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THE PROTECTION OF URACIL AND GUANINE RESIDUES IN OLIGONUCLEOTIDE SYNTHESIS

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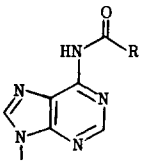
Summary: The protection of uracil and 2-N-acyl guanine residues with 4-O-phenyl [or 4-O-(2,4-dimethylphenyl)] and 6-O-(2-nitrophenyl) groups as in 7a [or 7b] and 9, respectively, is described. These O-aryl protecting groups, which appear to withstand the usual conditions of oligonucleotide synthesis, may readily be removed by treatment with 2-nitrobenzaldoximate ions.

In their studies on the synthesis of oligonucleotides by the phosphodiester approach, Khorana and his coworkers introduced[1] the use of N-acyl protecting groups for adenine, cytosine and guanine residues [as in 1, 2 and 3, respectively] but left uracil[2] and thymine[1] residues unprotected [as in 4a and 4b, respectively]. Despite subsequent progress in oligonucleotide synthesis, including the development of the phosphotriester approach[3], no significant improvements in the methodology of base-residue protection have been reported in the literature prior to this report. Indeed, virtually every worker in the field[4] has followed Khorana's original initiative[1,2].



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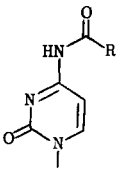

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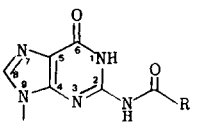

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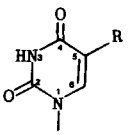
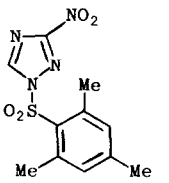


4 a, R = H
b, R = Me



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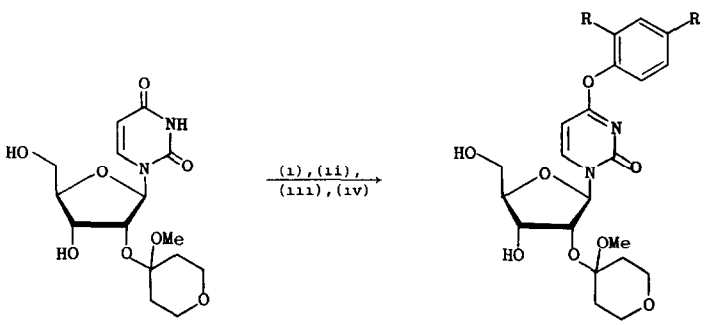
We and no doubt other investigators had suspected[5] for a number of years that guanine residues were inadequately protected by 2-N-acylation [as in 3] against phosphorylation and other side-reactions in oligonucleotide synthesis. However, we had not even considered the possibility of the occurrence of side-reactions involving other base residues until very recently, in the course of a study directed towards the synthesis of the 3'-terminal decaribo-

nucleoside nonaphosphate[6] of yeast alanine transfer ribonucleic acid ($tRNA^{Ala}$), we found[7] that 2-N-acyl guanine and uracil residues [as in 3 and 4a] could both undergo modification [at C-6 and C-4, respectively] during the second phosphorylation step of the phosphotriester approach when 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT, 5)[6,8] or the corresponding tetrazole derivative[9] was used as the condensing agent. We have subsequently found that the use of o-chlorophenyl phosphorodi-(1,2,4-triazolide)[10] in the first phosphorylation step of the phosphotriester approach can also lead[11] to related side-reactions. Thymine residues [as in 4b] appear[7] to be less susceptible to these side-reactions. While we successfully completed[6] the synthesis of the above decaribonucleoside nonaphosphate using the conventional methodology of base residue protection, we were unable[12] to synthesize the next nonamer sequence [AUUCCGGAC], let alone complete the synthesis of the 3'-terminal nonadecaribonucleoside octadecaphosphate of yeast $tRNA^{Ala}$ in this way. We have therefore undertaken the development of new protecting groups for uracil and guanine residues in oligoribonucleotide synthesis.

