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A RAPID AND EFFICIENT SYNTHESIS OF RIBONUCLEOTIDES

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Whereas the chemical synthesis of deoxyribonucleotides of defined sequence has advanced sufficiently to produce long polymers including the structural genes for alanine and tyrosine tRNAs[1,2], the procedures for the chemical synthesis of ribonucleotides of defined sequence are still being developed and the synthesis of a tRNA-like molecule is a distant goal. There are two basic problems which block successful ribonucleotide synthesis. First, suitably protected ribonucleosides are available only after several synthetic steps and in low yields. Second, the condensation of protected nucleosides is a slow reaction and is accompanied by the formation of unwanted side products resulting in low yields. These two problems are addressed in this communication.

Efficient procedures have been developed by Ogilvie and coworkers[3,4] for the protection of the four common non-N-acylated ribonucleosides using the tert-butyldimethylsilyl group (TBDMS). However, when these silylated nucleosides were condensed using tri-isopropylbenzenesulphonyl chloride (TPS) in the phosphotriester approach, yields of diribonucleotides of less than 30% were obtained. The reactions were slow and extensive sulphonation of the 5'-hydroxyl of the incoming nucleoside was observed[4]. Ogilvie and coworkers[5] have improved the yields using the phosphite method described by Letsinger and Lunsford[6] to produce silyl protected diribonucleotides (50-63% yield) and a triribonucleotide (56% yield).

Arylsulphonamides have been shown by Narang and coworkers[7] to be efficient condensing reagents as an alternative to TPS. Several deoxyribonucleotide polymers have been synthesized using arylsulphonyltetrazolides and triazolides including the lactose operator from Eschericia coli[8], the gene for the hormone somatostatin[9] and several decamers for molecular cloning experiments[10,11].

A combination of the advantages of the silyl protecting group for preparing appropriately protected ribonucleosides and the arylsulphonyltetrazolides as condensing reagents has shown promise for the synthesis of ribonucleotides[12]. We report in this communication the application of this strategy to the synthesis of fully protected diribonucleotides including all four common ribonucleosides in much improved yields. The basis of the procedure is a simple and rapid formation of the internucleotide linkage using the phosphotriester method.

The 5'-O-monomethoxytrityl-N-acylated ribonucleosides were silylated as previously described[3,4] for the non-N-acylated ribonucleosides except that a

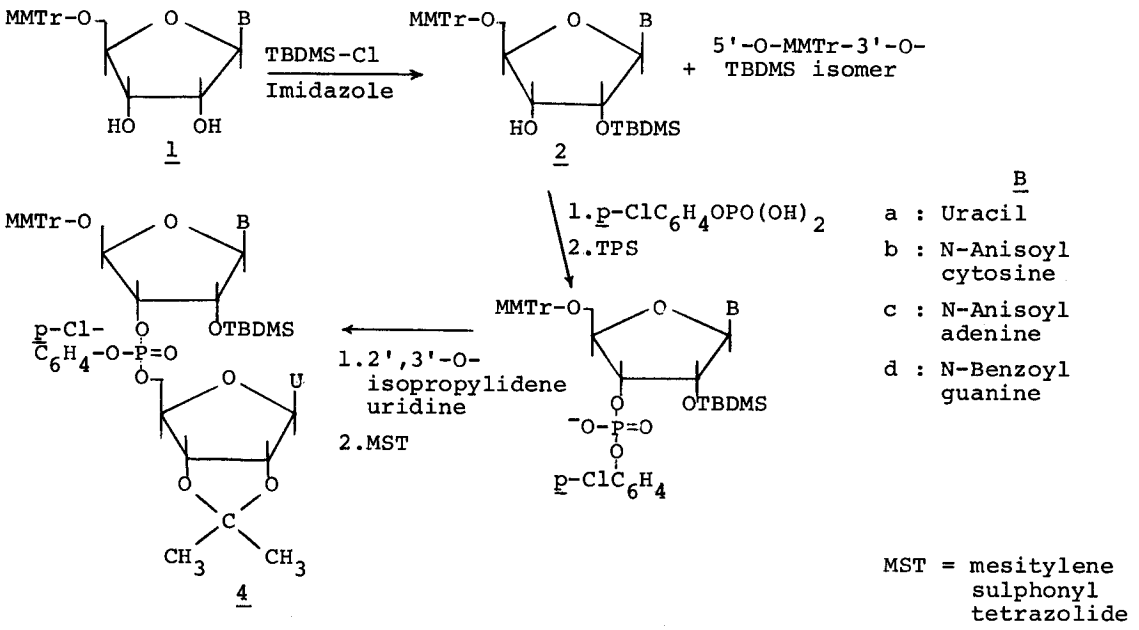
more convenient 'one flask' procedure was adopted. That is, the silylation reagents, TBDMS chloride and imidazole, were added to the pyridine solution just after the formation of the 5'-O-monomethoxytrityl (MMTr) derivatives with no intermediate work up. The yields and suitable solvent systems for separating the 2',5'- and 3',5'-protected isomers on silica gel plates are given in Table 1. The yields of the compounds 2a - 2d (the 2',5'-isomers) can be easily and substantially improved by allowing the unwanted 3',5'-isomer to equilibrate with the 2',5'-isomer followed by another purification step. The equilibration occurs readily in a weakly basic medium such as aqueous pyridine or during prolonged contact with silica gel. See Scheme 1 for details.

