The Protection of Thymine and Guanine Residues in Oligodeoxyribonucleotide Synthesis

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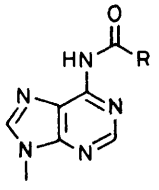
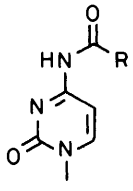
Thymidine is converted in four steps into its crystalline 4-*O*-phenyl derivative (**14b**) in 72% overall yield. 2'-Deoxyguanosine is converted in five steps into its 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl and crystalline 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl derivatives [(**19a**) and (**19b**), respectively] in *ca.* 39 and 42% overall yields, respectively. Compounds (**14b**), (**19a**), and (**19b**) are converted into the corresponding crystalline 5'-*O*-(9-phenyl-9*H*-xanthen-9-yl) derivatives (**15**), (**20a**), and (**20b**), respectively. The actions of N^1,N^1,N^3,N^3-tetramethylguanidinium 2-nitrobenzaldehyde oximate, 80% acetic acid, potassium carbonate in aqueous dioxane, pyridine, and triethylammonium toluene-*p*-thiolate on (**14b**), (**19a**), and (**19b**) have been investigated.

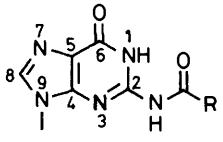
In their studies on the synthesis of oligodeoxyribonucleotides by the phosphodiester approach, Khorana and his co-workers [1] introduced the use of *N*-acyl protecting groups for adenine, cytosine, and guanine residues [as in (**1**), (**2**), and (**3**), respectively], but left thymine residues unprotected [as in (**4a**)]. Despite much subsequent progress in oligonucleotide synthesis, including the development of the phosphotriester approach,[2] no significant modification or improvement to the methodology of base-residue protection was then reported for over 20 years. Indeed, virtually every worker in the field followed Khorana's original initiative.[1]



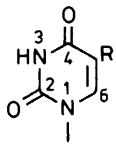
(**1**)



(**2**)



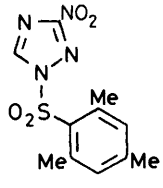
(**3**)



(**4**)

a, R = Me

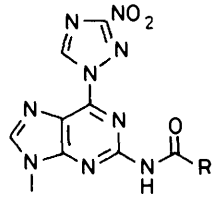
b, R = H



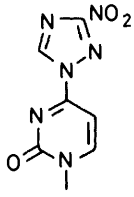
(**5**)

We, and no doubt other investigators, had realised [3] for a number of years that guanine residues were inadequately protected against phosphorylation and other side-reactions by 2-*N*-acylation [as in (**3**)]. However, we had not even considered the possibility of the occurrence of side-reactions involving other base-residues until we undertook the synthesis [4] of the 3'-terminal decaribonucleoside nonaphosphate of yeast alanine transfer ribonucleic acid ($tRNA^{Ala}$). We then found [5] that 2-*N*-acylguanine and uracil residues [as in (**3**) and (**4b**), respectively] could both undergo modifications [at C-6 and C-4, respectively, to give (**6**) and (**7**)] during the second phosphorylation step of the phosphotriester approach when 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole [(MSNT (**5**)] [4,6] was used as the condensing agent. While uracil [as in (**4b**)] underwent modification even more readily than 2-*N*-acyl guanine residues [as in (**3**)], thymine residues [as in (**4a**)] appeared [5] to be stable under the conditions of the MSNT (**5**)-promoted condensation reaction.

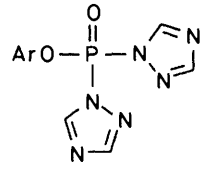
It is now clear that 2-*N*-acyl guanine, thymine, and uracil residues [as in (**3**), (**4a**), and (**4b**), respectively] are also susceptible to side-reactions during the first phosphorylation step of the phosphotriester approach when 2(or 4)-chlorophenyl phosphorodi-(1,2,4-triazolide) (**8**) is used [7,8] as the phosphorylating agent. While thymine [8] and uracil [7] residues both undergo 4-triazolation, to give (**9a**) and (**9b**), respectively, in the presence of the latter reagent (**8**), the nature of the modification [7] to 2-*N*-acylguanine residues has not yet been elucidated.



(**6**)

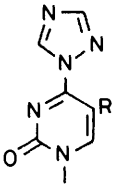


(**7**)



(**8**)

Ar = 2(or 4)-ClC_6H_4



(**9**)

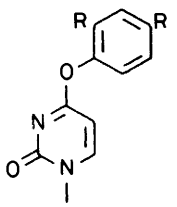
a, R = Me

b, R = H

Although the MSNT-promoted base modification reactions [5] and the modifications to thymine [9] and uracil [10] residues brought about by 2(or 4)-chlorophenyl phosphorodi-(1,2,4-

triazolide) (**8**) are apparently reversed during the unblocking of the internucleotide linkages with oximate ions,[6] the occurrence of any side-reactions during the phosphorylation steps in oligonucleotide synthesis is clearly undesirable and should, if possible, be completely avoided. An obvious way to achieve this *end would* be to protect thymine and uracil residues and to protect guanine residues in a different way.

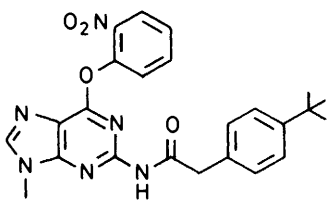
We were forced to look into this matter urgently when we encountered difficulties in some studies relating to the synthesis of the 3′-end of yeast $tRNA^{Ala}$. We then came to the conclusion[11,12] that it would be necessary to use additional protecting groups for the base-residues if the synthesis were to proceed beyond the 3′-terminal decaribonucleoside nonaphosphate stage. It seemed likely that additional protecting groups would both suppress the side-reactions and increase the lipophilicity of the fully protected oligonucleotides. We believed that the latter probable consequence of using additional protecting groups was also desirable in that it would lead to improved solubility properties and to the facilitation of the chromatographic purification of these intermediates. We have since come to the conclusion that additional base-protecting groups would very probably also be of value in oligodeoxyribonucleotide synthesis. This is particularly likely to be the case in the phosphotriester synthesis of oligodeoxyribonucleotides on solid supports when a large excess of phosphorylating agent is used at each stage and no purification steps are carried out until the end of the synthesis. We now report the results of our studies on the base protection of thymidine and on the additional base protection of 2′-deoxyguanosine.



