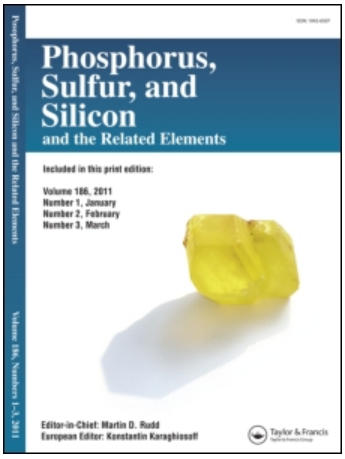

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Phosphoramidites as Synthons for Polynucleotide Synthesis

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PHOSPHORAMIDITES AS SYNTHONS FOR POLYNUCLEOTIDE SYNTHESIS

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Abstract Methods for the rapid synthesis of DNA and RNA are described. The procedures involve using nucleoside phosphoramidites as synthons and silica as a polymeric support. Additionally, a novel reaction involving nucleoside O-alkyl methylphosphonothioates is described.

Over the past 12 years, my laboratory has focused on developing procedures for synthesizing polynucleotides and then using these methods in combination with other developments in molecular biology and biochemistry to study various biological processes[1]. Because DNA synthesis methodologies have been extremely cumbersome[2], an initial objective was to develop high yielding and rapid procedures for synthesizing biologically active deoxyoligonucleotides[1]. This methodology, including recent modifications and improvements, is summarized in Figure 1. The

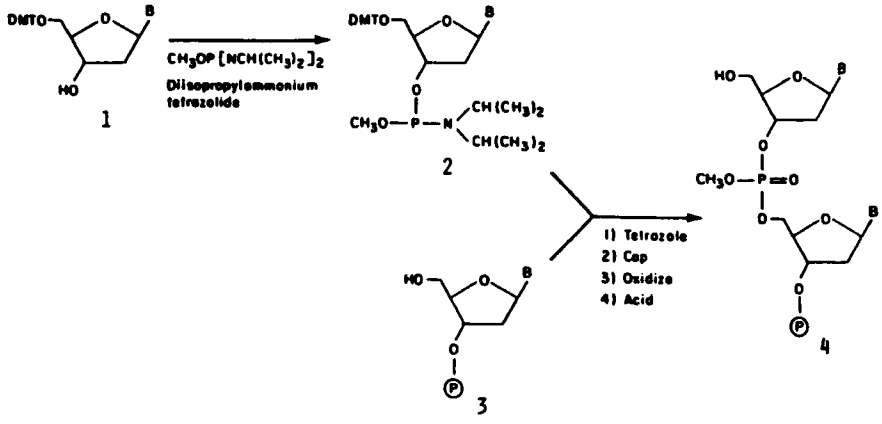

FIGURE 1 Steps in the synthesis of a dinucleotide using the current phosphoramidite procedure. Abbreviations: DMT, dimethoxytrityl; Ⓟ, silica support; B, thymine and appropriately protected cytosine, adenine or guanine; cap, acetic anhydride plus dimethylaminopyridine; oxidize, iodine in lutidine and water; acid, see text.

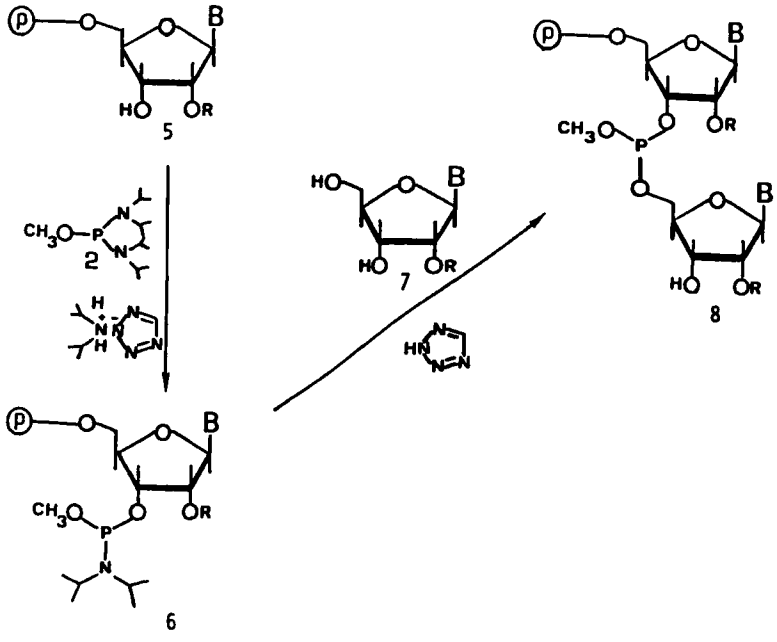

FIGURE 2 Regioselective RNA synthesis on silica supports. Abbreviations: B, uracil or appropriately protected cytosine, guanine and adenine; R, tetrahydropyranyl.

