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THE SYNTHESIS OF PHOSPHITE ANALOGUES OF RIBONUCLEOTIDES

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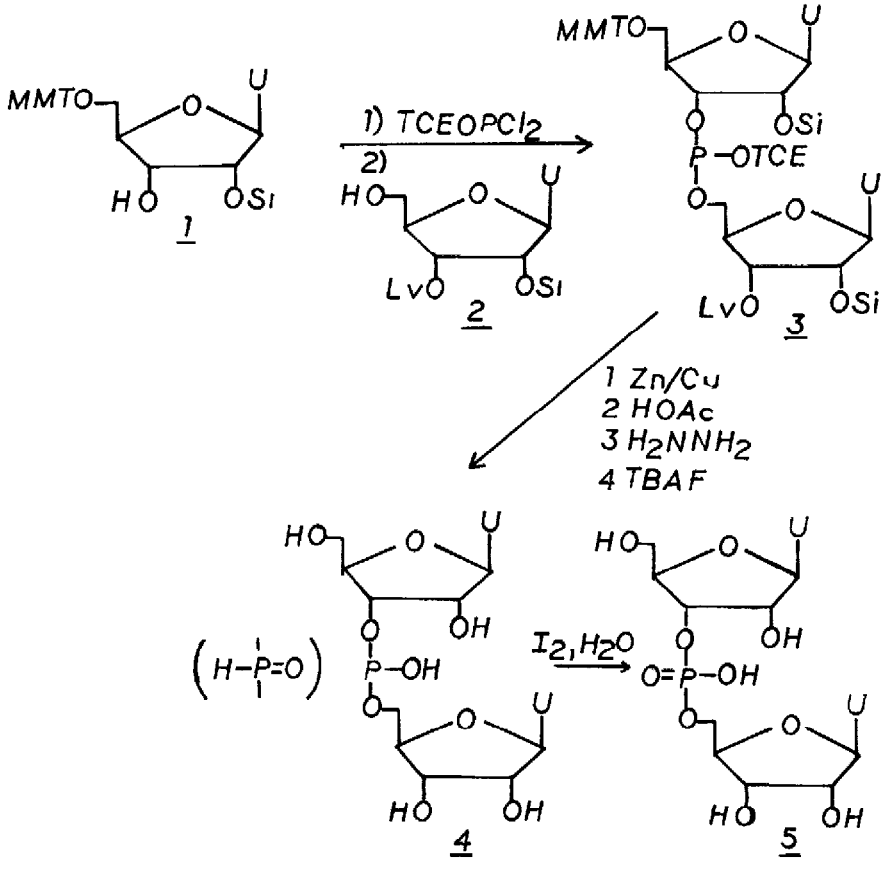
The synthesis of compounds having a phosphite bridge between nucleoside units, their characteristics toward phosphodiesterases, their conversion to normal nucleotides and their use in oligonucleotide synthesis is described

Since Letsinger's introduction of the chlorophosphite procedure (1) for the synthesis of deoxynucleotides, phosphites have been widely used as unisolated intermediates in the synthesi of oligonucleotides (2-5). We wish to describe the isolation and characterization of the phosphites themselves. These compounds are shown to be stable molecules which provide interesting analogues of natural nucleotides and which can be rapidly oxidized to natural nucleotides The elimination of the oxidation step until the last condensation step further simplifies the chlorophosphite procedure for producing oligonucleotides. In the accompanying paper in this issue, we show that the phosphites provide access to a wide variety of novel nucleotide analogues.

