Phosphotriester Approach to the Synthesis of Oligonucleotides: A Reappraisal

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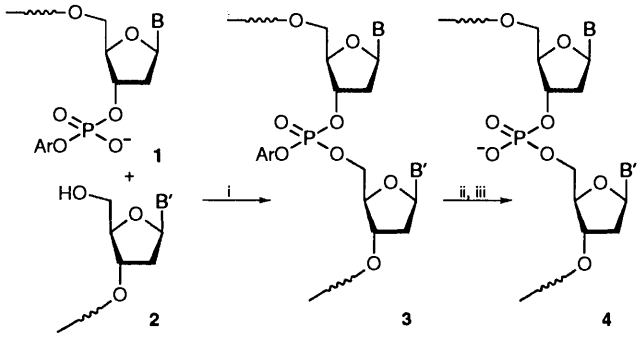
The phosphotriester approach to the synthesis of oligodeoxyribo- and oligoribo-nucleotides in solution has been reinvestigated. The efficacy of mesitylene-2-sulfonyl chloride (MSCl) **15a**, 2,4,6-triisopropylbenzenesulfonyl chloride (TrisCl) **15b**, 4-bromobenzenesulfonyl chloride **15c**, naphthalene-1-sulfonyl chloride **39**, and 2- and 4-nitrobenzenesulfonyl chlorides **40a** and **40b**, respectively, as activating agents has been examined. The latter arenesulfonyl chlorides have been used in conjunction with the following nucleophilic catalysts: 1-methylimidazole, 3-nitro-1*H*-1,2,4-triazole **19**, 5-(3-nitrophenyl)-1*H*-tetrazole **20a**, 5-(3,5-dinitrophenyl)-1*H*-tetrazole **20b**, 5-(1-methylimidazol-2-yl)-1*H*-tetrazole **21**, 5-[(1-methylimidazol-2-yl)methyl]-1*H*-tetrazole **22**, 4-ethoxypyridine 1-oxide **14a**, 4,6-dinitro-1-hydroxybenzotriazole **29a**, 1-hydroxy-4-nitro-6-(trifluoromethyl)benzotriazole **29b**, 1-hydroxy-5-phenyltetrazole **30a** and 1-hydroxy-5-(3-nitrophenyl)tetrazole **30b**. The rates of formation and yields of the fully protected dideoxyribonucleoside and diribonucleoside phosphates **37** and **47**, respectively, were determined using various combinations of activating agents and nucleophilic catalysts. Although 2- and 4-nitrobenzenesulfonyl chlorides **40a** and **40b**, respectively, proved to be the most powerful activating agents, their use in the deoxy-series led to the formation of by-products and hence to unsatisfactory isolated yields of the dideoxyribonucleoside phosphate **37**.

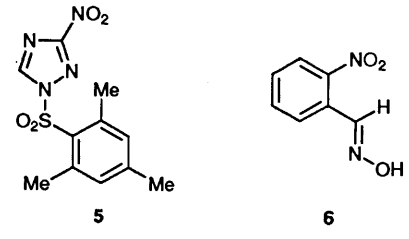
Prior to 1970, the chemical synthesis of oligonucleotides was effected mainly by a procedure in which the internucleotide linkages were left unprotected, that is by the *phosphodiester* approach. This method, which was introduced by H. G. Khorana and his co-workers,[1,2] was used in the synthesis of moderately sized oligodeoxyribonucleotides in solution by the latter workers in the course of their historic studies that led[3] to the elucidation of the genetic code. However, side-reactions, especially those involving guanine residues, and separation problems limited the effectiveness of this approach. It also proved to be of very limited value indeed in the synthesis of oligoribonucleotides.[4] Although the alternative *phosphotriester* approach[5] had been investigated in a preliminary manner[6] in the 1950s, it was not until the late 1970s that the main problems associated with its practical implementation had been solved.[7,8] It had by then superseded the phosphodiester approach and become the method of choice for the synthesis both of oligodeoxyribo- and oligoribo-nucleotides in solution; for a while, it was also the method of choice for the synthesis of oligodeoxyribonucleotides on a solid support.[9] A matter of crucial importance in the development of the phosphotriester approach to oligonucleotide synthesis was the choice of protecting group for the internucleotide linkages. It was found that aryl[10] (especially 2-chlorophenyl[11]) groups were the protecting groups of choice for this purpose but it proved to be critically important that they should be removed at the end of the synthesis by treatment with the conjugate bases of certain oximes,[12,13] particularly (*E*)-2-nitrobenzaldehyde oxime **6** and pyridine-2-carboxaldehyde oxime. A number of studies were carried out relating to the choice of phosphorylation method. One outcome of these studies was that 1-(mesityl-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT, **5**)[8,12] became established as the condensing agent of choice. The general procedure followed in the synthesis of oligodeoxyribonucleotides by the phosphotriester approach is indicated in outline in Scheme 1.

The phosphoramidite approach to the automated solid-phase synthesis of oligonucleotides was developed[14] in the early 1980s. This is essentially a variation of the phosphotriester approach in which a P^{III} acylating agent (Scheme 2a) is used and the protected internucleotide linkages are immediately oxidized from phosphite triesters to normal phosphotriesters (as in substrates **9**). This would appear, at present, to be the method of choice for the automated solid-phase synthesis of oligo- and poly-nucleotides. However, the phosphoramidite approach does not seem to be particularly suitable for the synthesis of oligonucleotides in solution. The same is true for the *H*-phosphonate approach (Scheme 2b) which is another procedure for the rapid synthesis of oligonucleotides on a solid support, that was also introduced[15,16] in the 1980s.

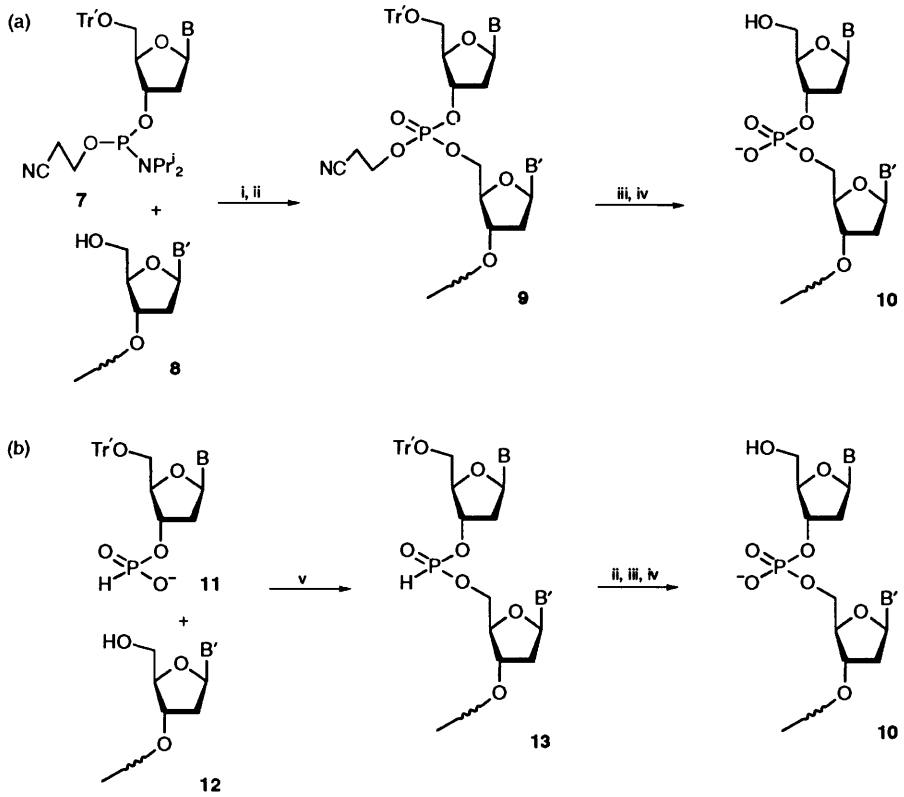


Ar = 2-chlorophenyl; B and B' are protected in substrates **1**, **2** and **3**, and unprotected base residues in product **4**

Scheme 1 *Reagents and conditions:* i, MSNT **5**, C_6H_5N; ii, **6**, $(Me_2N)_2C{=}NH$; iii, conc. aq. NH_3 (*d* 0.88)



The phosphotriester approach[5] (Scheme 1) is still by far the most versatile method of oligonucleotide synthesis and has a number of really significant advantages over the other methods that have subsequently been developed (Scheme 2). First, both the nucleotide building blocks (*e.g.*, **1**) and the phosphotriester intermediates (*e.g.*, **3**) are very stable and easy to handle. Secondly, monomer, dimer and very much larger nucleotide building blocks **1**[7,17] may be used. Thirdly, it is not necessary to use more than a slight excess of nucleotide building block. Fourthly, coupling reactions are not impeded by small



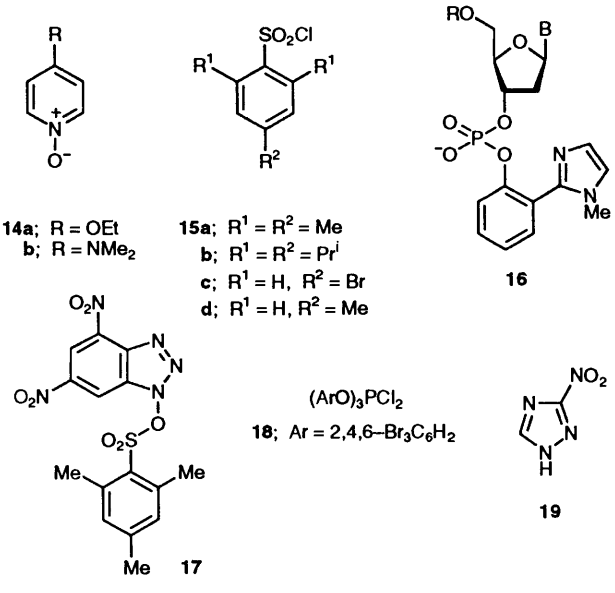
Tr′ = 4,4′-dimethoxytrityl or 9-phenylxanthen-9-yl; B and B′ are protected (except for thymine) in substrates **7–9**, **11–13**, and unprotected base residues in the products **10**

Scheme 2 *Reagents and conditions:* i, 1*H*-tetrazole, MeCN; ii I_2, 2,6-lutidine, aq., THF; iii, conc. aq. NH_3 (*d* 0.88); iv, aq. acid (pH ~ 2); v, Me_3CCOCl, C_5H_5N

quantities of moisture, which are removed by the excess of condensing agent (*e.g.*, MSNT **5**) usually present. Fifthly, the phosphotriester approach is the only method that is really suitable for the synthesis of oligodeoxyribo- and oligoribonucleotides in solution. It is therefore particularly useful for the synthesis of cyclic[18,19] and branched cyclic[20] oligonucleotides and for other, more sophisticated applications.

