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NECESSARY PROTECTION OF THE O^6-POSITION OF GUANINE DURING THE SOLID PHASE SYNTHESIS OF OLIGONUCLEOTIDES BY THE PHOSPHORAMIDITE APPROACH

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It has been observed that the use of O^6-protected deoxyguanosine phosphoramidites leads to a significant improvement in the automated synthesis of oligonucleotides on an insoluble support.

The introduction of deoxyribonucleoside-3'-phosphoramidite derivatives by Caruthers and Beaucage[1] has lead to the routine synthesis of short oligonucleotides (<30 bases) in many laboratories[2-3]. However, the synthesis of longer oligodeoxyribonucleotides is more difficult because very high coupling yields are required and no side reactions, such as depurination or base modification, can be permitted.

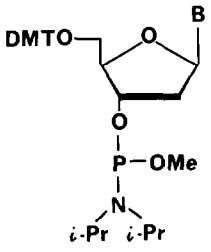
Phosphoramidite reagents have been widely used because they have been shown to produce exceptionally high coupling yields[4-5]. In addition, no account of base modification by these reagents has been reported. This is in contrast to oligodeoxyribonucleotide synthesis performed by any of the phosphate triester procedures. In these procedures, substantial modification of guanine bases has been well documented[6-9] and protection of the O^6-base position by a variety of protecting groups has been recommended[10-14].

In this letter we would like to present evidence which indicates that O^6-protection of guanine bases is also beneficial when phosphoramidite reagents are used to prepare sequences which contain a high guanine content.

A series of homogeneous oligodeoxyribonucleotides, each twenty four units in length, were prepared on controlled pore glass supports. Each oligodeoxyribonucleotide was prepared from one of the six different diisopropylaminophosphoramidites **1-6**. These phosphoramidite derivatives were prepared according to the standard literature procedures[3] and each was purified by silica gel chromatography. The compounds were further characterized by ^{31}P NMR (Table 1).

The synthesis cycle, which was performed by an automated DNA synthesizer[15], is shown in Table 2. This cycle contained a five minute coupling step which was sufficient to produce the high coupling efficiencies shown in Table 1.

At the end of the synthesis, each product was completely deprotected and the crude mixtures which were recovered were examined by electrophoresis (20%



1, B = Thymine

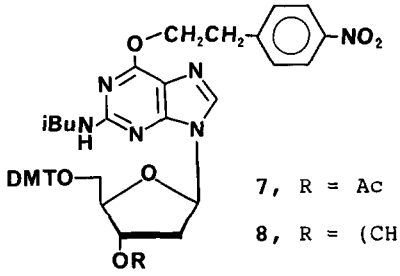
2, B = N^4-Benzoylcytosine

3, B = N^6-Benzoyladenine

4, B = N^6-(N-methyl-2-pyrrolidine amidine)adenine

5, B = N^2-Isobutyrylguanine

6, B = N^2-(N-methyl-2-pyrrolidine amidine)guanine



7, R = Ac

8, R = (CH_3O)-P-N(iPr)$_2$

TABLE 1. ^{31}P NMR Chemical Shifts and Average Coupling Efficiencies of Deoxyribonucleoside Diisopropylaminephosphoramidites.

PHOSPHOR-AMIDITE	^{31}P CHEMICAL SHIFT*	SEQUENCE PREPARED	AVERAGE YIELD**	OVERALL YIELD**
1	149.7, 149.1	$(Tp)_{23}T$	96.8%	47%
2	150.1, 149.4	$d[(Cp)_{23}C]$	97.5%	56%
3	149.8, 149.6	$d[(Ap)_{23}A]$	98.9%	78%
4	149.4, 149.3	$d[(Ap)_{23}A]$	98.8%	75%
5	149.2, 149.1	$d[(Gp)_{23}G]$	93.3%	20%
6	149.4, 149.3	$d[(Gp)_{23}T]$	94.9%	29%

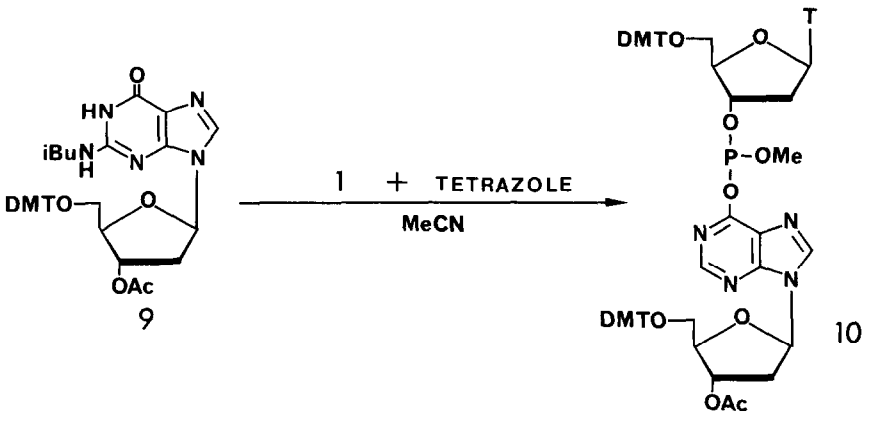
* - ppm (±0.1) downfield from 85% H_3PO_4 in $CDCl_3$

** - determined by trityl colour quantitation

TABLE 2. Automated DNA Synthesis Cycle.

