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NOVEL SOLID PHASE SYNTHESIS OF 2'-O-METHYLRIBONUCLEOSIDE 5'-TRIPHOSPHATES AND THEIR α-THIO ANALOGUES

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ABSTRACT

Simple, versatile and convenient syntheses of 2'-O-methyl-ribonucleoside 5'-triphosphates have been accomplished on controlled pore glass (CPG) in good yield (>60%) using 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (salicyl phosphorochloridite). Moderate yields were obtained for the corresponding α-thiotri-phosphates.

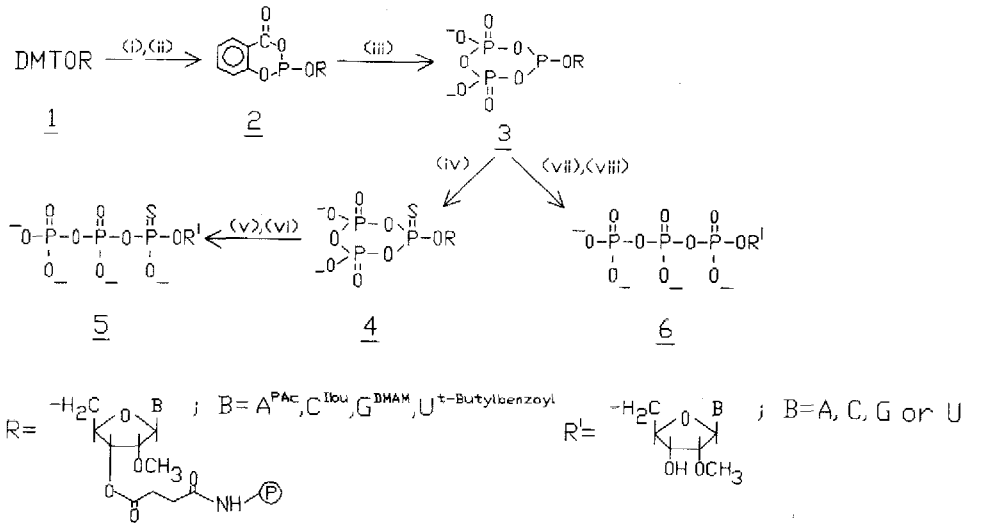
Since their introduction, nucleoside 5'-triphosphates and their α-thio analogues have been extremely useful in a wide variety of biochemical studies[1,2]. Recent years have witnessed the utility of 2'-O-methyloligoribonucleotides[3] in studying the mechanism of pre-mRNA splicing[4] and most importantly in the affinity purification of ribonucleoproteins by using the biotinylated oligomers in conjunction with streptavidin-agarose (for recent review and references see ref[5].). These studies depend upon the chemical synthesis of the modified oligonucleotides and will be hindered by inherent size limitations. Enzymatic RNA synthesis with DNA-template dependent RNA polymerases could overcome this problem[6] but would require the as yet unavailable 2'-O-methylribonucleoside 5'-triphosphates. For greater versatility of applications we decided to include the synthesis of 2'-O-methylribonucleoside 5'-(α-thiotriphosphates).

The biological importance of phosphorylated nucleosides and analogues in molecular biology and biochemistry has led to the development of improved methods for their synthesis. Most involve transformation of mononucleotides to reactive intermediates such as morpholidates[7], imidazolidates[8], phosphoramidates[9] or 8-quinolates[10] followed by displacement of the leaving group by pyrophosphate. Recently, Ludwig and Eckstein [11] demonstrated a new

synthesis of nucleoside 5'-triphosphates and their α-thio analogues. However, their method requires the protection of the ribose 2'- and 3'- hydroxyl groups and moreover leads to the formation of by products, some of which are difficult to remove by conventional DEAE-Sephadex chromatography.

Here we report a simple, convenient and rapid solid phase synthesis of 2'-O-methylribonucleoside 5'-triphosphates and their α-thio analogues. The 3'-hydroxyl group of the 5'-O-dimethoxytrityl-N-acyl-2'-O-methylribonucleoside is attached to an amino functionalised solid support (e.g. CPG) via a succinate linkage[5], which serves the dual purpose of anchor and protecting group. All the chemical manipulations are performed whilst the nucleoside is attached to the support, enabling excess reagents to be removed by simple washing under argon. In addition, the commercial availability of the fully protected 2'-O-methylribonucleoside functionalised supports renders this approach more attractive.