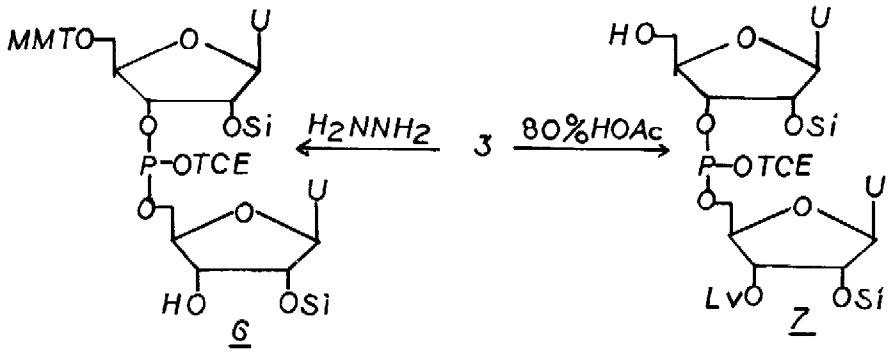
The synthesis of the phosphite derivative 3 illustrates the general procedure The first step involves the addition of 5'-methoxytrityl-2'-TBDMS uridine (1, 300 mg, 0 17 mmole) in THF (0.5 ml) to trichloroethylphosphorodichloridite (TCEOPCl$_2$, 78 µl, 1.1 eq) in THF (0.5 ml) containing collidine (0.28 ml, 2 mmole) at -78°C. After 10 min, 3'-levulinyl-2'-TBDMS uridine (2, ref 6, 172 mg, 0 38 mmole) in THF (1 ml) was added and the solution maintained at -78°C for 15 min. The solution was concentrated at reduced pressure and applied to TLC plates developed in ether chloroform ethanol (2.5 0 9 0 1). Compound 3 was isolated in 85% yield (mp 127-130°C, λ_{max}^{EtOH} 260 nm, R_f^{ether} 0 31, ^{31}P nmr, δ, -136 3, -136.7 ppm (H_3PO_4)) Compound 3 was further characterized by iodine oxidation to the phosphate ester which has been previously described (6, ^{31}P nmr, δ, +3 50, +3 08 (3 1))

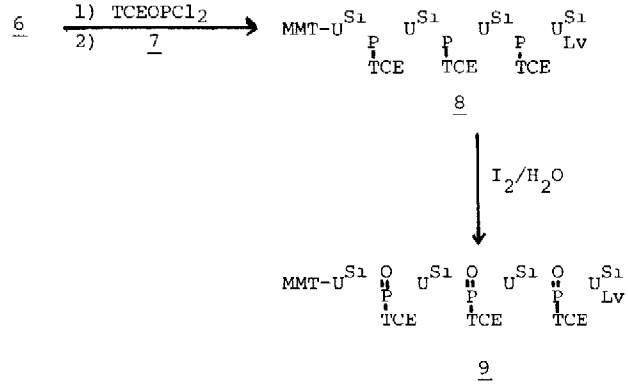
Compound 3 was treated successively with Zn/Cu in DMF at 50°C for 6 h, 80% HOAc at 80°C for 15 min, hydrazine in pyridine-acetic acid (7) and TBAF (8) to give the free phosphite 4 as the sole product (4, R_f^A 0.30, R_M^{TP} 0 65 (see reference 8 for solvents) The phosphite 4 was degraded by snake venom phosphodiesterase to uridine and uridine 5'-phosphite, R_f^A 0 23, R_M^{TP} 0.52, which was identical to the compound reported by Holy (9)(uridine 5'-phosphate has values of R_F^A 0.11 and R_M^{TP} 1 05). The phosphite 4 was unaffected by spleen phosphodiesterase and by ribonuclease A conditions which routinely degrade normal nucleotides However, on treatment with iodine in water-pyridine (10 min) 4 was completely oxidized to the natural

nucleotide UpU (5, R_f^A 0 19, R_M^{TP} 0.51) which was completely degraded by all three enzymes to the expected products and in the correct ratios



In all syntheses of oligonucleotides using the chlorophosphite procedure reported to date oxidation of the intermediate 3 to the phosphate ester is done prior to isolation and subsequent chain extension While the oxidation is rapid, subsequent extraction procedures can be time consuming. We have found that compounds such as 3 can undergo chain extension directly and oxidation need not be carried out until the final step For example, 3 can be converted to 6 with hydrazine hydrate (5 min) and to 7 with 80% acetic acid (10 min, 80°C). The coupling of 6 (0.12 mmole) with $TCEOPCl_2$ (1 eq.) and 7 (0 095 mmole) according to the above procedure gave the tetranucleoside triphosphite (8) in 54% yield (mp 135 - 140°C, λ_{Max}^{EtOH} 261, R_f^{ether} 0 24) Compound 8 was oxidized by iodine/water (5 min, THF/pyridine solvent) to give the nucleotide 9 in quantitative yield (9, R_f^{ether} 0 04, identical to sample characterized in ref. 6).





The phosphite analogues of natural nucleotides are stable and can be isolated. They are resistant to spleen and ribonuclease A but are degraded by snake venom Thus, they provide a novel analogue system to natural nucleotides and are currently undergoing biological testing The phosphites also provide a direct route to numerous novel analogues of natural nucleotides (next article) Of equal importance in terms of the rapid synthesis of normal oligonucleotide is the further reduction in time and effort afforded by the elimination of the oxidation step until the final condensation step in chain synthesis.

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