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SOME ASPECTS OF REACTIVITY AND PROTECTION OF THE IMIDE FUNCTIONS OF URIDINE AND GUANOSINE IN NUCLEIC ACID SYNTHESIS.

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Abstract:

The chemistry of acyl and arenesulfenyl groups for the N^3 protection of uridine has been reviewed along with the preparation and properties of O^4 and O^6 pyridyl substituted uridines and guanosines, respectively. It has turned out that the pyridyl groups can both protect the uracil and the N^2-protected guanine moieties of ribonucleosides and act as good leaving groups in nucleophilic substitution reactions allowing site-specific modification to take place in sugar-modified ribonucleosides. A combination of appropriate O^4 aryl groups and N^3 and O^4 groups on uridine have constituted a new strategy for the site-specific modification in fully protected oligouridylic acid during the final deprotection step. Finally, our studies on the utility of O^6 vs. N^1 protection of an N^2-protected guanosine to prevent the side reactions of the guanine moiety have been reviewed in the light of our ^{15}N-NMR studies of different O^6 and N^1 protected guanosine derivatives.

The protection of the O^4/N^3 and O^6/N^1 imide functions of uridine and guanosine, respectively, is necessary in the synthesis of nucleic acids in order to avoid the formation of by-products during phosphorylation reactions[1-11]. Several protecting groups have been proposed[8-23]. These groups protect the desired functions and are simply removed at the end of the synthesis; however, they cannot be used as leaving groups for site specific modifications. The only exceptions are triazolyl and tetrazolyl groups[24-27] which have been employed for site-specific modifications at C-4 and C-6 of uracil (thymine) and guanine residues, respectively.

1.1. The N^3-acyl groups for protection of imide function of uridine.

In view of the convention of using acyl groups for protection of the exocyclic amino functions of adenine, guanine and cytosine[28], it was considered desirable to explore the use of similar groups for protection of the uracil moiety. That this had not previously been the practice was due mainly to the lack of a method for selectively introducing a group to the base whilst leaving the three hydroxyl functions unreacted. Uridine (1a) could be converted

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to N^3- acyluridines (2) - (6) in 50 - 87% yields using transient protection of the hydroxyl functions with trimethylsilyl groups[29,30] to give 1b (Scheme 1). Acylation could possibly take place on either the O^4 or the N^3 position. It has been shown by ^{13}C-NMR spectroscopy[31] that the acyl group is attached at the N^3. For N^3-substituted uridines the chemical shifts of C-2, C-4 and C-5 are similar to that of uridine whereas for O^4-substituted uridines different chemical shifts are obtained for these carbon atoms[31], as supported by the results of other workers[13,32].

The protecting group used for the uracil residue must be able to withstand all of the conditions necessary for chemical manipulations in nucleic acid chemistry, and yet be easily removed at the desired moment in order to yield uridine. To determine which of the five acyl uridines is most suitable for RNA synthesis, the compounds (2) - (6) were treated with a variety of reagents and their stabilities or labilities were estimated. This study made it clear that the N^3-acyl groups would be suitable for protection of uridine in syntheses involving groups such as 2-dibromomethylbenzoyl[33], levulinyl[34], t-butyldimethylsilyl[35-38], 2-(trimethylsilyl)ethoxycarbonyl[39], 1,1,3,3-tetraisopropyldisiloxyl[40] and a series of β-eliminating phosphate protecting groups[41-45]. These results also illustrated that apart from mesitoyl, which was far too stable to the deprotecting conditions, all of the acyl groups studied would be suitable for further use. We therefore used benzoyl group for the N^3 protection of uridine and showed its application in olgoribonucleotide synthesis[46] and in the synthesis of 2'-O- methyluridine (7)[16], a modified nucleoside occurring naturally in tRNA[47-56]. These studies clearly showed that the N^3-benzoyl group is a useful imide protecting group for uridine.

1.2. Preparation and application of arenesulfenyl groups for the protection of N^3 of uridines.

Substituted benzenesulfenyl groups have found considerable usage for protection of amino functions in peptide chemistry[57,58], and for protection of hydroxyl functions in DNA and RNA synthesis[59], both in solution and on a solid support. More recently the 2-nitrobenzenesulfenyl (Nbs) group has been used for protection of the exocyclic amino functions of guanine, cytosine and adenine residues in both DNA and RNA synthesis[60]. In all of these cases the removal conditions required are mild and give relatively short deprotection times. During the preparation of 3',5'-di-O-(2-nitrobenzene)sulfenyl thymidine Letsinger *et al.*[59] reported the formation of a *tris*-Nbs thymidine derivative, however, this compound was neither structurally assigned nor used for any synthetic purpose. This led us to explore the possible use of the Nbs group for uridine protection in RNA synthesis19.

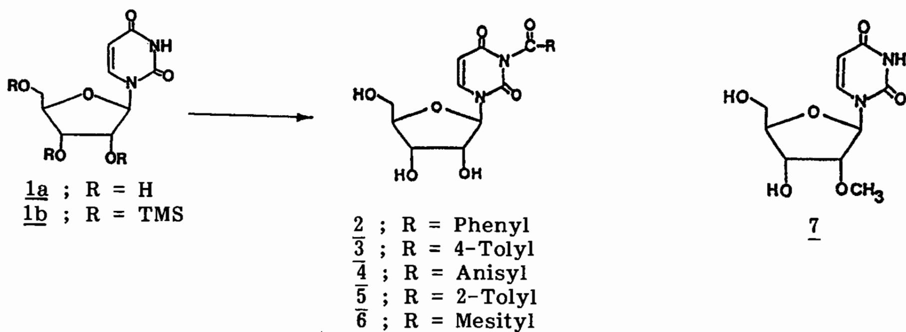
It was found necessary to have an ortho-nitro group in order to obtain an N^3-substituted uridine derivative, thus three groups were chosen for study; the 2-nitrobenzene (Nbs), 2,4-dinitrobenzene (Dnbs) and 2-nitro-4-methylbenzene (Nts) sulfenyl groups. The derivatives (8 - 10) were subjected to a series of reaction conditions likely to be encountered during a solution phase RNA synthesis. All three groups were found to be stable to the standard conditions used for removal of acid labile protecting groups (eg. Pixyl[61] and dimethoxytrityl[62,63]) and ß-eliminating groups (eg. Fmoc[64], ß-arylsulfonyl ethyl[41-44]). However, the stability of these groups to tetrabutylammonium fluoride in tetrahydrofuran, and to aqueous pyridine was less than that required for a successful RNA synthesis. These problems were overcome successfully as demonstrated by the synthesis of 2'-O-methyluridine (7) and the partially protected nucleosides 15 and 16 (Scheme 2). Methylation of compounds 11 and 12 was carried out using the iodomethane and silver oxide[53] to give 2'-O-methylated products 13 and 14 in 94 and 96% yields respectively; these compounds were then deprotected to give 2'-O-methyluridine (7) in 91 and 92% yields, respectively. This method for the synthesis of 2'-O-methyluridine is a considerable improvement on our previous method using acyl protecting groups[16], the overall yield being 71 or 76% depending on choice of arenesulfenyl group, compared to 56% using N^3-benzoyluridine, and 29% reported elsewhere in the literature[9].