(**10**)

a, R = H

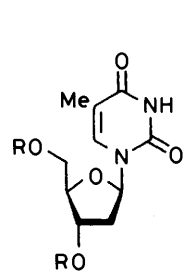
b, R = Me

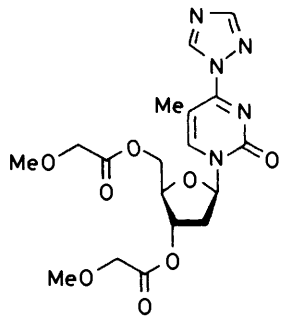


(**11**)

In connection with the above studies on oligoribonucleotide synthesis, we found[11,12] that aryl groups could be used to protect uracil and 2-*N*-acyl guanine residues on *O*-4 and *O*-6, respectively. In particular, we found[11,12] that phenyl and 2,4-dimethylphenyl were suitable groups for the protection of uracil [as in (**10a**) and (**10b**), respectively] and that 2-nitrophenyl was a suitable group for the further protection of 2-*N*-(4-t-butylphenylacetyl)guanine residues [as in (**11**)]. Side-reactions were not observed when these protecting groups were used, and the chromatographic purification of fully and partially protected oligonucleotides was greatly facilitated.[11,12] When 2,4-dimethylphenyl and 2-nitrophenyl groups were used for the protection of uracil and guanine residues [as in (**10b**) and (**11**), respectively], it became possible to extend the synthesis of the 3′-end of yeast $tRNA^{Ala}$ to the nonadecaribonucleoside octadecaphosphate stage.[11,12] The new base-protecting groups were stable under all the reaction conditions used in the assembly of the latter nonadecamer, and were removed during the course of the unblocking of the internucleotide linkages with N^1,N^1,N^3,N^3-tetramethylguanidinium 2-nitrobenzaldehyde oximate.[13] We were therefore prompted to investigate the use of aryl groups in the protection of thymidine and in the further protection of 2′-deoxyguanosine.

Our experience with the protection of uracil led us to



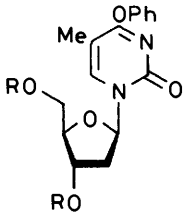


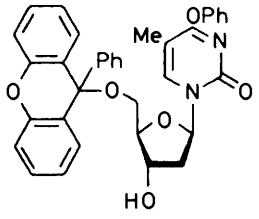
(**12**)

a, R = H

b, R = $MeOCH_2CO$

(**13**)





(**14**)

a, R = $MeOCH_2CO$

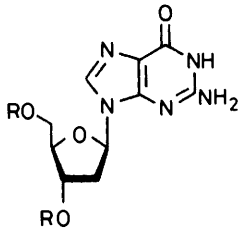
b, R = H

(**15**)

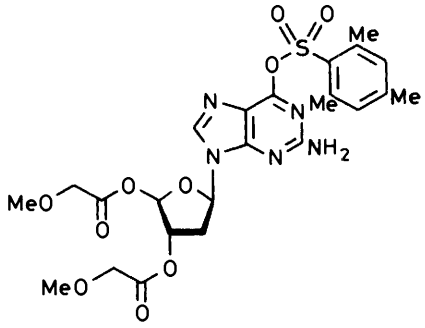
expect that phenyl would most probably also be a suitable group for the protection of thymine residues. Thymidine (**12a**) was treated with methoxyacetic anhydride in pyridine solution to give its 3′,5′-di-*O*-methoxyacetyl derivative[14] (**12b**). The crude material (**12b**) obtained was then allowed to react with the triazolating agent, prepared[15] from phosphoryl chloride (2 mol equiv.), 1,2,4-triazole (9 mol equiv.), and triethylamine (9 mol equiv.), in acetonitrile solution at room temperature. The product, which was assumed to be (**13**), was treated with 4 mol equiv. each of phenol and triethylamine in acetonitrile solution at room temperature. Finally, the resulting 4-*O*-phenylthymidine derivative (**14a**) was treated with 4M-methanolic ammonia for 40 min at room temperature to give 4-*O*-phenylthymidine (**14b**) itself. The latter compound (**14b**) was isolated as a colourless crystalline solid in 72% overall yield for the four steps starting from thymidine (**12a**). The results of some tests (see below) which have been carried out on (**14b**) have led us to believe that phenyl is indeed a suitable group for the protection of thymine residues in oligonucleotide synthesis, and some recent synthetic studies have added support to this view. Treatment of (**14b**) with 9-chloro-9-phenylxanthene gave 4-*O*-phenyl-5′-*O*-(9-phenyl-9*H*-xanthen-9-yl)thymidine[16] (**15**) as a crystalline solid. This derivative (**15**) is required as a building block in oligodeoxyribonucleotide synthesis.

As indicated above, we had used the 2-nitrophenyl group successfully in the further protection of guanine residues in oligoribonucleotide synthesis.[11,12] We therefore, in the first place, undertook the synthesis of 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) and its 9-phenyl-9*H*-xanthen-9-yl (pixyl) derivative (**20a**). 2′-Deoxyguanosine (**16a**) was converted into its 3′,5′-di-*O*-methoxyacetyl derivative[14] (**16b**) in good yield. The latter compound (**16b**) was allowed to react with mesitylene-2-sulphonyl chloride and triethylamine in acetonitrile solution at room temperature. The resulting 6-*O*-(mesitylene-2-sulphonyl) derivative[17] was then heated, under reflux, with an excess each of 2-nitrophenol and di-isoproylethylamine in pyridine solution to give (**18a**). Treatment of (**18a**) with phenylacetyl chloride in the presence of 2,6-lutidine

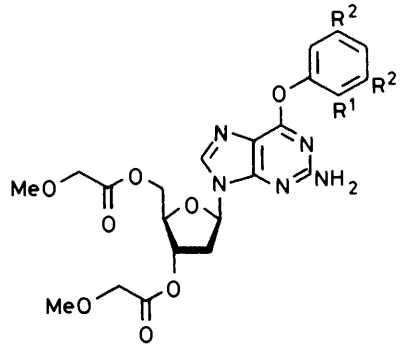
in acetonitrile solution at room temperature, followed by treatment of the products with 4M-ammonia in methanol solution for 20 min at room temperature gave (**19a**). The latter doubly protected 2′-deoxyguanosine derivative (**19a**) was isolated as a pure (t.l.c., ^{1}H n.m.r.) glass in 44% overall yield for the 4 steps starting from 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine (**16b**); when (**19a**) was treated[16] with 9-chloro-9-phenylxanthene in pyridine solution, the required building block (**20a**) was obtained and isolated as a crystalline solid in 67% yield.



