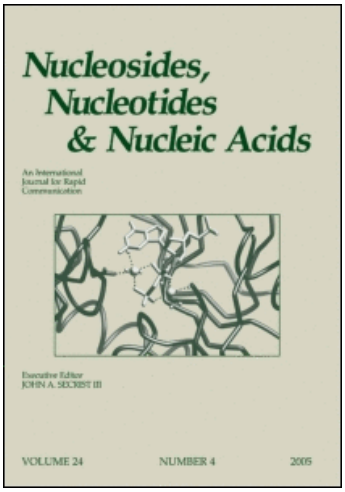

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Synthesis of Oligodeoxynucleotides Containing 5-Bromouracil and N6-Methyladenine

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SYNTHESIS OF OLIGODEOXYNUCLEOTIDES CONTAINING 5-BROMOURACIL AND N6-METHYLADENINE

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Summary : Oligodeoxynucleotides containing uracil, 5-bromouracil and N6-methyladenine were prepared by phosphotriester or amidite approaches and by a hybrid phosphotriester-phosphoramidite method.

Various oligonucleotides bearing the modified bases uracil, 5-bromouracil and N6-methyladenine have been prepared for biological purposes : the study of the effects of ionizing radiation on DNA [1] and of DNA *repair enzymes* [2].

With these objectives in mind, rather large quantities of various oligonucleotides bearing these modified bases were required and we wish to report the procedures we used to synthesize these products.

Silica gel solid phase synthesis was used to obtain the oligonucleotides bearing dU and BrdU. In the laboratory, at that time, only phosphotriester derivatives of dU and BrdU were available and also large quantities of the classical set of the phosphoramidite reagents for the natural nucleosides. Therefore, rather than prepare the corresponding phosphoramidites for dU and BrdU, a hybrid method for the assembly of the protected monomers on controlled pore glass support was performed. Phosphoramidite and phosphotriester methods [3,4] were used in turn on the support as described in the table. In this way, excellent yields of pure deprotected oligonucleotides were obtained (30 to 60 % for octanucleotides).

To analyse the influence of adenine methylation on the structure of the sequence GATC, the synthesis of d(GG^{m6}ATCC) and d(GG^{m6}ATATCC) were performed by phosphotriester and phosphoramidite methods. N6-methyl-2'-deoxyadenosine iodide salt was obtained and tritylated at the 5' position with satisfactory yield (55 %) [5].

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TABLE : Schedule for the condensation cycles

Operation	Time
Detritylation 2 % TCA in CH_2Cl_2	5'
Wash CH_3CN (5 x 3 ml)	
Drying with anhydrous acetonitrile (3 x 2 ml)	
Condensation :	
- Morpholino methoxy phosphine nucleoside 10 eq. + Tetrazole 40 eq. in dry acetonitrile (2 ml)	10'
or - Protected 3'-phosphodiester nucleoside 5 eq. + MSCL 10 eq. + 1-methyl imidazole 20 eq. in dry acetonitrile (2 ml)	20'
- Wash with CH_3CN (5 x 3 ml)	
Oxidation : I_2 1 g ; H_2O 32 ml ; THF 32 ml ; Lutidine 16 ml 2 x 3 ml (only in phosphoroamidite cycles)	2'
- Wash with THF (5 x 3 ml)	
Wash with CH_3CN (5 x 3 ml)	
Capping (CH_3CN/CH_3Im 4/1, 1.5 ml + CH_3CN/Ac_2O 4/1, 1.5 ml) two times	2'
Wash with CH_3OH (2 x 3 ml)	
Wash with CH_2Cl_2 (2 x 3 ml)	

Phosphoroamidite synthesis required the preparation of the corresponding phosphoramidite protected monomer. The one pot reaction sequence proposed by FOURREY et al was used [6]. 5'-O-[4,4'dimethoxytrityl]-N6-methyl- 2'-deoxyadenosine-3'-O-morpholino methoxyphosphine was obtained by reacting methoxy bis-triazolylphosphine with 5'-dimethoxytrityl-N6-methyl-2'-deoxyadenosine and then with trimethylsilyl morpholine in THF at low temperature. The quality of crude product obtained was checked by ^{31}P-NMR and FAB mass spectrometric analysis. No decrease in the efficiency in the phosphoramidite assembly cycle was observed when the N6-methyl-adenosine phosphoramidite unit was used in place of the protected N-benzoylated one. Deprotection was performed by thiophenoxide/triethylamine in tetrahydrofuran and then by concentrated ammonia.

Phosphotriester synthesis required the corresponding p-chlorophenyl phosphodiester derivative. The phosphorylation method proposed by CHATTOPADHYAYA and REESE for the natural nucleotides was adapted to give 5'-O-(4,4'-dimethoxy trityl)-N6-methyl-2'-deoxyadenosine-3'-p-chlorophenyl phosphate [7]. The nature and the purity of the protected monomer were checked by FAB mass spectrometry and ^{31}P-NMR. The assembly of the protected monomeric units was carried out with a BIOSEARCH synthesizer. The N6-methyl protected monomer was included in the assembly cycle in place of a N6-benzoylated protected deoxyadenosine unit (see phosphotriester cycle table). No difference in the detritylation reaction was ob-

served when the N6-methyl-adenosine unit was employed. Capping proved to be unnecessary. The oligonucleotides were deblocked with oximate and then by concentrated ammonia. They were purified by hplc on PARTISIL 10 SAX.

Characterization of the oligonucleotides bearing the modified nucleosides was performed by enzymatic hydrolysis with 3'-exonuclease and alkaline phosphatase and then analysis by h.p.l.c.

Methylation on both strands in the sequence GATC decreases the stability of the helix (B form). A detailed ^{1}H-NMR study showed that the preferred orientation of the adenine methyl group (methyl cis to N1) hinders base pair formation [8].

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