A general synthesis is illustrated in Scheme 1. A known amount of the nucleoside derivatised support, generally 100 μmol,is placed in a small glass column fitted with a septum at the top and equipped with a glass sinter and tap at the bottom (the volume should be about 5-10 ml). The support is treated with a 3% solution of trichloroacetic acid in dichloromethane (10 ml) for 2 min to remove the 5'-protecting group, then washed with dichloromethane followed by acetonitrile. A freshly prepared 1 M solution of salicyl phosphorochloridite in dry dioxan (1.1 ml) is then added to the support suspended in dry pyridine/dioxan (4 ml, 1:3 v/v). The vessel is gently agitated for 15 min, reagent removed under argon pressure and the support washed with dioxan followed by acetonitrile. Next, a mixture of 0.5 M solution of bis(tributylammonium) pyrophosphate in dry DMF (3 ml) and tri-n-butylamine (1 ml) is injected onto the support. After 20 min the support is washed extensively with dry DMF followed by acetonitrile. The oxidation reaction is performed with a solution of iodine/ water/pyridine/THF (5 ml, 3:2:20:75) for 30 min. Excess iodine solution is then removed by extensive washing with acetonitrile. The protecting groups and linkage are then cleaved by treating the support with 25% aqueous ammonia (20 ml) in a sealed vessel at 50°C for 2 h. In the synthesis of α-thiotriphosphates



Scheme 1. Reagents and conditions: (i), 3% TCA in CH_2Cl_2; (ii), salicyl phosphorochloridite; (iii), 0.5 M bis-tri-n-butylammonium pyrophosphate in DMF and tri-n-butylamine; (iv), sulphur; (v), dioxan-water; (vi) & (viii), 25% aq. ammonia; (vii), Iodine-water-pyridine-THF; PAc=phenoxyacetyl; Ibu=isobutyryl; DMAM=dimethyl-aminomethylidene, P=polymer support, DMT=4,4'-dimethoxytrityl.

the oxidation step is replaced by a sulphurisation step using a suspension of elemental sulphur (32 mg) in dry DMF (2 ml). The support is then treated with dioxan/water (5 ml, 1:1 v/v) for 45 min, washed, and then treated with ammonia.

The cooled ammoniacal solution of crude nucleoside triphosphate is evaporated to dryness, the residual syrup is dissolved in 5 ml of 0.2 M triethylammonium bicarbonate buffer (pH 7.8) and then purified by chromatography on a DEAE-Sephadex column (2 x 20 cm) at 4°C using a linear gradient from 0.2 to 1 M triethylammonium bicarbonate buffer. Pure fractions are pooled , evaporated in vacuo to dryness and residual buffer is removed by co-evaporation with dry methanol to give isolated yields of 60-65% for 2'-O-methylribonucleoside 5'-triphosphates and 40-45% for the α-thio analogues. Product purity is checked by ^{31}P NMR spectroscopy[12]. If required the nucleoside 5'-(α-thiotriphosphates) can easily be separated into pure diastereomers by reversed phase HPLC following a well established procedure[13].

We assumed that the moderate yield in the case of α-thiotriphosphates could be due to incomplete sulphurisation, however increasing the reaction time to 2 h or using a solution of sulphur in carbon disulphide/pyridine instead failed to improve the

yield. A more extensive study is required to find the optimal conditions for sulphurisation.

The method described here avoids the formation of major side products, such as branched triphosphate, tripolyphosphate and 1-thiocyclotriphosphate[11] and moreover can be modified for the synthesis of radioactive labeled analogues. Furthermore it can be used in conjunction with Omnifit equipment, which allows semi-automation and is therefore useful for multiple and/or large scale synthesis of these interesting compounds. A higher nucleoside loading more appropriate for large scale synthesis can be obtained by changing to a polystyrene based support. The commercial availability of the nucleoside derivatised supports makes this method less time consuming and moreover the desired product can be obtained in less then 24h.

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