

INCOMPATIBILITY OF ACID-LABILE 2' AND 5' PROTECTING GROUPS  
FOR SOLID-PHASE SYNTHESIS OF OLIGORIBONUCLEOTIDES

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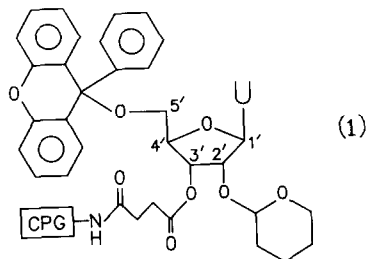
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**Abstract.** Cleavage of 2'-O-tetrahydropyranyl groups from ribonucleoside derivatives used in solid-phase oligoribonucleotide synthesis takes place to an unacceptable extent during treatment with protic acid to remove 5'-O-pixyl groups.

Much attention is now being directed to the development of reliable methods for solid-phase synthesis of oligoribonucleotides to match those already in routine use for oligodeoxyribonucleotides. The key to success lies in finding suitable protecting groups for the 2' and 5' hydroxyl functions. 2'-Protecting groups must remain stable throughout chain assembly and be specifically removed at the end of the synthesis, whereas, for synthesis in the 3' to 5'-direction, 5'-protecting groups must be removed at every cycle of nucleotide addition without side reactions.

Acid-labile tetrahydropyranyl (Thp)<sup>2</sup> and methoxytetrahydropyranyl (Mthp)<sup>3</sup> have proved to be the most suitable for 2'-protection, since they are not prone to migration from O-2' to O-3' and their removal can be effected without generation of alkoxide ions, which can subsequently attack neighbouring internucleotide bonds leading to isomerisation and chain cleavage.

Recent reports have suggested that highly acid-labile protecting groups such as 9-phenylxanthen-9-yl (pixyl) or di-O-(p-anisyl)phenylmethyl (dimethoxytrityl), which are used routinely in oligodeoxyribonucleotide synthesis, can be used as 5'-protecting groups for ribonucleosides concomitantly with the more moderately acid-labile 2'-protecting groups for synthesis by conventional solution chemistry and more recently by solid-phase methods. Whereas protecting groups of differential lability can sometimes be used to good effect in solution synthesis, the solid-phase method requires that 2'-protecting groups be stable to repeated acidic treatments. To test the suitability of such a combination of protecting groups, a uridine derivative containing the 5'-protecting group of highest lability (pixyl) and the 2'-protecting group of least lability (Thp) was prepared, attached to a solid support (controlled pore glass) and subjected to standard acidic conditions used for 5'-deprotection in the deoxy series.



Hence 2'-O-Thp-uridine<sup>1,11</sup> (low R<sub>F</sub> isomer on silica gel t.l.c. using methanol/chloroform (1:9)) was treated with 1.1 equivalents of pixyl chloride in pyridine to afford the 5'-O-pixyl derivative which was isolated in 44% yield. This was converted to the corresponding 3'-O-succinate derivative (61% yield) and attached to long chain alkylamine controlled pore glass essentially by standard methods to give support (1). 20mg aliquots of

(1) (0.2  $\mu$ mole of uridine) were packed in small glass columns in 1,2-dichloroethane (DCE) and treated for different times with 3% dichloroacetic acid in DCE in a continuous flow, semi-manual apparatus using a flow rate of 2 ml/min, followed by washing with DCE, pyridine and finally DCE. The support was removed from the column and treated with concentrated ammonia solution at room temperature for 16 h to cleave the nucleoside from the support. The supernatant was evaporated to dryness, dissolved in dioxane/water (1:1) and analysed by reversed phase h.p.l.c. on a Partisil 5 ODS column using a gradient of acetonitrile in 0.1M ammonium acetate buffer. As expected the 5'-O-pixyl group was removed completely by the first time point (30s) and only a single peak of 2'-O-Thp-uridine was seen. With longer times Thp groups were progressively lost, as witnessed by the appearance of a second peak corresponding to free uridine. After 120s a third minor peak was also visible eluting close to that of 2'-O-Thp-uridine. This is presumably the other stereoisomer of 2'-O-Thp-uridine, arising by acid-catalysed isomerisation of the Thp group.

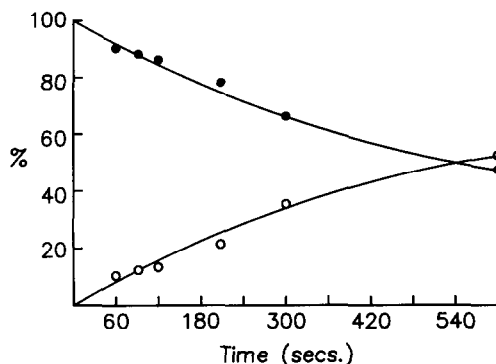


Figure. Time course of loss of Thp groups. Open circles: uridine  
Closed circles: 2'-O-Thp-uridine (both isomers).

From the Figure it can be seen that the removal of the Thp group occurs under these acidic conditions with a  $t_{1/2}$  of about 560s. Whilst some increase in this value might be achieved by adjustment of the acid strength or concentration, it is likely that considerable loss of Thp groups would take place during the course of oligoribonucleotide synthesis involving repeated acidic treatments, leading to 2'- to 3'-isomerisation and internucleotide cleavage. We conclude that such a combination of protecting groups is inappropriate for the attainment of routinely reliable solid-phase oligoribonucleotide synthesis. Compatibility of 2'- and 5'-protection will most likely be achieved by development of an alternative 2' protecting group stable to protic acids or a 5'-protecting group removable under non-acidic conditions. The latter approach is under investigation in this laboratory.

#### References and Notes.

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