The only really significant disadvantages of the conventional phosphotriester approach, in comparison particularly with the phosphoramidite approach,[21] are that coupling reactions proceed more slowly and, perhaps more importantly, in somewhat lower yield. The purpose of the present investigation was to attempt to modify the phosphotriester approach in such a way as to minimize or if possible eliminate these disadvantages. It is anticipated that if antisense[22] and other approaches to oligonucleotide-based chemotherapy develop in the future, there will be a demand for very large (perhaps multi-kilogram) quantities of specific synthetic oligonucleotides. As the phosphotriester approach can readily be carried out in solution as well as on a solid support, it may very well become the method of choice for the large-scale synthesis of oligodeoxyribo- and oligoribo-nucleotides and their analogues. However, this will most likely depend on there being improvements in phosphotriester coupling rates and more particularly in coupling yields.

A number of investigations directed towards speeding up and improving the efficiency of the phosphotriester approach have already been carried out. Efimov *et al.* reported[23] that certain 4-substituted pyridine 1-oxides (*e.g.*, **14a** and **14b**) act as powerful nucleophilic catalysts in coupling reactions involving mesitylene-2-sulfonyl chloride **15a** as the condensing agent, especially in dichloromethane solution. Efimov *et al.*[24] had previously recommended that 1-methylimidazole was a useful nucleophilic catalyst in phosphotriester condensations. Later,

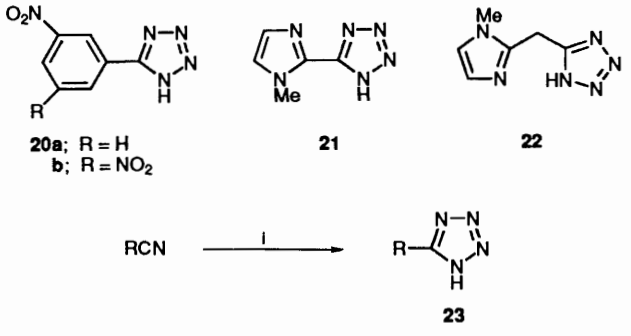


Froehler and Matteucci observed[25] enhanced nucleophilic catalysis when the 1-methylimidazole residue was attached directly to the aryl protecting group as in the nucleotide building block **16**. We ourselves found[26] that 1-(mesitylsulfonyloxy)-4,6-dinitrobenzotriazole **17** was approximately an order of magnitude more reactive as a condensing agent than was MSNT **5**. More recently, Hata and his co-workers reported[27] that the phosphorylating species obtained by preactivating the nucleotide building block with dichlorotris-(2,4,6-tribromophenoxy)phosphorane **18** and then adding 3-nitro-1*H*-1,2,4-triazole **19** was very reactive indeed. We wished

to ensure at the outset that any modifications that we made to the phosphotriester approach would be equally applicable both to solid-phase and solution synthesis. We therefore decided to separate the nucleophilic catalyst component (*e.g.*, **19**) from the activating agent (*e.g.*, **15**) rather than to use a composite reagent such as MSNT **5** or compound **17**. Despite the ingenuity of their idea, we did not favour Froehler and Matteucci's approach[25] as control of oximate ion promoted unblocking[12,13] by appropriate substition of the aryl group used for the protection of the internucleotide linkages is a very important aspect of phosphotriester synthesis. In this study, we have examined the efficacy of various combinations of nucleophilic catalysts and activating agents in solution, starting mainly from deoxyribonucleotide but also from ribonucleotide building blocks.

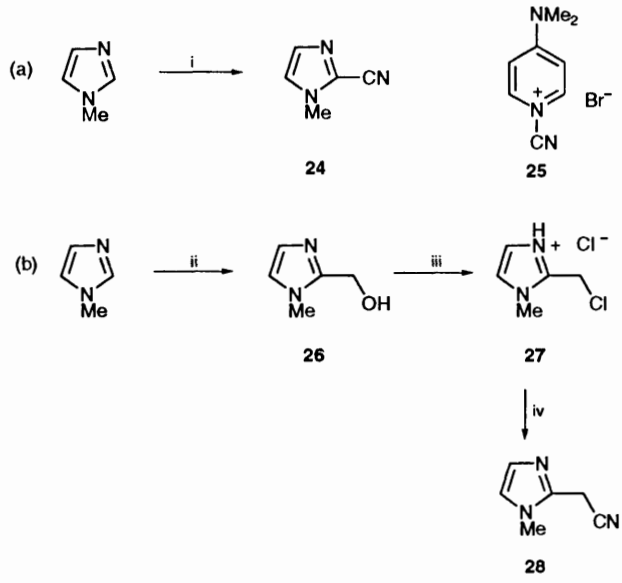
Results and Discussion

Preparation of Nucleophilic Catalysts.—1-Arenesulfonyl derivatives of 1*H*-tetrazole **23** (R = H) were found[28] to be effective condensing agents in the phosphotriester approach to oligodeoxyribonucleotide synthesis; although they have a similar reactivity, they have been much less widely used than corresponding derivatives of 3-nitro-1*H*-1,2,4-triazole **19**, such as MSNT **5**. However, it seemed possible that certain 5-substituted 1*H*-tetrazole derivatives **23** might prove to be superior nucleophilic catalysts compared with both 1*H*-tetrazole **23** (R = H) and 3-nitro-1*H*-1,2,4-triazole **19**. 5-(3-Nitrophenyl)-1*H*-tetrazole[29] **20a**, which is used as an activating agent in the phosphoramidite approach[30] to oligoribonucleotide synthesis,[31] 5-(3,5-dinitrophenyl)-1*H*-tetrazole[32] **20b** and the two 1-methylimidazole derivatives[33] **21** and **22** were selected as possible nucleophilic catalysts. It was hoped that a 1-methylimidazole side-chain might have a similar rate-enhancing effect on the condensation reaction when incorporated into the nucleophilic catalyst as it has when it is attached to the aryl group used[25] to protect the internucleotide linkages. Modification of the nucleophilic catalyst rather than the aryl group would bring the advantage that the 2-chlorophenyl protecting group, and hence control of the oximate ion-promoted unblocking[12,13] of the internucleotide linkages, could be retained.



Scheme 3 *Reagents and conditions:* i, NaN_3, NH_4Cl, DMF, 120–130 °C

5-Substituted 1*H*-tetrazole derivatives **23** are readily prepared[29] (Scheme 3) and in good yield (see Experimental section) by heating the corresponding nitriles with sodium azide and ammonium chloride in dimethylformamide (DMF) solution. 3-Nitro- and 3,5-dinitro-benzonitrile are commercially available. 2-Cyano- and 2-(cyanomethyl)-1-methylimidazole (**24** and **28**, respectively) were prepared by the procedures indicated in Scheme 4. It was necessary to use 1-cyano-4-(dimethylamino)pyridinium bromide[34] **25** in the preparation of 2-cyano-1-methylimidazole **24** (Scheme 4a and Experimental section) as the reaction between 1-methylimidazole and cyanogen bromide[34,35] led predominantly to 2-bromo-1-methylimidazole.

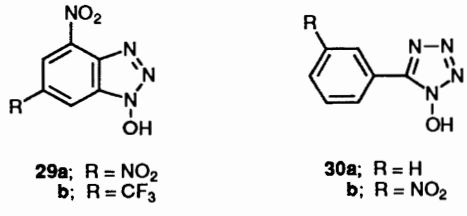


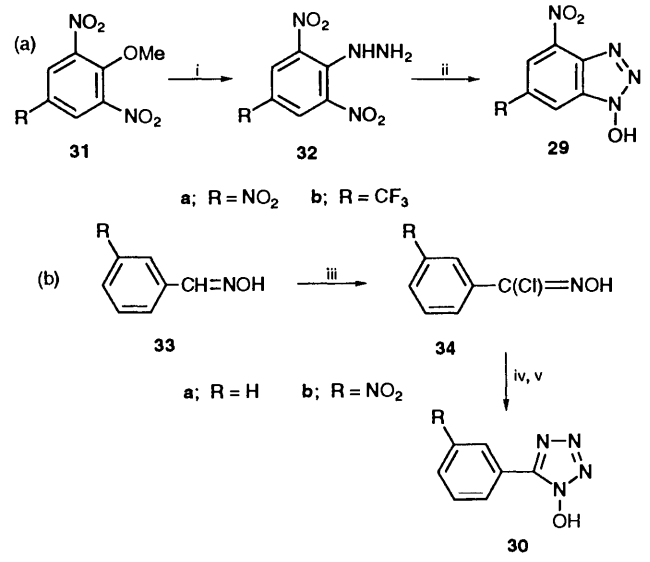
Scheme 4 *Reagents and conditions:* i, **25**, DMF; ii, paraformaldehyde, 160 °C; iii, $SOCl_2$, reflux; iv, NaCN, DMSO

In connection with the preparation of the 2-(cyanomethyl) derivative **28** (Scheme 4b and Experimental section), we found that the conversion of 1-methylimidazole into the corresponding 2-(hydroxymethyl) derivative **26** was better effected by heating it with neat paraformaldehyde than with aq. formaldehyde,[36] and we also found that the 2-(chloromethyl) derivative[37] **27** reacted with sodium cyanide in dimethyl sulfoxide (DMSO) solution to give virtually exclusively the desired 2-(cyanomethyl)-1-methylimidazole **28**. This was not the case when the salt **27** was treated with potassium cyanide in aq. ethanol.[37]

