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A NEW AND GENERAL PROCEDURE FOR THE PREPARATION OF DEOXYNUCLEOSIDE PHOSPHORAMIDITES

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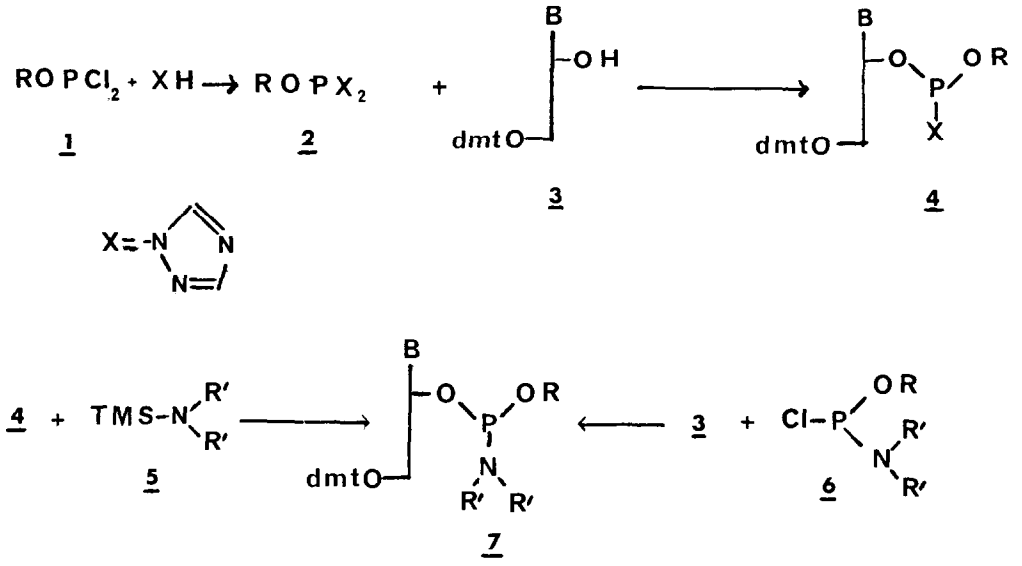
Summary: Nucleoside phosphoramidites can be obtained in a one pot reaction by reacting successively an alkyloxy (or aryloxy)bis-triazolylphosphine with a 3'-OH free deoxynucleoside and a trimethylsilylated secondary amine.

Two strategies are currently used for the synthesis of oligodeoxynucleotides depending on the P-O bond formation method . Besides the classical phosphotriester approach[1] LETSINGER pioneered a procedure with takes advantage of the very high reactivity of alkyloxydichlorophosphines 1 towards alcohols to give phosphite triesters which are subsequently oxidized to phosphates.[2] Application of this methodology to solution[3] as well as to solid phase[4] synthesis is now well established.

Recently, the introduction of methylphosphoramidite 7b building blocks in replacement of the very unstable chloromethoxyphosphine intermediates 4b (X = Cl) greatly enhanced the practicability of the phosphite strategy in solid phase synthesis.[5]

The current preparation of phosphoramidites 7a (or 7b) rests on the coupling of a 3' -OH free 5'-O-protected nucleoside 3 in the presence of a base with a chlorodialkylaminomethoxyphosphine 6 . Unfortunately , these reagents exhibit a poor stability upon storage even under a dry and inert atmosphere . Moreover, in view of their thermal unstability during purification by distillation which requires very high vacuum when R and R' represent a higher homologue of methyl they are not easy to prepare . Also phenyl substituted phosphines such as 6c and 6d are so far unknown.

