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STUDIES ON THE REACTION OF NUCLEOSIDE PHOSPHORODIESTERS WITH ARYL SULFONYL CHLORIDES

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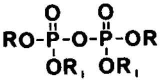
Abstract

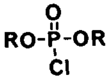
It was found that activation of phosphorodiesters by aryl sulfonyl chlorides does not stop at the stage of symmetrical pyrophosphates I but proceeds further with the formation of diester chlorophosphates II.

The reaction of phosphorodiesters, especially nucleoside aryl (or alkyl) phosphates with aryl sulfonyl chlorides has attracted considerable attention in several laboratories because of its importance for oligonucleotide synthesis via the phosphorotriester approach.[1] Detailed studies of the individual steps of phosphorotriester synthesis revealed that phosphorodiesters react with aryl sulfonyl chlorides producing the corresponding pyrophosphates of type I, which were postulated to be the reactive intermediates during the coupling reactions.[2]



$$RO-\underset{OR_1}{\overset{O}{\overset{\|}{P}}}-O-\underset{OR_1}{\overset{O}{\overset{\|}{P}}}-OR$$

Ia R=DMT-T-, R_1O=thiophenyl
(δ 13.8, 13.3, 12.5 ppm)
Ib R=DMT-T-, R_1=4-chlorophenyl
(δ -19.8, -20.2, -20.4 ppm)
Ic R=R_1=phenyl
(δ -25.5 ppm)
Id R=R_1=ethyl
(δ -13.5 ppm)



$$RO-\underset{Cl}{\overset{O}{\overset{\|}{P}}}-OR_1$$

IIa R=DMT-T-, R_1O=thiophenyl
(δ 29.8, 29.6 ppm)
IIb R=DMT-T-, R_1=4-chlorophenyl
(δ -2.2, -2.6 ppm)
IIc R=R_1=phenyl
(δ -6.2 ppm)
IId R=R_1=ethyl
(δ 2.7 ppm)

Abbreviations: DMT-T-, 5'-<u>O</u>-dimethoxytritylthymidine-3'-yl; TPS-Cl, 2,4,6-triisopropylbenzenesulfonyl chloride; BS-Cl, benzenesulfonyl chloride; NO_2BS-Cl, 4-nitrobenzenesulfonyl chloride; pyHCl, pyridinium hydrochloride.

However, during the activation process or coupling, at least an equimolar amount of a relatively strong nucleophile, i.e. chloride anion, is generated in the reaction mixture. This may react with a pyrophosphate to form another reactive species, namely diester chlorophosphate of type II. Such a reaction pathway has not been postulated for the formation of phosphorotriesters until now. Our studies on the reaction of nucleoside phosphodiesters with aryl sulfonyl chlorides indicate, that such a reaction pathway is possible, since we have found that diester chlorophosphates are formed from diesters upon the activation by aryl sulfonyl chlorides (3).

Reaction of 1 equiv. of TPS-Cl with 5'-O-dimethoxytritylthymidine S-phenyl 3'-phosphorothioate in pyridine produced Ia and no changes occurred in the reaction mixture during next 2 h as revealed by ^{31}P NMR spectroscopy. Addition of 6 equiv. of pyHCl did not cause any change. Addition of another 6 equiv. of TPS-Cl produced after 1 hr ca 20% of chlorophosphate IIa (two singlets at 29.8 and 29.6 ppm). The formation of IIa was found to be substantially faster when BS-Cl (9 equiv.) or NO_2BS-Cl (9 equiv.) were used instead of TPS-Cl. In the first reaction, after 30 min. ca 50% of IIa was formed and with NO_2BS-Cl the complete conversion of Ia into IIa occurred during 8 min.

The above experiments indicate that the equilibrium Ia $\rightleftharpoons$ IIa in the absence (or presence of a slight excess) of coupling agent is to the left, not because Cl^- is a poor nucleophile, but because the reaction of IIa with the phosphate anion, generated during an attack of Cl^- on Ia, is very fast. To shift the equilibrium toward IIa, a reactive coupling agent is needed, which can trap the phosphate anion. To prove that trapping of the phosphate anion is indeed the rate limiting step during the conversion of Ia into IIa, we have produced Ia from the appropriate diester and 1 equiv. of TPS-Cl, and afterwards added 3 equiv. of trimethylsilyl chloride. The ^{31}P NMR spectrum showed, that after 1.5 h ca 50% of Ia has been converted into the equimolar ammounts of IIa (29.8 and 29.6 ppm) and the silylated 5'-O-dimethoxytritylthymidine S-phenyl 3'-phosphorothioate (10.9 ppm). The same reaction with the addition of 3 equiv. of pyHCl was, as expected, even faster, and was completed after 1 h.

Using 9 equiv. of NO_2BS-Cl we were able to convert also Ib into IIb (80% after 1 h) and Ic into IIc (75% after 2 h). However, tetraethylpyrophosphate Id proved to be completly resistant to such a transformation and after 24 h not even traces of IId could be detected by ^{31}P NMR spectroscopy.

In conclusion, the results show that chloride anion can react with pyrophosphates Ia-c and that the chlorophosphates IIa-c can be detected in substantial amounts in reaction mixtures when a reactive trapping reagent for phosphate anions is used. Also, it seems likely that during the synthesis of phosphorotriesters using aryl sulfonyl chlorides as coupling agents, diester chlorophosphates of type II may be important reactive species.

Acknowledgements

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References

1. D. G. Knorre and V. F. Zarytova, Sov. Sci. Rev., Sect. B Chem. Rev., 6 , 347 (1984).
2. V. F. Zarytova and D. G. Knorre, Nucl. Acids Res., 12, 2091 (1984).
3. All diester chlorophosphates formed in the above reaction were identical with original samples of chlorophosphates prepared on different way.

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