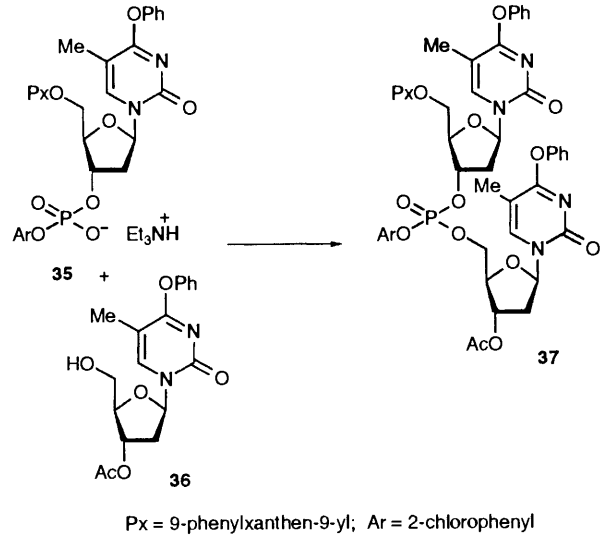
Previous studies by Efimov *et al.*[23] and by ourselves[26] (see below) prompted us to re-examine the promising nucleophilic catalytic activities of 4-ethoxypyridine 1-oxide **14a**, 1-hydroxy-4,6-dinitrobenzotriazole **29a**, and 1-hydroxy-4-nitro-6-(trifluoromethyl)benzotriazole **29b**. 4-Ethoxypyridine 1-oxide **14a** was prepared[38] by heating commercially available 4-nitropyridine 1-oxide with sodium ethoxide in ethanol, and the benzotriazole derivatives **29a** and **29b** were prepared (Scheme 5a and Experimental section) in 60 and 55% yield, respectively, from the corresponding phenylhydrazines **32a** and **32b** by a slight modification of Huisgen and Weberndörfer's procedure.[39] The phenylhydrazines **32a** and **32b** were prepared[40] from 2,4,6-trinitroanisole **31a** and 2,6-dinitro-4-(trifluoromethyl)anisole **31b**, respectively. The latter compound **31b** was in turn prepared by the action of sodium methoxide on commercially available 4-chloro-3,5-dinitrobenzotrifluoride in methanol solution.

Nucleophilic catalysts **14a**, **29a** and **29b** differ from 3-nitro-1*H*-1,2,4-triazole **19** and the above 5-substituted 1*H*-tetrazole derivatives **20a**, **20b**, **21** and **22** inasmuch as they must be (in the case of compound **14a**) or are likely to be (in the case of compounds **29a** and **29b**) attached to phosphorus by a P–O rather than by a P–N bond in the active phosphorylating species. It was therefore thought that 5-aryl-1-hydroxytetrazole derivatives **30**, which are structurally related to the 1-





Scheme 5 *Reagents and conditions:* i, N_2H_4·H_2O, EtOH, 0 °C; ii, N_2H_4·H_2O, NaOAc, aq. AcOH; then aq. HCl; iii, Bu^{t}OCl, CH_2Cl_2, $Me_2CH(OH)$, −12 °C; iv, NaN_3, aq. MeOH, room temp.; v, AcCl, MeCN, reflux; then recrystallization from 96% EtOH

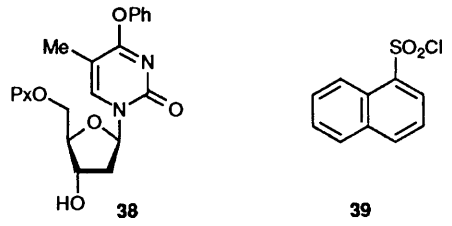


Px = 9-phenylxanthen-9-yl; Ar = 2-chlorophenyl

Scheme 6 *Reagents and conditions:* A solution of the arenesulfonyl chloride (0.90 mmol) in dry MeCN (1.5 cm^3) was added to a stirred solution of the salt **35** (~0.295 mmol), compound **36** (0.25 mmol), 1-methylimidazole (1.0 mmol) and usually (see Table 1) an additional nucleophilic catalyst (0.75 mmol) in C_5H_5N (2.5 cm^3) at room temp. The reactions were quenched and worked up (Experimental section) after an appopriate time.

hydoxybenzotriazole derivatives **29**, might also prove to be useful nucleophilic catalysts. 1-Hydroxy-5-phenyl-1*H*-tetrazole **30a** was prepared by the reported procedure[41] (Scheme 5b), and 1-hydroxy-5-(3-nitrophenyl)-1*H*-tetrazole **30b** was prepared in the same way (Scheme 5b and Experimental section), albeit in a rather modest yield.

Condensations in Oligodeoxyribonucleotide Synthesis.— Phosphotriester condensations in the deoxy-series (Scheme 6) were examined first. The 3′-phosphodiester component **35** was prepared as previously reported,[19] and the component with the free 5′-hydroxy function, compound **36**, was prepared (Experimental section) in two steps from 4-*O*-phenyl-5′-*O*-(9-phenylxanthen-9-yl)thymidine[42] **38** in 71% overall yield. The thymine base-residues in the two building blocks **35** and **36** were protected in the manner reported previously[42] in order to avoid possible side-reactions. All condensations (Scheme 6, Table 1



and Experimental section) were carried out on the same scale starting from 3′-*O*-acetyl-4-*O*-phenylthymidine **36** (0.25 mmol), the 3′-phosphodiester **35** (~1.18 mol equiv.), 1-methylimidazole (4 mol equiv.), arenesulfonyl chloride (3.6 mol. equiv.) and, except in four experiments (Table 1, entries nos. 1–4), an additional nucleophilic catalyst (3 mol equiv.). After work-up, the fully protected dinucleoside phosphate **37** was purified by short-column chromatography on silica gel, isolated as a solvent-free precipitated solid, and weighed. The theoretical yield of compound **37** was 0.277 g in all experiments. However, considering all of the manipulations involved, it may well be almost impossible to avoid small losses (say, 2–4%) of product during work-up, purification and isolation. Thus an isolated yield of 0.266 g or above may represent a virtually quantitative yield of fully protected dinucleoside phosphate in the condensation. It was clear from the results obtained (see Table 1 and below) that MSCl[43] **15a** and TrisCl[44] **15b** were the most effective activating agents examined. Although both 2- and 4-nitrobenzenesulfonyl chlorides **40a** and **40b** were found (see below) to effect faster condensations, their use also led to the occurrence of side-reactions and therefore to lower yields of the desired condensation product **37**. Although, if the phosphotriester approach in solution is to be used for the large-scale synthesis of oligonucleotides, it is desirable that the condensations should be as rapid as possible, it is crucially important that virtually quantitative yields of pure protected oligonucleotides should be obtained. If side-reactions occur, both impure products and diminished yields will inevitably result.

Very satisfactory isolated yields (~94 and 95%, respectively) of fully protected dinucleoside phosphate **37** were obtained with both MSCl **15a** and TrisCl **15b** as the activating agent and with 1-methylimidazole as the sole nucleophilic catalyst (Table 1, entries nos. 2 and 4, respectively). The reactions proceeded relatively slowly but, in both experiments, the 5′-hydroxy component **36** was completely consumed. Addition of 3-nitro-1*H*-1,2,4-triazole **19** to the TrisCl-promoted condensation (entry no. 8) increased its rate very marginally (*i.e.*, by a factor of ~1.5) and the high isolated yield (~96%) suggested that the condensation had proceeded virtually quantitatively. By comparing entries nos. 14 and 16 with entry no. 6, it can be seen that 5-(3-nitrophenyl)-1*H*-tetrazole **20a** and especially 5-(3,5-dinitrophenyl)-1*H*-tetrazole **20b** are somewhat more effective nucleophilic catalysts than is 3-nitro-1*H*-1,2,4-triazole **19**. Rather disappointingly, it appears that the two tetrazole derivatives **21** and **22** with covalently attached 1-methylimidazole side-chains (entries nos 18 and 20) are not more effective nucleophilic catalysts than was 3-nitro-1*H*-1,2,4-triazole (entry no. 6). It can also be seen (entry no. 6 and footnote[a]) that no advantage is gained by using MSNT **5** rather than a mixture of MSCl **15a** and 3-nitro-1*H*-1,2,4-triazole **19.** The observation by Efimov *et al.*[23] that 4-ethoxypyridine 1-oxide **14a** (entries nos. 21–23) promotes rapid condensations has been confirmed. Thus, after 4 min with MSCl **15a** as the condensing agent (entry no. 23), no 5′-hydroxy compound **36** remained. However, in our hands, the latter nucleophilic catalyst **14a** did not prove to be particularly useful as, due to side-reactions, the isolated yield (~86%; entry no. 23) of fully protected dinucleoside phosphate **37** was not especially high. In accord with our earlier studies,[26] 1-hydroxy-4,6-dinitrobenzotriazole **29a** was found

Table 1 Preparation of 3′-*O*-acetyl-4-*O*-phenylthymidin-5′-yl 2-chlorophenyl 4-*O*-phenyl-5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl phosphate **37**

Entry	Arenesulfonyl chloride	Additional catalyst	Reaction time (*t*/min)	Isolated yield (g)	Yield (%)
1	**15a**		20	0.234	84.6
2	**15a**		30	0.259	93.6
3	**15b**		60	0.243	87.9
4	**15b**		90	0.264	95.4
5	**15a**	**19**	10	0.207	74.8
6	**15a**	**19**	20	0.256	92.6[a]
7	**15b**	**19**	20	0.229	82.8
8	**15b**	**19**	60	0.266	96.2
9	**15d**	**19**	10	0.137	49.5
10	**15d**	**19**	20	0.226	81.7
11	**15c**	**19**	10	0.189	68.3
12	**39**	**19**	10	0.179	64.7
13	**15a**	**20a**	10	0.224	81.0
14	**15a**	**20a**	20	0.261	94.4
15	**15a**	**20b**	4	0.226	81.7
16	**15a**	**20b**	10	0.263	95.1
17	**15a**	**21**	10	0.240	86.8
18	**15a**	**21**	20	0.254	91.8
19	**15a**	**22**	10	0.241	87.1
20	**15a**	**22**	20	0.255	92.2
21	**15a**	**14a**	1	0.211	76.3
22	**15a**	**14a**	2	0.230	83.2
23	**15a**	**14a**	4	0.237	85.7
24	**15a**	**29a**	1	0.120	43.4
25	**15a**	**29a**	2	0.227	82.1
26	**15a**	**29a**	4	0.258	93.3
27	**15a**	**29a**	10	0.260	94.0
28	**15b**	**29a**	4	0.234	84.6
29	**15b**	**29a**	10	0,265	95.8
30	**39**	**29a**	2	0.232	83.9
31	**39**	**29a**	4	0.248	89.7
32	**15a**	**29b**	2	0.203	73.4
33	**15a**	**29b**	4	0.241	87.1
34	**15a**	**29b**	10	0.255	92.2
35	**15a**	**30a**	10	0.241	87.1
36	**15a**	**30b**	4	0.249	90.0

[a] When mesitylene-2-sulfonyl chloride **15a** (0.90 mmol) and 3-nitro-1*H*-1,2,4-triazole **19** (0.75 mmol) were replaced by MSNT **5** (0.75 mmol), the fully protected dinucleoside phosphate **37** (0.252 g, 91%) was isolated after a reaction time of 30 min.

