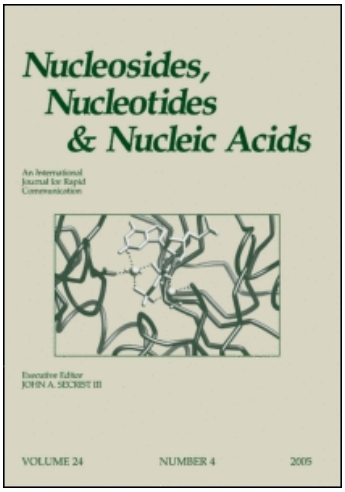

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APPROACH TO THE SYNTHESIS OF NATURAL AND MODIFIED OLIGONUCLEOTIDES BY THE PHOSPHOTRIESTER METHOD USING O-NUCLEOPHILIC INTRAMOLECULAR CATALYSIS

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□ *An approach to the solid phase synthesis of natural and modified oligonucleotides using phosphotriester technique has been developed. Particularly, this method allows the synthesis of ribo- and deoxyribo-oligonucleotides containing various 2′-modified mononucleotides as well as stereodefined nucleotide phosphorothioate analogues.*

Keywords Oligoribo- and deoxyribonucleotides; stereospecific phosphotriester synthesis; phosphorothioate analogues

INTRODUCTION

In recent times, synthetic siRNAs are widely used as powerful molecular tools to easily and rapidly assay gene function in a wide range of biological systems, and they are even being considered as therapeutics. In this connection, the effective synthesis of natural and modified oligoribonucleotides attracts a considerable interest. Nowadays, phosphoramidite method is routinely used for the synthesis of oligonucleotides. However, by virtue of its chemical peculiarities this method does not allow one to obtain some types of modified oligonucleotides, particularly derivatives containing strong electrophilic centers (e.g., azide, or N-oxide groups), due to the proceeding side reactions. Earlier, we have developed an alternative approach to the automated chemical synthesis of oligodeoxyribonucleotides based on the rapid solid phase phosphotriester method with O-nucleophilic intramolecular catalysis.[1] Later, it was shown that this method can be successfully applied for the synthesis of oligodeoxyribonucleotides containing 2′-azido- and 2′-amino-2′-deoxyuridine residues.[2] Now, we extended this approach to the synthesis of stereospecific phosphorothioate analogues of

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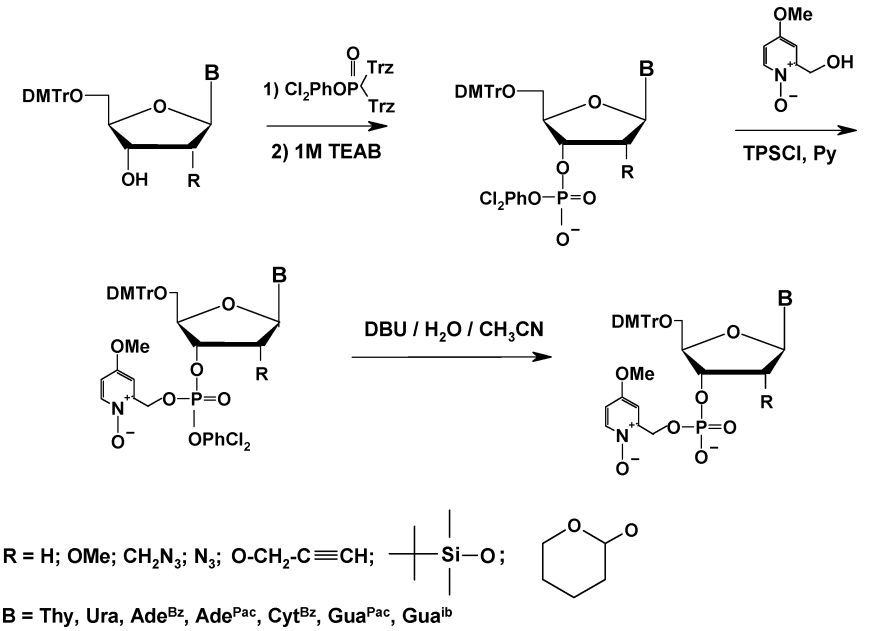