Attempts to demonstrate the use of the arenesulfenyl groups in oligoribonucleotide synthesis were unsuccessful because of the instability of the 2',5'-bis-protected nucleosides 15 and 16 during phosphorylation reaction[19].

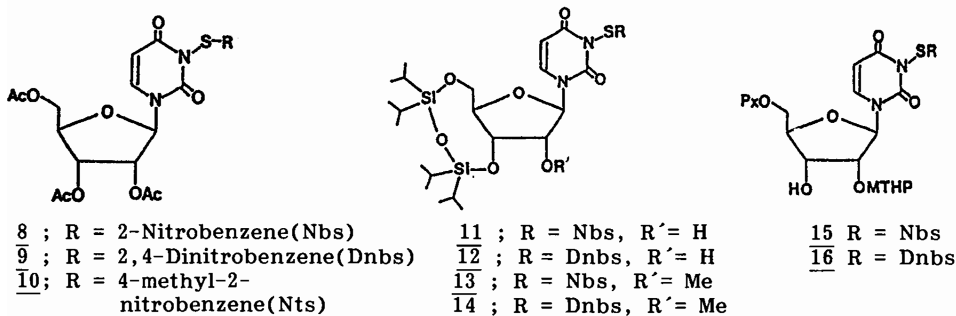
1.3. Pyridyl groups for the protection of the O^4 and O^6 imide functions of uridine and guanosine respectively.

The imide functions of uridine and guanosine have many similarities in their reactivity to the reagents used during oligonucleotide synthesis[3,65]. Work carried out in this laboratory[66,67] has clearly demonstrated that protection of the imide of guanosine by use of a group attached to the N^1 position is insufficient, because such N^1-protected guanosine derivatives have enhanced nucleophilicity at N^7, promoting the electrophilic attack at this site during a phosphorylation or a methylation reaction, which then leads to ring opening of the imidazole system. Necessity thus dictated that the O^6-function must be protected in order to prevent the formation of by-products due to side reactions on an N^2-protected guanosine[1-11].

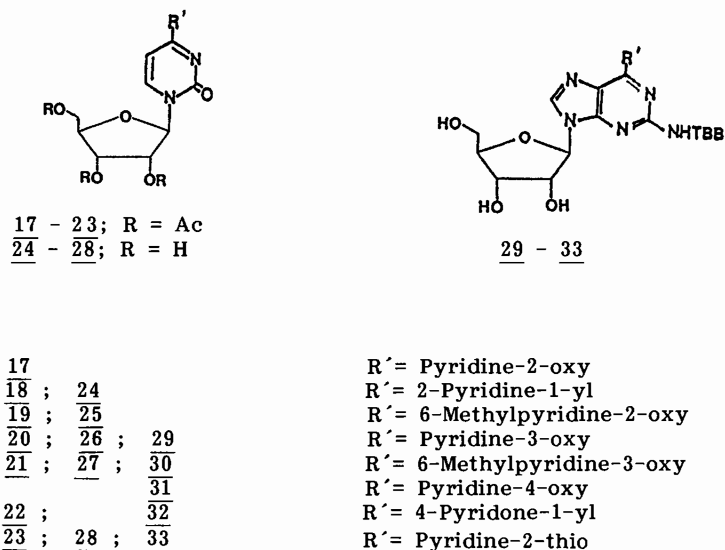
Substituted pyridyl groups have been found to be a very attractive solution for the protection of the O^4 of uracil and the O^6 of N-2 protected guanine residues of ribonucleosides. They are stable and can be stored indefinitely at room temperature. These pyridyl groups are also found to be good leaving groups



Scheme 1



Scheme 2



Scheme 3

for nucleophilic substitution reactions by amines, thiolates and oximate; the rate of these substitution reactions could be increased by almost 1000-fold by conversion of the pyridyl moiety to its methiodide.

