosine (28) is first acetylated on the ribosyl residue (29) and then the Mitsunobu reaction applied to give 30. Subsequent reaction with p-nitrophenylethyl chloroformate acylates the 2-amino group (31) and treatment with ammonia unblocks the sugar moiety (32). Reaction with monomethoxytrityl chloride leads to selective protection of the 5'-hydroxy group (33) and subsequent treatment with 1-methyl-3-p-nitrophenylethoxycarbonylimidazolium chloride in presence of 4-dimethylaminopyridine forms the corresponding 3'-carbonate 34. Acid catalysed detritylation finally gives rise to the 3'-terminal synthone 35.

The 3'-O-p-nitrophenylethoxycarbonyl derivatives of the common four 2'-deoxyribonucleosides as well as the 2',3'-di-O-carbonates of the ribonucleosides have been prepared analogously and are used as valuable building blocks in oligonucleotide synthesis.

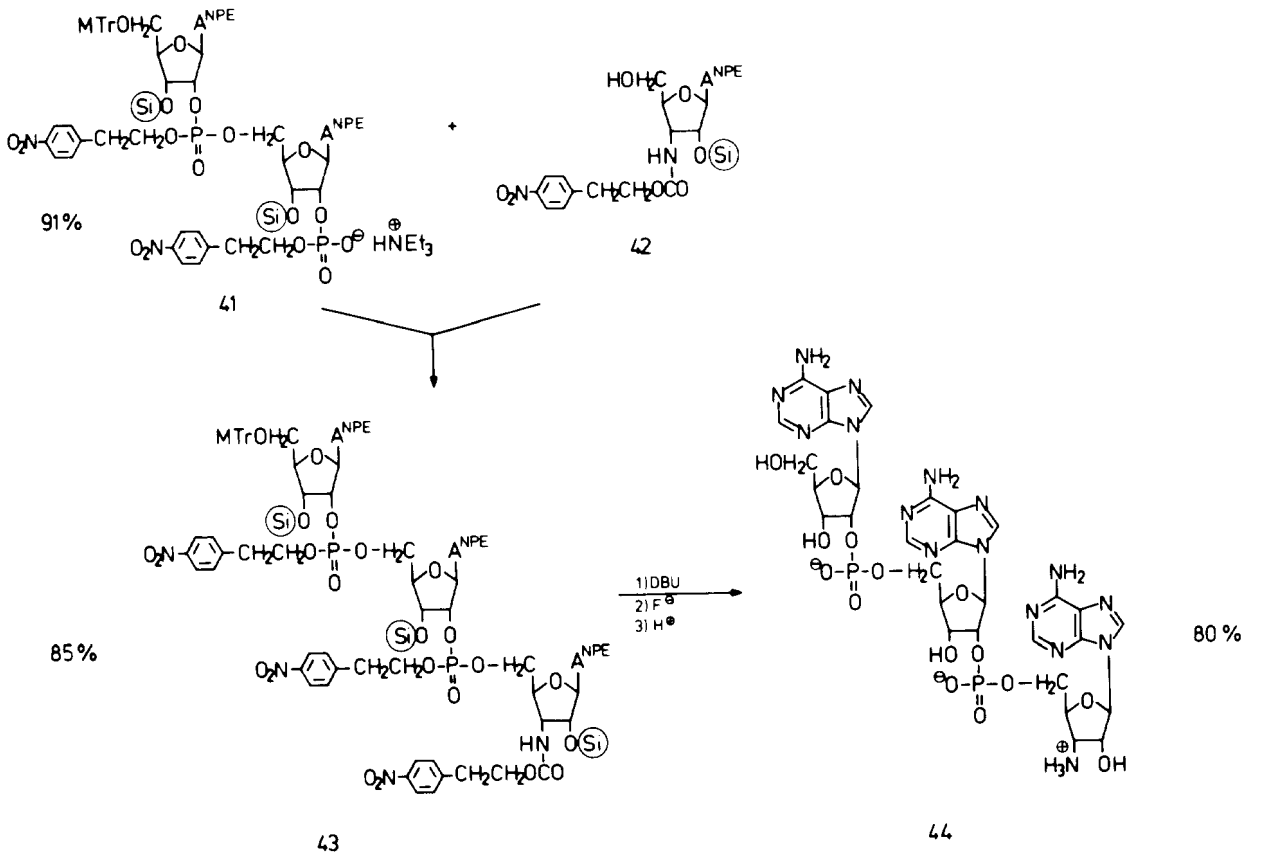


B =				
YIELD a	94	91	83	85
b	84	91	63	91

It has furthermore been recognized[13] that the p-nitrophenylethyl group can successfully be applied in Caruthers'[14,15] most effective phosphoramidite approach. Solution synthesis of all 16 combinations of the protected most common di-2'-deoxynucleoside phosphotriesters works well with the 5'-O-dimethoxytrityl-2'-deoxyribonucleoside-3'-p-nitrophenylethylphosphoromorpholidites and 3'-O-benzoyl-2'-deoxyribonucleosides followed by subsequent oxidation of the intermediary phosphites.