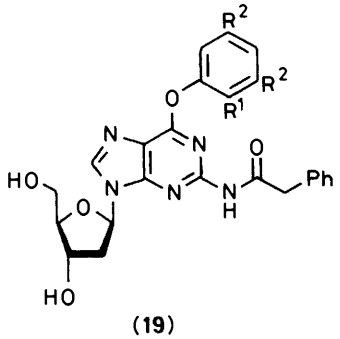
(**16**)
a; R = H
b; R = $MeOCH_2CO$



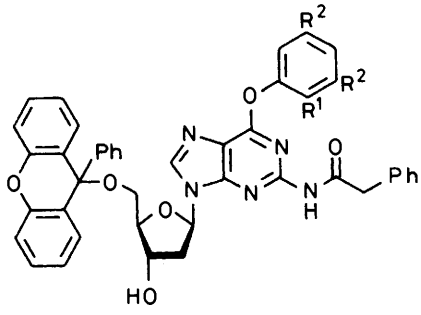
(**17**)



(**18**)
a; $R^1 = NO_2$, R^2 = H
b; R^1 = H, R^2 = Cl



(**19**)
a; $R^1 = NO_2$, R^2 = H
b; R^1 = H, R^2 = Cl

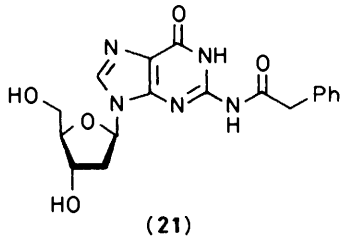


(**20**)
a; $R^1 = NO_2$, R^2 = H
b; R^1 = H, R^2 = Cl

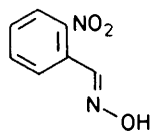
The tests carried out (see below) on 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) suggest that the 2-nitrophenyl group is suitable for the further protection of guanine residues in oligodeoxyribonucleotide synthesis. However, we believed that it would be more satisfactory if it were possible to obtain a doubly protected 2′-deoxyguanosine derivative (**19**) as a crystalline solid. We further believed that, in order to minimise the possibility of nucleophilic displacement at the 6-position, it would probably be better if the 6-*O*-aryl protecting group were derived from a phenol which was less acidic than 2-nitrophenol (pK_a 7.23).[18] We finally hoped that it would be possible to improve the general procedure for the conversion of 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine (**16b**) into a doubly protected 2′-deoxyguanosine derivative (**19**); we particularly hoped to improve the second step, involving the displacement of mesitylene-2-sulphonate ion with the conjugate base of a phenol. With all of these points in mind, we undertook the preparation of 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**). The pK_a of 3,5-dichlorophenol is reported[19] to be 8.19.

The conversion of (**16b**) into (**17**) appeared to be improved marginally by the addition of 0.05 molecular equivalents of 4-dimethylaminopyridine.[20] As indicated above, it had been necessary to heat (**17**) with 2-nitrophenol and di-isopropylethylamine in boiling pyridine solution in order to effect the displacement of mesitylene-2-sulphonate ion. These rather drastic conditions led to some darkening of the reaction medium. However, we found that if (**17**) were first allowed to react with an excess of 1-methylpyrrolidine (*ca.* 1 ml/mmol) in acetonitrile solution at room temperature for 30 min and 3,5-dichlorophenol (3 mol equiv.) was then added, the 6-(3,5-dichlorophenyl) derivative (**18b**) was readily obtained without heating and in good yield. This approach which presumably involves an intermediate quaternary ammonium salt is based on the recent use[21] of trimethylamine as a catalyst in related transformations. 1-Methylpyrrolidine (b.p. 81—83 °C), unlike triethylamine, appears to be as effective a catalyst as trimethylamine (b.p. 3 °C) but is much easier to handle under ordinary laboratory conditions. The two-step conversion of (**18b**) into (**19b**) was carried out under the conditions used (see above) for the conversion of (**18a**) into (**19a**). 6-*O*-(3,5-Dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) was isolated as a crystalline compound in 48% overall yield for the 4 steps starting from 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine (**17**). When (**19b**) was treated with 9-chloro-9-phenylxanthene[16] in pyridine–acetonitrile solution at room temperature, the corresponding 5′-*O*-(9-phenyl-9*H*-xanthen-9-yl) derivative (**20b**) was obtained and isolated as a crystalline solid in 59% yield.

As indicated above, aryl-protecting groups are removable[11,12] from 4-*O*-aryluracil and 6-*O*-aryl-2-*N*-acylguanine residues under the conditions used for the unblocking of



(21)



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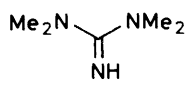


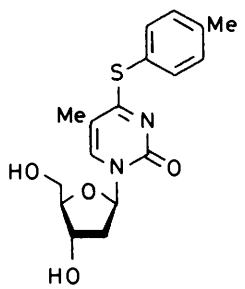
(23)

internucleotide linkages with N^1,N^1,N^3,N^3-tetramethylguanidinium 2-nitrobenzaldehyde oximate.[13] It was clearly important to determine the approximate rates for the removal of the aryl-protecting groups from 4-*O*-phenylthymine [as in (**14**)] and from 6-*O*-aryl-2-*N*-phenylacetylguanine residues [as in (**19a**) and (**19b**)] under the latter conditions. The unblocking reactions were conveniently monitored by t.l.c. When a 0.05M-solution of 4-*O*-phenylthymidine (**14b**) in dioxane was treated with 10 mol equiv. of *syn*-2-nitrobenzaldehyde oxime (**22**) and 9 mol equiv. of N^1,N^1,N^3,N^3-tetramethylguanidine (**23**) (TMG) at room temperature, times for half and complete conversions of the substrate (**14b**) into thymidine (**12a**) were *ca.* 10 and 60 min, respectively. The unblocking of (**14b**) proceeded at a marginally faster rate in acetonitrile solution. Under the same conditions, also in acetonitrile solution, 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) was found to be half and completely converted into 2-*N*-phenylacetyl-2′-deoxyguanosine (**21**) in *ca.* 4 and 25 min, respectively; however, this unblocking reaction was found to proceed more slowly [50 and 100% conversion into (**21**) in *ca.* 20 and 90 min, respectively] in acetonitrile–dioxane (1:1, v/v) solution. The same solvent effect was observed in the unblocking of 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**). In acetonitrile solution, (**19b**) was found to be half and completely converted into (**21**) in *ca.* 4 and 35 min, respectively; in acetonitrile–dioxane (1:1, v/v) solution, the times for half and complete reactions were *ca.* 18 and 150 min, respectively.

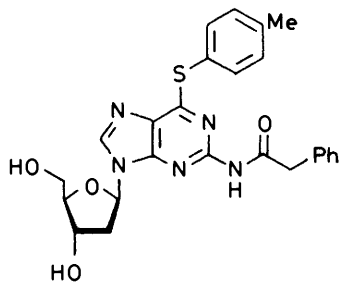
While there could be little doubt regarding the interpretation of the t.l.c. data obtained in the course of the above experiments, we thought that it would be a wise precaution to carry out a second series of unblocking reactions in which products were actually isolated and characterised. The unblocking of 4-*O*-phenylthymidine (**14b**) was examined first. The latter compound was first converted into its 3′,5′-di-*O*-acetyl derivative (**14**; R = Ac) which was then treated with an excess of *syn*-2-nitrobenzaldehyde oxime (**22**) and TMG (**23**) in dioxane solution. The products were purified by chromatography and 3′,5′-di-*O*-acetylthymidine (**12**; R = Ac), identical with authentic material, was isolated as a crystalline solid in 58.5% yield. A much higher isolated yield (79.5%) of (**12**; R = Ac) was obtained in a second experiment in which unblocking with *syn*-2-nitrobenzaldehyde oxime (**22**) and TMG (**23**) was carried out prior to acetylation. Similarly, 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) was treated first with an excess each of *syn*-2-nitrobenzaldehyde oxime (**22**) and TMG (**23**) in acetonitrile–dioxane (1:1, v/v) solution, and then with concentrated aqueous ammonia. Acetylation of the products gave 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**16**; R = Ac), identical with authentic material, in at least 85% isolated yield.