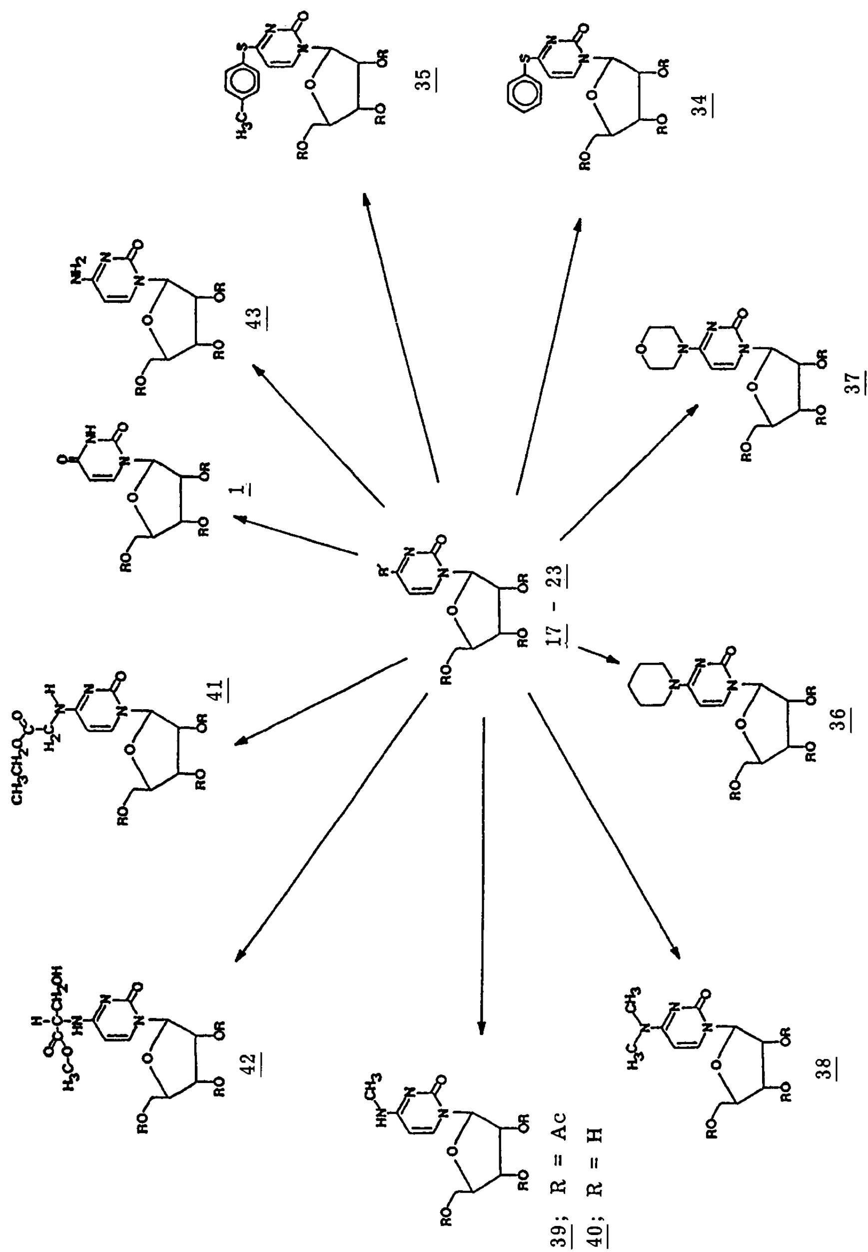
2-Pyridone (pK_a ca. 11 and 5.23), 6-methyl-2-pyridone, 4-pyridone (pK_a 11.09 and 3.07), 2-pyridinethiol (pK_a 9.97 and 5.27), 3-hydroxypyridine (pK_a 8.72 and 4.86) and 6-methyl-3-hydroxypyridine were considered as suitable candidates[68].

1.3.1. Preparation of pyridyl derivatives of uridine and guanosine.

The C-4-triethylammonium derivative of uridine[20,21], was reacted with substituted pyridines, to give the corresponding C-4-pyridyluridines (17) to (28) (Scheme 3). 4-pyridone, as expected[72], gave the N-substituted product 22 in 91 % yield and the 2-pyridone gave a mixture of O- and N-substituted products 17 and 18 in 1:2 ratio while 6-methyl-2-pyridone and 2-pyridinethiol gave almost quantitatively the O-and S-substituted products 19 and 23 respectively. On the other hand, 3-hydroxy- and 6-methyl-3-hydroxypyridines, gave the expected[71,72] O-substituted products 20 and 21 in 89 and 80 % yields respectively. The O-6 protected pyridyl derivatives of guanosine (29 - 33)[66], (Scheme 3) were also prepared. Both sets of compounds were studied in order to explore their utility in oligoribonucleotide synthesis[73].

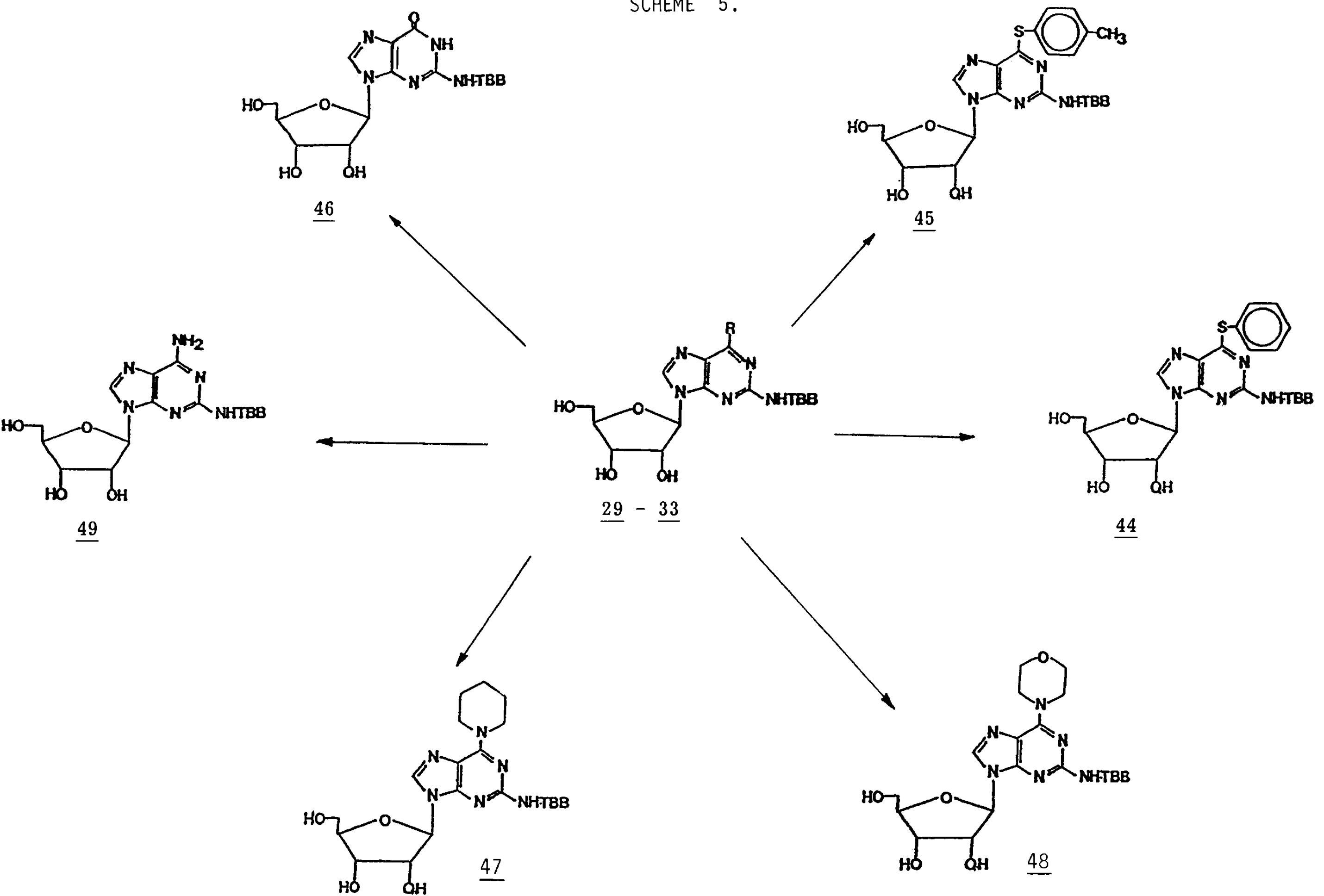
1.3.2. Nucleophilic substitution reactions of pyridyl derivatives of uridine and guanosine.

