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REACTION BETWEEN 4-NITROBENZALDOXIMATE ION AND PHOSPHOTRIESTERS

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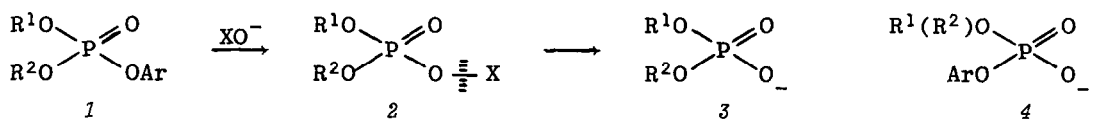
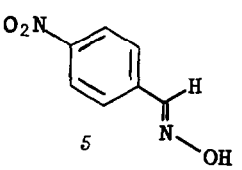
A major problem which has arisen in the synthesis of oligonucleotides by the phosphotriester approach has been the significant amount of internucleotide cleavage[1] accompanying the removal of otherwise satisfactory aryl protecting groups. Thus treatment of a phenyl-protected dinucleoside phosphate (represented by *1a*) with hydroxide ion (Scheme 1; X = H) leads[2], even under the most favourable conditions so far found, to 97-98% of the desired product (represented by *3*) and *ca.* 2-3% of internucleotide cleavage products (represented by *4*). This clearly becomes a serious problem in the unblocking of protected oligonucleotides containing a comparatively large number (say, 15 or more) residues[2].

Scheme 1



1 *2* *3* *4*

a; Ar = Ph
b; Ar = 2 - ClC_6H_4
c; Ar = 4 - $O_2NC_6H_4$



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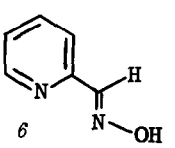


6

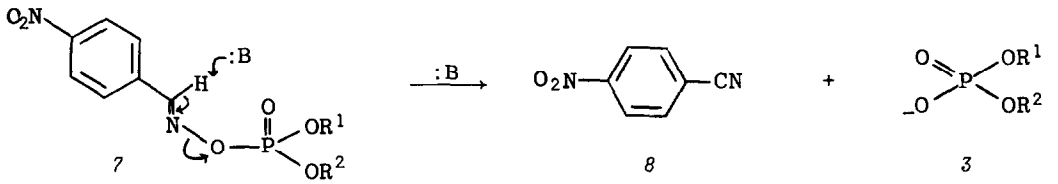
This problem may be partially solved by the use of an aryl protecting group (e.g. 2-chlorophenyl, as in *1b*) derived from a phenol more acidic than phenol itself. However, recent studies[3] have indicated that, if cleavage is to be kept at or below 0.5% per internucleotide linkage, the pK_a of the phenol involved must not be higher than *ca.* 7.5. Unfortunately, the phosphotriester intermediates then become very sensitive to alkaline hydrolysis and extremely difficult to handle[3].

An alternative and much more satisfactory approach to the problem is to use a nucleophile other than hydroxide ion in the unblocking step. Thus we have very recently found[4] that the conjugate bases of *syn*-4-nitrobenzaldoxime (*5*) and *syn*-pyridine-2-carboxaldoxime (*6*) react with 2-chlorophenyl esters of oligonucleotides (represented by *1b*) at convenient rates to give products with unblocked internucleotide linkages (represented by *3*) with a negligible amount (probably < 0.5% per phosphotriester group) of concomitant internucleotide cleavage. The

present paper is concerned with a study of the mechanism of the reaction between the 4-nitrobenzaldoximate ion and phosphotriesters.

In related studies between oximate ions and (a) isopropyl methylphosphonofluoridate[5] and (b) diphenyl 4-nitrophenyl phosphate[6], it was assumed that the corresponding oxime esters were involved as intermediates. However, in neither study was such an intermediate detected and no firm evidence as to whether its formation or its decomposition was rate determining under the particular experimental conditions was obtained. Finally, in the case of aldoximate ions, the mechanism of the decomposition of the putative oxime ester intermediates has not been elucidated. Indeed, in the study involving diphenyl 4-nitrophenyl phosphate (*1c*; $R^1 = R^2 = Ph$), the conclusion reached[6] with regard to the last point appears to be incorrect (see below).