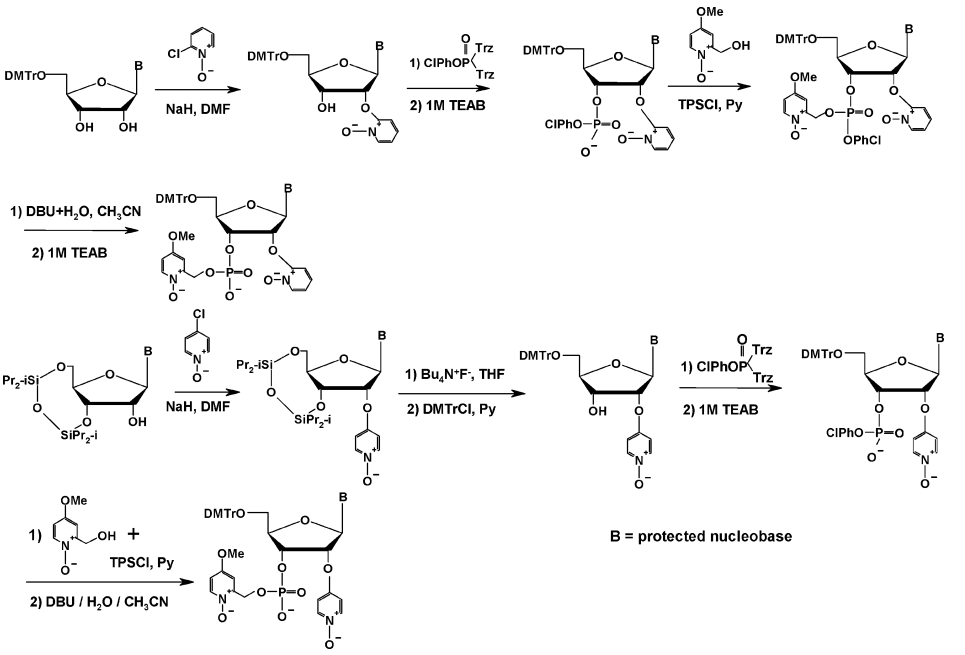
FIGURE 1 General scheme for the synthesis of protected monomers for the synthesis of natural and modified oligonucleotides by the solid phase phosphotriester method using O-nucleophilic intramolecular catalysis.

oligodeoxyribonucleotides and oligoribonucleotides as well as natural and 2′-modified oligoribonucleotides.

RESULTS AND DISCUSSION

The general scheme for the synthesis of the mononucleotides containing O-nucleophilic P-protective group is depicted in Figure 1. The effectiveness of the procedure was demonstrated on the synthesis of natural and 2′-modified RNA fragments. The examples of the synthesis of 2′-modified monomers for the phosphotriester oligonucleotide synthesis, protected monoribonucleotides containing 2- or 4-O-pyridine N-oxide groups in 2′-position are shown in Scheme 1. The investigation of hybridization properties of oligodeoxyribonucleotides bearing the insertions of these monomers revealed that the incorporation of a modified residue into the middle of oligonucleotide chain resulted in some destabilization of the complexes formed by this oligonucleotide with the complementary nucleic acid targets as compared with the stability of natural DNA/DNA and DNA/RNA duplexes (Figure 2).

Also, it was shown that, similar to the procedure recently published for the synthesis of stereodefined dithymidine phosphorothioates in solution,[3]