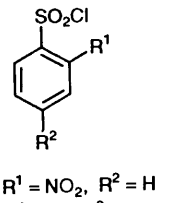
to be a very active nucleophilic catalyst. Thus with MSCl **15a** as the condensing agent (entry no. 26), the fully protected dinucleoside phosphate **37** was obtained in ~93% isolated yield after 4 min, and with TrisCl **15b** as the condensing agent (entry no. 29), it was obtained in ~96% isolated yield after 10 min. Also in accord with our earlier studies,[26] it was confirmed that 1-hydroxy-4-nitro-6-(trifluoromethyl)benzotriazole **29b** (entries nos. 32–34) was also an effective nucleophilic catalyst and that it was less active than the 4,6-dinitro compound **29a**. While the related 5-aryl-1-hydroxytetrazoles **30a** and **30b** (entries nos. 35 and 36) are effective nucleophilic catalysts, neither of them is as active as 1-hydroxy-4,6-dinitrobenzotriazole **29a** (entry no. 26). Finally, neither 4-bromobenzenesulfonyl chloride **15c** (entry no. 11) nor naphthalene-1-sulfonyl chloride **39** (entry no. 12) was found to be as effective an activating agent as MSCl **15a** (entry no. 5) in the presence of 3-nitro-1*H*-1,2,4-triazole **19**. Naphthalene-1-sulfonyl chloride **39** (entry no. 31) was also less effective than was MSCl **15a** (entry no. 26) in the presence of 1-hydroxy-4,6-dinitrobenzotriazole **29a**.

In summary, what are in practice virtually quantitative yields of the fully protected dideoxyribonucleoside phosphate **37** were obtained when TrisCl **15b** was used as the activating agent (*a*) in the absence of an additional nucleophilic catalyst (entry no. 4), (*b*) in the presence of 3-nitro-1*H*-1,2,4-triazole **19** (entry no. 8) and (*c*) in the presence of 1-hydroxy-4,6-dinitrobenzotriazole **29a** (entry no. 29). The times required for the latter reactions were 90, 60 and 10 min, respectively. Exceptionally high yields were also obtained when MSCl **15a** was used as the activating agent in the presence of (*a*) 5-(3-nitrophenyl)-1*H*-tetrazole **20a** (entry no. 14, 20 min reaction time), (*b*) 5-(3,5-dinitrophenyl)-1*H*-tetrazole **20b** (entry no. 16, 10 min reaction time) and (*c*) 1-hydroxy-4,6-dinitrobenzotriazole **29a** (entry no. 27, 10 min reaction time).

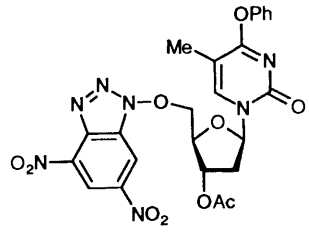
When the condensation between the phosphodiester **35** and the 5′-hydroxy compound **36** was carried out according to the procedure indicated in Scheme 6 with 2-nitrobenzenesulfonyl chloride **40a** as the condensing agent and 1-hydroxy-4,6-dinitrobenzotriazole **29a** as the additional nucleophilic catalyst, the reaction was complete within 2 min. The products were worked up and chromatographed in the usual way (Experimental section) to give the fully protected dinucleoside phosphate **37** in 83% isolated yield and a crystalline compound, identified as the benzotriazole derivative **41**, in 12% isolated yield. When the phosphodiester component **35** was omitted from the above reaction mixture and the products were worked up after 25 min, the benzotriazole derivative **41** was obtained in 75% isolated yield. The latter two experiments were then repeated except that 2-nitrobenzenesulfonyl chloride **40a** was replaced by 4-nitrobenzenesulfonyl chloride **40b**. When the phosphodiester component **35** was included in the reaction mixture, the fully protected dinucleoside phosphate **37** was obtained in 82% and the benzotriazole derivative **41** in 14% isolated yield. When the phosphodiester component **35** was omitted from the reaction mixture, the benzotriazole derivative **41** was obtained in 77% isolated yield. Finally, the condensation between the phosphodiester component **35** and the 5′-hydroxy compound **36** was carried out (Scheme 6) with 2-nitrobenzenesulfonyl chloride **40a** as the activating agent and 3-nitro-1*H*-

Table 2 Preparation of 2-chlorophenyl 2',3'-di-*O*-acetyl-4-*O*-(2,4-dimethylphenyl)uridin-5'-yl 4-*O*-(2,4-dimethylphenyl)-2'-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-5'-*O*-(9-phenylxanthen-9-yl)uridin-3'-yl phosphate **47**

Entry	Arenesulfonyl chloride	Additional catalyst	Reaction time (*t*/min)	Isolated yield (g)	Yield (%)
1	**15a**		75	0.266	93.9
2	**15b**		220	0.267	94.2
3	**15a**	**19**	45	0.267	94.2
4	**15b**	**19**	150	0.268	94.6
5	**15a**	**29a**	15	0.252	88.9
6	**15b**	**29a**	25	0.256	90.3
7	**15a**	**20b**	30	0.259	91.4



40a; $R^1 = NO_2$, $R^2 = H$
b; $R^1 = H$, $R^2 = NO_2$



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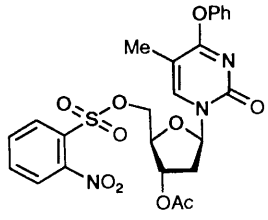

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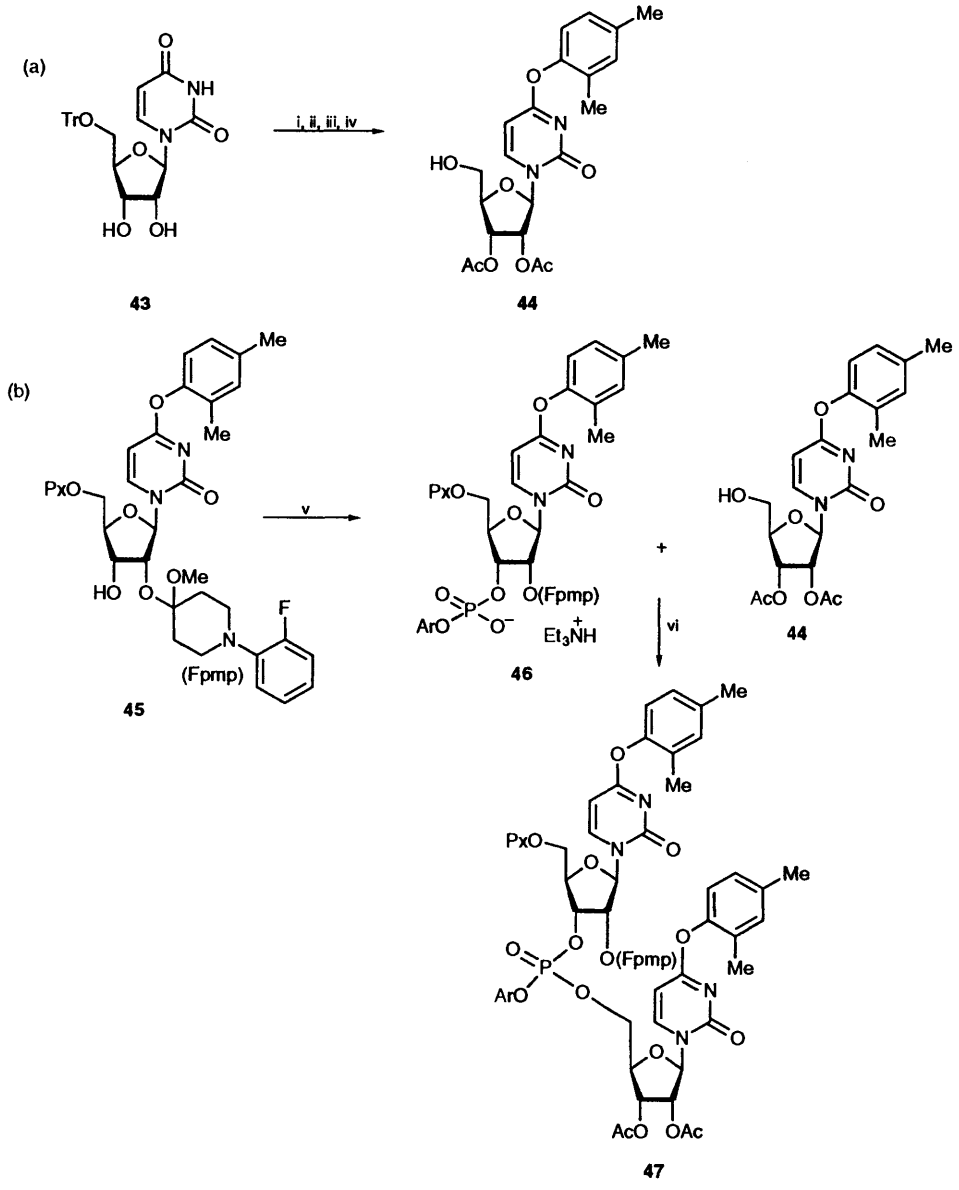
1,2,4-triazole **19** as the additional nucleophilic catalyst. After 2 min, the products were worked up and chromatographed to give the fully protected dinucleoside phosphate **37** in 67% isolated yield and a by-product which, on the basis of its ^1H NMR spectrum, was believed to be the 5'-*O*-(2-nitrophenylsulfonyl) derivative **42**, in 23% isolated yield. It would seem likely that the latter compound **42** was also formed initially in the reactions involving 2-nitrobenzenesulfonyl chloride **40a** and 1-hydroxy-4,6-dinitrobenzotriazole **29a**, and that the conjugate base of compound **29a** reacted rapidly with it to give compound **41**. 2- and 4-Nitrobenzenesulfonyl chlorides **40a** and **40b** are insufficiently selective to be useful activating agents. Fortunately, MSCl **15a** and especially TrisCl **15b** are very selective indeed; when they are used as activating agents, negligible quantities of by-products are obtained. The use of 4-nitro-1-(4-nitrophenylsulfonyl)imidazole as an activating agent in oligodeoxyribonucleotide synthesis has previously been reported by Leach *et al.*[45]