It is well established[74] that mutagenic and carcinogenic modifications, such as alkylation, occur at the C-4 site of pyrimidine and the C-6 site of guanine residues of nucleic acids. In order to study the properties of such modified nucleosides in synthetic oligonucleotide chemistry, an efficient and specific method for their introduction is required. A further requirement is the study of both the modified and non-modified nucleosides/nucleotides, which should ideally be formed from the same precursor. A survey of pertinent pyridine chemistry[69,71] made it clear that a pyridyl group and especially its methiodide would constitute a good leaving group in a nucleophilic substitution reaction, allowing pyridyl groups both as protecting groups for uracil and guanine residues and also as useful building blocks for any site-specific modifications[24-27]. This was demonstrated when compounds 17 - 23 and 29 - 33 were subjected to nucleophilic substitution reactions using a variety of thiolates and amines as nucleophiles (Schemes 4 and 5 respectively). The reaction of benzenethiolate and 4-methylbenzethiolate ion on the latter substrates gave 34, 35, 44 and 45, respectively in 71 - 93 % yields while the reaction with piperdine, morpholine and dimethylamine gave 36, 37, 38, 47 and 48 in 87 - 96% yields. A comparison of half-lives in the latter reactions, along with the



Scheme 4

SCHEME 5.



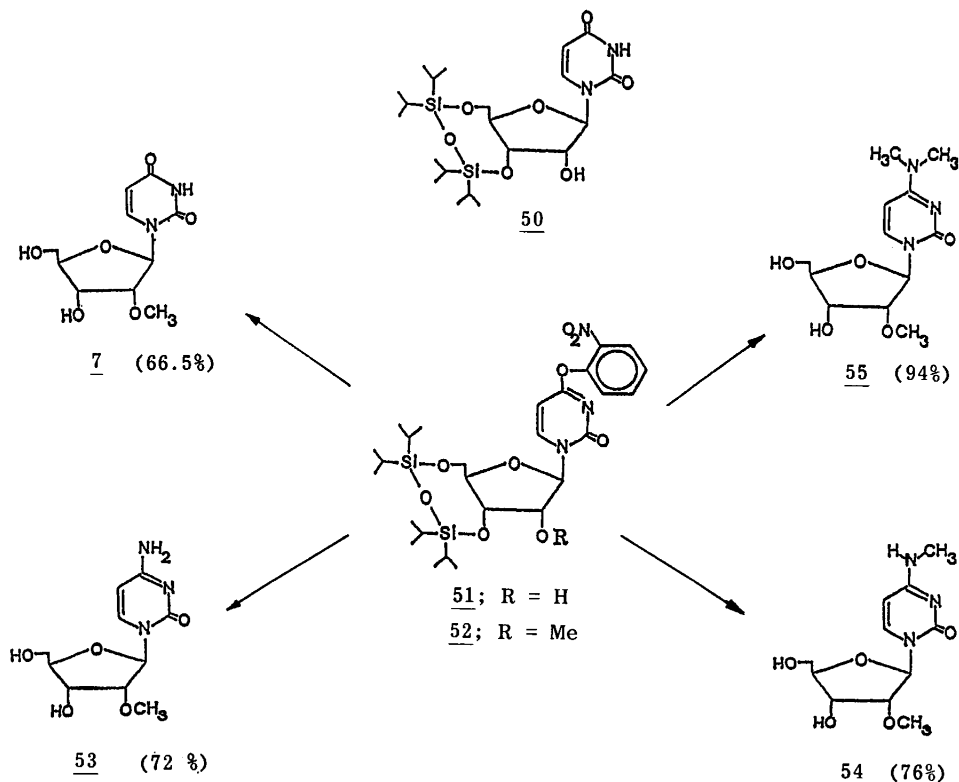
reactions with different primary amines clearly revealed that the nucleophilic substitution reactions of 17 to 23 and 29 to 33 with primary amines were several fold slower than with secondary amines as one would expect from a comparison of their respective pK_a[68]. However, compounds 40, 41 and 42 were easily obtained in 64 - 93 % yields when the methiodides of 20 and 21 were subjected to the similar reactions ($t_{1/2}$ ca. 10 min) with methylamine, glycine ethyl ester and serine methyl ester.

1.3.3. Synthesis of an mRNA fragment of Alanyl-tRNA synthetase gene in E. Coli using the 6-methyl-3-pyridyl group for the protection of the imide functions of uridine and guanosine.