Scheme 2



As we considered 2-chlorophenyl diethyl phosphate (*1b*; $R^1 = R^2 = Et$) to be a good model for a protected oligonucleotide, we first examined the reaction between a 0.23 *M* solution of the latter substrate (*1b*; $R^1 = R^2 = Et$) and tenfold excesses of N^1,N^1,N^2,N^2-tetramethylguanidine (TMG) and *syn*-4-nitrobenzaldoxime (*5*) in dioxan-D_2O (1:1 v/v) at 20°. The progress of the reaction was monitored by ^{31}P n.m.r. spectroscopy: the disappearance of the substrate (*1b*; $R^1 = R^2 = Et$; δ - 7.51) was accompanied by the appearance of the diethyl phosphate anion (*3*; $R^1 = R^2 = Et$; δ - 0.54). The reaction was 64% complete after 40 min and 91% complete after 165 min. When a *ca.* 0.4 *M* solution of *1b* ($R^1 = R^2 = Et$) in dichloromethane was treated with 3 molecular equivalents each of *5* and triethylamine at 20°, a much slower reaction ensued and 76% of substrate (*1b*; $R^1 = R^2 = Et$; δ - 7.17, in CH_2Cl_2 - CD_2Cl_2) remained after 146 hr. Treatment of *1b* ($R^1 = R^2 = Et$) with 10 molecular equivalents each of *5* and TMG in dioxan-water (1:1 v/v) at 20° and work-up of the products after 50 hr gave 4-nitrobenzonitrile (*8*) as a crystalline solid, m.p. 146-147°, in 74% isolated yield.

Although the high yield of nitrile (*8*) obtained may be regarded as strong evidence for diethyl phosphate ion (*3*; $R^1 = R^2 = Et$) being formed by the mechanism indicated in Scheme 2 and hence for the intermediacy of the oxime ester (*7*; $R^1 = R^2 = Et$) in the reaction between the conjugate base of *5* and *1b* ($R^1 = R^2 = Et$), the presence of *7* ($R^1 = R^2 = Et$) was not detected by ^{31}P n.m.r. spectroscopy. Reaction between *5* and diethyl phosphorochloridate in the presence of 5-chloro-1-ethyl-2-methylimidazole in acetonitrile solution gave *7* ($R^1 = R^2 = Et$) in high yield. The latter compound was isolated as an impure solid [^{31}P n.m.r. spectrum: δ - 1.41 (CH_2Cl_2 - CD_2Cl_2), - 0.47 (dioxan - D_2O; 1:1 v/v); ^1H n.m.r. spectrum ($CDCl_3$): δ 1.40 (6H, dt, $J \sim 1.3$ and 7 Hz), 4.31 (4H, m), 7.89 (2H, d, $J \sim 9$ Hz), 8.28 (2H, d, $J \sim 9$ Hz), 8.46 (1H, s)], m.p. 36-42°, following Kugelrohr distillation of the products[7]. When a *ca.* 0.4 *M* solution of *7* ($R^1 = R^2 = Et$) in CH_2Cl_2 - CD_2Cl_2 was treated with 2 molecular equivalents each of *5* and triethylamine at 26.5°, 50% conversion to diethyl phosphate anion (*3*; $R^1 = R^2 = Et$;

δ - 1.01, in CH_2Cl_2 - CD_2Cl_2) occurred after 15 min and the reaction was nearly 80% complete after 30 min. In a separate experiment, *7* ($R^1 = R^2 = Et$) was treated with 2 molecular equivalents each of *5* and triethylamine in dichloromethane solution at 20° and the products were worked-up after 3 hr to give 4-nitrobenzonitrile (*8*) as a crystalline solid in 85% isolated yield.

By consideration of the relative rates (see above) of the reactions of (i) *1b* ($R^1 = R^2 = Et$) and (ii) *7* ($R^1 = R^2 = Et$) with *syn*-4-nitrobenzaldoxime(*5*)-triethylamine in dichloromethane solution and the nature of the products obtained, it may be concluded that the reaction between 4-nitrobenzaldoximate ion and the phosphotriester (*1b*; $R^1 = R^2 = Et$) involves a slow, rate-determining nucleophilic attack at phosphorus to give the oxime ester *7* ($R^1 = R^2 = Et$) followed by a fast (*ca.* 2-3 orders of magnitude faster under the above conditions) base-catalyzed elimination reaction (Scheme 2) to give 4-nitrobenzonitrile (*8*) and diethyl phosphate anion (*3*; $R^1 = R^2 = Et$). It therefore appears that the conjugate base of *5* (and of *6* also)[4] is an ideal nucleophile (XO^-, Scheme 1) for unblocking aryl-protected oligonucleotides. While the first step (*1* → *2*) proceeds virtually exclusively with displacement of arylate (e.g. 2-chlorophenoxide) ion, the second step (*2* → *3*) proceeds with fission of the O-X bond and cannot therefore lead to internucleotide cleavage. With regard to the monitoring (e.g. by thin-layer chromatography) of the unblocking reaction, it is further advantageous that the first step should be rate-determining.