Scheme



dmt = 4,4'-dimethoxytrityl TMS = trimethylsilyl

Compounds

1, 2, 4, 8 a: R = CH_3
b: R = o-C_6H_4Cl

5 a: R',R' = $(CH_2CH_2)_2O$
b: R' = CH_3

6, 7 a: R = CH_3, R',R' = $(CH_2CH_2)_2O$
b: R = R' = CH_3
c: R = o-C_6H_4Cl, R',R' = $(CH_2CH_2)_2O$
d: R = o-C_6H_4Cl, R' = CH_3

Table. ^{31}P NMR δ values (ppm) of phosphoramidites 7a[10]

B			B		
T	144.98	144.73	A^{Bz}	145.21	144.96
C^{Bz}	145.45	145.14	G^{Ibu}	145.59	144.96

Accordingly, it was highly desirable to explore a new efficient synthesis of phosphoramidites 7. We herein describe a simple and general access to this class of compounds which might prompt the study of the influence of various R and R' groups on the reactivity and stability of phosphoramidites 7.[6]

The one-pot reaction sequence leading to phosphoramidites 7 is outlined in the Scheme. In the first step the nucleoside 3 is added slowly to the bis-triazolide 2[4a, 7] to give the intermediate 4 which upon reaction with a N,N-dialkyltrimethylsilylamine 5 provides the expected phosphoramidite 7. The use of the bis-triazolide 2 instead of the corresponding dichlorophosphine 1 is recommended in order to minimize the formation of the unwanted 3',3'-dinucleoside phosphite 8.[7]

The preparation of the thymidine derivative 7a illustrates the typical reaction conditions: dichloromethoxyphosphine[8] 1a (2 equivalents) was added to a THF solution of triazole (7 equivalents) and N,N-diisopropylethylmine (8 equivalents). To this solution which was cooled at -78°C was added dropwise one equivalent of 5'-O-dimethoxytritylthymidine 3 (B = thyminyl) in THF. This resulted in the immediate disappearance of the nucleoside as checked by silica gel TLC (ethyl acetate). At this stage N-trimethylsilylmorpholine 5a[9] (8 equivalents) was added and the cooling bath removed. When the solution reached room temperature it was extracted as usual with ethyl acetate to give the phosphoramidite 7a in yield over 90%.

Compound 7a was characterized by ^{1}H and ^{31}P NMR. The ^{1}H NMR spectrum (benzene d_6) showed H-3' as a broad triplet centered at 4.75 ppm, the chemical shifts and patterns of other signals were in agreement with the proposed structure. The ^{31}P NMR spectrum[10] of a 7a exhibited only two singlets at 144.98 and 144.73 ppm, respectively, as expected for two diastereoisomers. Addition of methanol and tetrazole[5] to a solution of 7a resulted in the complete disappearance of the two signals which were replaced by a new signal at 141.27 ppm which indicated the exclusive formation of the dimethyl phosphite 4a (X = OCH_3).

The same reaction conditions were used to prepare the corresponding phosphoramidite derivatives 7a of the three other deoxynucleosides having B = C^{Bz}, A^{Bz} and G^{Ibu}. As in the case of 7a (B = T) their ^{31}P NMR spectra were measured and the observed δ values are given in the Table. It could be inferred from these spectra (absence of signal at $\sim$ 140 ppm) that the formation of 3',3'-dinucleoside phosphite 8a had been completely avoided.

In the same manner, by adding N,N-dimethyltrimethylsilylamine 5b to the triazolide derivative 4a (B = T) the phosphoramidite 7b (B = T) could be obtained in high yield.

In order to establish further the versatility of the method we pre-

pared in situ from o-chlorophenoxydichlorophosphine 1b,[11] the corresponding bis-triazolide 2b, which upon successive reaction with 5'-O-dimethoxytrityl-thymidine 3 followed by reaction with the silylated secondary amine 5a (and 5b) provided the arylphosphoramidite 7c (and 7d), a derivative which should be difficult to obtain otherwise. In both cases the main reaction product was contaminated by a small amount (< 15%) of 3',3'-dinucleoside phosphite 8b. However, complete purification of 7c (and 7d) could be achieved by short column chromatography over silica gel (elution with hexane:ethyl acetate:triethylamine/49:49:2). Under these conditions phosphoramidites 7c (and 7d) were obtained in 50% overall yield.

In conclusion, we have developed a general and efficient route for the preparation of phosphoramidites 7. In particular this method gives an access to the new derivatives 7 (R = o-C_6H_4Cl) and an investigation of their reactivity is currently in progress in this laboratory.

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