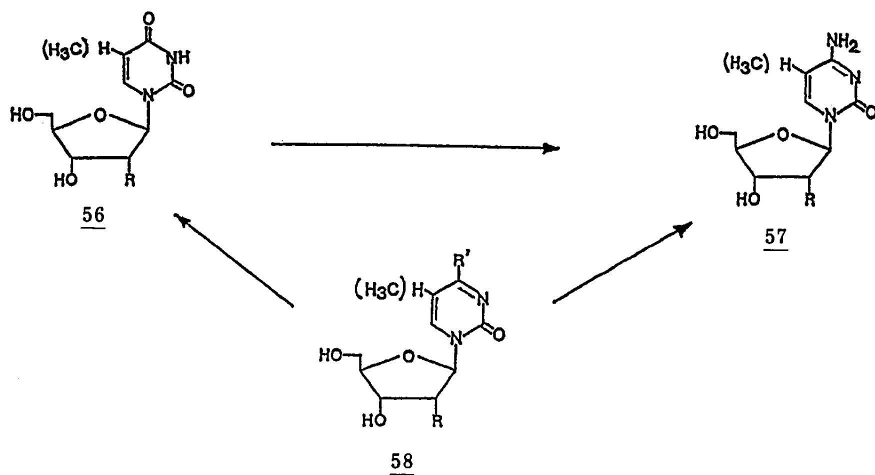
Having shown that the 6-methyl-3-pyridyl group was suitable as an imide protecting group for RNA synthesis, we synthesised[75] a pentaribonucleotide fragment, 5'-GGUGU-3', corresponding to part of the alanyl tRNA synthestase gene in Escherichia Coli. The successful synthesis of this pentaribonucleotide clearly show that the 6-methyl-3-pyridyl is an efficient protecting group for the imide functions of uridine and guanosine, able to protect the aglycones from any subsequent electrophilic attack during phosphorylation and condensation reactions. Subsequent work[76] has also demonstrated the ease with which modified nucleotides may be obtained by varying the deprotection conditions.

1.4 Synthesis of $O^{2'}$-methyluridine, $O^{2'}$-methylcytidine $N^4,O^{2'}$-dimethyl cytidine and $N^4,N^4,O^{2'}$-trimethylcytidine from a common O^4-(2-nitrophenyl)uridine derivative.

Subtle modification of nucleic acid structure is critical in controlling the mechanism of protein biosynthesis and for the effective functioning of nucleic acid molecules in complex biochemical process[77]. In Nature these modifications are often methylation of the base and/or the 2'-hydroxyl function of the pentofuranose moiety of specific nucleosides in nucleic acids[74,78,80]. Chemical modifications of nucleic acids have therefore attracted increasing attention in Nucleic acid research[74,78-86] and in mutagenesis[82] and carcinogenesis studies[83]. To this end, we have reported facile syntheses of $O^{2'}$-methyluridine (7), $O^{2'}$-methylcytidine (53), $N^4,O^{2'}$-dimethylcytidine (54) and $N^4,N^4,O^{2'}$-trimethylcytidine (55) from a common precursor 51 (Scheme 6)[87]. These compounds have previously been prepared using multi-step synthetic routes[9,16,19,53-56,77,84,85] with, in most cases, poor overall yields. The main problem in the synthesis of methylated nucleosides is the side-reaction on the pyrimidine base during the methylation of the 2'-hydroxyl function. We reasoned that a protected intermediate 51, readily available from 50, should be able to perform a dual role in the



Scheme 6



R = OH or H

R′= Leaving group

Scheme 7

synthesis of the target molecules 7 and 53 - 55. The O^4 protecting group of compound 51 not only protects the heterocyclic base during the methylation of the sugar hydroxyl function but it also constitutes a good leaving group in the displacement reactions with a nitrogen or oxygen nucleophile.

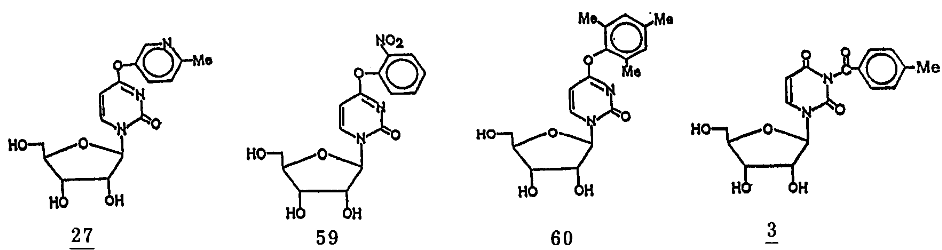
Treatment of 51 with iodomethane and silver oxide gave the 2'-O-methylated compound 52 which upon treatment with either oximate, ammonia, methylamine or dimethylamine, gave the products 7, 53, 54 and 55 in good overall yields (Scheme 6). This work demonstrated that an appropriate aryl group at C-4[14] can protect uridine against electrophilic attack, and yet can be used as a good leaving group for further modifications of the nuleoside.

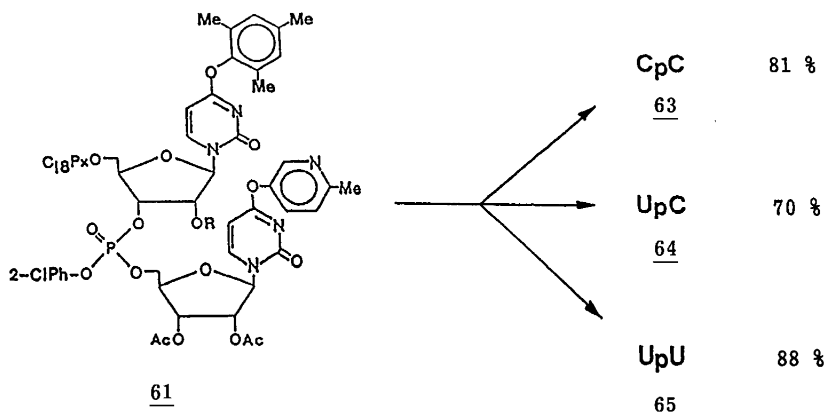
1.5 Site-specific modification of the pyrimidine residue during the deprotection of the fully-protected diuridylic acid.