One of the remaining problems in oligodeoxyribonucleotide synthesis is the tendency[22] of purine 2′-deoxyribonucleosides (and especially of 6-*N*-acyl derivatives of 2′-deoxyadenosine) to undergo glycosidic cleavage during the removal of acid-labile protecting groups. It was, therefore, important to examine the stability of the above base-protected 2′-deoxyribonucleoside derivatives [(**14b**), (**19a**), and (**19b**)] in acidic solution. Not surprisingly, 4-*O*-phenylthymidine (**14b**) was found to be relatively stable in 80% acetic acid [acetic acid–water (4:1, v/v)] solution at room temperature inasmuch as only *ca.* 30% degradation occurred after 43 h. Fortunately, both of the doubly protected 2′-deoxyguanosine derivatives [(**19a**) and (**19b**)] proved to be more stable to acidic hydrolysis than the singly protected 2-*N*-phenylacetyl-2′-deoxyguanosine (**21**). Thus, after treatment for 19 h with 80% acetic acid at room temperature, (**19a**), (**19b**), and (**21**) had undergone *ca.* 30—40, *ca.* 65, and *ca.* 90% hydrolysis, respectively. All of the latter compounds were much more resistant to acid-catalysed degradation than 6-*N*-benzoyl-2′-deoxyadenosine which underwent *ca.* 90% hydrolysis in 2 h under the same conditions.

Mildly basic work-up conditions are commonly used in oligonucleotide synthesis.[4,23] We were, therefore, glad to find that (**14b**), (**19a**), and (**19b**) were all completely unchanged after they had been treated with 0.2M-potassium carbonate in dioxane–water (4:1, v/v) solution for 3 h at room temperature. Pyridine is widely used as a solvent for condensation reactions in oligonucleotide synthesis. We were, therefore, also glad to find that (**14b**), (**19a**), and (**19b**) were all completely unchanged after 48 h at room temperature in pyridine solution.



(24)



(25)

The final test that (**14b**), (**19a**), and (**19b**) were subjected to was treatment with toluene-*p*-thiol and triethylamine in acetonitrile solution. We recently recommended the use of the 2,4-dinitrobenzyl group[24] for the protection of 3′-terminal phosphodiester functions in oligonucleotide synthesis and showed that the latter protecting group could be removed in less than 1 min by treating a 0.02M-solution of substrate with 4 mol equiv. each of toluene-*p*-thiol and triethylamine in acetonitrile solution at room temperature. We have, therefore, examined the stabilities of (**14b**), (**19a**), and (**19b**) under approximately the same reaction conditions. When 4-*O*-phenylthymidine [(**14b**), 0.05M-solution in acetonitrile] was treated with 4 mol equiv. each of toluene-*p*-thiol and triethylamine for 3 h at room temperature, it underwent *ca.* 30% conversion into a product believed to be (**24**). In the same way, when 6-*O*-(2-nitrophenyl)- and 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosines [(**19a**) and (**19b**), respectively] were treated with toluene-*p*-thiol and triethylamine for 3 h under identical experimental conditions, they underwent *ca.* 40 and 65%, respectively, conversion into a product believed to be (**25**).

Considering that the 2,4-dinitrobenzyl protecting group[24] is removed from phosphotriester functions within 1 min at even lower concentrations than those used in the above stability tests, it seems probable that side-reactions involving especially 4-*O*-

phenylthymine [as in (**14b**)] and 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetylguanine [as in (**19a**)] would occur to a negligible extent during this unblocking process. Preliminary studies[25] on oligodeoxyribonucleotide synthesis involving phenyl-protected thymine residues support this conclusion. The surprising observation that 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) is somewhat more susceptible than (**19a**) to attack by toluene-*p*-thiolate ions may perhaps be rationalised in steric terms.

Following our demonstration that the 2-nitrophenyl group was suitable for the further protection of guanine residues in oligoribonucleotide synthesis, there has recently been a number of other reports in the literature relating to guanine protection. Thus Hata and his co-workers have suggested[26] several new (mainly acyl) protecting groups for this purpose, and Jones and his co-workers[27] have investigated the use of 6-*O*-alkyl groups for the further protection of guanine residues in oligodeoxyribonucleotide synthesis. One of these alkyl groups, 2-(4-nitrophenyl)ethyl, which was originally introduced by Pfleiderer *et al.* for the protection of internucleotide linkages in oligonucleotide synthesis,[28] has also recently been recommended[29] by the latter workers for the *O*-4 protection of uracil and thymine residues as well as for the *O*-6 protection of guanine residues. It has also very recently been suggested that uracil residues should be protected on *N*-3 by acyl groups.[30,31]

In conclusion, we believe that the advantages provided by the suppression of side-reactions and, with regard to synthesis in solution, the increased lipophilicity of intermediates, will lead in the future to the protection of uracil and thymine residues and the further protection of guanine residues becoming more widespread in oligonucleotide synthesis.

Experimental

^{1}H N.m.r. spectra were measured at 250 MHz with a Bruker WM 250 spectrometer; tetramethylsilane was used as an internal standard. U.v. absorption spectra were measured with a Cary 17 recording spectrophotometer. T.l.c. was carried out on Merck silica gel 60 F_{254} plates which were developed in systems A [$CHCl_3$–MeOH (9:1, v/v)] and B [$CHCl_3$–MeOH (4:1, v/v)]. Merck silica gel H was used for short column chromatography.[32] Acetonitrile, triethylamine, and pyridine were dried by heating, under reflux, with calcium hydride; these solvents were then distilled at atmospheric pressure. Acetonitrile and pyridine were stored over 4A molecular sieves.

4-O-*Phenylthymidine* (**14b**).—Methoxyacetic anhydride[33] (8.36 g, 51.6 mmol) was added to a stirred suspension of thymidine (5.0 g, 20.6 mmol) in pyridine (100 ml) at room temperature. After 30 min, water (2 ml) was added and the reaction solution was stirred for a further period of 10 min. The products were then concentrated under reduced pressure and redissolved in chloroform (100 ml). The resulting solution was extracted with saturated aqueous sodium hydrogen carbonate (2 × 40 ml) and the aqueous layers were back extracted with chloroform (40 ml). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give 3′,5′-di-*O*-methoxyacetylthymidine as a gum.

