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THE SYNTHESIS OF PHOSPHORAMIDATES FROM SILYLPHOSPHITES AND AZIDES

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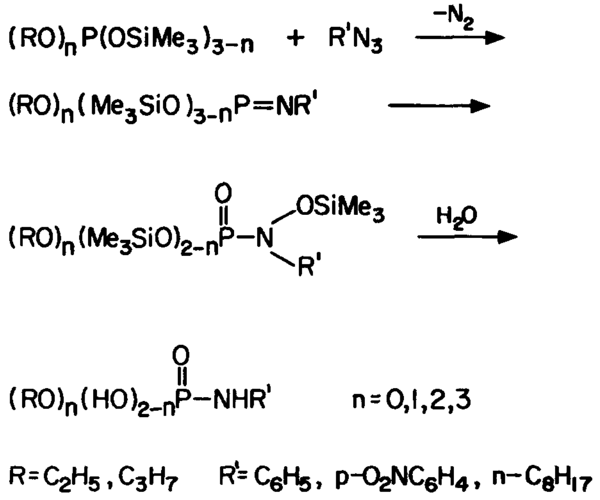
Phosphoramidates are of interest as analogues of biologically-important phosphates. Amino acid derivatives of nucleotides are being investigated as intermediates in protein synthesis.[1] Oligoazanucleotides, which are P-N analogues of natural oligonucleotides, are under study as templates for DNA-dependent enzymes by us as well as by other workers.[2]

We have found that phosphoramidates can be obtained with particular ease by condensing azides with silylphosphites as shown in Scheme 1. Mono- and diesters of phosphorus acid do not react with azides at normal temperatures. However, the silyl derivatives of these esters react with azides at room temperature with elimination of nitrogen. Particularly important



$$(RO)_nP(OSiMe_3)_{3-n} + R'N_3 \xrightarrow{-N_2}$$

$$(RO)_n(Me_3SiO)_{3-n}P{=}NR' \longrightarrow$$

$$(RO)_n(Me_3SiO)_{2-n}\overset{O}{\overset{\|}{P}}-N\begin{matrix}OSiMe_3\\R'\end{matrix} \xrightarrow{H_2O}$$

$$(RO)_n(HO)_{2-n}\overset{O}{\overset{\|}{P}}-NHR' \qquad n=0,1,2,3$$

$$R=C_2H_5, C_3H_7 \qquad R'=C_6H_5,\ p\text{-}O_2NC_6H_4,\ n\text{-}C_8H_{17}$$

SCHEME I

is our finding that the trimethylsilyl group migrates from oxygen to nitrogen in the postulated phosphoimine intermediate so readily that no alkyl migration is observed. The reaction of phosphines with organic azides was discovered by Staudinger.[3] Phosphite triesters were shown to behave similarly by Kabachnik and Gilyarov.[4] 5-Azidonucleosides have been reacted with trialkyl and triaryl phosphites.[5,6] Oligoazanucleotides have been obtained in moderate yields by condensing 5'-azido-5'-deoxythymidine with thymidine diethyl-3'-phosphite.[7] Elimination of both nucleoside and ethyl groups from the phosphoimine was observed. The reaction of dimethyl trimethylsilylphosphite with phenylazide has been reported.[8a] Nucleoside silylphosphites were first described and used in nucleotide syntheses by Hata and Sekine.[8b]