first step is conversion of protected deoxynucleosides to **2** by phosphitylation with bis(diisopropylamino)methoxyphosphine. Activation of the phosphine using diisopropylammonium tetrazolide is specific since 3'-3' dinucleotides or dinucleotide 3'-phosphinic acids are not produced. The next step is condensation of **2** with **3**, a deoxynucleoside linked via an ester to a silica support[1], using tetrazole as a catalyst (one minute). The remaining steps for addition of one nucleotide involve acylation of unreacted **3**, oxidation of phosphite to phosphate, and removal of the dimethoxytrityl ether with dichloroacetic acid. The resulting deoxydinucleotide, compound **4**, can be recycled through the same chemistry to produce deoxyoligonucleotides containing 100-200 monomers.

More recently this chemistry has been adapted to the synthesis of oligoribonucleotides. The approach (Figure 2) involves condensation of **5**, a ribonucleoside esterified to silica through the 5'-hydroxyl, with excess bis(diisopropylamino)methoxyphosphine to form **6** in high yield. This synthesis requires one hour. Because diisopropylammonium tetrazolide, a weak acid salt, is used to activate the phosphine, the product is stable toward hydrolysis or oxidation. Once the synthesis is complete, the solution is filtered from the support and a 2' protected nucleoside (compound

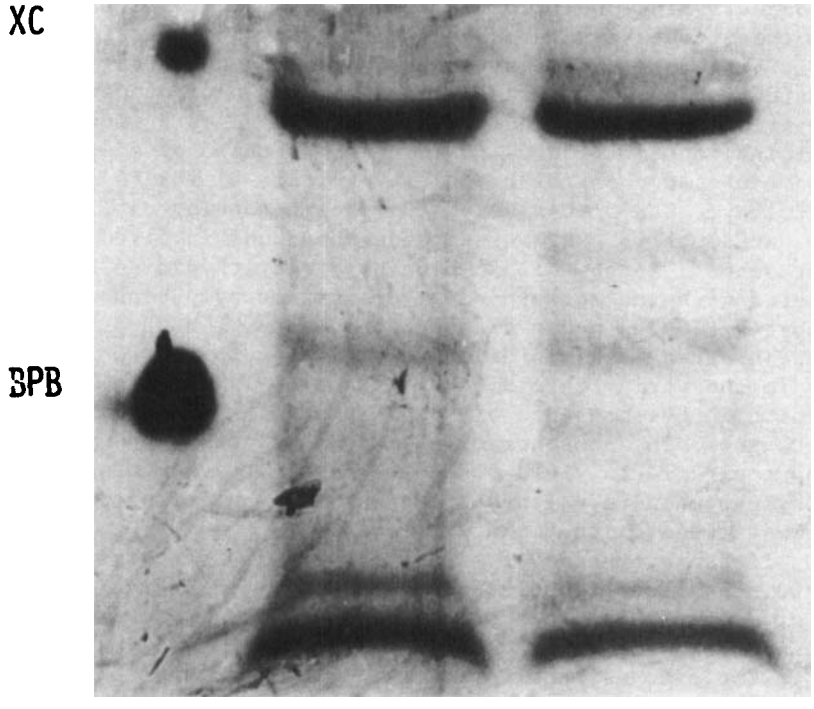

FIGURE 3 Purification of synthetic RNA by polyacrylamide gel electrophoresis. Abbreviations: XC, xylenecyanol blue; BPB, bromophenol blue.

7) and tetrazole is added. Tetrazole being much more acidic than the tetrazolide salt, activates the phosphoramidite which leads to regioselective condensation with the 2'-protected nucleoside to form **8**, a 3'-5' dinucleoside phosphite attached to the support. Oxidation of phosphite to phosphate completes the steps required for addition of one nucleotide. Therefore these synthons are quite simple since only protection on the 2'-hydroxyl and the exocyclic amino groups are needed. The still unresolved problem of identifying compatible protecting groups on the 2', 3', and 5'-hydroxyls ceases to exist.

A typical result is shown in Figure 3. After synthesis, the oligoribonucleotide is treated to remove protecting groups and then purified by polyacrylamide gel electrophoresis. Two main products are observed in the crude reaction mixture. The fast migrating compound is a marker, uridine 2' and 3'-phosphate. The second major band is CAAUAUUUACCUUUG, the product of the synthesis. Enzymatic analysis confirmed that internucleotide linkages were exclusively 3'-5'.

During the course of investigating chiral phosphate analogs of nucleotides, we centered our interest on methylphosphono-

thioates. Since O-Alkyl methylphosphonothioates are chiral, do not racemize easily in aqueous solutions, and have four chemically different moieties, they may be important reagents for elucidating enzymatic reactions involving nucleotides or be potential inhibitors for proteins that possess a wide range of medicinal applications. In relation to these studies, we observed an unexpected chemical reactivity (Figure 4) for 5'-O-dimethoxytritylthymidine-3'-O-alkoxymethylphosphonothioates (**11a,b**).