Condensations in Oligoribonucleotide Synthesis.—Following the above studies in the deoxy-series (Table 1), a more limited investigation was carried out in the ribose-series (Table 2). The 5'-hydroxy compound **44** (Scheme 7a and Experimental section) was prepared in four steps from 5'-*O*-trityluridine[46] **43** and was isolated in 46% overall yield. The 3'-phosphodiester component **46** was prepared in the usual way[17] (Scheme 7b, step v) from the corresponding 2',5'-protected nucleoside derivative[47] **45**. All of the condensations (Scheme 7, Table 2 and Experimental section) were carried out on the same scale, starting from the 5'-hydroxy compound **44** (0.2 mmol), the 3'-phosphodiester component **46** (~1.6 mol equiv.), 1-methylimidazole (4.0 mol equiv.), the arenesulfonyl chloride (4.8 mol equiv.) and, except in the first two experiments (Table 2, entries nos. 1 and 2), an additional nucleophilic catalyst (4.0 mol equiv.). As in the above condensations in the deoxy-series (Scheme 6 and Table 1), the fully protected dinucleoside phosphate **47** was purified by short-column chromatography on silica gel, isolated as a solvent-free precipitated solid, and weighed. The theoretical yield of **47** was 0.283 g.

Despite the fact that larger excesses each of the phosphodiester component **46**, the arenesulfonyl chloride activating agent, and the additional nucleophilic catalyst were used in the ribose-series, the rates of the condensations were appreciably (~2–3-times) slower than those observed in the deoxy-series (Table 1). However, very satisfactory isolated yields (~94% or greater) of the fully protected dinucleoside phosphate **47** were obtained when MSCl **15a** or TrisCl **15b** was used as the activating agent both in the absence of an additional nucleophilic catalyst (Table 2, entries nos. 1 and 2) and when 3-nitro-1*H*-1,2,4-triazole **19** was added (entries nos. 3 and 4). When more active nucleophilic catalysts such as 1-hydroxy-4,6-dinitrobenzotriazole **29a** (entries nos. 5 and 6) and 5-(3,5-dinitrophenyl)-1*H*-tetrazole **20b** (entry no. 7) were added, the condensation rate increased but, due presumably to the occurrence of side-reactions, the isolated yield of fully protected dinucleoside phosphate **47** decreased. It would seem reasonable to assume that the slower condensation reactions in the ribose-series were due to the phosphorylating species being more sterically hindered. The success of the phosphotriester approach depends on the intermediate phosphorylating species acylating the 5'-hydroxy component very much faster than does the arenesulfonylating species. Any factor such as steric hindrance that leads to a decrease in the rate of phosphorylation is bound to make arenesulfonylation of the 5'-hydroxy component a more competitive reaction. Nevertheless, despite their relative slowness, the condensations effected by MSCl **15a** and TrisCl **15b** in the presence of 3-nitro-1*H*-1,2,4-triazole **19** (entries nos. 3 and 4) were very satisfactory indeed, and concomitant side-reactions occurred only to a negligible extent.

Concluding Remarks.—Although the present investigation has been concerned with the synthesis of oligodeoxyribo- and oligoribo-nucleotides by the phosphotriester approach in solution, it seems likely that this methodology could readily be adapted to automated solid-phase synthesis. In automated synthesis involving nucleoside phosphoramidite building blocks[14,48] (*e.g.*, compound **7**), solutions of the latter in acetonitrile, contained in appropriate bottles, and a solution of the activator (*e.g.*, 1*H*-tetrazole), also contained in an appropriate bottle, are attached to the synthesizer. In automated solid-phase synthesis by the phosphotriester approach, it is expected that each 'phosphoramidite bottle' would contain a solution of the appropriate phosphodiester component (*e.g.*, **35** or **46**), 1-methylimidazole and the additional nucleophilic catalyst in dry pyridine, and the 'activator bottle' would contain a solution of MSCl **15a** or TrisCl **15b** probably in dry acetonitrile. Coupling times would generally be much longer than is usual in the phosphoramidite approach (Scheme 2a) but coupling yields would be expected to be high, especially in the deoxy-series. Therefore, despite the fact that it is particularly economical and also has other advantages (see above), the phosphotriester method is un-



Scheme 7 *Reagents and conditions:* i, Ac_2O, C_5H_5N, room temp.; ii, $POCl_3$, 1*H*-1,2,4-triazole, Et_3N, MeCN, room temp.; iii, 2,4-dimethylphenol, Et_3N, MeCN, room temp.; iv, CF_3CO_2H, pyrrole, CH_2Cl_2, room temp.; v, reagent prepared from 2-$ClC_6H_4OPOCl_2$, 1*H*-1,2,4-triazole, Et_3N, THF; then aq. Et_3N; vi, a solution of the arenesulfonyl chloride (0.96 mmol) in dry MeCN (1.2 cm^3) was added to a stirred solution of the salt **46** (~0.32 mmol), diacetate **44** (0.20 mmol), 1-methylimidazole (0.80 mmol) and usually (see Table 2) an additional nucleophilic catalyst (0.80 mmol) in C_5H_5N (2.0 cm^3) at room temp. The reactions were quenched and worked up (Experimental section) after appropriate times.

likely to be preferred to the phosphoramidite approach in the solid-phase synthesis of small quantities of oligodeoxyribo- and oligoribo-nucleotides until even more effective activating agents and nucleophilic catalysts are found. However, at present, the real potential of the phosphotriester approach would appear to lie in large-scale synthesis in solution, probably by the 'filtration' method,[49] when economy of building blocks and coupling efficiency rather than coupling rates are likely to be of paramount importance. It seems likely that MSCl **15a** and TrisCl **15b** will then be the activating agents of choice and that 3-nitro-1*H*-1,2,4-triazole **19**, 1-hydroxy-4,6-dinitrobenzotriazole **29a** and 5-(3,5-dinitrophenyl)-1*H*-tetrazole **20b** will be the additional nucleophilic catalysts of choice.

Experimental

M.p.s were measured with a Büchi melting point apparatus and are uncorrected. H NMR spectra, unless otherwise stated, were measured at 360 MHz with a Bruker AM 360 spectrometer; ^{13}C NMR spectra were measured at 90.6 MHz with the same spectrometer. Tetramethylsilane was used as internal standard, and *J*-values are given in Hz. ^{31}P NMR spectra were measured at 101.3 MHz with a Bruker WM 250 spectrometer; 85% phosphoric acid was used as an external standard. IR spectra were measured with a Perkin-Elmer 983 G spectrometer, and UV spectra were measured with a Perkin-Elmer Lambda-3 spectrophotometer. Merck silica gel 60 F_{254} TLC plates were developed in solvent systems A [chloroform–methanol (9:1 v/v)] and B [chloroform–methanol (19:1 v/v)]. Merck silica gel H was used for short-column chromatography. Acetonitrile, triethylamine, and pyridine were dried by heating, under reflux, over calcium hydride and were then distilled; 1-methylimidazole, DMSO, and DMF were dried by distillation over calcium hydride under reduced (water-pump) pressure. Arenesulfonyl chlorides and other reagents and starting materials were purchased from the Aldrich Chemical

Company and, when appropriate, were recrystallized before use.

5-(3,5-*Dinitrophenyl*)-1H-*tetrazole* **20b**.—3,5-Dinitrobenzonitrile (5.07 g, 26.3 mmol), sodium azide (2.10 g, 32.3 mmol), ammonium chloride (2.10 g, 39.3 mmol) and dry DMF (15 cm^3) were stirred and heated together at 120–130 °C for 30 min. The cooled products were diluted with water (30 cm^3), then filtered, and the filtrate was acidified (to pH ~ 5) with dil. hydrochloric acid. The crystalline precipitate was collected by filtration, washed with water, dried, and recrystallized from ethanol–water to give 5-(3,5-dinitrophenyl)-1*H*-tetrazole **20b** (5.30 g, 85%), m.p. 179–180 °C (lit.,[32] 180–181 °C); δ_C[$(CD_3)_2SO$] 120.3, 126.9, 128.1, 148.8 and 154.9.

1-*Methyl*-1H-*imidazole*-2-*carbonitrile* **24**.—Cyanogen bromide (5.3 g, 50.0 mmol) was added, under nitrogen, to a stirred solution of 4-(dimethylamino)pyridine (6.1 g, 49.9 mmol) in dry DMF (100 cm^3) at −10 °C (ice–salt-bath). An exothermic reaction ensued and a pale yellow precipitate was obtained. The stirred reaction mixture was recooled to 10 °C, 1-methylimidazole (1.59 cm^3, 1.64 g, 20.0 mmol) was added and the reactants were heated at 40 °C for 16 h. The products were then poured into 0.1 mol dm^{-3} aq. sodium hydrogen carbonate (600 cm^3) and the resulting solution was extracted with ethyl acetate (3 × 200 cm^3). The extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure to give 1-methyl-1*H*-imidazole-2-carbonitrile **24** (1.66 g, 77%) as an oil; δ_H[$(CD_3)_2SO$] 3.86 (3 H, s), 7.24 (1 H, d, *J* 1.0) and 7.66 (1 H, d, *J* 0.8); δ_C[$(CD_3)_2SO$] 33.9, 111.2, 120.9, 126.2 and 130.5.