SCHEME 1

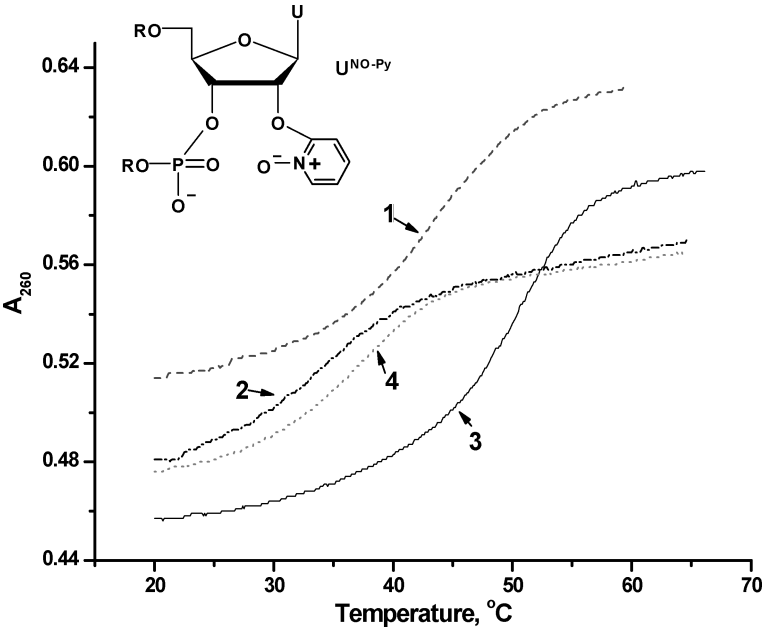