Table 1. Yields of the protected nucleosides and diribonucleotides and the solvent systems for their purification

Compound	Yield (%)	Solvent system*
2a	44+	Ether-hexane (3:1)
2b	45+	Ether
2c	35+	Ether
2d	45+	Chloroform-ethanol (20:1)
4a	95	Ether-hexane (4:1)
4b	95	Ether
4c	93	Ether
4d	78#	Chloroform-ethanol (15:1)

+ See text for increasing these yields
# Conditions for this synthesis were not optimized
* For developing silica gel plates

Anhydrous 5'-O-monomethoxytrityl-2'-O-TBDMSuridine and the respective N-acylated ribonucleosides (2a-2d) (0.2 mmol) were phosphorylated using p-chlorophenyl phosphate (0.4 mmol) and TPS (0.8 mmol) in a pyridine solution for four hours. The ice cooled reaction mixture was quenched with water and extracted with chloroform, which was back washed with water (3 X 2 ml). Most of the solvent was removed under reduced pressure and the residue was dried by co-distillation of pyridine (3 X 2 ml). After adding 2',3'-O-isopropylidene uridine (0.3-0.4 mmol) the reactants were again dried by pyridine co-distillation (3 X 3 ml). A pyridine solution of the residue (5 ml) and mesitylenesulphonyl-tetrazolide (0.6 mmol, freshly prepared) was stirred for 3-4 hours. The reaction mixture was cooled in ice, quenched with water and extracted with chloroform. The organic layer was washed with triethylammonium bicarbonate buffer (3 X 3 ml, pH 7.5, 0.1 M) and then with water (1 X 3 ml). The solvents were removed by evaporation and the residual pyridine was removed by co-distillation with toluene. A chloroform solution of the residue was applied to two silica gel plates (Brinkmann MN P/UV254 silica gel, 20 X 20 cm) and developed in suitable solvent systems (Table 1). After removing a part of the eluting solvents (chloroform-methanol, 10:1), the residue was co-distilled



Scheme 1

with heptane to produce a solid in all cases. The yields of compounds 4a - 4d (Scheme 1) are recorded in Table 1. Somewhat lower yields (80-85%) were obtained when the cyanoethyl group was substituted for the p-chlorophenyl group as a protecting group for the internucleotide phosphate. All four diribonucleotides were deprotected and characterized in the usual manner. The monomethoxytrityl group was removed by treatment with 80% acetic acid at room temperature. The N-acyl groups were removed by treatment with 9 M ethanolic ammonia. Finally, treatment with n-butylammonium fluoride produced the isopropylidene containing dinucleotides which were purified by paper chromatography and characterized by electrophoresis and enzymatic degradation.

Stawinski and coworkers[13] have recently used a modified phosphotriester approach and tetrazole based condensing reagents to achieve the synthesis of ribonucleotides. Their procedure involved the prior isolation of the 3'-phosphorylated derivatives in only 40 - 65% yields while the yields in the condensation reaction are in the range of 55 - 80%.

The simple methodology described in this communication does not involve the isolation of the 3'-phosphorylated products (3a - 3d) and this results in much improved yields of the diribonucleotides. The efficiency of the procedure described is illustrated by the fact that a diribonucleotide can be prepared in one day from the suitably protected ribonucleosides which can be prepared in a one flask reaction mixture. The advantages of this method are being applied to

the synthesis of larger fragments corresponding to the sequence of yeast phenylalanine tRNA.

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