5-(1-*Methyl*-1H-*imidazol*-2-*yl*)-1H-*tetrazole* **21**.—1-Methyl-1*H*-imidazole-2-carbonitrile (8.88 g, 82.9 mmol), sodium azide (8.05 g, 0.124 mol), ammonium chloride (6.62 g, 0.124 mol) and dry DMF (90 cm^3) were stirred and heated together at 120–130 °C for 16 h. The cooled products were filtered and the filtrate was evaporated under reduced pressure. The residue was crystallized from methanol to give 5-(1-methyl-1*H*-imidazol-2-yl)-1*H*-tetrazole **21** (9.80 g, 78%) (Found: C, 40.1; H, 3.9; N, 55.9. Calc. for $C_5H_6N_6$: C, 40.0; H, 4.0; N, 56.0%), m.p. 275–278 °C (decomp.) (lit.,[33] 279–281 °C); δ_H[$(CD_3)_2SO$], 4.18 (3 H, s), 7.58 (1 H, d, *J* 1.8) and 7.74 (1 H, d, *J* 1.7); δ_C[$(CD_3)_2SO$] 35.9, 121.5, 124.7, 136.5 and 149.0.

(1-*Methyl*-1H-*imidazol*-2-*yl*)*methanol* **26**.—1-Methyl-1*H*-imidazole (29.13 cm^3, 30.0 g, 0.365 mol) and paraformaldehyde (30.0 g, equivalent to ~ 1.0 mol of formaldehyde) were heated together at 160 °C for 2 h. The cooled products, which solidified, were crystallized from methanol to give (1-methyl-1*H*-imidazol-2-yl)methanol **26** (23.7 g, 58%) as crystals, m.p. 103–105 °C (lit.,[36] 91–92.5 °C); δ_H[$(CD_3)_2SO$], 3.64 (3 H, s), 4.46 (2 H, d, *J* 4.9), 5.32 (1 H, t, *J* 5.3), 6.75 (1 H, d, *J* 1.1) and 7.06 (1 H, d, *J* 1.1).

(1-*Methyl*-1H-*imidazol*-2-*yl*)*acetonitrile* **28**.—(1-Methyl-1*H*-imidazol-2-yl)methanol (10.0 g, 89.2 mmol) was added, with shaking and exclusion of moisture, over a period of 15 min to cooled (ice-bath) thionyl dichloride (12.3 cm^3, 20.1 g, 0.169 mol). The reactants were then heated, under reflux, for 15 min. The cooled solution was evaporated under reduced pressure and the residue was crystallized from ethanol (25 cm^3) to give 2-(chloromethyl)-1-methyl-1*H*-imidazole hydrochloride **27** (12.50 g, 84%) as crystals, m.p. 165–167 °C.

A solution of compound **27** (6.68 g, 40.0 mmol) in warm DMSO (50 cm^3) was added to a solution of sodium cyanide (10.0 g, 0.204 mol) in hot DMSO (150 cm^3). The reaction mixture was allowed to cool to room temperature and was stirred for 2 h. The products were then evaporated under reduced pressure and the residue was partitioned between saturated aq. sodium hydrogen carbonate (50 cm^3) and chloroform (50 cm^3). The aqueous layer was separated and re-extracted with chloroform (3 × 25 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure to give (1-methyl-1*H*-imidazol-2-yl)acetonitrile **28** (3.00 g, 62%) as a solid, m.p. 30–33 °C; δ_H[$(CD_3)_2SO$] 3.88 (3 H, s), 4.82 (2 H, s), 7.69 (1 H, d, *J* 2.0) and 7.79 (1 H, d, *J* 2.0); δ_C[$(CD_3)_2SO$] 14.5, 34.3, 113.8, 124.3 and 136.8.

5-[(1-*Methyl*-1H-*imidazol*-2-*yl*)*methyl*]-1H-*tetrazole* **22**.—(1-Methyl-1*H*-imidazol-2-yl)acetonitrile **28** (10.0 g, 82.6 mmol), sodium azide (8.05 g, 0.124 mol), ammonium chloride (6.62 g, 0.124 mol) and dry DMF (90 cm^3) were stirred and heated together at 120–130 °C for 17 h. The cooled products were filtered and the filtrate was evaporated under reduced pressure. The residue was crystallized from methanol to give 5-[(1-methyl-1*H*-imidazol-2-yl)methyl]-1*H*-tetrazole **22** (11.40 g, 84%) (Found: C, 44.0; H, 5.0; N, 51.05. Calc. for $C_6H_8N_6$: C, 43.9; H, 4.9; N, 51.2%), m.p. 190–191 °C (lit.,[33] 191–192 °C); δ_H[$(CD_3)_2SO$] 3.69 (3 H, s), 4.47 (2 H, s), 7.02 (1 H, s) and 7.28 (1 H, s); δ_C[$(CD_3)_2SO$] 21.6, 32.9, 122.3, 123.8, 142.7 and 153.2.

4,6-*Dinitrobenzotriazol*-1-*ol* **29a** (carried out by Dr K. G. Devine).—*p*-Anisic acid (20.0 g, 0.131 mol) was added in portions to a stirred mixture of fuming nitric acid (*d* 1.5, 160 cm^3), conc. sulfuric acid (80 cm^3) and fuming sulfuric acid (30% SO_3; 80 cm^3) at 0 °C (ice–water-bath). The reactants were heated at 70 °C for 2 h, cooled to room temperature, and then poured onto ice (2.0 kg). The precipitated solid was collected by filtration, washed with ice-cold water, and crystallized from methanol to give 2,4,6-trinitroanisole **31a** (30.0 g, 93%), m.p. 68 °C.

A solution of hydrazine hydrate (2.0 cm^3, 41.2 mmol) in absolute ethanol (20 cm^3) was added dropwise to a stirred solution of 2,4,6-trinitroanisole **31a** (10.0 g, 41.1 mmol) in ethanol (60 cm^3) under nitrogen at 0 °C (ice–water-bath). After 1 h, the products were evaporated under reduced pressure and the residue was crystallized from glacial acetic acid to give[40] 2,4,6-trinitrophenylhydrazine **32a** (6.80 g, 68%) as red crystals, m.p. 210–212 °C.

The latter material **32a** (5.00 g, 20.6 mmol), hydrazine hydrate (1.0 cm^3, 20.6 mmol), sodium acetate trihydrate (10.04 g, 73.8 mmol), glacial acetic acid (4.95 cm^3, 86.5 mmol) and water (60 cm^3) were heated together, under reflux, under nitrogen for 4 h. Activated charcoal (5.0 g) was then added and the hot products were filtered. The filtrate was allowed to cool to room temp. and was then acidified with 2 mol dm^{-3} hydrochloric acid (20 cm^3). 4,6-Dinitrobenzotriazol-1-ol **29a** (2.80 g, 60%) (Found, in material recrystallized from 0.2 mol dm^{-3} hydrochloric acid: C, 32.2; H, 1.3; N, 30.9. Calc. for $C_6H_3N_5O_5$: C, 32.0; H, 1.3; N, 31.1%) was obtained as a yellow crystalline precipitate, m.p. 185–186 °C [lit.,[39] 185–190 °C (decomp.)] δ_C[$(CD_3)_2SO$] 114.7, 117.0, 129.7, 136.2, 136.9 and 144.5.

4-*Nitro*-6-(*trifluoromethyl*)*benzotriazol*-1-*ol* **29b** (carried out by Dr K. G. Devine).—A solution of sodium methoxide in methanol (~ 4.4 mol dm^{-3}; 29.4 cm^3, ~ 0.13 mol) was added dropwise under nitrogen to a stirred solution of 4-chloro-3,5-dinitrobenzotrifluoride (20.0 g, 73.9 mmol) in methanol (84 cm^3) at room temperature. A deep purple colour developed immediately. After 30 min, 2.0 mol dm^{-3} hydrochloric acid (100 cm^3) was added and the products were filtered. The filtrate was concentrated under reduced pressure and was then dried over P_2O_5 to give a pale yellow solid (16.4 g). The latter material (10.93 g) was treated with hydrazine hydrate (2.0 cm^3, 41.2 mmol) according to the procedure described above in the preparation of 4,6-dintrobenzotriazol-1-ol **29a**. Crystallization of the product from glacial acetic acid gave 2,6-dinitro-4-

(trifluoromethyl)phenylhydrazine **32b** (7.0 g, 53% overall yield based on 4-chloro-3,5-dinitrobenzotrifluroride), m.p. 124 °C; δ_H[$(CD_3)_2SO$] 4.85 (2 H, s), 8.41 (2 H, s) and 9.67 (1 H, s).

2,6-Dinitro-4-(trifluoromethyl)phenylhydrazine **32b** (5.48 g, 20.6 mmol), hydrazine hydrate (1.0 cm^3, 20.6 mmol), sodium acetate trihydrate (10.04 g, 73.8 mmol), glacial acetic acid (4.95 cm^3, 86.5 mmol), ethanol (40 cm^3) and water (20 cm^3) were heated together, under reflux, under nitrogen for 5 h. Activated charcoal (5 g) was added and the hot products were filtered. The cooled filtrate was concentrated under reduced pressure to ~one-third volume and was then acidified with 2 mol dm^{-3} hydrochloric acid (20 cm^3). 4-*Nitro*-6-(*trifluoromethyl*)*benzotriazol*-1-*ol* **29b** (2.80 g, 55%) [Found, in material recrystallized from 0.2 mol dm^{-3} trifluoroacetic acid (TFA): C, 34.1; H, 1.3; N, 22.1. $C_7H_3F_3N_4O_3$ requires C, 33.9; H, 1.2; N, 22.6%] was obtained as a yellow crystalline precipitate, m.p. 163–164 °C; δ_C[$(CD_3)_2SO$] 116.5, 118.6, 122.8 (q, $J_{C,F}$ 273), 126.5 (q, $J_{C,F}$ 34.0), 130.1, 135.8 and 138.0.

5-(3-*Nitrophenyl*)-1*H*-*tetrazol*-1-*ol* **30b**.—Aq. sodium hydroxide (2.5 mol dm^{-3}; 220 cm^3, 0.55 mol) was added to a stirred mixture of 3-nitrobenzaldehyde (25.90 g, 0.171 mol), hydroxylamine hydrochloride (60.0 g, 0.86 mol) and water (220 cm^3) at room temperature. The reactants were heated, under reflux, for 20 min, and then acidified (to pH ~4) with dil. hydrochloric acid. The precipitated 3-nitrobenzaldehyde oxime was collected by filtration and recrystallized from water (25.1 g, 88%).

