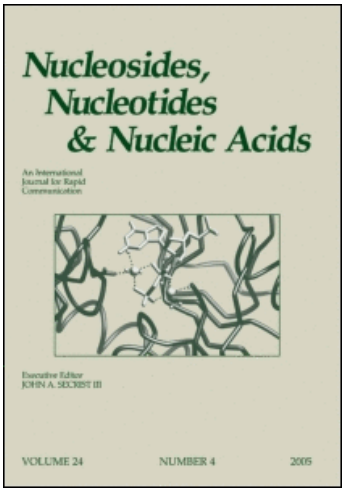

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CHEMICAL SYNTHESIS OF RNA-FRAGMENT ANALOGUES THAT HAVE PHOSPHOROTHIOATE LINKAGES OF IDENTICAL CONFIGURATION

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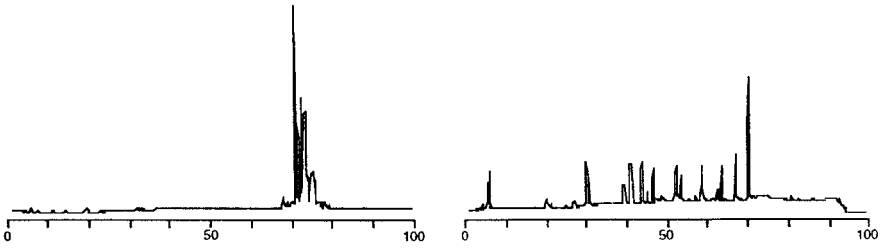
Abstract: Oligouridine all-*R*p-phosphorothioates were synthesized using the H-phosphonate approach followed by sulfurisation with S_8 and treatment with nuclease P1. The degree of stereoselection in the couplings is reported for different 2'-*O*-silyl protecting groups. The influence of the heterocyclic base was also investigated.

The interest in modified oligonucleotides has boomed over the last few years not least because of the potential of "antisense" oligonucleotides as therapeutic agents for diseases such as cancer and AIDS. The kind of modified oligonucleotide that has been most widely used over the years is when the phosphodiesters are replaced by chiral phosphorothioate linkages. Despite this type of oligomer being one of the most explored it is only recently that it has been possible to obtain homogenous stereochemistry around the phosphorus centers in chemically synthesized oligodeoxyribonucleoside phosphorothioates.[1] For RNA-fragments even less has been achieved and only dimers and trimers have been made in stereocontrolled reactions.[2,3]

We have developed a method for the synthesis of oligoribonucleoside phosphorothioates where all phosphorus linkages have *R*P configuration.[4] The method consists of three stages, first an oligoribonucleoside H-phosphonate fragment is assembled by automated synthesis on a solid support.[5] The use of the bulky *tert*-butyldimethylsilyl protecting group for the 2'-hydroxyl influences the couplings to *H*-phosphonodiester and results in predominant formation of *S*P-configured *H*-phosphonate linkages.[2] The second stage relies upon the stereospecific oxidation with elemental sulphur,[2] which affords a phosphorothioate RNA fragment enriched in *R*P-configured phosphorothioate linkages. After cleavage from support and deprotection, the oligonucleotide is digested with nuclease P1 from *Penicillium citrinum*, an enzyme which preferentially degrades internucleotidic phosphorothioates having *S*P

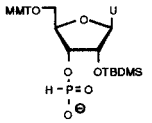
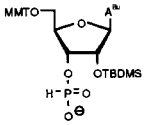
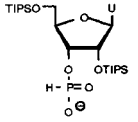
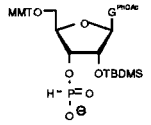
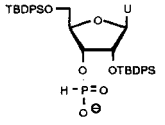
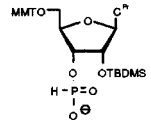


An isomeric mixture of oligoribonucleoside phosphorothioates (from synthesis of 8-mer, prepurified by HPLC to remove minor quantities of shorter fragments)

After treatment with Nuclease P1 for 24 hours

FIGURE 1

TABLE 1. The selectivity was determined by integration of ^{31}P-NMR signals. All couplings were performed in acetonitrile/pyridine (3/1) with pivaloyl chloride as coupling agent and 2',3'-O-dibenzoyluridine as alcohol.

H-phosphonate monomer	S_P / R_P stereoselectivity	*H*-phosphonate monomer	S_P / R_P stereoselectivity
	85 / 15		84 / 16
	84 / 16		86 / 14
	86 / 14		72 / 28

configuration.[6] The enzymatic degradation results in a collection of all R_P oligoribonucleoside phosphorothioates, differing only in length (see Fig 1).
The full-length RNA fragment is separated from shorter fragments by reversed phase HPLC and characterised by resistance towards nuclease P1 digestion and susceptibility towards digestion by snake venom phosphodiesterase (SVPD, *Crotalus adamanteus*), an enzyme that catalyses hydrolysis of R_P-phosphorothioates.[7]

The practical limit of the method is set by the stereoselectivity of the *H*-phosphonate couplings. The use of the *tert*-butyldimethylsilyl (TBDMS) protecting group ensures a stereoselectivity of 85/15 in favour of S_P-configured *H*-phosphonate linkages. With this stereoselectivity we have been able to prepare stereochemically homogenous phosphorothioate analogues of oligouridylic acids up to 16 nucleotides in length. The stereoselectivity in the *H*-phosphonate coupling step most likely depends on the bulkiness of the silyl protecting group in the 2' position since no selectivity is observed with deoxyribo-derivatives. In a model study, two other silyl protecting groups for the 2'-hydroxyl of uridine were investigated (see Table 1). Both the triisopropylsilyl (TIPS) and the *tert*-butyldiphenylsilyl (TBDPS) groups gave a stereoselectivity comparable to that obtained with the *tert*-butyldimethylsilyl protecting group. As a first step towards preparation of mixed sequence phosphorothioate RNA by our chemo-enzymatic approach we have also investigated the influence of the heterocyclic base on the key coupling step. Results obtained with the common RNA monomers are given in Table 1. The A and G monomers display a stereoselectivity in the coupling step comparable to that for the U monomer whereas the C monomer gives lower selectivity ($\approx$ 72 %). We will continue to compare the influence of the 2'-*O*-protecting group in order to improve on the stereoselectivity. This is particulary important for the cytidine derivative that now limits the size of oligomers that could be made.

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