Triethylamine (25.9 ml, 186 mmol) was added dropwise over a period of 10 min to a stirred mixture of 1,2,4-triazole (12.83 g, 186 mmol) and phosphoryl chloride (3.79 ml, 40.7 mmol) in acetonitrile (50 ml) at 0 °C (ice-bath). A solution of the above crude 3′,5′-di-*O*-methoxyacetylthymidine in acetonitrile (100 ml) was added to the resulting mixture which was then stirred at room temperature for 16 h. Triethylamine (25.9 ml, 186 mmol) and water (4 ml) were added and, after 10 min, the products were concentrated under reduced pressure, redissolved in chloroform (100 ml), and the solution was extracted with saturated aqueous sodium hydrogen carbonate (2 × 40 ml). The aqueous layers were back extracted with chloroform (40 ml), and the dried ($MgSO_4$), combined organic extracts were evaporated under reduced pressure. The residual gum was dissolved in acetonitrile (100 ml), and phenol (7.77 g, 82.6 mmol) and triethylamine (11.5 ml, 82.5 mmol) were added. The resulting solution was then stirred at room temperature. After 5 h, the products were concentrated under reduced pressure and redissolved in chloroform (100 ml). The solution obtained was extracted with saturated aqueous sodium hydrogen carbonate (2 × 40 ml) and the aqueous layers were back extracted with chloroform (40 ml). The combined organic layers were dried ($MgSO_4$), evaporated under reduced pressure and the residue was dissolved in 4M-methanolic ammonia (100 ml) at room temperature. After 40 min, the products were concentrated under reduced pressure and the residue was chromatographed on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (90:10, v/v), were combined and concentrated under reduced pressure. Crystallisation of the residue from ethyl acetate gave 4-O-*phenylthymidine* (Found: C, 60.2; H, 5.7; N, 8.8. $C_{16}H_{18}N_2O_5$ requires C, 60.4; H, 5.7; N, 8.8%) as colourless crystals, m.p. 160—162 °C (4.756 g, 72% overall yield, based on thymidine); R_F 0.18 (system A), 0.59 (system B); $\lambda_{max.}$ (95% EtOH) 291 (ε 7 400), $\lambda_{min.}$ 249 nm (ε 2 600); δ_H[$(CD_3)_2SO$] 2.0 (1 H, m), 2.07 (3 H, d, *J* 0.9 Hz), 2.22 (1 H, m), 3.62 (2 H, m), 3.83 (1 H, m), 4.24 (1 H, m), 5.13 (1 H, m), 5.26 (1 H, d, *J* 4.1 Hz), 6.09 (1 H, t, *J* 6.2 Hz), 7.18 (2 H, m), 7.27 (1 H, m), 7.45 (2 H, m), and 8.21 (1 H, m).

4-O-*Phenyl*-5′-O-(9-*phenyl*-9H-*xanthen*-9-*yl*)*thymidine* (**15**).—A solution of 9-chloro-9-phenylxanthene[16] (0.761 g, 2.6 mmol) in pyridine (20 ml) was added dropwise over a period of 60 min to a stirred solution of 4-*O*-phenylthymidine (0.637 g, 2.0 mmol) in pyridine (20 ml) at room temperature. Water (0.2 ml) was added to the products which were then concentrated to small volume under reduced pressure. The residue was dissolved in chloroform (50 ml) and the resulting solution was extracted with saturated aqueous sodium hydrogen carbonate (25 ml). The aqueous layer was back extracted with chloroform (25 ml), and the combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure. The residual glass was fractionated by short-column chromatography: the appropriate fractions, eluted with $CHCl_3$–EtOH (96:4, v/v), were combined and evaporated under reduced pressure. Crystallisation of the residue from ethyl acetate gave 4-O-*phenyl*-5′-O-(9-*phenyl*-9H-*xanthen*-9-*yl*)*thymidine* (Found: C, 70.7; H, 5.6; N, 4.2. $C_{35}H_{30}N_2O_6{\cdot}CH_3CO_2C_2H_5$ requires C, 70.7; H, 5.8; N, 4.2%) as colourless crystals, m.p. 174—178 °C (0.75 g, 56.5%); R_F (system A) 0.45; δ_H[$(CD_3)_2SO$] 1.79 (3 H, s), 2.1—2.4 (2 H, m), 3.17 (2 H, m), 3.94 (1 H, m), 4.26 (1 H, m), 5.34 (1 H, d, *J* 4.1 Hz), 6.14 (1 H, t, *J* 6.2 Hz), 7.1—7.55 (18 H, m), and 8.02 (1 H, s).

3′,5′-*Di*-O-*methoxyacetyl*-2′-*deoxyguanosine* (**16b**).—This preparation was first carried out by Dr. S. Sibanda. Methoxyacetic anhydride[33] (11.88 g, 73.3 mmol) was added to a stirred suspension of 2′-deoxyguanosine (4.9 g, 18.3 mmol) in dimethylformamide (90 ml) and pyridine (40 ml) at room temperature. After 2 h, methanol (10 ml) was added and the reaction solution was stirred for a further period of 30 min. The products were then evaporated under reduced pressure (oil pump) and the residue was crystallised from ethanol to give 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine (Found: C, 46.6; H, 5.1; N, 17.2. $C_{16}H_{21}N_5O_8$ requires C, 46.7; H, 5.15; N, 17.0%) as colourless crystals, m.p. 174—178 °C (6.01 g, 79.7%);* R_F

* A higher yield (6.66 g, 88%) of solid product is obtained if the residue is triturated with ether and then washed with ether and ethanol, rather than recrystallised from ethanol.

(system A) 0.095; δ_H[$(CD_3)_2SO$] 2.93 (1 H, m), 3.15 (1 H, m), 3.31 (3 H, s), 3.34 (3 H, s), 4.10 (2 H, d, *J* 2.4 Hz), 4.12 (2 H, s), 4.24 (1 H, m), 4.33 (2 H, m), 5.41 (1 H, m), 6.13 (1 H, dd, *J* 5.9, 8.7 Hz), 6.53 (2 H, br s), 7.94 (1 H, s), and 10.69 (1 H, br s).

6-O-(2-*Nitrophenyl*)-2-N-*phenylacetyl*-2′-*deoxyguanosine* (**19a**).—Triethylamine (1.0 ml, 7.2 mmol) was added to a stirred suspension of 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine (1.50 g, 3.65 mmol) and mesitylene-2-sulphonyl chloride (1.59 g, 7.27 mmol) in dry acetonitrile (20 ml) at room temperature. After 3 h, triethylamine (1.0 ml, 7.2 mmol) was added, and the products were poured into ice-cold saturated aqueous sodium hydrogen carbonate (50 ml). The resulting mixture was extracted with dichloromethane (2 × 60 ml). The dried ($MgSO_4$) dichloromethane extracts were evaporated under reduced pressure and the residue was fractionated by short-column chromatography. The appropriate fractions, eluted with $CHCl_3$–EtOH (96:4, v/v) were combined and evaporated under reduced pressure. The brown glass obtained was dissolved in pyridine (18 ml) and di-isopropylethylamine (3.8 ml, 21.8 mmol) and 2-nitrophenol (1.515 g, 10.9 mmol) were added. The reactants were heated, under reflux, for 1 h, cooled, and evaporated under reduced pressure. The residue was dissolved in chloroform (60 ml) and the solution was extracted with saturated aqueous sodium hydrogen carbonate (30 ml). The aqueous layer was back extracted with chloroform (30 ml), and the combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was fractionated by short-column chromatography: the appropriate fractions, eluted with $CHCl_3$–EtOH (96:4, v/v), were combined and evaporated under reduced pressure to a glass (1.41 g, *ca.* 73% for the two steps).