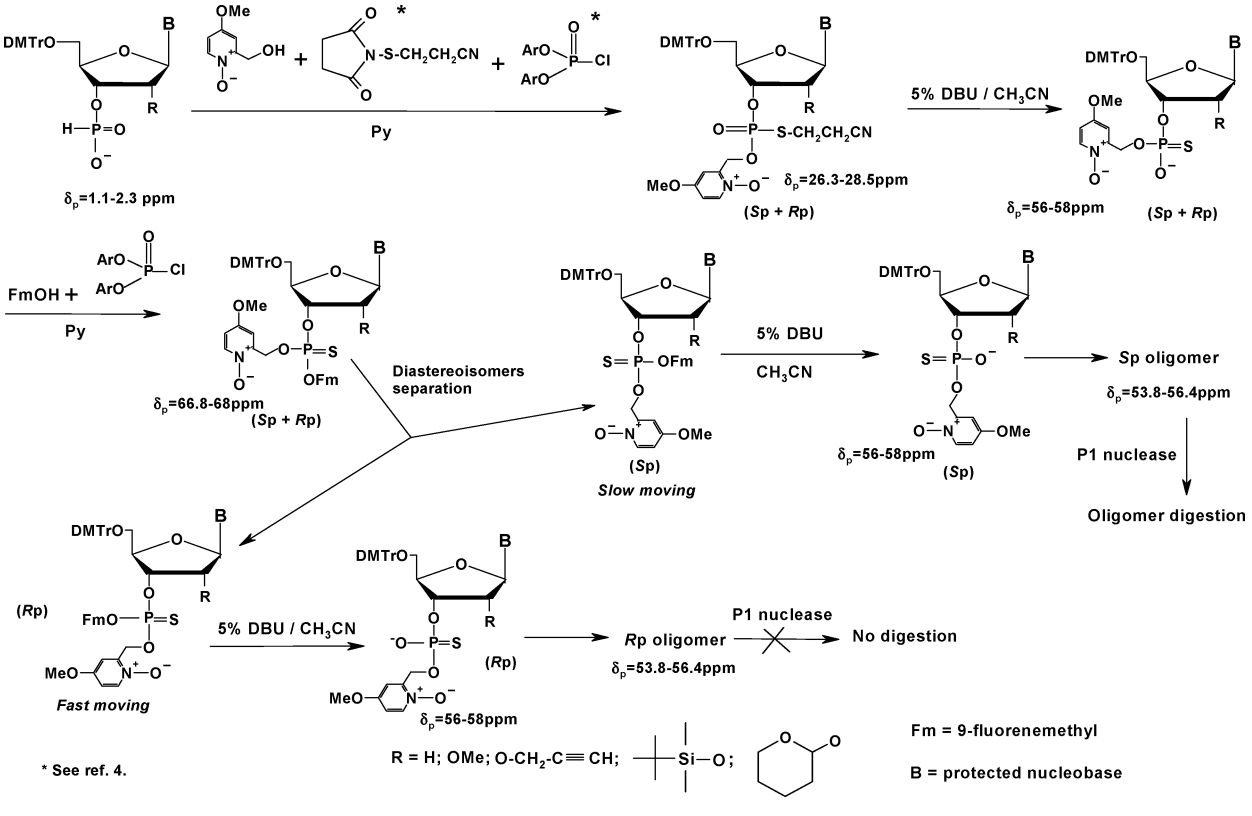
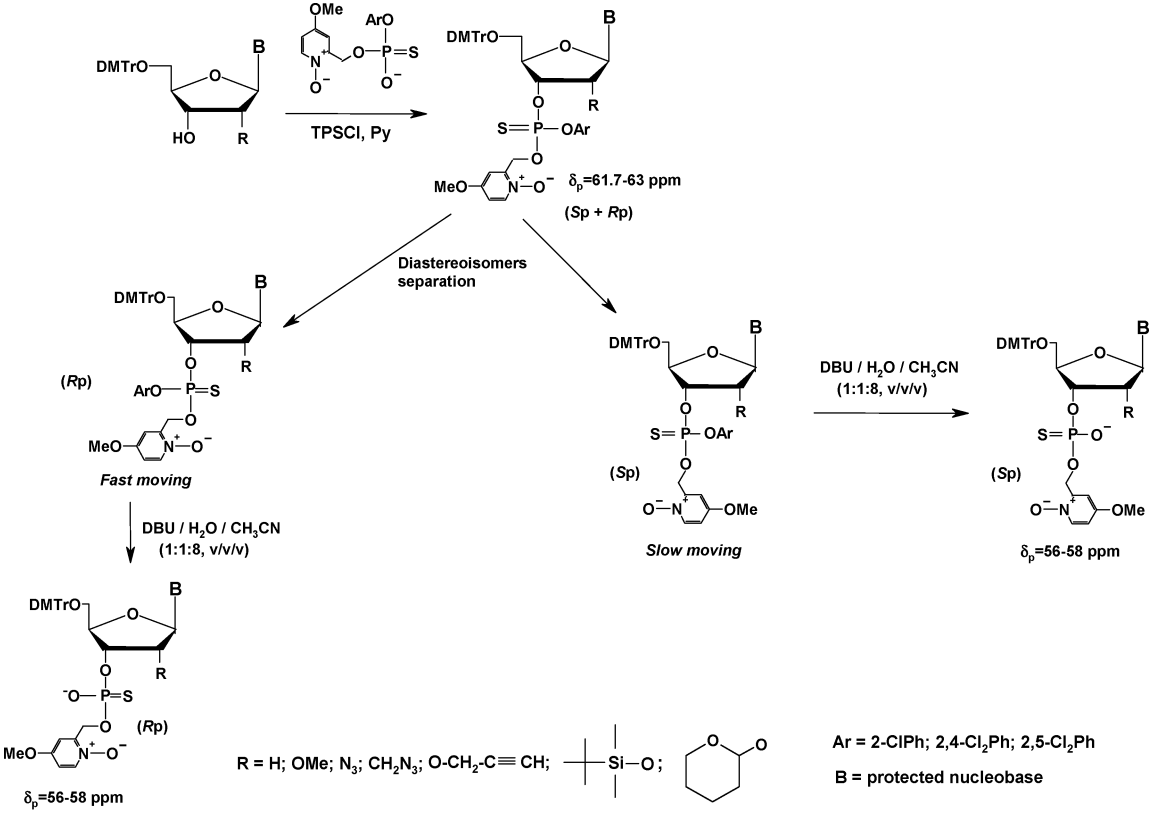