Attempts to synthesize these compounds involved two approaches. Method A: 5'-Dimethoxytritylthymidine (**9**) was reacted with the corresponding O-alkyl methylphosphonochlorothioates (**10a,b**) under Einhorn conditions[3]. Method B: Compound **9** was converted with bis(diisopropylamino)methylphosphane to 5'-dimethoxytritylthymidine-3'-diisopropylaminomethylphosphonoamidite in the presence of a weakly acidic ammonium salt or ion exchanger. The phosphoramidite was then condensed with 3-hydroxyproprionitrile to yield 5'-O-dimethoxytritylthymidine-3'-O-alkoxymethylphosphonite which was subjected to sulfurization with elemental sulfur to yield **11b**.

If the synthesis of either **11a** or **11b** is attempted in dry pyridine at room temperature (method A), tlc analysis within the first 30 min shows that products are initially formed which possess a lower R_f than compound **9** in various solvent systems. After three hours, the initially formed compounds have been converted into **12** which has a higher R_f than **9**. If method B is used, **12** is obtained after oxidation with elemental sulfur in 2,6-lutidine or pyridine. Thus the O-alkoxymethylphosphonothioate moiety is eliminated from **11a,b** irrespective of the synthetic

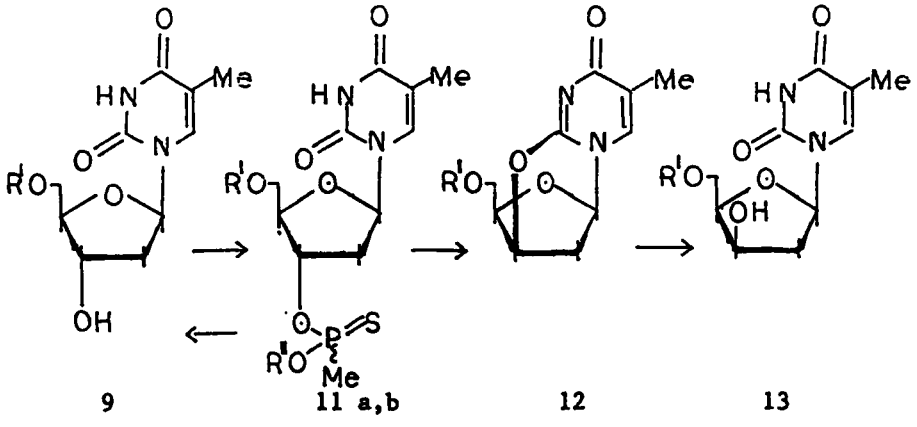

FIGURE 4 Reactions of 5'-O-Dimethoxytritylthymidine-3'-O-alkoxymethylphosphonothioates. **11a**, R" = octyl; **11b**, R" = β-cyanoethyl; R' = dimethoxytrityl.

method. This was especially the case in pyridine containing either 5% triethylamine or Hünig's base where compound **12** was the only observed product.

Removal of the O-alkyl methylphosphonothioate moiety in the absence of a nucleophile suggests an elimination rather than a hydrolysis mechanism. This is also supported by a faster rate of phosphonothioate cleavage than what is expected from hydrolysis[4]. The product of this reaction, compound **12**, is also hydrolyzable with water and forms **13**. These observations lead to the following mechanistic proposal. First the thymine N^3 proton (pK_a:9.93) is removed in the presence of Hünig's base or triethylamine ($pK_a \approx 10$). The resulting anion can then nucleophilicly attack the 3' carbon of the ring through the O^2 moiety. This leads to nucleophilic displacement of O-alkyl methylphosphonothioate concommitant with the formation of a 2,3'-anhydronucleoside (**12**) which has a different UV spectrum than the acyclic precursor. Compound **12** hydrolyzes in basic, aqueous solution to **13**, the correponding deoxyxylonucleoside. This mechanistic scheme was further proved by the synthesis of 5'-dimethoxytrityl-3-N(2-methoxyethoxymethyl)-3'-O-n-octyl methylphosphonothioate which is stable under the conditions that lead to cleavage for the N^3 unprotected nucleoside. Therefore the N^3 proton is essential for the removal of O-alkyl methylphosphonothioate moieties on the 3'-carbon.

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References

1. M. H. Caruthers, Science 230, 281-285 (1985).
2. V. Amarnath and A. D. Broom, Chem. Rev. 77, 183-217 (1977).
3. H. Henecka, Houben Weyl Methods of Organic Chemistry Vol. VIII, 359-680, 1952.
4. B. A. Connolly, B. V. L. Potter, F. Eckstein, A. Pingoud and L. Grotjahn, Biochemistry 23, 3443-3453 (1984).