2,6-Lutidine (0.35 ml, 3.0 mmol) and phenylacetyl chloride (0.2 ml, 1.5 mmol) were added to a stirred solution of the above material (0.135 g, *ca.* 0.25 mmol) in acetonitrile (2.5 ml) at room temperature. After 20 min, water (0.1 ml) was added and, after a further period of 10 min, the products were evaporated under reduced pressure. Dichloromethane (20 ml) was added and the mixture was washed with cold M-sulphuric acid (10 ml), followed by saturated aqueous sodium hydrogen carbonate (30 ml). The latter washings were back extracted with chloroform (20 ml), and the combined, dried ($MgSO_4$) chloroform layers were evaporated under reduced pressure. The residue was fractionated by short-column chromatography: the appropriate fractions, eluted with $CHCl_3$–EtOH (96:4, v/v), were evaporated under reduced pressure and the residue was redissolved in 4M-methanolic ammonia (5 ml) at room temperature. After 20 min, the products were concentrated and then fractionated by short-column chromatography. The fractions containing 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine, eluted with $CHCl_3$–MeOH (90:10, v/v), were combined and evaporated under reduced pressure. Yield of glass, 0.078 g (*ca.* 61%; *ca.* 44% overall, for the four steps starting from 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine); R_F 0.20 (system A), 0.63 (system B); $\lambda_{max.}$ (95% EtOH) 265, $\lambda_{min.}$ 240 nm; δ_H[$(CD_3)_2SO$] 2.31 (1 H, m), 2.76 (1 H, m), 3.57 (2 H, m), 3.66 (2 H, s), 3.87 (1 H, m), 4.44 (1 H, m), 4.92 (1 H, t, *J* 5.5 Hz), 5.34 (1 H, d, *J* 4.1 Hz), 6.36 (1 H, t, *J* 6.7 Hz), 7.15—7.4 (5 H, m), 7.57 (1 H, m), 7.67 (1 H, m), 7.87 (1 H, m), 8.22 (1 H, m), 8.60 (1 H, s), and 10.60 (1 H, s).

6-O-(2-*Nitrophenyl*)-2-N-*phenylacetyl*-5′-O-(9-*phenyl*-9H-*xanthen*-9-*yl*)-2′-*deoxyguanosine* (**20a**).—A solution of 9-chloro-9-phenylxanthene[16] (0.99 g, 3.38 mmol) in pyridine (20 ml) was added dropwise over a period of 60 min to a stirred solution of 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (1.01 g, 2.0 mmol) in pyridine (20 ml) at room temperature. After a further period of 1 h, water (0.2 ml) was added and the products were worked up and fractionated according to the procedure described above in the preparation of the corresponding 4-*O*-phenylthymidine derivative (**15**). Crystallisation of the glass obtained, following the concentration of the appropriate chromatographic fractions, from benzene gave 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-5′-*O*-(9-phenyl-9*H*-xanthen-9-yl)-2′-deoxyguanosine (Found: C, 66.8; H, 4.6; N, 10.6. $C_{43}H_{34}N_6O_8 \cdot 0.5H_2O$ requires C, 66.9; H, 4.6; N, 10.9%) as crystals, m.p. 118—138 °C (1.04 g, 67%); R_F 0.44 (system A); δ_H[$(CD_3)_2SO$] 2.33 (1 H, m), 2.79 (1 H, m), 3.19 (2 H, m), 3.56 (1 H, d, *J* 15.1 Hz), 3.67 (1 H, d, *J* 15.1 Hz), 4.00 (1 H, m), 4.52 (1 H, m), 5.32 (1 H, d, *J* 4.6 Hz), 6.33 (1 H, t, *J* 6.4 Hz), 6.73 (1 H, m), 7.0—7.4 (17 H, m), 7.58 (1 H, m), 7.67 (1 H, m), 7.88 (1 H, m), 8.23 (1 H, m), 8.38 (1 H, s), and 10.47 (1 H, s).

6-O-(3,5-*Dichlorophenyl*)-2-N-*phenylacetyl*-2′-*deoxyguanosine* (**19b**).—Mesitylene-2-sulphonyl chloride (9.30 g, 42.5 mmol), triethylamine (7.4 ml, 53 mmol), and 4-dimethylaminopyridine (0.104 g, 0.85 mmol) were added to a stirred suspension of 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine (7.0 g, 17.0 mmol) in acetonitrile (85 ml) at room temperature. After 90 min, the products were poured into ice-cold saturated aqueous sodium hydrogen carbonate (75 ml), and the mixture was extracted with dichloromethane (3 × 30 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting oil was triturated with light petroleum (b.p. 30—40 °C; 3 × 50 ml) to give a solid, which was then dissolved in chloroform. Evaporation of this solution under reduced pressure gave a glass.

1-Methylpyrrolidine (17.0 ml, 0.163 mol) was added to a stirred solution of the latter material in acetonitrile (17 ml) at room temperature. After 30 min, 3,5-dichlorophenol (8.3 g, 51 mmol) was added and, after a further period of 2.5 h, chloroform (75 ml) was added and the resulting solution was extracted with saturated aqueous sodium hydrogen carbonate (100 ml). The aqueous layer was back extracted with chloroform (50 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short-column chromatography: the appropriate fractions, eluted with $CHCl_3$–EtOH (98:2, v/v) were combined and evaporated under reduced pressure. After the residual gum had been triturated with light petroleum (b.p. 30—40 °C; 2 × 50 ml), it was dissolved in acetonitrile (80 ml) and 2,6-lutidine (23.7 ml, 0.203 mol) was added. Phenylacetyl chloride (13.5 ml, 0.102 mol) was then added dropwise to the cooled (ice-bath) solution, and the reaction was allowed to proceed for a further 5 min. Water (3 ml) was added and, after 10 min, the products were poured into ice-cold saturated aqueous sodium hydrogen carbonate solution (100 ml). The solution was extracted with dichloromethane (3 × 60 ml), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was triturated with light petroleum (b.p. 30—40 °C; 3 × 60 ml) and then dissolved in 4M-methanolic ammonia (100 ml) at room temperature. After 30 min, the products were concentrated under reduced pressure and fractionated by short-column chromatography. The appropriate fractions, eluted with $CHCl_3$–EtOH (92:8, v/v), were combined and evaporated under reduced pressure. The glass obtained was dissolved in chloroform (30 ml) and the resulting solution was added dropwise to light petroleum (b.p. 30—40 °C; 300 ml) to give 6-O-(3,5-*dichlorophenyl*)-2-N-*phenylacetyl*-2′-*deoxyguanosine* as a colourless solid (4.32 g, 48% overall yield for the four steps starting from 3′,5′-di-*O*-methoxyacetyl-2′-deoxyguanosine). Crystallisation of this material from acetonitrile–water gave colourless crystals (Found: C, 52.9; H, 4.0; N, 13.1. $C_{24}H_{21}Cl_2N_5O_5 \cdot H_2O$ requires C, 52.6; H, 4.2; N, 12.8%), m.p. 118—126 °C; R_F 0.23 (system A), 0.66 (system B); $\lambda_{max.}$ (95%

EtOH) 276 (ε 17 400), $\lambda_{infl.}$ 223 (ε 30 900), $\lambda_{min.}$ 249 nm (ε 13 300), δ_H[$(CD_3)_2SO$] 2.30 (1 H, m), 2.74 (1 H, m), 3.56 (2 H, m), 3.73 (2 H, s), 3.86 (1 H, m), 4.43 (1 H, m), 4.92 (1 H, t, *J* 5.5 Hz), 5.34 (1 H, d, *J* 4.1 Hz), 6.36 (1 H, t, *J* 6.9 Hz), 7.2—7.4 (5 H, m), 7.53 (1 H, m), 7.60 (2 H, m), 8.59 (1 H, s), and 10.68 (1 H, s).