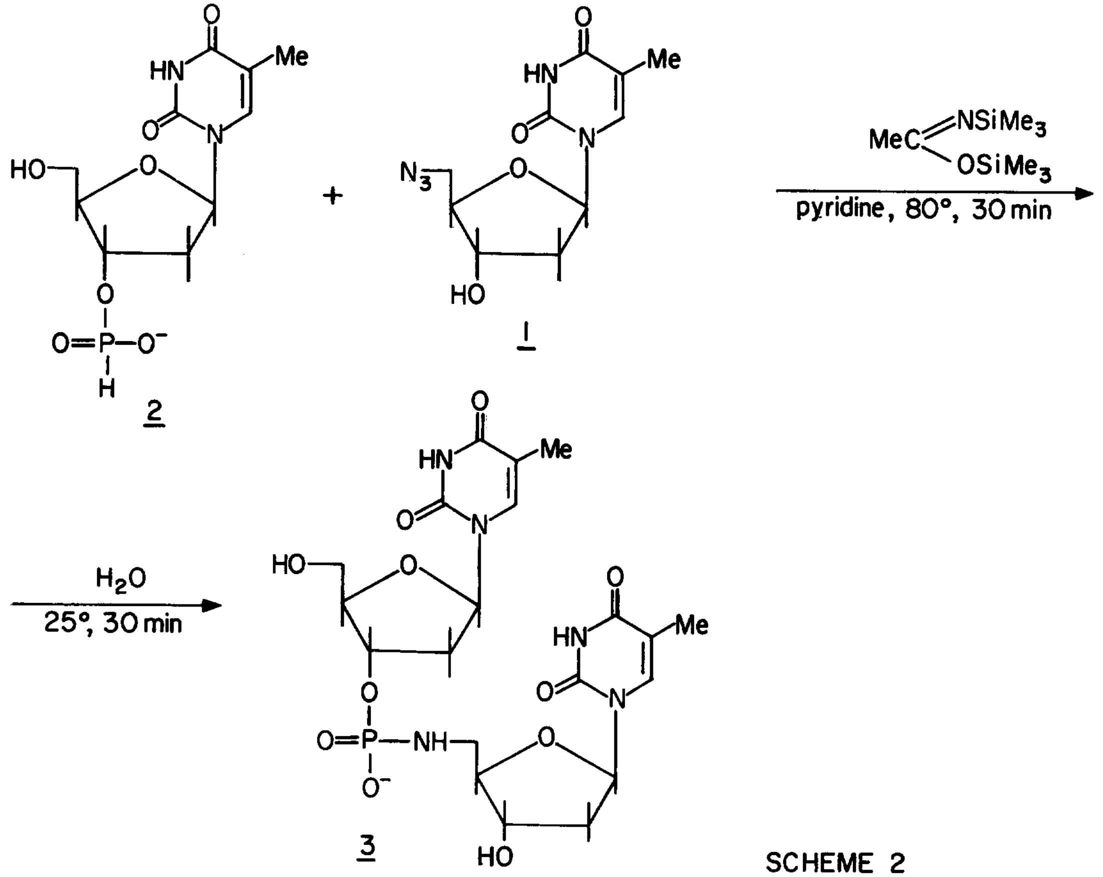
In a typical example of the new method the silylation reaction and the azide condensation are conducted simultaneously. A 0.02M solution of the phosphite in pyridine is treated with two moles of bis-(trimethylsilyl)-acetamide (BSA) and one mole of azide. The mixture is heated at 80° under nitrogen until the evolution of gas ceases (20-30 minutes). The reaction also goes to completion at 25° in 2-3 days. The isolated yields of the phosphoramidates shown in Scheme 1 are 90-99%. The identity of the products was established by comparison of their IR and UV spectra and chromatographic mobilities with those of authentic samples prepared by published procedures.

Silylation is the rate-limiting step. Infrared spectroscopy showed practically no silylation at room temperature either by BSA or by chlorotrimethylsilane. However, the reaction of the silylphosphite present in low concentration with the azide occurs so readily that only stoichiometric amounts of silylating agent are required.

The usefulness of various silylating agents was determined by heating a 0.2M solution of diethyl phosphite with two moles of the silylating agent in acetonitrile for 30 minutes at 70°, adding one mole of p-nitrophenylazide at 25° and determining the rate of formation of diethyl N-p-nitrophenylphosphoramidate. Bis-(trimethylsilyl)-acetamide and bis-(trimethylsilyl)-trifluoroacetamide gave quantitative yields of phosphoramidate within 3 hours. Practically no phosphoramidate was obtained when either N-(trimethylsilyl)-imidazole or hexamethyldilazane were used. Moderate yields were realized with chlorotrimethylsilane plus an equimolar amount of triethylamine. These results reflect more than the extent of silylphosphite formation. For example, hexamethyldisilazane gave diethyl trimethylsilylphosphite in 90% yield under the conditions given above as estimated by IR spectroscopy.

Significant solvent effects were observed for the silylphosphite-azide reaction. The rate of nitrogen evolution was measured for the reaction of pure diethyl trimethylsilylphosphite and p-nitrophenylazide (0.2M) in various anhydrous solvents at 25°. The rates were highest for acetonitrile and pyridine, slower in dioxane (50% reaction in 2 days), and imperceptible in tetrahydrofuran and hexamethylphosphorictriamide. A rapid reaction occurred in dimethylformamide, but the reaction mixture was heavily contaminated by a product of the reaction of DMF with BSA.

Application of the silylphosphite-azide method to the synthesis of oligoazanucleotides was demonstrated by the preparation of thymidyl-(3'-5')-5'-amino-5'-deoxythymidine, 3, as shown in Scheme 2. Equimolar amounts of 5'-azido-5'-deoxythymidine, 1, and thymidine-3'-phosphite, 2,[9] gave 3 in nearly quantitative yield as estimated from paper chromatography and in an isolated yield of 82%, by aqueous extraction of the ammonium salt of 3 from the reaction mixture. The product gave the correct elemental analysis and was identical with the major product obtained from the reaction of 5'-amino-5'-deoxythymidine with thymidine-3'-phosphate in the presence of triphenylphosphine and 2,2'-dipyridyl disulfide.[10] The method is being extended to the synthesis of other dinucleoside phosphoramidates and to the homopolymerization of 5'-azidonucleoside-3'-phosphites.



SCHEME 2

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