Our conclusions relating to the mechanism of the conversion of oxime ester (*7*) to phosphodiester product (*3*) indicated in Scheme 2 differ from those reached by Bunton and Ihara[6]. The latter workers[6] examined the reaction between diphenyl 4-nitrophenyl phosphate (*1c*; $R^1 = R^2 = Ph$) with various oximate ions (including the conjugate base of *5*) both in the absence and presence of cetyltrimethylammonium bromide (CTAB) and were unable to detect nitriles in the products. They concluded[6] that the conversion of oxime esters [such as *7* ($R^1 = R^2 = Ph$)] to diphenyl phosphate ion (*3*; $R^1 = R^2 = Ph$) involved nucleophilic attack on phosphorus and that, in the case of CTAB-catalyzed reactions between oximate ions and *1c* ($R^1 = R^2 = Ph$), the first step [i.e. the formation of oxime ester] was not rate-determining.

The oxime ester (*7*; $R^1 = R^2 = Ph$) was obtained in modest yield by treating *5* with diphenyl phosphorochloridate in the presence of 5-chloro-1-ethyl-2-methylimidazole in acetonitrile solution; it was isolated as an analytically pure crystalline solid [^{31}P n.m.r. spectrum: δ - 12.20 (CH_2Cl_2 - CD_2Cl_2), - 11.66 (dioxan - D_2O), - 12.40 ($CDCl_3$); ^1H n.m.r. spectrum ($CDCl_3$): 7.30 (10H, m), 7.85 (2H, d, $J \sim 9$ Hz), 8.30 (2H, d, $J \sim 9$ Hz), 8.47 (1H, s)], m.p. 118°. Treatment of diphenyl 4-nitrophenyl phosphate (*1c*; $R^1 = R^2 = Ph$; δ - 18.63 in CH_2Cl_2 - CD_2Cl_2) with 3 molecular equivalents each of *5* and TMG in acetonitrile-water (4:1 v/v) solution and work-up of the products after 1 hr gave 4-nitrobenzonitrile (*8*) in 94% yield (81% after recrystallization; m.p. 146-148°). When the oxime ester (*7*; $R^1 = R^2 = Ph$) was treated with 2 molecular equivalents of TMG in dichloromethane solution at 20° and the products worked-up after 10 min, crystalline *8* was isolated in 92% yield.

The reaction between a *ca.* 0.1 *M* solution of the oxime ester (*7*; $R^1 = R^2 = Ph$) and 2 molecular equivalents each of TMG and *5* in CH_2Cl_2 - CD_2Cl_2 solution was confirmed by ^{31}P n.m.r.

spectroscopy to be very fast indeed and to give diphenyl phosphate ion (*3*; $R^1 = R^2 = Ph$; δ - 11.39) quantitatively within 4 min at 26°. Although the base-catalyzed decomposition of *7* ($R^1 = R^2 = Ph$) is extremely facile, it may be monitored by n.m.r. spectroscopy if a comparatively weak base is used. Thus the reaction between a *ca.* 0.14 *M* solution of *7* ($R^1 = R^2 = Ph$) and a twofold excess of *N*-methylmorpholine in CH_2Cl_2 - CD_2Cl_2 solution at 26° was found to be 37, 84 and 95% complete after 6, 22 and 36 min, respectively. On the other hand when a *ca.* 0.14 *M* solution of diphenyl 4-nitrophenyl phosphate (*1c*; $R^1 = R^2 = Ph$) was heated, under reflux, with 10 molecular equivalents each of *syn*-4-nitrobenzaldoxime (*5*) and *N*-methylmorpholine in dichloromethane solution, *ca.* 50 and 70% conversion to diphenyl phosphate ion (*3*; $R^1 = R^2 = Ph$) occurred after 112 and 340 min, respectively. It is especially noteworthy that *ca.* 5% of the intermediate oxime ester (*7*; $R^1 = R^2 = Ph$) could be detected in the products by ^{31}P n.m.r. spectroscopy after both 112 and 340 min.

From a consideration of all of the above results, it may be concluded that the reactions between the conjugate base of *syn*-4-nitrobenzaldoxime (*5*) and 2-chlorophenyl diethyl and diphenyl 4-nitrophenyl phosphates (*1b*; $R^1 = R^2 = Et$ and *1c*; $R^1 = R^2 = Ph$, respectively) proceed by essentially the same mechanism. Presumably it is possible to detect the intermediate (*7*) only in the reaction between 4-nitrobenzaldoximate ion and diphenyl 4-nitrophenyl phosphate (*1c*; $R^1 = R^2 = Ph$) because the relative rates of nucleophilic attack on phosphorus and base-catalyzed decomposition of the oxime ester (*7*; $R^1 = R^2 = Ph$) are closer than in the reaction between 4-nitrobenzaldoximate ion and 2-chlorophenyl diethyl phosphate (*1b*; $R^1 = R^2 = Et$).

Acknowledgements

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