6-O-(3,5-*Dichlorophenyl*)-2-N-*phenylacetyl*-5′-O-(9-*phenyl*-9H-*xanthen*-9-*yl*)-2′-*deoxyguanosine* (**20b**).—A solution of 9-chloro-9-phenylxanthene[16] (0.702 g, 2.4 mmol) in acetonitrile (20 ml) was added dropwise over a period of 1 h to a stirred solution of 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (1.06 g, 2.0 mmol) in pyridine (20 ml) at room temperature. After a further period of 1 h, water (0.1 ml) was added to the stirred products. After 10 min, the solution was concentrated under reduced pressure and the residue was dissolved in chloroform (30 ml). The solution was extracted with saturated aqueous sodium hydrogen carbonate (30 ml) and the aqueous layer was back extracted with chloroform (30 ml). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residual glass was fractionated by short-column chromatography: the appropriate fractions, eluted with $CHCl_3$–EtOH (96:4, v/v), were combined and concentrated under reduced pressure. Crystallisation of the residue from ethanol gave 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-5′-*O*-(9-phenyl-9*H*-xanthen-9-yl)-2′-deoxyguanosine (Found: C, 64.6; H, 4.2; N, 8.5. $C_{43}H_{33}Cl_2N_5O_6$·0.5H_2O requires C, 64.9; H, 4.3; N, 8.8%) as crystals, m.p. 125—135 °C (0.937 g, 59%); R_F 0.50 (system A); δ_D[$(CD_3)_2SO$] 2.33 (1 H, m), 2.78 (1 H, m), 3.18 (2 H, m), 3.65 (1 H, d, *J* 14.8 Hz), 3.75 (1 H, d, *J* 14.9 Hz), 3.99 (1 H, m), 4.53 (1 H, m), 5.33 (1 H, d, *J* 4.5 Hz), 6.33 (1 H, t, *J* 6.2 Hz), 6.79 (1 H, m), 7.0—7.4 (17 H, m), 7.55 (1 H, m), 7.60 (2 H, m), 8.37 (1 H, s), and 10.58 (1 H, s).

Determination of Approximate Rates of Removal of Aryl Protecting Groups from Base Residues with N^1,N^1,N^3,N^3-*Tetramethylguanidinium* 2-*Nitrobenzaldehyde Oximate*.—(*a*) A solution of 0.9M-N^1,N^1,N^3,N^3-tetramethylguanidine (TMG) (0.5 ml, 0.45 mmol) in dioxane was added to a stirred solution of 4-*O*-phenylthymidine (**14b**) (0.016 g, 0.05 mmol) and *syn*-2-nitrobenzaldehyde oxime (0.088 g, 0.5 mmol) in dioxane (0.5 ml) at room temperature. The reaction was monitored by t.l.c. and the conversion of the substrate to thymidine, which was the sole product detected, was found to be half-complete in under 10 min, and complete in 60 min.

(*b*) The reaction between 4-*O*-phenylthymidine (**14b**) and 2-nitrobenzaldehyde oximate ions was repeated under the same conditions described in (*a*) above except that acetonitrile was used as solvent instead of dioxane. The conversion of the substrate into thymidine was found to be half-complete in *ca.* 7 min and complete in *ca.* 60 min.

(*c*) A solution of 0.9M-TMG (0.5 ml, 0.45 mmol) in dioxane was added to a stirred solution of 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) (0.025 g, 0.05 mmol) and *syn*-2-nitrobenzaldehyde oxime (0.083 g, 0.5 mmol) in acetonitrile (0.5 ml) at room temperature. The reaction was monitored by t.l.c. (system B), and the conversion of the substrate into 2-*N*-phenylacetyl-2′-deoxyguanosine, which was the sole product detected, was found to be half-complete in *ca.* 20 min and complete in 90 min.

(*d*) The reaction between 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) and 2-nitrobenzaldehyde oximate ions was repeated under the same conditions described in (*c*) above except that acetonitrile was used as solvent instead of dioxane–acetonitrile (1:1, v/v). The conversion of the substrate into 2-*N*-phenylacetyl-2′-deoxyguanosine was found to be half-complete in 4 min and complete in 25 min.

(*e*) A solution of 0.9M-TMG (0.5 ml, 0.45 mmol) in dioxane was added to a stirred solution of 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) (0.027 g, 0.05 mmol) and *syn*-2-nitrobenzaldehyde oxime (0.083 g, 0.5 mmol) in acetonitrile (0.5 ml) at room temperature. The reaction was monitored by t.l.c. (system B), and the conversion of the substrate into 2-*N*-phenylacetyl-2′-deoxyguanosine, which was the sole product detected, was found to be half-complete in *ca.* 18 min and complete in *ca.* 150 min.

(*f*) The reaction between 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) and 2-nitrobenzaldehyde oximate ions was repeated under the same conditions described in (*e*) above except that acetonitrile was used as solvent instead of dioxane–acetonitrile (1:1, v/v). The conversion of the substrate into 2-*N*-phenylacetyl-2′-deoxyguanosine was found to be half-complete in *ca.* 4 min and complete in 35 min.

Conversion of 4-O-*Phenylthymidine into* 3′,5′-*Di*-O-*acetylthymidine*.—(*a*) A solution of 4-*O*-phenylthymidine (**14b**) (0.637 g, 2.0 mmol) and acetic anhydride (1.13 ml, 12.0 mmol) in anhydrous pyridine (10 ml) was stirred at room temperature. After 2 h, water (1.0 ml) was added and, after a further period of 15 min, the products were partitioned between chloroform (30 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure and the residue was redissolved in dioxane (10 ml). *syn*-2-Nitrobenzaldehyde oxime (0.997 g, 6.0 mmol) and TMG (0.63 ml, 5.0 mmol) was added to the resulting solution which was then stirred at room temperature. After 60 min, the products were evaporated under reduced pressure and partitioned between chloroform (30 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The dried ($MgSO_4$) chloroform layer was evaporated under reduced pressure and the residue was fractionated by short column chromatography on silica gel. The appropriate fractions were combined and evaporated under reduced pressure. The residue was triturated with light petroleum (b.p. 30—40 °C, 3 × 20 ml) and then crystallised from ethanol (yield 0.382 g, 58.5%). The latter material was identical [^{1}H n.m.r., m.p., t.l.c. (system A)] with authentic 3′,5′-di-*O*-acetylthymidine[34] (**12**; R = Ac).