The latter compound (16.6 g, 0.10 mol) was dissolved in dichloromethane and propan-2-ol (125 cm^3) was added. The resulting solution was cooled to −12 °C (ice–salt-bath) and *tert*-butyl hypochlorite[50] (14.5 cm^3, ~0.12 mol) was added.[51] After 15 min, when TLC (system **B**) revealed that no starting material remained, the products were evaporated under reduced pressure and recrystallized from benzene to give the chloro oxime (18.3 g, 91%).

Aq. sodium azide (3.55 g, 54.6 mmol in 50 cm^3) was added dropwise to a stirred solution of the above chloro oxime (10.0 g, 49.9 mmol) in methanol (150 cm^3) at room temperature. After 6 h, the products were concentrated under reduced pressure and the residue was extracted with ethanol (100 cm^3). The extracts were evaporated, the residue was dissolved in pyridine (40 cm^3), and the solution was reevaporated to leave a residual solid (9.2 g). Acetyl chloride (1.42 cm^3, 20 mmol) was added dropwise to a solution of this material (2.07 g) in acetonitrile at −20 °C. The reaction mixture was then heated, under reflux, for 12 h. The products were evaporated under reduced pressure and the residue was crystallized from 96% ethanol to give 5-(3-*nitrophenyl*)-1H-*tetrazol*-1-*ol* **30b** (0.73 g, 31% based on the chloro oxime **34b**) (Found: C, 41.0; H, 2.4; N, 33.7. $C_7H_5N_5O_3$ requires C, 40.6; H, 2.4, N, 33.8%), m.p. 165–167 °C; δ_C[$(CD_3)_2SO$] 121.7, 124.2, 125.7, 131.0, 133.4, 144.0 and 147.9.

3′-O-*Acetyl*-4-O-*phenylthymidine* **36**.—Acetic anhydride (0.80 cm^3, 8.5 mmol) was added to a stirred solution of 4-*O*-phenyl-5′-*O*-(9-phenylxanthen-9-yl)thymidine[42] (0.492 g, 0.86 mmol) in anhydrous pyridine (10 cm^3) at room temperature. After 4 h, methanol (10 cm^3) was added dropwise to the products. After a further period of 1.5 h, the products were evaporated under reduced pressure and the residue was dissolved in chloroform (12 cm^3). The resulting solution was extracted with saturated aq. sodium hydrogen carbonate (2 × 10 cm^3) and the combined aqueous layers were back-extracted with chloroform (3 × 10 cm^3). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. Pyrrole (0.9 cm^3, 13 mmol) and then 2% dichloroacetic acid in dichloromethane (15 cm^3, 3.6 mmol of Cl_2CHCO_2H) were added to a stirred solution of the residue in dichloromethane (15 cm^3) at room temperature. After 10 min, the products were extracted with saturated aq. sodium hydrogen carbonate (30 cm^3). The organic layer was retained and the aqueous layer was extracted with chloroform (3 × 10 cm^3). The organic layers were combined, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions which were eluted with chloroform–ethanol (93:7 v/v) were combined, and evaporated under reduced pressure. Crystallization of the residual glass from ethyl acetate–ethanol gave the *title compound* **36** (0.22 g, 71%) (Found C, 60.0; H, 5.6; N, 7.8. $C_{18}H_{20}N_2O_6$ requires C, 60.0; H, 5.6; N, 7.8%), m.p. 223–224 °C; R_f 0.39 (system **B**); δ_H[$(CD_3)_2SO$] 2.06 (3 H, s), 2.08 (3 H, s), 2.21 (1 H, m, 2.38 (1 H, m), 3.67 (2 H, m), 4.08 (1 H, m), 5.23 (1 H, m), 5.28 (1 H, *t*, *J* 5.1), 6.14 (1 H, dd, *J* 5.7 and 8.1), 7.19 (2 H, m), 7.29 (1 H, m), 7.45 (2 H, m) and 8.19 (1 H, s).

General Procedure for Oligodeoxyribonucleotide Coupling: Preparation of 4-O-*Phenyl*-5′-O-(9-*phenylxanthen*-9-*yl*)*thymidin*-3′-*yl* 3′-O-*Acetyl*-4-O-*phenylthymidin*-5′-*yl* 2-*Chlorophenyl Phosphate* **37**.—A solution of the triethylammonium salt of 4-*O*-phenyl-5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl 2-chlorophenyl hydrogen phosphate **35** (0.255 g, 0.294 mmol), 3′-*O*-acetyl-4-*O*-phenylthymidine **36** (0.09 g, 0.25 mmol), 1-methylimidazole (0.12 cm^3, 1.5 mmol) and, if included (Table 1), additional nucleophilic catalyst (0.75 mmol) in dry pyridine (5 cm^3) was concentrated to small volume under reduced pressure (bath temperature <25 °C). This process was repeated twice more and the residue was dissolved in dry pyridine (2.5 cm^3). 1-Methylimidazole (0.08 cm^3, 1.0 mmol) was added followed by a solution of arenesulfonyl chloride (0.90 mmol) in dry acetonitrile (1.5 cm^3). After the appropriate period of time, the reaction mixture was quenched with saturated aq. sodium hydrogen carbonate (0.5 cm^3). Chloroform (10 cm^3) was added and the resulting solution was transferred to a separatory funnel containing 0.2 mol dm^{-3} aq. triethylammonium hydrogen carbonate (pH 7.5, 50 cm^3). The organic layer was separated and the aqueous layer was extracted with chloroform (5 × 5 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. The residue was coevaporated with toluene (20 cm^3) and was then fractionated by short-column chromatography on silica gel: the appropriate fractions were eluted with chloroform–ethanol (197.5:2.5 v/v), combined, and evaporated under reduced pressure. A solution of the residue in chloroform (2.0 cm^3) was added dropwise to stirred light petroleum (boiling range 30–40 °C; 50 cm^3) to give the fully protected dinucleoside phosphate **37** as a precipitate, R_f 0.50 (system **B**). The latter material was dried *in vacuo* over phosphorus pentaoxide and weighed. The results obtained are indicated in Table 1.

1-(*Mesityl*-2-*sulfonyl*)-3-*nitro*-1H-1,2,4-*triazole* (*MSNT*, **5**) *as Condensing Agent*.—The general procedure for oligodeoxyribonucleotide coupling described above was followed except that the nucleophilic catalyst was omitted from the pyridine solution and mesitylene-2-sulfonyl chloride **15a** (0.197 g, 0.90 mmol) was replaced by MSNT **5** (0.222 g, 0.75 mmol). The products were quenched after 30 min and worked up in the same way and fractionated to give the fully protected dinucleoside phosphate **37** (0.252 g, 91%).

2-*Nitrobenzenesulfonyl Chloride* **40a** *as Condensing Agent in the Presence of* 4,6-*Dinitrobenzotriazol*-1-*ol* **29a**.—(*a*) The above general procedure for oligodeoxyribonucleotide coupling was followed with 2-nitrobenzenesulfonyl chloride (0.20 g, 0.90 mmol) as the coupling agent and 4,6-dinitrobenzotriazol-1-ol **29a** (0.169 g, 0.75 mmol) as the nucleophilic catalyst. After 2

min, when no 5'-hydroxy compound **36** remained, the reactants were quenched with saturated aq. sodium hydrogen carbonate (0.5 cm^3), worked up, and chromatographed as above on silica gel. Elution of the column with chloroform–ethanol (125:1 v/v), combination of the appropriate fractions, concentration, and precipitation gave the fully protected dinucleoside phosphate **37** (0.230 g, 83%), R_f 0.50 (system **B**). Subsequent elution of the column with chloroform–ethanol (250:3 v/v), combination, and evaporation of the appropriate fractions gave a residue. Crystallization of this material from methanol–diisopropyl ether gave 3'-O-*acetyl*-5'-O-(4,6-*dinitrobenzotriazol*-1-*yl*)-4-O-*phenylthymidine* **41** (0.017 g, 12%) (Found: C, 50.7; H, 3.7; N, 17.1. $C_{24}H_{21}N_7O_{10}$ requires: C, 50.8; H, 3.7; N, 17.3%) as crystals, m.p. 183–184 °C, R_f 0.48 (system **B**): $\delta_H[(CD_3)_2SO]$ 1.98 (3 H, s), 2.10 (3 H, s), 2.44 (2 H, m), 4.63 (1 H, m), 5.10 (2 H, m), 5.39 (1 H, m), 6.22 (1 H, dd, *J* 6.3 and 8.1), 7.17 (2 H, m), 7.30 (1 H, m), 7.46 (2 H, m), 7.96 (1 H, m), 8.96 (1 H, d, *J* 1.9) and 9.30 (1 H, d, *J* 2.0).

(*b*) A solution of 3'-*O*-acetyl-4-*O*-phenylthymidine **36** (0.09 g, 0.25 mmol), 4,6-dinitrobenzotriazol-1-ol **29a** (0.169 g, 0.75 mmol) and 1-methylimidazole (0.12 cm^3, 1.5 mmol) in dry pyridine (5 cm^3) was evaporated under reduced pressure. The residue was redissolved in dry pyridine (5 cm^3) and the solution was reevaporated. After this process had been repeated once more, the residue was dissolved in dry pyridine (2.5 cm^3). 1-Methylimidazole (80 mm^3, 1.0 mmol), followed by a solution of 2-nitrobenzenesulfonyl chloride (0.20 g, 0.90 mmol) in dry acetonitrile (1.5 cm^3) were then added to the stirred, resulting solution at room temperature. After 25 min, the reaction was quenched by the addition of saturated aq. sodium hydrogen carbonate (0.5 cm^3), and the products were worked up, and then chromatographed on silica gel as in (*a*) above. Appropriate fractions, which were eluted with chloroform–ethanol (250:3 v/v), were combined, and evaporated under reduced pressure. Crystallization of the residue from methanol–diisopropyl ether gave 3'-*O*-acetyl-5'-*O*-(4,6-dinitrobenzotriazol-1-yl)-4-*O*-phenylthymidine **41** (0.107 g, 75%), m.p. 183–184 °C, identical (TLC, ^1H NMR) with the material described in (*a*) above.