FIGURE 2 Comparison of the stability of complexes formed by the 16-mer oligodeoxyribonucleotide containing the insertion of uridine 2′-modified with 2-O-pyridine N-oxide $d(T)_9$-U^{NO-Py}-$(T)_6$ (1, 2) and $d(T)_{16}$ (3, 4) with $d(A)_{16}$ (1, 3) or $r(A)_{16}$ (2, 4) targets in 0.1 M NaCl/0.02 M Tris-HCl (pH 7.5)/0.01 M $MgCl_2$.



SCHEME 2



SCHEME 3

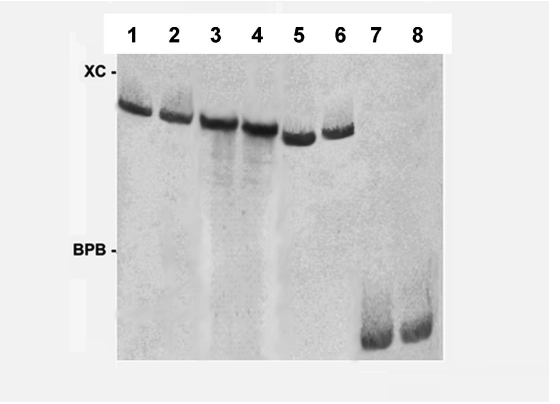


FIGURE 3 Analysis of ^{32}P-labeled stereospesific (*S*p and *R*p) oligodeoxyribonucleotide phosphorothioates PS-d(CACGTTGAGGGGCATCGT) by electrophoresis in 20% denaturing polyacrylamide gel: *S*p PS-oligonucleotide (1); *S*p PS-oligonucleotide treated with SVPD at 37°C for 24 hours (2); *R*p PS-oligonucleotide (3); *R*p PS-oligonucleotide treated with P1 nuclease at 37°C for 4 hours (4); natural oligonucleotide d(CACGTTGAGGGGCATCGT) (5); PS-oligonucleotide of mixed Sp-Rp type obtained using phosphoramidite method and bis(p-toluenesulfonyl)disulfide as sulfurizing agent [5] (6); digestion of *S*p PS-oligonucleotide with P1 nuclease at 37°C for 4 hours (7); digestion of *R*p PS-oligonucleotide with SVPD at 37°C for 24 hours (8).

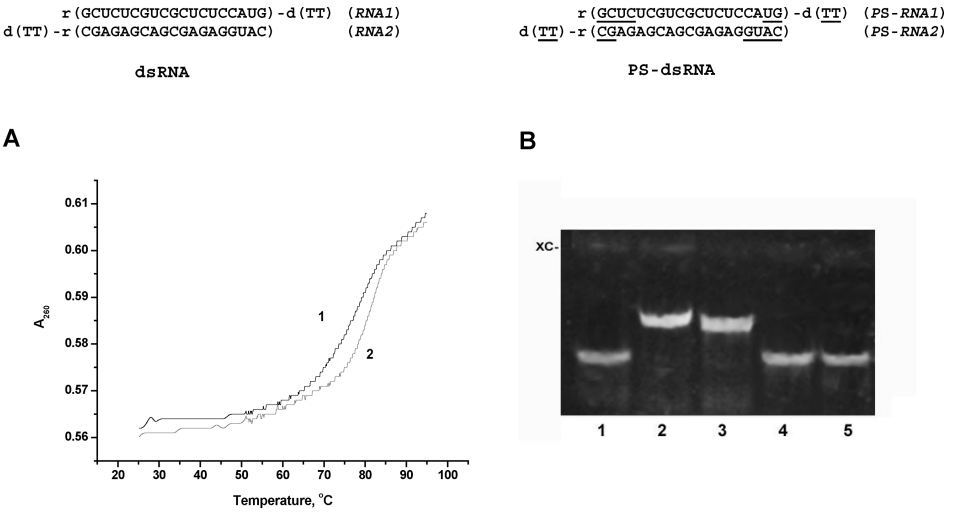


FIGURE 4 PS-modified dsRNA designed to inhibit production of green fluorescent protein (GFP) in eukaryotic cells containing stereodefined *R*p phosphorothioate nucleotide residues (nucleotides with PS linkages are underlined). **A** - Melting curves of PS-dsRNA (1) and the control natural dsRNA (2). **B** – Analysis of duplexes formed by oligoribonucleotides and their PS-analogues by native electrophoresis in 12% polyacrylamide gel: 1 – ss*RNA-1*; 2 – PS-dsRNA; 3 – natural dsRNA; 4 – *PS-RNA1* and 5 - *PS-RNA2* (staining with ethidium bromide).

our approach allows automatic stereo-specific synthesis of P-chiral phosphorothioate DNA and RNA analogues on a solid phase. Two routes for the synthesis of P-chiral phosphorothioate monomers were developed. Using the first route, the synthesis is started from a protected nucleoside H-phosphonate (Scheme 2), whereas in the second route a protected nucleoside is used as the starting compound (Scheme 3). Using such P-chiral monomers, the solid phase synthesis of several stereospecific phosphorothioate oligoribo- and oligodeoxyribonucleotides by the solid phase phosphotriester method with O-nucleophilic intramolecular catalysis has been accomplished, and their properties were investigated (Figures 3 and 4). It should be noted that no epimerization at the phosphorus center during the condensation and deprotection steps as well as no formation of unwanted phosphodiester bonds resulted from P-S-bond activation by the condensing agent was observed.

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