Approaches to Oligoribonucleotide Synthesis *via* Phosphotriester Intermediates

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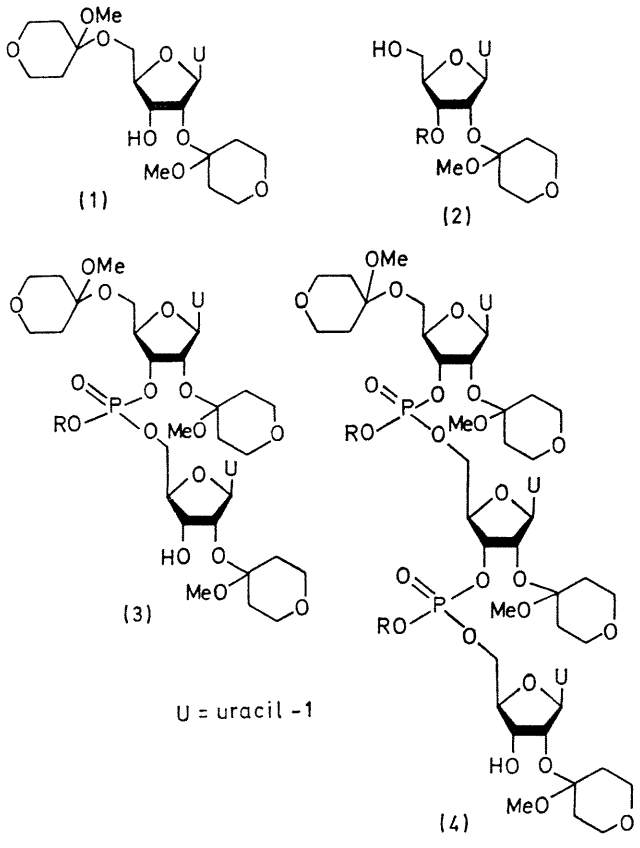
Summary Methods are described for the synthesis of uridylyl-(3′ → 5′)-uridine and uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridine, in good yields; a phosphotriester approach has been adopted with the internucleotidic linkages protected by aryl protecting groups.

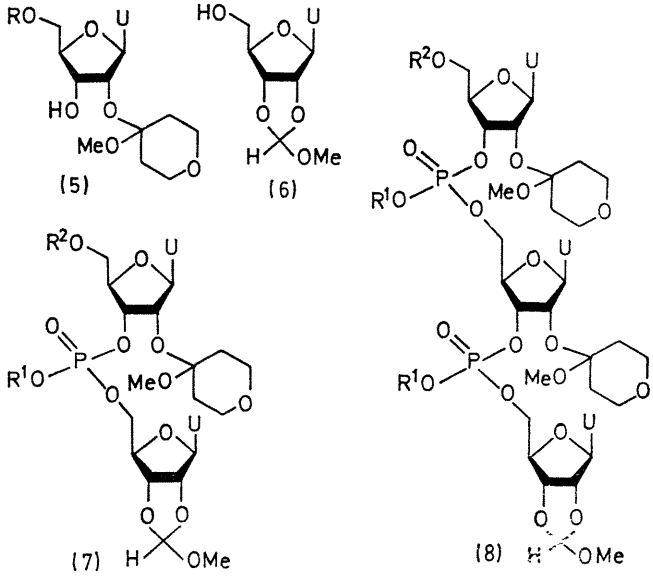
MOST of the published work on the chemical synthesis of oligonucleotides has involved the use of intermediates with unprotected internucleotidic linkages.[1] Such intermediates are often difficult to purify, and can only be handled on a comparatively small scale; their use can also lead to undesirable side reactions. Accordingly, the possibility of effecting the synthesis of oligonucleotides *via* phosphotriester intermediates has been investigated,[2–5] mainly in the deoxy-series,[2–4] but there are two previous studies[5] in the ribose series.

The most crucial decision in the planning of an oligonucleotide synthesis by a phosphotriester approach lies in the choice of the protecting group for the internucleotidic linkages. We previously demonstrated[4] that the phenyl group was suitable for this purpose in the deoxy-series, and we now report that phenyl (or substituted

phenyl) is equally suitable in the ribose series. We also report two phosphorylation procedures.



