Tetrahedron Letters,Vol.24,No.9,pp 951-954,1983 0040-4039/83/090951-04$03.00/0
Printed in Great Britain

DEALKYLATION OF NUCLEOSIDE ARYLMETHYL 2-CHLOROPHENYL PHOSPHATES: THE 2,4-DINITROBENZYL PROTECTING GROUP

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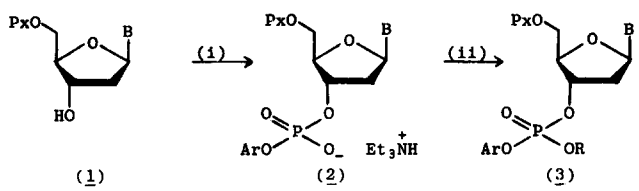
Summary: Following a study of the effect of benzyl substitution on the rates of nucleophilic dealkylation of phosphotriesters, 2,4-dinitrobenzyl is proposed as a temporary protecting group for 3'-terminal phosphodiester functions in oligonucleotide synthesis.

Kinetic data indicate[1] that the effect of nuclear substitution on the rates of displacement reactions of benzyl halides is complex. Many years ago, Bennett and Jones showed[2] that, while the relative rates of the reactions between benzyl, 4-chlorobenzyl, 2-chlorobenzyl and 4-nitrobenzyl chlorides, and potassium iodide in acetone solution at 20°C were 1, 2.12, 3.98 and 6.19, respectively, the relative rates of hydrolysis [acetone-water (1:1 v/v), 84.5°C] of the same substrates were 1, 0.573, 0.297 and 0.106, respectively. Thus the order of reactivity of the substrates is reversed in the two reactions.

Scheme 1



(1) (2) (3)

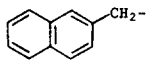
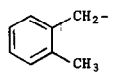
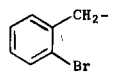
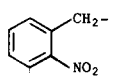
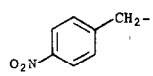
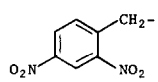
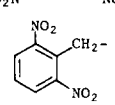
a; B = thymin-1-yl; Ar = 2-ClC$_6$H$_4$; Px = 9-phenylxanthen-9-yl(pixyl)[3]

Reagents: (i)(a) 2-chlorophenyl phosphorodi-(1,2,4-triazolide)[4] - 1-methylimidazole/tetrahydrofuran, (b) triethylamine-water; (ii) 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole(MSNT)[5] - ROH/pyridine.

Like benzyl halides, phosphotriesters containing at least one benzyl residue are susceptible to attack by certain nucleophiles. Debenzylation then occurs and the corresponding phosphodiesters are obtained. While nucleophilic reagents such as tertiary amines[6] (including pyridine[7]), lithium chloride[8] and potassium thiocyanate[9] have been used for this purpose, thiophenate ion[10] has been shown to be particularly effective. We have prepared (Scheme 1) a number of 5'-O-(9-phenylxanthen-9-yl)thymidine 3'-arylmethyl(2-chlorophenyl) phosphates[11] (3a) with the intention of studying the effect of benzyl substitution on the rates of nucleophilic dealkylation. For reasons that will become clear below, the nucleophiles which we chose were (a) toluene-*p*-thiolate ion and (b) pyridine. The results obtained are given in Table 1.

All of the experiments were carried out at 20°C with an initial substrate concentration of 0.02*M*. The half-times ($t_{\frac{1}{2}}$) and the times for the completion (t_{∞}) of the reactions were

Table 1. Nucleophilic reactions of 5'-O-pixylthymidine 3'-phosphotriesters (3a)

	Substrate (3a) R-	Reaction with 4-MeC_6H_4SH/NEt_3[a] $t_{\frac{1}{2}}$(min)	t_∞(min)	Reaction with pyridine[b] $t_{\frac{1}{2}}$(hr)	Ratio of half-times[c]
(1)	CH_3-	45	-	12	16
(2)	$PhCH_2$-	30	-	12	24
(3)		5	60	5	60
(4)		7	90	3	26
(5)		4	45	10	150
(6)		5	60	68	820
(7)		2	20	40	1,200
(8)		-[d]	~1	120	~43,000
(9)		-[d]	~1	45	~16,000

[a] These reactions were carried out at 20°C in acetonitrile solution. The initial concentrations of substrate (3a), toluene-*p*-thiol and triethylamine were 0.02, 0.08 and 0.08 *M*, respectively. The reactions were monitored by t.l.c. and the approximate times for half and complete reactions ($t_{\frac{1}{2}}$ and t_∞, respectively) are given.
[b] These reactions were carried out at 20°C with an initial substrate (3a) concentration of 0.02 *M*; they were monitored by t.l.c. and the approximate times for half reaction ($t_{\frac{1}{2}}$) are given.
[c] These ratios are the quotients of the $t_{\frac{1}{2}}$ values of the pyridine and toluene-*p*-thiol/triethylamine reactions.
[d] It is estimated that $t_{\frac{1}{2}}$ of this reaction was not more than 10 sec.