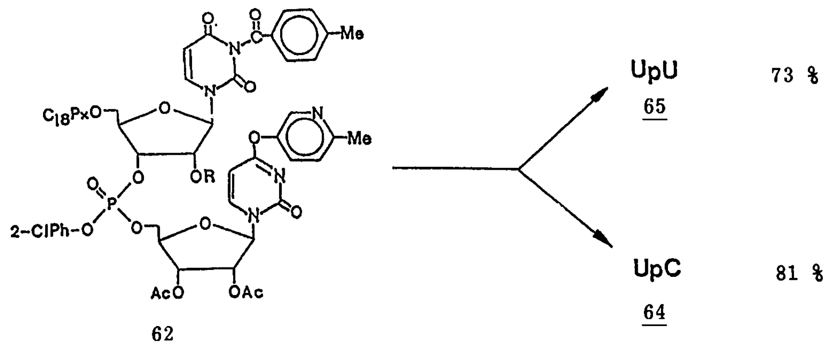
Despite the tremendous development[86] in the synthesis of specific sequences of DNA and RNA molecules, adequate chemical methodologies are not available for site-specific modification of aglycones of DNA and RNA. The only method which has been so far reported[24-27] for the selective modification of uracil or thymine (56) to cytidine or 5-methylcytidine (57) employs either a triazolyl or a tetrazolyl group at the C-4 position of the pyrimidine. An oxygen or a nitrogen nucleophile can then convert compound 58 (R'=triazolyl or tetrazolyl) to either a uracil (thymine) or cytosine (5-methylcytosine) nucleoside (Scheme 7). However, this procedure is limited by the fact that while it employs a C-4 substituted uracil (thymine) building block for the specific modification at the C-4 center, it leaves other uracil (thymine) moieties in the nucleic acid unprotected, thus allowing the formation of by- products due to side reactions[3-11].

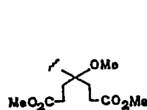
We have developed[76] two different procedures for site-specific modification of uracil to cytosine in which all uracil residues have been appropriately protected in such a way that it was possible to induce a specific modification to one of the protected uracil block to cytosine. For this purpose, we considered four different types[14-16,73] of O^4 and N^3 protected uracil building blocks, as shown in 3, 27, 59 and 60, for the synthesis of fully protected diuridylic acids 61 and 62, which could be converted to either CpC (63), UpC (64) or UpU (65) depending upon deprotection condition[76].

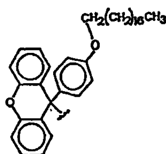
It was shown in our laboratory that the O^4-(6-methyl-3-pyridyl)[73] (MePy) protected block 27 was smoothly converted to cytidine quantitatively with 3 M ammonia in dry methanol, while the O^4-(2-nitrophenyl)[14] protected block 59, gave a 7:3 mixture of cytidine and the 4-methoxypyrimidone derivative (R' = OMe in 58), respectively in the latter reaction condition. The O^4-(2,4,6- trimethylphenyl) (TMP)[15] derivative 60 was completely stable under the above condition. On the other hand, the attack of a nitrogen or an

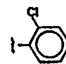












R

$C_{18}Px$

2-ClPh

Scheme 8

oxygen nucleophile converted blocks 27, 59 and 60 to the corresponding cytidine or uridine derivatives quantitatively. A consideration of reactivity of 27 versus the stability of 60 clearly suggested that a fully protected diuridylic acid U*pU+ (* = TMP, + = MePy) 61, depending upon the deprotection condition, should give either UpU, UpC or CpC. Compound 61 was therefore subjected[76] to deprotection in three different ways: treatment with 4-nitrobenzaldoximate ion[88] to give UpU (65), (2) treatment with tetrabutylammonium fluoride[89], followed by ammonia in methanol and then oximate[88] to give UpC (64) and (3) treatment with fluoride ion[89] followed by aqueous ammonia to give CpC (63) (Scheme 8).

Similarly, a fully protected diuridylic acid U§pU+ (§ = 4-toluoyl group at N-3[16], + = 6-methyl-3-pyridyl at O-4[73]) (62) was deprotected[76] in two different ways: (1) treatment with 4-nitrobenzaldoximate ions[88] in aqueous dioxane followed by the usual work-up and purification step gave UpU (65) in 73% yield, while (2) treatment with tetrabutylammonium fluoride[89] in pyridinetetrahydrofuran-water (1:8:1, v/v/v)[89], followed by the treatment with 3 M ammonia in dry methanol gave UpC (64) in 81% yield (Scheme 8).

This work[76] clearly demonstrated that a judicious choice of a O^4 protecting group for uracil moiety can serve as a good leaving group for the site-specific modification as well as to protect its urethane function from electrophilic attack.

1.6 Why should the imide function of the N^2-protected guanine be protected at O^6 but not at N^1 ?

It has been shown[1-11] that the N^2-protected guanine residue is susceptible to sulfonation by condensing agents and to phosphorylation by activated nucleotides at the O^6 position of the lactam. Several groups of workers have, therefore, proposed a variety of O^6-protecting groups[2-5] for N^2-protected guanosine residues. It is commonly believed that the N^2-protected guanine residue is adequately inert toward the attack of electrophiles as long as the lactam function is protected. What was not clear from the literature was how the protecting groups at O^6 or N^1 affect the nucleophilicity of the N^7-nitrogen and why the lactam function should be protected as the O^6 derivative, not as the N^1 derivative. In order to study this aspect of the lactam protection of guanine, we have prepared[66] 1,2-di-N-(2,2,2-trichloro-t-butyloxycarbonyl)guanosine derivative 66. Compound 66 should be normally considered to be a completely inert toward electrophiles. However, when treated with o-chlorophenylphosphoro-bis-(1,2,4-triazolide) 67, it gave a phosphoramidate quantitatively which was isolated after a quick work-up and chromatography in 23% yield[66]. Spectroscopic data (^1H, ^{13}C and ^{31}P NMR,