STEP	REAGENT	DURATION	ELAPSED TIME
1	Acetonitrile wash	60 sec	1.0 min
2	3% Trichloroacetic acid/Dichloroethane	90 sec	2.5 min
3	Acetonitrile wash	60 sec	3.5 min
4	Nucleotide/Tetrazole addition	12 sec	3.7 min
5	Recycle	288 sec	8.5 min
6	0.1M I_2 H_2O/PYR/THF 1:2:7	30 sec	9.0 min
7	0.25M Ac_2O/DMAP/THF	60 sec	10.0 min

SCHEME 1



polyacrylamide gel). The sequences which contained one of the following bases: thymine; N^4-benzoylcytosine; N^6-benzoyladenine; or N^6-(N-methyl-2-pyrrolidine amidine)adenine[16]; all gave only one sharp and predominant band as the major component. It is interesting to note that no significant chain cleavage, as a result of depurination, occurred with either of the adenine containing sequences.

However, both the N^2-isobutyrylguanine and the N^2-(N-methyl-2-pyrrolidine amidine)guanine containing sequences were very unsatisfactory. The desired 24-unit long sequence could not be detected. Instead, there were many shorter products producing a smear on the gel. This smear was indicative of substantial chain degradation.

We believe that this poor result was due to the formation of a phosphite side product (e.g. Scheme 1) on the O^6-position of the guanine base. The base modified nucleoside **10** could not be isolated due to the instability of the phosphite linkage. However, the ^{31}P NMR spectrum of an acetonitrile solution of **1** (1 eq.), **9** (1 eq.) and tetrazole (3 eq.) showed the appearance of new phosphorus signals at 133.95 and 133.79 ppm, corresponding to the two diastereomers of compound **10**. These signals did not occur when the O^6-(4-nitrophenylethyl)-protected nucleoside **7**[13] was substituted for **9** in the NMR experiment.

Further proof that the O^6-position of guanine was interfering in the synthesis, was obtained by repeating the **d[(Gp)$_{23}$G]** synthesis with the O^6-protected derivative **8**. The resulting product was completely deprotected and run on a 20% polyacrylamide electrophoresis gel. This time the crude material contained only a single major product and there was virtually no sign of lower molecular weight fragments.

The structure of the material obtained from this latter synthesis was further confirmed, as being free from base modifications, by digestion with snake venom phosphodiesterase and alkaline phosphatase. Analysis of the digest by HPLC showed only the presence of deoxyguanosine.

This result shows that much better synthetic material can be obtained when guanine bases are protected on both the N^2- and O^6-positions. This observation should prove to be very important in the future synthesis of long oligonucleotides, such as gene fragments, or in the synthesis of shorter oligonucleotides which contain a high percentage of guanine bases.

Acknowledgements

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References

1, S.L. Beaucage and M.H. Caruthers, Tetrahedron Lett. **22**, 1859-1862 (1981).

2, H.G. Gassen and A. Lang, ed., "Chemical and Enzymatic Synthesis of Gene Fragments. A Laboratory Manual", Verlag Chemie, Weinheim, 1982.

3, M.J. Gait, ed., "Oligonucleotide Synthesis - A Practical Approach", IRL Press Ltd., Oxford, 1984.

4, S.P. Adams, K.S. Kavka, E.J. Wykes, S.B. Holder, and G.R. Gallupi, J. Am. Chem. Soc. **105**, 661-663 (1983).

5, M.S. Urdea, J.P. Merryweather, G.T. Mullenbach, D. Coit, U. Heberlein, P. Valenzuela, and P.J. Barr, Proc. Natl. Acad. Sci USA **80**, 7461 (1983).

6, C.B. Reese and A. Ubasawa, Tetrahedron Lett. **21**, 2265-2268 (1980).

7, H.P. Daskalov, M. Sekine, and T. Hata, Bull. Chem. Soc. Jap. **54**, 3076-3083 (1981).

8, S. DeBernardini, F. Waldmeir, and C. Tamm, Helv. Chim. Acta **64**, 2142-2147 (1981).

9, C.B. Reese and P.K. Skone, J. Chem. Soc. Perkin Trans. I, 1263 (1984).

10, S.S. Jones, C.B. Reese, S. Sibanda, and A. Ubasawa, Tetrahedron Lett. **22**, 4755-4758 (1981).

11, B.L. Gaffney and R.A. Jones, Tetrahedron Lett. **23**, 2257-2260 (1982).

12, M. Sekine, J. Matsuzaki, M. Satoh, and T. Hata, J. Org. Chem. **47**, 571-573 (1982).

13, F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, and W. Pfleiderer, Tetrahedron **40**, 59-72 (1984).

14, T. Kamimura, M. Tsuchiya, K. Urakami, K. Koura, M. Sekine, K. Shinozaki, K. Miura, and T. Hata, J. Am Chem. Soc. **106**, 4554-4557 (1984).

15, R.T. Pon and K.K. Ogilvie, Nucleosides and Nucleotides, 3, 485-500 (1984).

16, L.J. McBride and M.H. Caruthers, Tetrahedron lett. **24**, 2953-2956 (1983).

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