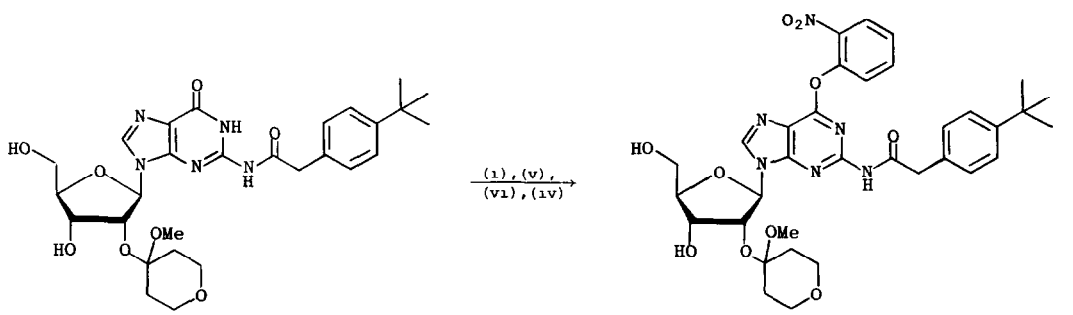
Schemes

I



6 → 7 a, R = H; b, R = Me

II



8 → 9

Reagents (i) $(MeOCH_2CO)_2O/C_5H_5N$; (ii) 3-nitro-1,2,4-triazole-$(PhO)_2POCl/C_5H_5N$, (iii) (a) PhOH or (b) 2,4-$Me_2C_6H_3OH$-Et_3N/MeCN, (iv) NH_3/MeOH; (v) $MeSO_2Cl$-Et_3N/CH_2Cl_2; (vi) 2-$O_2NC_6H_4OH/C_5H_5N$.

We decided to investigate the possibility of using 4-O-aryl groups for the protection of uracil residues and 6-O-aryl groups for the further protection of 2-N-acyl guanine residues. 2'-O-Methoxytetrahydropyranyluridine[13] (6) was converted into its crystalline 4-O-phenyl and 4-O-(2,4-dimethylphenyl)-derivatives [7a and 7b; m.p.'s 159-160° and 115°C, respectively][14] by the four-step procedure indicated in Scheme I. The overall yields of 7a and 7b were 71

and 77%, respectively. 2-N-(4-t-Butylphenylacetyl)-2'-O-methoxytetrahydropyranylguanosine (8) was converted into its 6-O-(2-nitrophenyl)-derivative[15] (9) in 37% overall yield, by the four-step procedure indicated in Scheme II.

The protection of uracil and 2-N-acyl guanine residues with 4- and 6-O-aryl groups, respectively, would be expected to suppress the side-reactions which are presumably initiated by electrophilic attack during phosphorylation. However, in order to be useful, the new protecting groups must be able to withstand mildly basic conditions and relatively weak nucleophiles (e.g. morpholine, see below), and they must be easily removable at the end of the oligonucleotide synthesis. Building blocks (7a, 7b and 9) were therefore subjected to three main tests. First, they were treated with 0.2 M-potassium carbonate in dioxan-water (4:1 v/v) at room temperature and found to be completely unchanged (by t.l.c.) after 3 hr. Secondly, 0.01 M-solutions of all three compounds in tetrahydrofuran-water (98:2 v/v) were treated with 5 molecular equivalents of morpholine at room temperature. While 7a and 7b were completely unchanged (t.l.c.) after 5 hr, 9 was *ca.* 5% converted into what is assumed to be the corresponding 6-morpholino compound after 3 hr. Finally, when 0.05 M-solutions of 7a, 7b and 9 in anhydrous dioxan solution were treated with 10 molecular equivalents of *syn*-2-nitrobenzaldoxime and 9 molecular equivalents of N^1,N^1,N^3,N^3-tetramethylguanidine at room temperature, they were quantitatively converted back into 6, 6 and 8, respectively, after *ca.* 15, 120 and 150 min. When preparative scale (i.e. starting with *ca.* 0.5 mmol of substrate) regeneration experiments were carried out with 7b and 9 under the latter conditions, 6 and 8 were recovered as pure crystalline solids in 83 and 93% yields, respectively.