(*b*) *syn*-2-Nitrobenzaldehyde oxime (0.997 g, 6.0 mmol) and TMG (0.63 ml, 5.0 mmol) were added to a suspension of 4-*O*-phenylthymidine (**14b**) (0.637 g, 2.0 mmol) in anhydrous dioxane (10 ml). The reactants were warmed gently and the resulting solution was stirred at room temperature for 60 min. Water (15 ml) was added and the products were extracted with chloroform (6 × 15 ml) and ether (6 × 15 ml). The aqueous layer was neutralised (pH 6, indicator paper) with dilute hydrochloric acid and re-extracted with chloroform (6 × 15 ml) and ether (6 × 15 ml). Pyridine (10 ml) was then added to the concentrated (reduced pressure) aqueous layer, and the resulting solution was evaporated under reduced pressure. This process was repeated twice more. The residue was then dissolved in pyridine (10 ml) at room temperature and acetic anhydride (1.13 ml, 12 mmol) was added. After 2 h, water (1.0 ml) was added and, after a further period of 15 min, the products were partitioned between chloroform (30 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The aqueous layer was back extracted with chloroform (40 ml) and the organic layers were combined, dried, and evaporated under reduced pressure. Crystallisation of the residue from ethanol gave 3′,5′-di-*O*-acetylthymidine (**12**; R = Ac) (0.421 g in two crops, 64.5%), identical [^{1}H n.m.r., m.p., t.l.c. (system A)] with authentic material.[34] Evaporation of the mother liquors and trituration of the residue with light petroleum (b.p. 40—60 °C) (3 × 20 ml) gave a solid (0.098 g) which was found [^{1}H n.m.r., t.l.c. (system A)] to be almost pure 3′,5′-di-*O*-acetylthymidine (**12**; R = Ac). The total yield of the latter compound was 0.519 g (79.5%).

Conversion of 6-O-(3,5-*Dichlorophenyl*)-2-N-*phenylacetyl*-2′-*deoxyguanosine into* 3′,5′-*Di*-O-*acetyl*-2′-*deoxyguanosine*.—A solution of 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) (1.06 g, 2.0 mmol), *syn*-2-nitrobenzaldehyde oxime (0.997 g, 6.0 mmol) and TMG (0.63 ml, 5.0 mmol) in dioxane (5 ml) and acetonitrile (5 ml) was stirred at room temperature for 2.5 h. The products were then concentrated under reduced pressure and aqueous ammonia (*d* 0.88; 10 ml) was added to the residue. The resulting mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. The residue obtained was dissolved in water (20 ml) and the resulting solution was extracted with chloroform (6 × 15 ml) and ether (6 × 15 ml). The aqueous layer was neutralised (pH 6, indicator paper) with dilute hydrochloric acid, re-extracted with chloroform (6 × 15 ml) and ether (6 × 15 ml), and evaporated under reduced pressure. Pyridine (10 ml) was then added and the resulting solution was evaporated under reduced pressure. This process was repeated twice more. The residue was suspended in dimethylformamide (10 ml) and pyridine (4.28 ml) and acetic anhydride (2.14 ml, 22.7 mmol) was added. The reactants were stirred at room temperature for 46 h. Methanol (20 ml) was then added and, after 30 min, the products were concentrated under reduced pressure (oil-pump). After it had been triturated with ether (2 × 20 ml), the residue was crystallised from aqueous ethanol to give 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (0.467 g, 66.5%), identical [^{1}H n.m.r., m.p., t.l.c. (system A)] with authentic material.[35] Evaporation of the mother liquors and trituration of the residue with ether (3 × 20 ml) gave a solid (0.19 g) which was found [^{1}H n.m.r., t.l.c. (system A)] to be mainly 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**16**; R = Ac). The total yield of the latter compound was estimated to be at least 0.6 g (85%).

Action of Acid on Protected Thymidine and 2′-*Deoxyguanosine Derivatives*.—The substrates (0.005 mmol) were dissolved in acetic acid–water (4:1, v/v; 0.2 ml) at room temperature and the reactions were monitored by t.l.c. (system B). 4-*O*-Phenylthymidine (**14b**) had undergone *ca.* 30% decomposition after 43 h, 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) had undergone *ca.* 30—40% decomposition after 19 h, and 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) had undergone *ca.* 65% decomposition after 19 h. Under the same conditions, 2-*N*-phenylacetyl-2′-deoxyguanosine and 6-*N*-benzoyl-2′-deoxyadenosine underwent *ca.* 90% decomposition in 19 h and in under 2 h, respectively.

Action of Potassium Carbonate on Protected Thymidine and 2′-*Deoxyguanosine Derivatives in Aqueous Dioxane Solution*.—M-Aqueous potassium carbonate (0.1 ml) was added to a solution of the substrate (0.005 mmol) in dioxane (0.4 ml) at room temperature. After 3 h, t.l.c. (system B) revealed that 4-*O*-phenylthymidine (**14b**), 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) and 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) were all completely unchanged.

Stabilities of Protected Thymidine and 2′-*Deoxyguanosine Derivatives in Pyridine Solution*.—The substrates (0.005 mmol) were dissolved in anhydrous pyridine (0.2 ml) at room temperature. After 48 h, t.l.c. (system B) revealed that 4-*O*-phenylthymidine (**14b**), 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) and 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) were all completely unchanged.

Action of Toluene-p-*thiolate Ions on Protected Thymidine and* 2′-*Deoxyguanosine Derivatives in Anhydrous Acetonitrile Solution*.—0.4M-Triethylamine in acetonitrile (0.5 ml, 0.2 mmol) was added to a stirred solution of substrate (0.05 mmol) and toluene-*p*-thiol (0.026 g, 0.2 mmol) in acetonitrile (0.5 ml) at room temperature. After 3 h, t.l.c. (systems A and B) suggested that 4-*O*-phenylthymidine (**14b**), 6-*O*-(2-nitrophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19a**) and 6-*O*-(3,5-dichlorophenyl)-2-*N*-phenylacetyl-2′-deoxyguanosine (**19b**) were all completely unchanged. The t.l.c. evidence was, however, misleading. The products were concentrated under reduced pressure and then triturated with hexane (6 × 5 ml). The ^{1}H n.m.r. spectra [$(CD_3)_2SO$] of the residues thus obtained suggested that (**14b**) had undergone *ca.* 30% conversion most probably into the corresponding 4-*p*-tolylthio-derivative, and that (**19a**) and (**19b**) had respectively undergone *ca.* 40 and 65% conversion most probably to the corresponding 6-*p*-tolylthio-derivative.

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