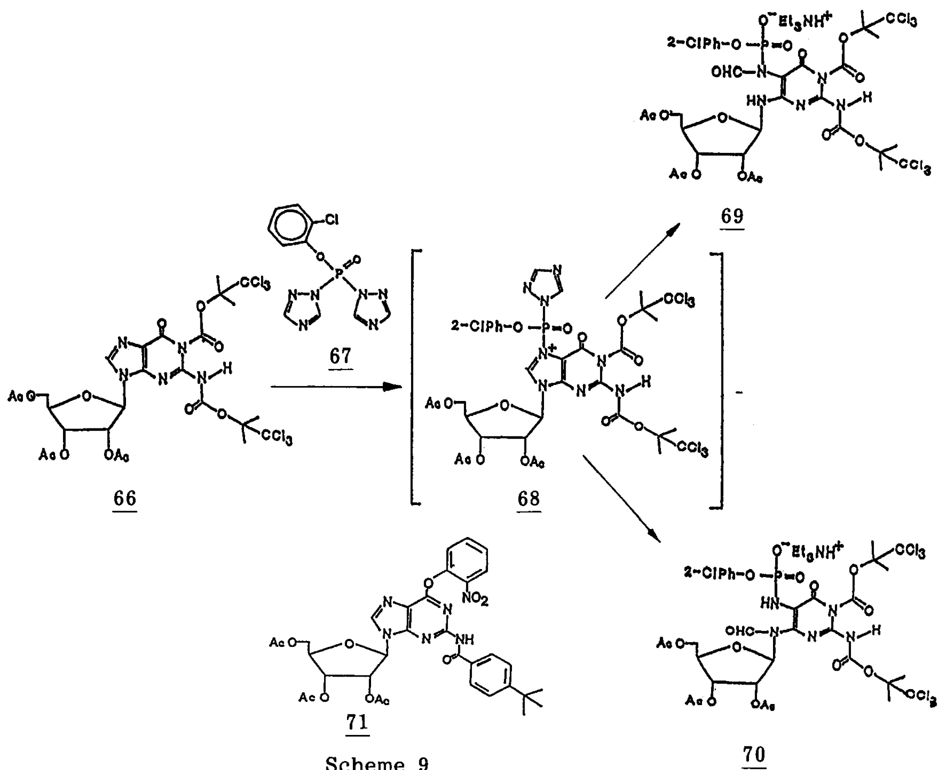
IR and UV) suggested that the structure of the phosphoramidate is either of the isomers 69 or 70 which is presumably formed through the intermediacy of 68. It should be noted that when 2-N-(4-t-butylbenzoyl)-6-O-(2-nitrophenyl)-2',3',5'-tri-O-acetyl-guanosine 71 was subjected to a similar phosphorylation condition, it was found to be completely inert[66]. It thus emerges from the foregoing study that the protection of the lactam function on the N^1 clearly enhances the nucleophilic character of the N^7 while the conventional O^6-protection reduces its reactivity which is also now substantiated by our ^{15}N-NMR study[67].

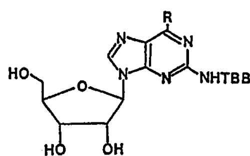
1.7 ^{15}N-NMR study of protected guanosine derivatives.

We have recently reported[67] our ^{15}N-NMR studies of a few O^6 protected guanosine derivatives (3-pyridyl-[73], 2-nitrophenyl-[14], 4-nitrophenylethyl-[22]) and shown how these O^6 protecting groups affect the electron density distribution of the resulting purine system. We have subsequently examined the effect of such redistributed electron density in the protected guanine residues on their chemical reactivities toward electrophilic reaction conditions such as protonation and methylation. Such a study has enabled us to define the "electronic criteria" that a O^6 protecting group of an appropriately substituted guanine derivative must fulfill in order to protect the structural integrity of the guanine residue.

The ^{15}N chemical shifts of the N^7 is expected to be influenced by its effective nuclear charge, its symmetry in the valence electrons and by its effective excitation energy ($n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$)[91]. Table 1 shows that a protecting group or a substituent either at O^6 or N^1 of guanosine has a definite effect on the nitrogen chemical shifts of purines. However, a comparison of the N^7 chemical shifts amongst 46, 29 and 72-76 in neutral DMSO and in their acidic solutions (Table 1) clarifies that the reactivity of the N^7 toward electrophiles can not be even qualitatively estimated from the ^{15}N chemical shifts alone in any of these media but from the magnitude of the difference in δ ^{15}N shift.

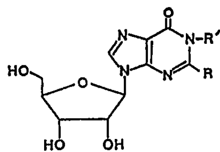
What is important is to understand how a protecting group on O^6 actually influences the nucleophilicity of N^7, and which of the O^6 protecting groups (O^6-alkyl or O^6-aryl) are safe to use for guanosine protection under electrophilic reaction conditions. The effect of the C^2-amino substituent is obvious through the comparison of chemical shifts of guanosine (72) and inosine (74) (Table 1). With one equivalent of acid (CF_3COOH) in inosine (74), the N^7 moves by almost 11 ppm[92] while the N^7 in guanosine, under a similar acidic condition, moves upfield by 75 ppm, establishing the electron-donating effect of the C^2-amino function. On the other hand, the N^7 in N^2-TBB-guanosine (46), under a similar acidic condition, was shielded by 25 ppm. The





Scheme 9

COMPOUNDS	29	30	31	33	75	76	32	77
R =								

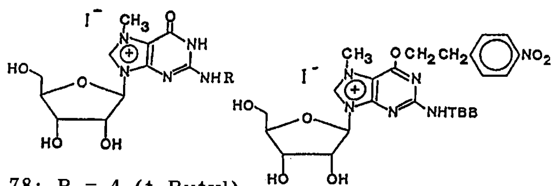


72; R = NH_2, R′= H

46; R = 4-(t-Butyl)benzamido R′= H

73; R = NH_2, R′= CH_3

74; R = R′= H



78; R = 4-(t-Butyl)-benzamido

79; R = H

80

Scheme 10

nucleophilic character of the N^7 in 46 is therefore much poorer than in 72. This reduced nucleophilic reactivity is also supported by methylation studies with 72 and 46 by methyl iodide in dimethylformamide at 20° C which showed that the half-lives of N^7-methylation of 72 and 46, to give the corresponding N^7 methylated products, are 50 and 165 min respectively.