In one of our synthetic approaches, *o*-chlorophenyl, which is *ca.* 6 times as base-labile as phenyl,[6] was chosen as the protecting group. 2′,5′-Di-*O*-methoxytetrahydropyranyluridine[7] (**1**) (1·0 mmol) was phosphorylated with *o*-chlorophenyl phosphate (1·1 mmol) and 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPS) (2·2 mmol) in anhydrous pyridine solution at 20°, as previously described.[8] After 5 h, when t.l.c. indicated that the reaction had gone to completion, 2′-*O*-methoxytetrahydropyranyluridine[9] (**2**; R = H) (1·2 mmol) and more TPS (0·55 mmol) were added, and the reaction was continued for a further 16 h.† Work-up and chromatography (on SilicAR CC7) of the products gave the partially-protected dinucleoside phosphate (**3**; R = *o*-ClC_6H_4) as a glass which was homogeneous on t.l.c. [75—80%, based on (**1**)]. Phosphorylation of (**3**; R = *o*-ClC_6H_4) with *o*-chlorophenyl phosphate and TPS followed, after 7 h, by the addition of (**2**; R = H) and more TPS gave (**4**; R = *o*-ClC_6H_4), which was isolated as a homogeneous glass (t.l.c.) (73—76%). When (**3**; R = *o*-ClC_6H_4) and (**4**; R = *o*-ClC_6H_4) were treated with an excess of 0·1M-NaOH–aqueous dioxan (**4**:1, v/v) to remove the aryl protecting groups‡ and then submitted to acidic hydrolysis[9] (6 h, pH 2, 20°), uridylyl-(3′ → 5′)-uridine (UpU) and uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridine (UpUpU), respectively, were obtained as the *sole nucleotide products*. The latter compounds were paper-chromatographically (2 systems) and electrophoretically (0·1M-sodium borate buffer, pH 8·8) homogeneous; they underwent total digestion in the presence of pancreatic ribonuclease to give uridine 3′-phosphate and uridine in the expected proportions. Although there was no evidence that either product was contaminated with material containing 3′ → 3′-internucleotidic linkages, in future it is intended to use 3′-protected extension units, such as 2′-*O*-methoxytetrahydropyranyl-3′-*O*-methoxyacetyluridine (**2**; R = MeO·CH_2·CO), and thereby avoid this possibility.



In another approach, 2′-*O*-methoxytetrahydropyranyl-5′-*O*-*p*-chlorophenoxyacetyluridine[10] (**5**; R = *p*-ClC_6H_4·O·CH_2·CO) (1·0 mmol) in pyridine solution was treated with phenyl phosphate (1·05 mmol) and TPS (2·4 mmol), followed after 5 h by 2′,3′-*O*-methoxymethylideneuridine[11] (**6**) (1·1 mmol) and more TPS (0·3 mmol). After a further 16 h, the products were treated with an excess of 0·1M-NaOH–aqueous dioxan at 20° for 30 s, worked-up, and chromatographed. The partially protected dinucleoside phosphate (**7**; R^1 = Ph, R^2 = H) was then isolated as a colourless solid [82%, based on (**5**)]. In the same way, the partially protected trinucleoside diphosphate (**8**; R^2 = H) was obtained [over 60%, based on (**7**; R^1 = Ph, R^2 = H)] from (**5**; R = *p*-ClC_6H_4·O·CH_2·CO) (1·4 mmol), phenyl phosphate (1·25 mmol), TPS (2·8 + 0·3 mmol), and (**7**; R^1 = Ph, R^2 = H) (1·0 mmol). Removal of the protecting groups§ from (**7**; R^1 = Ph, R^2 = H) and (**8**; R^1 = Ph,

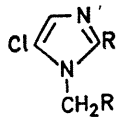
† This procedure for phosphotriester synthesis from a nucleoside phosphodiester derivative, an arenesulphonyl chloride, and a nucleoside was first used in the deoxy-series by Letsinger and his collaborators;[2] it has also been reported[5] to be effective in the ribose series.