The great advantage of the NPE and NPEOC groups as universal blocking groups for a broad variety of commonly used substituents becomes especially obvious when preparative scale syntheses of oligonucleotides are in demand. Easy deprotection of the blocking groups, simple work-up and purification guarantee this way high yields of the free oligonucleotides. A necessity to favour the preparative approach can be seen in the recent findings of low molecular weight inhibitors of protein synthesis of the 2',5'-oligoadenylate type[16] and their structural analogs as potential antiviral[17] and antitumor[18] agents. A modified adenylate trimer, the adenylyl-2',5'-adenylyl-2',5'-3'-amino-3'-deoxyadenosine (44), has been synthesized by our strategy from 3'-O-tert.-butyldimethylsilyl-5'-O-monomethoxytrityl-N^6-p-nitrophenylethoxy-



carbonyl-adenosine (36) and 2'-tert.butyldimethylsilyl-N^6,3'-di-p-nitrophenylethoxycarbonyl-3'-amino-3'-deoxyadenosine (42) respectively. 36 was first phosphorylated to 37 which was used as the common precursor for selective deprotection to the corresponding phosphodiester 38 and the 5'-OH derivative 39. Both components yielded on condensation the fully protected dimer 40, which was then transferred by the oxime method into the 2'-terminal phosphodiester 41 and subsequently coupled with 42 to the trimer 43. The final deblocking consisted of only three steps, namely DBU-, $F^{\ominus}$- and $H^{\oplus}$-treatment to give adenylyl-2',5'-adenylyl-2',5'-3'-amino-3'-deoxyadenosine (44) in 80 % yield.

REFERENCES

1 J.H. van Boom, Heterocycles 7, 1197 (1977).

2 C.B. Reese, Tetrahedron 34, 3143 (1978).

3 R.L. Letsinger and W.B. Lunsford, J.Am.Chem.Soc. 98, 3655 (1976).

4 K.K. Ogilvie, N. Theriault and K.L. Sadana, J.Am.Chem.Soc. 99, 7741 (1977).

K.K. Ogilvie, S.L. Beaucage, A.L. Schifman, N.Y. Theriault and K.L. Sadana, Can.J.Chem. 56, 2768 (1978).

5 H. Kössel and H. Seliger, Fortschr.Chem.Org. Naturstoffe 32, 297 (1975).

6 V. Amarnath and A.D. Broom, Chem.Rev. 77, 183 (1977).

7 F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala and W. Pfleiderer, Tetrahedron 40, 59 (1984).

8 E. Uhlmann and W. Pfleiderer, Helv.Chim. Acta 64, 1688 (1981).

9 C.B. Reese and L. Zard, Nucleic Acids Res. 9, 4611 (1981).

10 F. Himmelsbach and W. Pfleiderer, Tetrahedron Lett. 1982, 4793.

11 H. Köster, K. Kulikowski, T. Liese, W. Heikens and V. Kohli, Tetrahedron 37, 363 (1981).

12 D. Mitsunobu, Synthesis 1 (1981).

13 A.H. Beiter and W. Pfleiderer, Tetrahedron Lett. 1984, 1975.

14 S.L. Beaucage and M.H. Caruthers, Tetrahedron Lett. 1981, 1859.

15 L.J. McBride and M.H. Caruthers, Tetrahedron Lett. 1983, 245.

16 P.F. Torrence, Molec. Aspects. Med. 5, 129 (1982).

17 Y. Devash, A. Gera, D.H. Willis, M. Reichman, W. Pfleiderer, R. Charubala, I. Sela and R.J. Suhadolnik, J.Biol.Chem. 259, 3482 (1984).

18 D.H. Willis, W. Pfleiderer, R. Charubala and R.J. Suhadolnik, Fed.Proc. 42, 443 (1983) (Abstr.).