The ^{15}N chemical shifts of the C^6 substituted guanosine derivatives 29, 75 and 76 along with the result of protonated and methylated derivatives are also shown in Table 1. Since all guanosine derivatives in this work have a N^2-TBB group, the observed ^{15}N chemical shifts, in protonation and methylation experiments are attributed to the electronic effect of the C^6 substituent. The data for N^1-methylguanosine (73) are also shown for comparison. The N^7 in compounds 29 and 75 moved only 0.5 and 1 ppm upfield, respectively, upon protonation. Compound 75 was completely resistant to methylation reaction conditions (vide supra); the N^7 of 29 was also found to be completely resistant to methylation while its pyridyl-nitrogen, as expected, was methylated to give 77. The N^7 of C^6-(4-nitrophenylethyl-) derivative 76, upon protonation, moved upfield by 5 ppm, suggesting that the N^7 is not completely deactivated. This is also corroborated by the fact that the 76 can be methylated (vide supra) at N^7 to give 80 with a half-life of 300 min. (compare half-lives of methylation of 79 and 46 which are respectively 50 and 165 min.). Compound 80, which was also isolated and characterized as a pure compound, has ^{15}N shifts which are very similar to N^7-methyl-N^2-TBB-guanosine (78) (Table 1).

It appears from these data that there is a considerable reduction of the nucleophilicity of N^7 with most of the C^6 substituted guanosine derivatives. This is most probably due to the delocalization of the electron rich imidazole system to the π-deficient pyrimidine system with an overall reduction of the nucleophilic character of the N^7. However, the present data suggests an "electronic criteria" that a O^6 protecting group of guanosine should fulfill. That such a group should not be electron-releasing to the resulting fully-aromatic purine system. This is substantiated by the fact that the O^6-alkyl substituent of 76, as the O^6 protecting group of the guanine moiety, has made the N^7 more susceptible to electrophilic reagents than the electron-withdrawing O^6-aryl group of 29 or 75.

It also should be noted that the N^7 of N^1-methylguanosine (73), upon protonation with one equivalent of acid, moved upfield by 99.5 ppm while the N^7 of guanosine, in a similar protonation experiment, moved only by 75 ppm (Table 1). This clearly shows that the N^1 methylation enhances the nucleophilicity of the N^7. It is also established[66] that an N^1-acyl group of a fully-protected guanosine derivative 66 enhances the nucleophilicity of the N^7 and gave[66] a N^7 phosphorylated product 69 or 70. All of these

TABLE 1.
Study of the ^{15}N chemical shifts of guanosine derivatives upon protonation and methylation at 313 K.

Compound	Concentration of acid	N1	N3	N7	N9	N2	N-substituent
72	0 equiv.	-233.9	-215.2	-134.4	-211.1	-307.7	
	1.0 equiv.	-232.7	-218.1	-211.0	-204.4	-301.4	
	2.0 equiv.	-232.8	-218.3	-214.8	-204.3	-301.3	
46	0 equiv.	-226.6	-195.0	-132.1	-207.4	-248.3	
	1.0 equiv.	-226.3	-196.3	-156.8	-205.3	-248.0	
	2.0 equiv.	-226.3	-196.8	-166.3	-204.5	-248.0	
29	0 equiv.	-157.7	-160.9	-141.5	-210.6	-243.5	-61.9
	1.0 equiv.	-157.0	-160.8	-142.0	-209.6	-243.1	-113.1
	2.0 equiv.	-158.3	-161.1	-142.8	-209.5	-243.1	-159.2
30	0 equiv.	-156.3	-160.8	-140.9	-210.2	-243.3	-62.9
31	0 equiv.	-154.2	-159.4	-141.1	-210.1	-243.1	-73.8
32	0 equiv.	-155.4	-159.3	-141.7	-209.7	-243.4	-224.9
33	0 equiv.	-129.4	-158.6	-139.5	-210.8	-243.3	-65.5
73	0 equiv.	-237.2	-215.8	-133.3	-212.3	-300.4	
	1.0 equiv.	-235.7	-217.0	-232.8	-208.3	-297.0	
	2.0 equiv.	-235.1	-217.8	-	-207.1	-296.0	
74	0 equiv.	-206.6	-167.1	-131.9	-206.6	-	
	1.0 equiv.	-206.2	-167.3	-141.2	-205.6	-	
	2.0 equiv.	-205.9	-167.4	-147.7	-204.9	-	
75	0 equiv.	-157.3	-160.5	-141.1	-209.9	-243.5	-13.5
	1.0 equiv.	-157.4	-160.8	-142.1	-209.9	-243.4	-13.5
	2.0 equiv.	-157.5	-161.0	-143.5	-209.8	-243.4	-13.5
76	0 equiv.	-166.1	-158.0	-140.3	-210.6	-243.1	-10.5
	1.0 equiv.	-166.5	-157.9	-145.5	-210.2	-243.1	-10.6
	2.0 equiv.	-167.1	-157.9	-150.0	-209.4	-243.0	-10.6
77	0 equiv.	-159.6	-160.5	-141.5	-209.4	-242.9	-177.3
78	0 equiv.	-223.3	-198.0	-219.1	-203.5	-246.9	-
79	0 equiv.	-231.9	-217.9	-220.8	-207.1	300.1	
	1.0 equiv.	-232.2	-218.1	-220.9	-207.2	300.3	
80	0 equiv.	-170.3	-154.7	-225.7	-205.3	-242.9	-10.7

observations suggest that if the lactam function of the N^2-protected guanine is protected at N^1, subsequent electrophilic reactions at the N^7 can not be prevented.

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