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FACILE REMOVAL OF NEW BASE PROTECTING GROUPS USEFUL IN OLIGONUCLEOTIDE SYNTHESIS

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SUMMARY : New base protecting groups are proposed in place of benzoyl and isobutyryl groups. They are phenoxy acetyl for adenine and guanine and isobutyryl for cytosine. They are cleaved in aqueous ammonia in a shorter time at lower temperature. These easily removable groups are useful in the synthesis of modified oligonucleotides, containing fragile bases.

During oligonucleotide synthesis, the protection of the exocyclic amino functions of adenine, guanine and cytosine is a crucial problem. This has been solved very early by Khorana and coworkers who proposed the use of the benzoyl group for the protection of adenine and cytosine (1) and of the isobutyryl group for guanine (2). These groups have been adopted by most of the scientific community and by all the suppliers of DNA synthesis products. However, Köster et al. (3) showed in a comprehensive study that N-deacylation of N-isobutyryl deoxyguanosine in sodium hydroxyde is 30 times slower than that of N-benzoyl deoxycytidine.

This raises the following problem : isobutyryl guanine is so stable that the overnight heating in ammonia, as proposed, is not sufficient to ensure a complete removal of this group on a DNA chain (4). Furthermore, this treatment requires such drastic conditions that it leaves no hope to synthesize oligonucleotides containing fragile bases such as saturated pyrimidines. Hence, we have decided to develop a new set of protecting groups, allowing deprotection in the easy one step ammonia procedure at lower temperature and for a shorter reaction time.

SYNTHESIS OF N-ACYL NUCLEOSIDES

Various acyl groups were introduced on the exocyclic amino function of adenine, guanine and cytosine, according to the classical two step reaction outlined in scheme 1.

The rate of hydrolysis of these products in concentrated ammonia was evaluated by TLC and compared with that of usual protected nucleosides (Table I), at room temperature in order to get significative figures.

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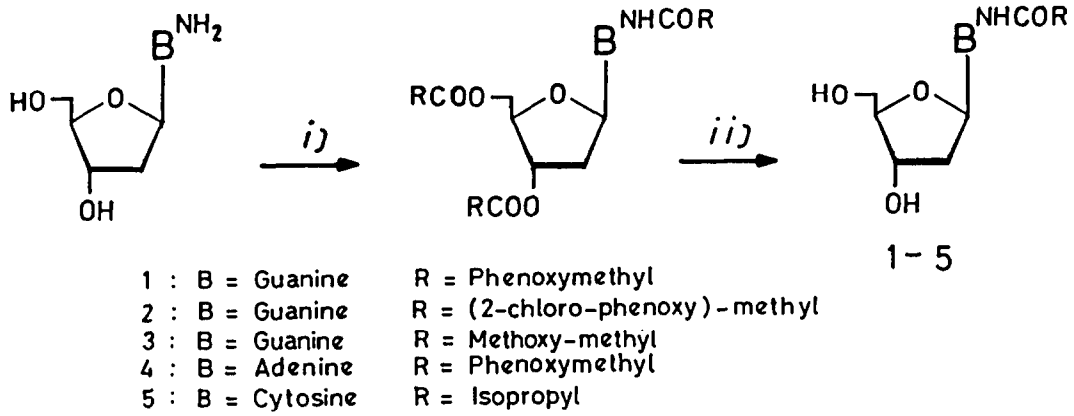


Scheme 1 : General procedure for the preparation of base protected nucleosides. i) Acyl chloride or anhydride in pyridine. ii) Triethylamine/water/Pyridine.

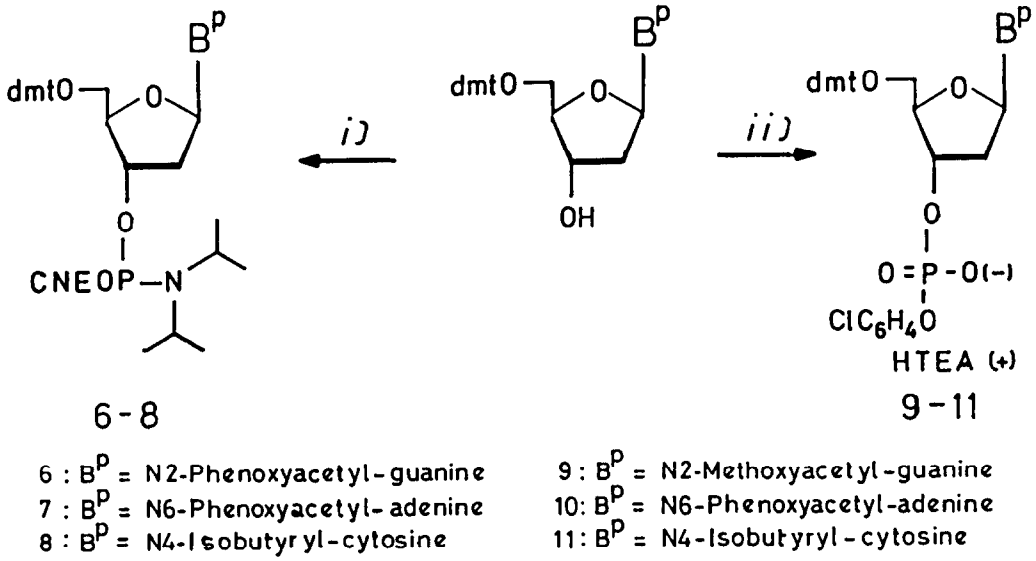
Compounds	Yield	t 1/2 (6)	Compounds	Yield	t 1/2 (6)
1	58 %	15 min.	ibu dG		10 hrs
2	15 %	8 min.	bz dA		8 hrs
3	50 %	45 min.	bz dC		2 hrs
4	65 %	7 min.			
5	60 %	30 min.			