‡ The times required for complete hydrolysis of the phosphotriester functions of (**3**; R = *o*-ClC_6H_4) and (**4**; R = *o*-ClC_6H_4) at 20° were 90 and 180 min, respectively.

§ Before alkaline hydrolysis of the phosphotriester groups, both (**7**) and (**8**) (R^1 = Ph, R^2 = H) were tetrahydropyranylated on their terminal 5′-hydroxy-groups. When this step was omitted with (**7**; R^1 = Ph, R^2 = H), the UpU obtained was contaminated with *ca.* 5% of uridylyl-(5′ → 5′)-uridine.

R^2 = H) gave UpU and UpUpU, respectively, as the sole nucleotide products.

Phenyl phosphorodichloridate had been used successfully as the phosphorylating agent in the deoxy-series.[4] However, in the ribose series, no success was achieved with this reagent in the presence of either 2,6-lutidine or pyridine. However, the partially protected dinucleoside phosphate (**7**; R^2 = H) was isolated (89%) from the products of the reaction between (**5**; R = *p*-ClC_6H_4·O·CH_2·CO), phenyl phosphorodichloridate, and (**6**) in the presence of 5-chloro-1-methylimidazole[12] (**9a**), in acetonitrile solution. Although both phosphorylation steps were complete within 190 min, the product was contaminated with a non-nucleotidic impurity which was difficult to remove. When 5-chloro-1-ethyl-2-methylimidazole[12] (**9b**) was used instead of (**9a**), the isolated product (**7**; R^2 = H) was pure but it was obtained in lower yield, and both steps of the phosphorylation were slower. Thus, while it is clear that the imidazole derivatives (**9a** and **9b**) are effective catalysts in phosphorylations with phenyl phosphorodichloridate, it may be concluded that phenyl (or substituted phenyl) phosphate activated by TPS in pyridine solution is a more suitable



(9) a; R = H
b; R = Me

phosphorylating system for the synthesis of oligoribonucleotides *via* phosphotriester intermediates. Indeed, it seems likely that it will be possible to synthesize oligoribonucleotides of moderate size by the latter procedure.

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[1] See, for example, R. Lohrmann, D. Söll, H. Hayatsu, E. Ohtsuka, and H. G. Khorana, *J. Amer. Chem. Soc.*, 1966, **88**, 819; S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, 1967, **89**, 2158.
[2] R. L. Letsinger and V. Mahadevan, *J. Amer. Chem. Soc.*, 1965, **87**, 3526; R. L. Letsinger and K. K. Ogilvie, *ibid.*, 1967, **89**, 4801.
[3] F. Eckstein and I. Rizk, *Angew. Chem. Internat. Edn.*, 1967, **6**, 695, 949.
[4] C. B. Reese and R. Saffhill, *Chem. Comm.*, 1968, 767.
[5] T. Neilson, *Chem. Comm.*, 1969, 1139; G. W. Grams and R. L. Letsinger, *J. Org. Chem.*, 1970, **35**, 868.
[6] J. C. M. Stewart, Ph.D. Thesis, Cambridge University, 1969, p. 56.
[7] D. P. L. Green, T. Ravindranathan, C. B. Reese, and R. Saffhill, *Tetrahedron*, 1970, **26**, 1031.
[8] R. Lohrmann and H. G. Khorana, *J. Amer. Chem. Soc.*, 1966, **88**, 829.
[9] C. B. Reese, R. Saffhill, and J. E. Sulston, *Tetrahedron*, 1970, **26**, 1023.
[10] J. H. van Boom, G. R. Owen, J. Preston, T. Ravindranathan, and C. B. Reese, *J. Chem. Soc.* (*C*), in the press.
[11] B. E. Griffin, M. Jarman, C. B. Reese, and J. E. Sulston, *Tetrahedron*, 1967, **23**, 2301.
[12] O. Wallach, *Annalen*, 1877, **184**, 1; *ibid.*, 1882, **214**, 257; F. F. Blicke and H. C. Godt, jun., *J. Amer. Chem. Soc.*, 1954, **76**, 3653.