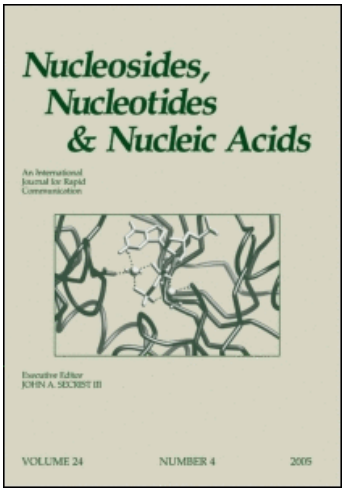

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A New 5'-Protecting Group for Use in Solid-Phase Synthesis of Oligoribonucleotides

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A NEW 5'-PROTECTING GROUP FOR USE IN SOLID-PHASE SYNTHESIS OF OLIGORIBONUCLEOTIDES

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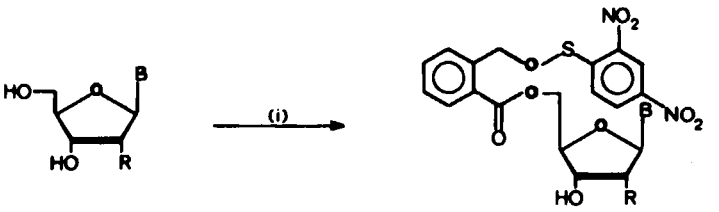
ABSTRACT. The 2-(2,4-dinitrobenzenesulphenyloxymethyl)benzoyl (DNBSB) group is proposed as a protecting group for the 5'-position of nucleosides. The DNBSB group may be removed under mild non-acidic conditions and may have potential in solid-phase synthesis of oligoribo- and oligodeoxyribonucleotides.

We wish to develop a route for solid-phase synthesis of oligoribonucleotides that involves assembly of nucleotide units in a 3' to 5' direction, similar to that in routine use for oligodeoxyribonucleotides.[1] To this end we have chosen to use acid-labile tetrahydropyranyl (THP)[2] or methoxytetrahydropyranyl (MTHP)[3] for 2'-hydroxyl protection of the nucleotide units and to investigate possible alternative protecting groups for the 5'-position. Such groups must be removed sequentially after each nucleotide addition under conditions where no other protecting group is affected.

We have ruled out the use of acid-labile pixyl or dimethoxytrityl groups for 5'-protection, since we found substantial danger of concomitant loss of THP groups during the sequential acidic treatments required to elaborate an oligonucleotide chain.[4] Instead we have been searching for a 5'-protecting group which can be introduced selectively on to the 5'-position of nucleosides and which can be removed under very mild conditions that do not interfere with oligonucleotide assembly. Although several potentially useful groups have been proposed as 5'-protecting groups[5-8] none as yet have been reported for use in solid-phase oligoribonucleotide synthesis. We now describe a new 5'-protecting group which may have potential usefulness in solid-phase synthesis.



1 a: R=H . B=T
b: R=OR', R'=THP, B=U
c: R=OR', R'=THP, B=C^{Bz}

2 a: R=H . B=T
b: R=OR', R'=THP, B=U
c: R=OR', R'=THP, B=C^{Bz}

Reagents: (i) DNBSB acid pivaloyl chloride, diisopropylethylamine, dioxane then 4-dimethylaminopyridine, pyridine

FIGURE. Preparation of DNBSB nucleosides

The 2-(2,4-dinitrobenzenesulphenyloxymethyl)benzoyl group (DNBSB) is based on the idea of a 'protected' protecting group originally proposed for nucleosides by Reese[5,6] but avoids the use of metal ions in the deprotection scheme. The group combines the attributes of the known rapid intramolecularly catalysed deacylation afforded by the 2-hydroxymethylbenzoyl moiety[6] with the 2,4-dinitrobenzenesulphenyl group[9] to protect the hydroxyl function. The preparation of 2-(2,4-dinitrobenzenesulphenyloxymethyl)benzoic acid[10] is accomplished in two steps from phthalide by ring opening with tetra-n-butylammonium hydroxide followed by reaction with 2,4-dinitrobenzenesulphenyl chloride in anhydrous 1,2-dichloroethane. Reaction of thymidine, 2'-O-tetrahydropyranyluridine and 2'-O-tetrahydropyranyl-4-N-benzoylcytidine (1a-c) with DNBSB acid together with pivaloyl chloride in anhydrous pyridine yielded after silica gel column chromatography and crystallisation moderate yields (25-33%) of the desired 5'-O-DNBSB nucleosides (2a-c)[10] (FIG).

The DNBSB group is completely removed from nucleosides (2a-c) using 50mM p-toluenethiol and 200mM diisopropylethylamine in acetonitrile/water (90:10 v/v) within 2 minutes. Release of the

2,4-dinitrobenzenesulphenyl group in the first step of the reaction gives rise to a yellow colour which can be quantitated by UV spectroscopy (λmax 456nm).

In order to determine whether these conditions of removal would be compatible with solid-phase synthesis the oligonucleotides, $d[T]_{14}A$ was prepared by the phosphoramidite procedure[11] on a manual flow system, but using 5'-O-pixylthymidine-3'-O-(2-cyanoethyl-N,N-diisopropylamino)phosphite as monomer unit. After each acidic deprotection step the glass support was washed (3 minutes) with 50mM p-toluene thiol and 200mM diisopropylethylamine in acetonitrile/water (90:10). High coupling yields were obtained and there was no evidence of significant side reactions. Similarly $d[T]_6C$ was prepared by the H-phosphonate procedure[12,13] on a manual flow system but using triethylammonium 5'-O-pixylthymidine-3'-O-H-phosphonate as monomer unit and after each coupling step the intermediate phosphonate was oxidised to the phosphodiester using 2% iodine in pyridine/water (98:2) for 2.5 minutes. Once again after each acidic deprotection step the glass support was washed (3 minutes) with p-toluenethiol and diisopropylethylamine in acetonitrile/water (90:10). Yields were identical to those obtained to a control synthesis without the p-toluenethiol treatment.

Thus far it has not been possible to obtain 5'-O-DNBSB nucleoside 3'-O-(2-cyanoethyl,N,N-diisopropylamino)phosphites in sufficiently pure form. However, we have recently prepared 5'-O-DNBSB-2'-O-tetrahydropyranyl-4-N-benzoylcytidine-3'-O-H-phosphonate in 74% yield from 1c by the phosphonylation procedure previously described.[13] This phosphonate showed no evidence of decomposition after 24h in pyridine solution as judged by tlc analysis. Attempts at oligonucleotide synthesis using such phosphonates are in progress.

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