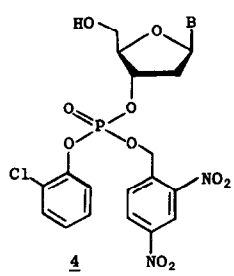
estimated by t.l.c.[13] In the first series of experiments, the substrates (3a) were treated with four molecular equivalents each of toluene-*p*-thiol and triethylamine in acetonitrile solution. It can be seen from Table 1 that the rates of dealkylation of the methyl and benzyl esters (3a; R = CH_3 and $PhCH_2$, respectively; entries nos. 1 and 2) are similar, and that all of the substituted benzyl esters (entries nos. 3-9) undergo dealkylation more rapidly. In each case, the product obtained is 5'-O-(9-phenylxanthen-9-yl)thymidine 3'-(2-chlorophenyl) phosphate (2a). While the effects of *ortho*-methyl, -bromo, and -nitro substituents (entries nos. 4, 5, and 6, respectively) on the rate of toluene-*p*-thiolate ion promoted dealkylation are all quite large, the effect of the *para*-nitro substituent (entry no. 7) is even larger.[14] The 2,4-dinitro- and 2,6-dinitro-benzyl esters (3a; R = 2,4- and 2,6-$(O_2N)_2C_6H_3$, respectively; entries nos. 8 and 9) undergo dealkylation extremely rapidly at, as far as can be estimated, rates almost 200 times as fast as that of the benzyl ester (3a; R = $PhCH_2$; entry no. 2).

No attempt will be made here to rationalize the relative effects of different substituents on the rates of the reactions between toluene-*p*-thiolate ion and benzyl derivatives. There are indeed several reports in the literature relating to the accelerating effects,

particularly of *ortho*-substituents,[15] on the rates of the reactions between soft nucleophiles (e.g. lithium benzenethiolate) and benzyl halides. It can be seen from Table 1 that the effect of the substituents on the rates of the reactions between pyridine and 5'-O-(9-phenylxanthen-9-yl)thymidine 3'-arylmethyl(2-chlorophenyl) phosphates (3a) is far more complex. While the introduction of an *ortho*-methyl and, to some extent, an *ortho*-bromo substituent (entries nos. 4 and 5, respectively) leads to an increase in the rate of pyridine promoted dealkylation, the introduction of an *ortho*-nitro substituent (entry no. 6) has the opposite effect. As a *para*-nitro substituent (entry no. 7) also slows down the rate of pyridine promoted dealkylation, it is not surprising that the 2,4-dinitrobenzyl ester (3a; R = 2,4-$(O_2N)_2$-C_6H_3; entry no. 8) is particularly stable to attack by pyridine. Indeed, the latter phosphotriester intermediate undergoes pyridine promoted dealkylation 10 times more slowly than the corresponding benzyl ester (3a; R = $PhCH_2$; entry no. 2).

The relative stability of the 2,4-dinitrobenzyl ester (3a; R = 2,4-$(O_2N)_2C_6H_3$) in pyridine solution is particularly interesting as the latter undergoes toluene-*p*-thiolate ion promoted dealkylation (see Table 1 and above) nearly 200 times *more rapidly* than the benzyl ester (3a; R = $PhCH_2$). The ratios of the half-times of the pyridine and toluene-*p*-thiolate ion promoted dealkylation reactions are given in the final column of Table 1. This ratio, which is greatest for the 2,4-dinitrobenzyl ester (3a; R = 2,4-$(O_2N)_2C_6H_3$; entry no. 8) and smallest for the methyl ester (3a; R = Me; entry no. 1), varies over a range of more than three orders of magnitude.

We undertook this study in the course of a search for a suitable temporary protecting group for 3'-terminal phosphodiester functions[16] (as in the mononucleotide derivative 2a) in oligonucleotide synthesis. The main requirements for such a temporary protecting group include stability under mildly basic and mildly acidic conditions, and also under phosphorylation conditions. In connection with the last requirement, it is important that the protecting group should be stable to pyridine which is commonly used as a solvent in oligonucleotide synthesis. A final and essential requirement is that the protecting group should be removable under very mild conditions which do not affect fully-protected oligonucleotides in any other way.



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The particular stability of the 2,4-dinitrobenzyl group (Table 1, entry no. 8) in pyridine solution, coupled with its high susceptibility to attack by toluene-*p*-thiolate ion,[17] clearly make it the most suitable 3'-terminal phosphodiester protecting group of those examined in the present study. The 2,4-dinitrobenzyl group, which appears to meet all of the other requirements for a 3'-terminal protecting group considered above, has already been used successfully in the synthesis both of oligoribo-[19] and oligodeoxyribo[20]-nucleotides. In our opinion, the 2,4-dinitrobenzyl group is to be preferred to 2-cyanoethyl[16,21] and to the other protecting groups[22] which have previously been used for this purpose. 2,4-Dinitrobenzyl

alcohol is a relatively easily accessible compound,[23] and the required mononucleotide building blocks (3; R = 2,4-$(O_2N)_2C_6H_3$ or 4) may be prepared[24] in good yields. Finally, if suitable synthetic procedures can be devised, 2,4-dinitrobenzyl should prove to be superior to methyl as a protecting group for internucleotide linkages in the phosphite-triester approach[10,26] to oligonucleotide synthesis.

Acknowledgement. We thank the Science and Engineering Research Council and G.D. Searle and Co. Ltd. (C.A.S.E. Studentship to C.C.) for generous support of this work.

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(Received in UK 17 December 1982)