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SYNTHESIS OF PHOSPHOROMONOAMIDATE DIESTER NUCLEOTIDES VIA THE PHOSPHITE-AZIDE COUPLING METHOD

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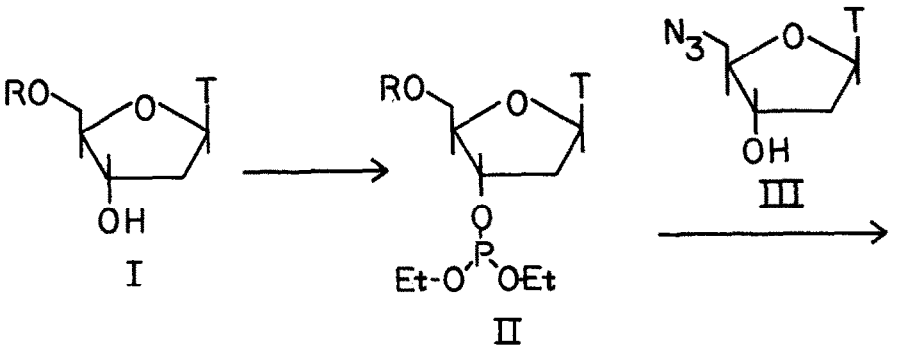
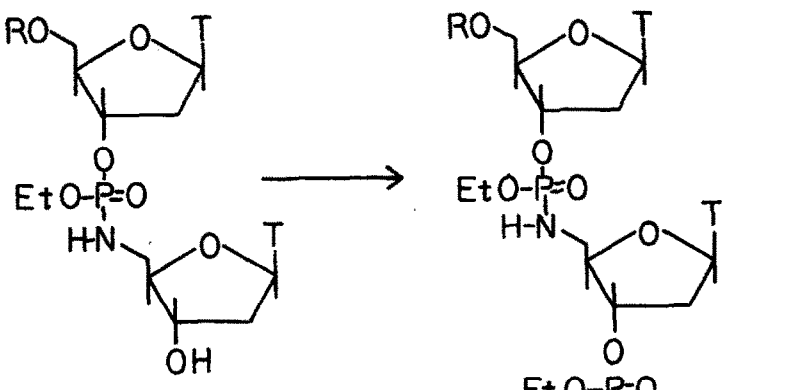
There has been increasing interest in the synthesis of oligonucleotides and analogs for potential biological investigation. The synthetic methods generally employed involve coupling with highly active condensing agents such as carbodiimides or aryl sulfonyl halides to give either diester or triester nucleotides[1,2]. We have been interested in the synthesis of phosphoramidate DNA[3,4], and we now wish to report a new method for the synthesis of phosphoromonoamidate diester nucleotides involving the highly specific coupling reaction between phosphites and azides.

It has been reported by two groups that trialkyl and triaryl phosphites will react with aliphatic azides to give phosphoromonoamidate diesters[5,6]. We have found that the 3'-hydroxyl of 5'-α-naphthylcarbamoylthymidine (Ia) reacted with one equivalent of diethyl phosphorochloridite in anhydrous pyridine gave quantitative conversion (as determined by TLC) to the corresponding phosphite (IIa) in less than two minutes. The addition of 5'-azido-5'-deoxythymidine (III) resulted in the visible evolution of nitrogen and the formation of the phosphoramidate dinucleotide IVa at room temperature.[7] The product was readily purified by either regular column, short column or thin layer chromatography on silica gel and was precipitated from tetrahydrofuran-heptane. The reaction was inhibited by water and the selective elimination of an ethyl group over the nucleoside from the mixed phosphite was enhanced by the addition of an excess of anhydrous lithium chloride to the reaction mixture. When an excess of lithium chloride was added the yield of the dinucleotide was 61-66%. Omission of the

lithium chloride resulted in the formation of large amounts of the diethyl phosphoramidate VI. The identity of VI was confirmed by an independent synthesis[6].





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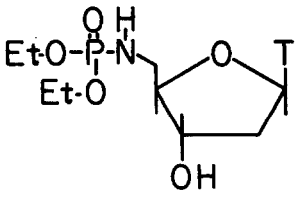
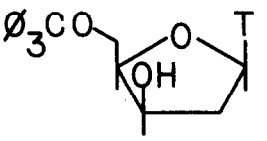
a α-naphthylcarbamoyl

b trityl

In the reaction of Ib with diethyl phosphorochloridite followed by 5'-azide-5'-deoxythymidine in the absence of lithium chloride, the major product isolated was the 5'-trityl-2'-deoxyxylose (VII). This compound was synthesized by an independent route and proved identical to the material isolated from the reaction mixture. It was determined that this



VI



VII

compound was not formed through the anhydronucleoside, since the anhydronucleoside proved to be stable under the reaction conditions.

The reaction is believed to procede by initial formation of the phosphite imine, with elimination of nitrogen, followed by its conversion to the phosphoramidate by a mechanism similar to that of the Michaelis-Arbuzov reaction.

The dinucleotide IVa, in anhydrous pyridine, reacted cleanly with one equivalent of diethyl phosphorochloridite to give quantitative conversion (TLC) to the corresponding phosphite. The reaction of this phosphite with a 50% excess of 5'-azido-5'-deoxythymidine at room temperature in the presence of a ten fold excess of lithium chloride gave the trinucleotide Va in 48% yield.

The phosphite-azide coupling procedure for the formation of phosphoromonoamidate diester nucleotides offers several advantages over the conventional coupling methods. The spontaneous coupling of the phosphite with the azide requires no coupling or activating agents; the coupling conditions are compatable with unprotected hydroxyl and amino functions in the azido compounds, eliminating the need for the selective protection and subsequent deblocking of the 3'-hydroxyl for further chain elongation; and the reaction is highly selective and procedes in good yield.

Other dialkyl and diaryl phosphorochloridites have been used; and the details of this work will be published later.

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