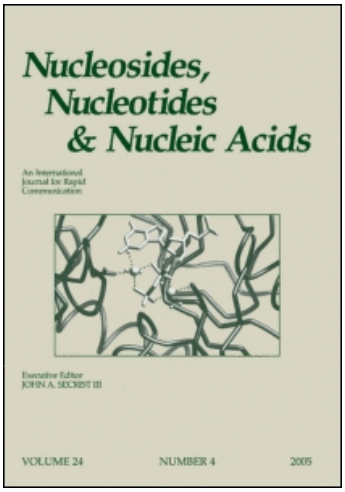

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Synthesis of RNA Fragments Using the H-Phosphonate Method and 2'-(2'-Chlorobenzoyl) Protection

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SYNTHESIS OF RNA FRAGMENTS USING THE H-PHOSPHONATE METHOD AND 2'-(2-CHLOROBENZOYL) PROTECTION

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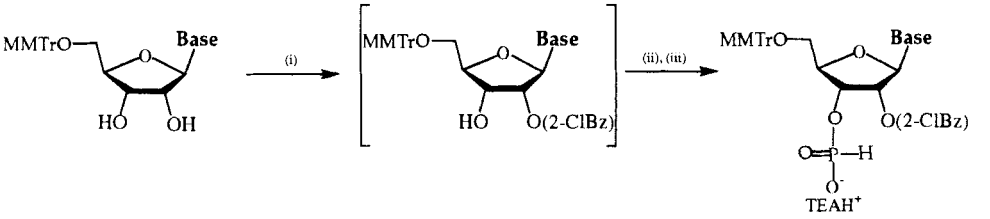
Abstract: The performance of 2'-(2-chlorobenzoyl) protected ribonucleoside H-phosphonates in the synthesis of oligoribonucleotides has been studied.

Recently we developed a straightforward one pot synthesis of protected ribonucleoside hydrogenphosphonates bearing the 2'-O-(2-chlorobenzoyl) group[1]. The key procedure in our approach is the selective 2'-benzoylation[2] of the cis-2',3'-diol system of ribonucleosides **1** using a slight excess of 2-chlorobenzoyl chloride at -78° (Scheme). The phosphonylation is performed in the same reaction media without isolation of the 2'-protected ribonucleoside **2**.



1 **2** **3**

Base = Ura, N^6-n-butyrylAde, N^4propionylCyt, N^2phenoxyacetylGua. Reagents: (i) 2-Chlorobenzoyl chloride 1.1 eq. (ii) PCl_3, imidazole, triethylamine (iii) 1M Triethylammonium bicarbonate aq.

Scheme

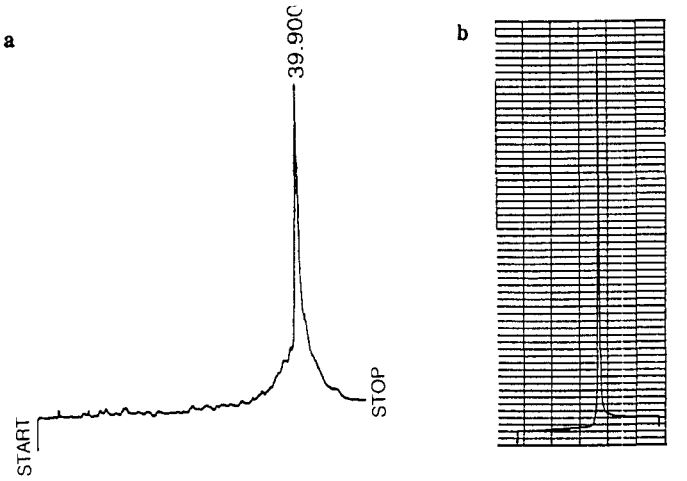


Fig 1. HPLC profiles of ApUpGpApApGpApApUpApCpCpCpApUpG; a) anion exchange separation of crude oligonucleotide, b) reverse phase analysis of the fraction separated by anion exchange.

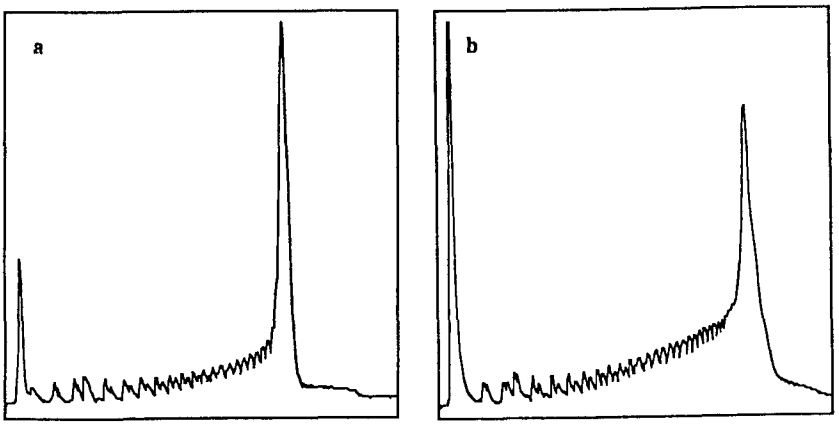


Fig 2. Anion exchange HPLC of crude a) $(Up)_{24}U$, b) $(Up)_{34}U$.

Thus our method provides a fast, simple and inexpensive route for synthesis of protected ribonucleoside H-phosphonates, synthons for oligoribonucleotide synthesis. The problem of 2'-3' isomerization is practically solved, because the work up of 2'-O-aroyl ribonucleosides **2**, which can lead to acyl migration, is eliminated. It has been found that the target products **3** can be completely purified from the undesired 3'-O-(2-chlorobenzoyl) isomers by simple silica gel column chromatography[1].

A problem when using the 2-chlorobenzoyl protection is concomitant cleavage of internucleotidic phosphodiesters during the ammonolysis step. We found that 8 hours treatment with 32 % aq. ammonia-ethanol, 3:1 at RT allowed complete deblocking of 2-chlorobenzoyl groups with acceptably low degradation. To achieve complete deblocking of oligoribonucleotides we used N-protecting groups[3] previously successfully combined with 2'-*t*-butyldimethylsilyl protection[4] and removable under conditions mentioned above.

The efficiency of the method is demonstrated on short RNA fragments. Typical results from synthesis of short mixed sequences are shown in Fig. 1. Purity of oligoribonucleotides synthesised were confirmed by PAGE, and enzymatic degradation followed by HPLC analysis also gave the correct ratio of nucleosides. The possibility to prepare longer oligoribonucleotides was studied on oligouridylic acids (Fig. 2). Results when analysing crude oligoadenylic and oligocytidylic acids were qualitatively similar. The results show that the approach is efficient enough to synthesise oligoribonucleotides up to 30-35 nucleotides long. Further studies on the synthesis of RNA fragments of this length are in progress.

In summary, the approach is relatively efficient and has the specific advantage of fast preparation of building blocks and low overall cost. This makes the method particularly interesting for large scale synthesis which is most important for medical applications.

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