Table I

Taking into account the yield of synthesis and the ease of deprotection of the corresponding amides, this Table shows that compounds 1, 3, 4 and 5 can be successfully used for the synthesis of oligonucleotides. Final deprotection of such protected oligonucleotides should take from 4 to 6 hours ($t_{1/2}$ x 8) at room temperature, depending on which group is used to protect the guanine residue.

PREPARATION OF FULLY PROTECTED MONOMERS

After protection of the 5' hydroxyl function of the sugar moiety by the 4,4'-dimethoxytrityl group, both phosphotriester and phosphoramidite monomers were synthesised according to scheme 2 (5).



6 : B^P = N2-Phenoxyacetyl-guanine
7 : B^P = N6-Phenoxyacetyl-adenine
8 : B^P = N4-Isobutyryl-cytosine

9 : B^P = N2-Methoxyacetyl-guanine
10: B^P = N6-Phenoxyacetyl-adenine
11: B^P = N4-Isobutyryl-cytosine

Scheme 2 : Preparation of fully protected monomers for DNA assembling. i) Bis(diisopropylamino)cyanoethyl phosphine + diisopropylammonium tetrazolidate in dry dichloromethane. ii) chlorophenyl bis triazolidate in CH_3CN/Pyridine.

USE IN OLIGONUCLEOTIDE SYNTHESIS

Compounds 6, 7 and 8 were tested for the synthesis of homopentadecamers according to the cyanoethyl phosphoramidite method (7). The fully protected 15-mers linked to the silica support were treated by concentrated ammonia for 4 hrs at room temperature.

Compounds 9, 10, 11 and the corresponding derivatives of dT and dU were used to synthesize four heptadecamers containing the U mutation at four central positions instead of the normal DNA bases. Efimov's procedure (8) was employed and oligonucleotides were finally deprotected in 6 hours within the same conditions.

The crude deprotection mixtures were desalted by size exclusion on Sephadex and 5'-end labelled by ^{32}P ATP and T4 polynucleotide kinase. They were length separated and purified by polyacrylamide gel electrophoresis, showing that no problem was encountered.

To examine whether these deprotection conditions were leading to a fully deprotected DNA fragment or not, an oligonucleotide was prepared according to the phosphoramidite approach. The crude controlled pore glass support, containing the protected tetramer, was reacted with concentrated aqueous ammonia for 4 hours. The deprotection mixture was then size separated using a Waters I 125 "Protein Analysis Column" with .025 M triethylammonium acetate as the eluent (9), so that if unwanted partially protected compounds were present

in the mixture, they should not be separated. The product obtained in this way was enzymatically treated by a mixture of snake venom phosphodiesterase and alkaline phosphatase, and analysed by reversed phase liquid chromatography (C18). In the limit of sensitivity of this methodology, it was not possible to detect any protected nucleoside in the mixture.

In conclusion, the protection groups described here are removed very easily, allowing both base deprotection and cleavage from the support in the same short time. In this way, these last reactions can be performed automatically with ammonia at room temperature within four hours. Furthermore, fragile bases which are of great interest in mutagenesis studies can be introduced in modified oligonucleotides.

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5. ^{1}H and ^{31}P NMR data of compounds 6-11. ^{1}H chemical shifts are in ppm relative to TMS as an internal standard. Solvent was CD_3OD for triester synthons, CD_3CN for compounds 7 and 8, and CD_3COCD_3 for compound 6. ^{31}P chemical shifts are in ppm, measured in the same solvents with 85 % H_3PO_4 as an external standard.

Compound	H_1'	H_3'	H_5	H_6	H_2	H_8	^{31}P
6	6.45(t)	4.88(m)				8.12(s)	146.2
7	6.44(t)	4.93(m)			8.28(s)	8.56(s)	155.3
8	6.14(t)	4.60(m)	7.12(d)	8.21(d)			155.3
9	6.36(t)	5.19(m)				8.03(s)	-5.0
10	6.56(t)	5.28(m)			8.42(s)	8.53(s)	-5.5
11	6.22(t)	5.20(m)	7.56(d)	8.19(d)			-5.3

6. The half-life deprotection times were estimated by t.l.c. on silicagel plates eluted in $CHCl_3$-MeOH 80-20 v/v.

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