The above experiments with 0.2 M-potassium carbonate clearly demonstrate that the O-aryl protecting groups will not be lost from 4-O-phenyl- and 4-O-(2,4-dimethylphenyl)-uracil residues [as in 7a and 7b, respectively] or from 6-O-(2-nitrophenyl)-2-N-acyl guanine residues [as in 9] during work-up and other manipulations requiring mildly basic conditions. The experiments with morpholine show that the 2-dibromomethylbenzoyl (DBMB) protecting group[16] can be used in conjunction with the latter O-aryl groups. The morpholinolysis conditions required to cleave the 2-formylbenzoyl group [obtained[16] following Ag^+-promoted hydrolysis of the DBMB group] are such that 4-O-phenyl- and 4-O-(2,4-dimethylphenyl)-uracil residues [as in 7a and 7b, respectively] will be completely unaffected and the modification of 6-O-(2-nitrophenyl)-2-N-acyl guanine residues [as in 9] will be negligible (±0.5%). If mild alkaline hydrolysis is used instead of morpholinolysis to remove 2-formylbenzoyl groups, no modification whatsoever of any of the base residues will occur. Finally, the conditions required for 2-nitrobenzaldoximate ion removal of the O-aryl groups from 7a, 7b and 9 are such that complete regeneration of the uracil and 2-N-acyl guanine residues will occur under the conditions which are used to remove 2-chlorophenyl protecting groups[17] from the internucleotide linkages at the end of an oligoribonucleotide synthesis.

When it became clear that phenyl and 2,4-dimethylphenyl groups were both satisfactory for the protection of uracil residues and that 2-nitrophenyl groups were satisfactory for the protection of 2-N-acyl guanine residues, we repeated the synthesis of the 3'-terminal decaribonucleoside nonaphosphate [UCGUCCACCA] of yeast $tRNA^{Ala}$ using 7b and 9 as building blocks, instead of 6 and 8, respectively. By means of the same synthetic strategy as was used before[6], higher yields of the corresponding protected and partially-protected intermediates were obtained in the reactions involving protected uracil and 2-N acyl guanine residues [as in 7b

and 9, respectively]. It seems likely that the improved yields were due partly to the suppression of side-reactions and partly to the enhanced lipophilicity of the intermediates which in turn led to their being easier to fractionate by short column chromatography[18]. The purity of the isolated decaribonucleoside nonaphosphate, following removal of all of the protecting groups in the usual way and chromatography of the products on DEAE-Sephadex, was very high. Full details of the synthesis of the latter decaribonucleoside nonaphosphate and indeed of the 3'-terminal nonadecaribonucleoside octadecaphosphate sequence [AUUCCGGACUCGUCCACCA] of yeast $tRNA^{Ala}$, using the new O-aryl base protecting groups, will be reported shortly.

Finally, we have prepared[19] 4-O-phenylthymidine and 2-N-acetyl-6-O-(2-nitrophenyl)-2'-deoxyguanosine and propose to investigate their use in the synthesis of oligodeoxyribonucleotides.

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[14] Satisfactory spectroscopic and microanalytical data were obtained for these compounds.
[15] Compound 9 has not been obtained crystalline; however, its constitution is firmly based on spectroscopic (n.m.r., m.s.) data and on its having been prepared from a pure crystalline 2-O-methoxytetrahydropyranyl derivative, 8. Preliminary experiments have indicated that aryl groups derived from phenols (e.g. phenol itself and 2,4-dichlorophenol) with pK_a's higher than that of 2-nitrophenol are removed too slowly by oximate ion treatment to be suitable for the protection of 2-N-acyl guanine residues in oligonucleotide synthesis.
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