4-*Nitrobenzenesulfonyl Chloride* **40b** *as Condensing Agent in the Presence of* 4,6-*Dinitrobenzotriazol*-1-*ol* **29a**.—The two experiments [(*a*) and (*b*)] described above were repeated on exactly the same scale and in exactly the same way except that 2-nitrobenzenesulfonyl chloride was replaced by 4-nitrobenzenesulfonyl chloride (0.20 g, 0.90 mmol). In experiment (*a*), in which the triethylammonium salt of 4-*O*-phenyl-5'-*O*-(9-phenylxanthen-9-yl)thymidin-3'-yl 2-chlorophenyl hydrogen phosphate **35** (0.255 g, 0.294 mmol) was included in the reaction mixture, the isolated products were the fully protected dinucleoside phosphate **37** (0.226 g, 82%) and 3'-*O*-acetyl-5'-*O*-(4,6-dinitrobenzotriazol-1-yl)-4-*O*-phenylthymidine **41** (0.02 g, 14%). In experiment (*b*), in which compound **35** was excluded from the reaction mixture, compound **41** (0.109 g, 77%) was the only isolated product.

2-*Nitrobenzenesulfonyl Chloride* **40a** *as the Condensing Agent in the Presence of* 3-*Nitro*-1H-1,2,4-*triazole* **19**.—The above general procedure for oligodeoxyribonucleotide coupling was followed with 2-nitrobenzenesulfonyl chloride **40a** (0.20 g, 0.90 mmol) as the coupling agent and 3-nitro-1*H*-1,2,4-triazole **19** (0.085 g, 0.75 mmol) as the additional nucleophilic catalyst. After 2 min, when no compound **36** remained, the reactants were quenched with saturated aq. sodium hydrogen carbonate (0.5 cm^3), worked up, and chromatographed as above on silica gel. Elution of the column with chloroform–ethanol (125:1 v/v), combination of the appropriate fractions, concentration, and precipitation gave the fully protected dinucleoside phosphate **37** (0.185 g, 67%), R_f 0.50 (system **B**). Subsequent elution of the column with chloroform–ethanol (250:3 v/v), combination, and evaporation of the appopriate fractions gave a solid residue (0.032 g, 23%) believed to be 3'-*O*-acetyl-5'-*O*-(2-nitrophenylsulfonyl)-4-*O*-phenylthymidine **42**; R_f 0.43 (system **B**); $\delta_H(CDCl_3)$ 2.08 (3 H, s), 2.16 (3 H, s), 2.21 (1 H, m), 2.64 (1 H, m), 4.24 (1 H, m), 4.57 (1 H, dd, *J* 2.4 and 10.9), 4.70 (1 H, dd, *J* 2.2 and 10.9), 5.23 (1 H, m), 6.45 (1 H, dd, *J* 5.2 and 9.0), 7.15 (2 H, m), 7.23 (1 H, m), 7.39 (2 H, m), 7.8–7.9 (4 H, m) and 8.20 (1 H, m).

2',3'-*Di*-O-*acetyl*-4-O-(2,4-*dimethylphenyl*)*uridine* **44**.—Uridine (10.0 g, 41 mmol), chlorotriphenylmethane (12.0 g, 43.0 mmol) and pyridine (120 cm^3) were stirred together with the exclusion of moisture at 100 °C. After 1 h, the cooled products were evaporated under reduced pressure and the residue was partitioned between chloroform (250 cm^3) and dil. sulfuric acid (2.0 mol dm^{-3}; 500 cm^3). The chloroform layer was separated, and washed with saturated aq. sodium hydrogen carbonate (250 cm^3). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the residue was recrystallized from benzene to give 5'-*O*-(triphenylmethyl)uridine **43** (16.9 g, 85%), m.p. 193–195 °C (lit.,[46] 200 °C); R_f 0.60 (system **A**).

Acetic anhydride (18.85 cm^3, 0.20 mol) was added to a stirred solution of 5'-*O*-(triphenylmethyl)uridine **43** (9.73 g, 20.0 mmol) in dry pyridine (200 cm^3) at room temperature. After 5 h, methanol (100 cm^3) was added and, after a further period of 1.5 h, the products were concentrated under reduced pressure. A solution of the residue in chloroform (150 cm^3) was extracted with saturated aq. sodium hydrogen carbonate (3 × 100 cm^3) and the combined aqueous layers were back-extracted with chloroform (3 × 100 cm^3). The dried ($MgSO_4$), combined organic layers were concentrated under reduced pressure to give a glass (10.6 g). 3-Nitro-1*H*-1,2,4-triazole **19** (3.98 g, 34.9 mmol) and diphenyl phosphorochloridate (7.37 cm^3, 35.6 mmol) were added to a stirred solution of the latter glass (10.0 g) in dry pyridine (120 cm^3) at room temperature. After 40 h, solid potassium hydrogen carbonate (8.0 g) and water (8.0 cm^3) were added. After a further period of 10 min, the products were concentrated to small volume under reduced pressure, dissolved in chloroform (200 cm^3), and washed with saturated aq. sodium hydrogen carbonate (200 cm^3). The dried ($MgSO_4$) solution was concentrated under reduced pressure and the residue was dissolved in dry acetonitrile (120 cm^3) at room temperature. 2,4-Dimethylphenol (6.41 g, 52.5 mmol) and triethylamine (7.36 cm^3, 52.8 mmol) were added to the stirred solution. After 2.5 h, the products were concentrated to small volume and partitioned between chloroform (200 cm^3) and saturated aq. sodium hydrogen carbonate (3 × 100 cm^3). The aqueous extracts were back-extracted with chloroform (3 × 100 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure to give a glass (7.45 g). Freshly distilled pyrrole (9.0 cm^3, 0.13 mol), followed by an anhydrous solution of TFA (2.0 cm^3, 26.0 mmol) in dichloromethane (150 cm^3) was added to a stirred solution of the latter glass (7.0 g) in dichloromethane (150 cm^3) at room temperature. After 30 min, saturated aq. sodium hydrogen carbonate (300 cm^3) was added and the aqueous layer was separated, and back-extracted with chloroform (3 × 100 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions which were eluted with chloroform–ethanol (97:3 v/v) were combined, and evaporated under reduced pressure. Crystallization of the residue from ethyl acetate–ethanol gave the *title compound* **44** [3.54 g, 46% based on 5'-*O*-(triphenylmethyl)uridine as starting material] (Found:

C, 58.1; H, 5.6; N, 6.3. $C_{21}H_{24}N_2O_8$ requires C, 58.3; H, 5.6; N, 6.5%), m.p. 199–200 °C; R_f 0.41 (system **B**); $\delta_H[(CD_3)_2SO]$ 2.03 (3 H, s), 2.05 (3 H, s), 2.08 (3 H, s), 2.29 (3 H, s), 3.63 (1 H, m), 3.73 (1 H, m), 4.20 (1 H, m), 5.33 (1 H, m), 5.39 (1 H, m), 5.45 (1 H, t, *J* 4.9), 6.04 (1 H, d, *J* 4.8), 6.40 (1 H, d, *J* 7.4), 6.98 (1 H, m), 7.05 (1 H, m), 7.12 (1 H, m) and 8.41 (1 H, d, *J* 7.4).

Triethylammonium Salt of 2-Chlorophenyl 4-O-(2,4-*dimethylphenyl*)-2′-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-5′-O-(9-*phenylxanthen*-9-*yl*)*uridin*-3′-*yl Hydrogen Phosphate* **46**.—2-Chlorophenyl phosphorodichloridate (4.87 cm³, 29.6 mmol), 1*H*-1,2,4-triazole (4.42 g, 64.0 mmol), triethylamine (8.2 cm³, 58.8 mmol and tetrahydrofuran (THF) (256 cm³) were stirred together at room temperature. After 20 min, a solution of 4-*O*-(2,4-dimethylphenyl)-2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-5′-*O*-(9-phenylxanthen-9-yl)uridine[47] **45** (8.00 g, 9.85 mmol) and 1-methylimidazole (3.12 cm³, 39.1 mmol) in THF (40 cm³) was added, and the resulting mixture was stirred for 30 min. Triethylamine (24 cm³) and water (40 cm³) were then added. After the resulting solution had been stirred for 10 min, it was concentrated under reduced pressure. A solution of the residue in chloroform (300 cm³) was extracted successively with saturated aq. sodium hydrogen carbonate (200 cm³) and water (200 cm³). The combined aqueous layers were back-extracted with chloroform (100 cm³). The dried ($MgSO_4$), combined organic layers were evaporated under reduced pressure and the residue was redissolved in chloroform (40 cm³). The resulting solution was added dropwise to stirred light petroleum (boiling range 30–40 °C; 2.5 dm³) to give the title compound **46** as a solid (9.60 g, 88%); $\delta_P[(CD_3)_2SO]$ −6.7.

General Procedure for Oligoribonucleotide Coupling: Preparation of 2-*Chlorophenyl*-2′,3′-*Di*-O-*acetyl*-4-O-(2,4-*dimethylphenyl*)*uridin*-5′-*yl* 4′-O-(2,4-*dimethylphenyl*)-2′-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-5′-O-(9-*phenylxanthen*-9-*yl*)*uridin*-3′-*yl Phosphate* **47**.—A solution of the triethylammonium salt of 2-chlorophenyl 4-*O*-(2,4-dimethylphenyl)-2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-5′-*O*-(9-phenylxanthen-9-yl)uridin-3′-yl hydrogen phosphate **46** (0.353 g, 0.32 mmol), 2′,3′-di-*O*-acetyl-4-*O*-(2,4-dimethylphenyl)uridine **44** (0.0865 g, 0.20 mmol), 1-methylimidazole (96 mm³, 1.2 mmol) and, if included (Table 2), additional nucleophilic catalyst (0.80 mmol) in dry pyridine (5 cm³) was concentrated to small volume under reduced pressure (bath temperature < 25 °C). This process was repeated twice more and the residue was dissolved in dry pyridine (2.0 cm³). 1-Methylimidazole (64 mm³, 0.8 mmol) was added, followed by a solution of arenesulfonyl chloride (0.96 mmol) in dry acetonitrile (1.2 cm³). After the appropriate period of time, the reaction mixture was quenched with saturated aq. sodium hydrogen carbonate (0.5 cm³). The products were then worked up and chromatographed as in the above preparation of the fully protected di-2′-deoxyribonucleoside phosphate **37**. The required fully protected diribonucleoside phosphate **47** was, like compound **37**, isolated by precipitation as a solid, R_f 0.58 (system **B**). The yields obtained are indicated in Table 2.

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