Tetrahedron Letters, Vol.22, No.20, pp 1915 - 1918, 1981
Printed in Great Britain

0040-4039/81/201915-04$02.00/0

2-PHENYLSULFONYLETHYL, A NEW PHOSPHATE PROTECTING GROUP: ITS APPLICATION IN THE SYNTHESIS OF DODECATHYMIDYLIC ACID.

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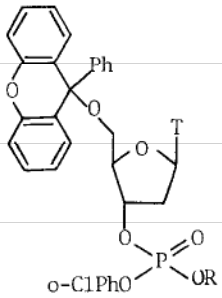
Summary: 2-Phenylsulphonylethyl group has been employed for the first time to protect 3'-terminal phosphodiester to the triester level in the synthesis of dodecathymidylic acid using the phosphotriester approach.

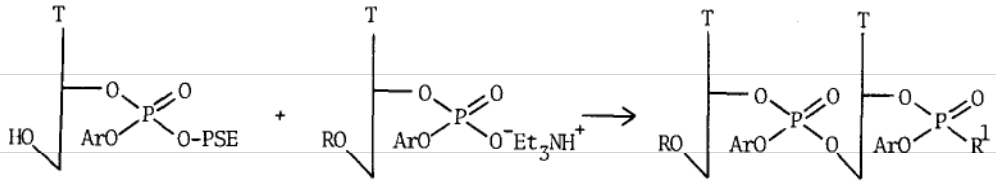
2-Arylthioethyl group was first introduced by Narang[1] and then Khorana[2] attempted to use this phosphate protecting group in the phosphodiester approach. The novelties of this phosphate protecting group were considered to lie in the facts that (i) the oligodeoxynucleotides with this group would be sufficiently stable during the removable of acid and base labile protecting groups and (ii) yet, after the conversion of sulfide to the sulfoxide or sulfone oxidation state, it would be removable by base induced β-elimination of the phosphate without any side reactions. Narang used 2M NaOH to activate such a β-elimination of the sulfoxide[1] which resulted in the removal of N-acyl protecting groups. Khorana then improved on this observation by using 1M NaOH for the removal of arylsulfonyl derivative[2]. In the phosphotriester approach[3] with the aryl protecting group at the internucleotide junctions, any one of the above conditions is clearly very drastic beside the fact that attempts to oxidize 2-arylthioethyl group led to the oxidations of adenine and guanine residues. Thus 2-arylthioethyl group, as such, for phosphate protection can not be employed successfully. We, therefore, concentrated on exploring the stabilty of the 2-phenylsulfinylethyl- and 2-phenylsulphonylethyl phosphotriester derivatives (1) and (2) respectively[4]. The 2-phenylsulfinylethyl group in (1) could be removed very slowly with Et_3N (15 equiv.) in dry pyridine ($t_{1/2}$ Ca. 26h at 20°C). However, the result of removal of 2-phenylsulfonylethyl group from (2) was very encouraging by the fact that it could be removed completely under 3h at 20°C using only 2 equiv. of Et_3N in dry pyridine(10ml/mmol) compared to at least 15 equiv. of Et_3N in pyridine for the removal of 2-cyanoethyl protecting group in (3)[5]. Furthermore, We observed that 2-phenylsulfonylethyl group in (2) was stable under the conditions which are normally employed in oligodeoxyribonucleotide synthesis[5,6,7] despite the relatively mild conditions employable for its removal. Thus the 5'-O-(9-phenylxanthen-9-yl)- (Pixyl) group[7,8] could be easily removed using 4-toluenesulfonic acid, H_2O in 2% ethanol-$CHCl_3$ to obtain the corresponding 5'-hydroxy compound (4) quatitatively and both (2) and (4) were reosonably stable during column chromatographic conditions over silica gel[6,7]. These observations led us to synthesize dodecathymidylic acid using 2-phenylsulfonylethyl group



(1), R = $-CH_2-CH_2-\overset{\overset{O}{\uparrow}}{S}-Ph$

(2), R = $-CH_2-CH_2-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-Ph$

(3), R = $-CH_2-CH_2-CN$



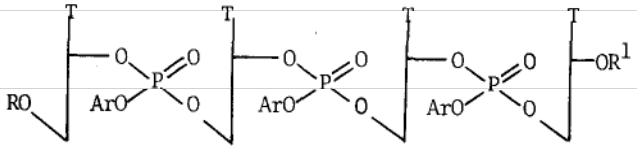
(4) (5), R = Px (6), R = Px, R^1 = O-PSE

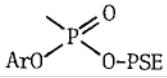
(9), R = H, R^1 = O-PSE

(11), R = Px, R^1 = $O^-Et_3NH^+$

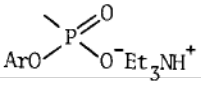
(7) (8), R = Px

(10), R = H



(12), R = Px, R^1 = 

(13), R = Px, R^1 = Bz

(14), R = Px, R^1 = 

(15), R = H, R^1 = Bz

PSE = 2- Phenylsulphonylethyl

Px = 9-Phenylxanthen-9-yl

Ar = o-Chlorophenyl

to demonstrate its application in oligodeoxyribonucleotide synthesis following the phosphotriester approach[3,4,9]. Thus, We condensed a slight excess of (5) with (4) in pyridine in presence of a large excess (5 equiv.) of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS-NT)[10] under a standard condition[7] to obtain (6), R_f=0.53[11], in 83% yield(powder) after usual work-up and chromatography[7]. Similarly, We obtained (8) in 86% yield, R_f=0.68[11], by reacting (5) with (7) in pyridine soln. in presence of MS-NT. The (6) and (8) could be depixylated[7,8] in quantitative yields to the corresponding 5'-hydroxy compounds (9) and (10) respectively by 4-toluenesulfonic acid,H_2O in 2% EtOH-$CHCl_3$ at 20°C. The 2-phenylsulfonylethyl group from (6) was then deprotected to obtain the phosphodiester triethylammonium salt (11) using Et_3N (2 equiv.) in dry pyridine (10ml/mmol) for 3h at 20°C. The deprotection of (6) to (11) was smooth, clean and quatitative. At the end of the reaction, the volatile matters were removed using a rotavapor. The residue was dissolved in small volume of $CHCl_3$ and then this solution was precipitated from a mixture of Et_2O-petether (30-50°),1:1 v/v to remove 2-phenylsulfonylethylene completely. The dry powder of (11) was then used, in slight excess (1.1 equiv.), for further condensations with (9) and (10), using standard procedures[7], to obtain (12) and (13), R_f=0.48&0.58 respectively[11], in 73.0 and 71.0% yields(powders) respectivly. The comparison of isolated % yields of (12) and (13) adequately support that 2-phenylsulfonylethyl group at 3'-terminal phosphotriesters is, indeed, stable during the condensation reactions, work-up procedures and column chromatographic conditions which are normally necessary to synthesize and purify a fully protected oligodeoxyribonucleotide fragment. The 2-phenylsulphonylethyl group from (12) was then deprotected to the phosphodiester salt (14) using an identical stoichiometry of Et_3N (i.e 2 equiv.) in pyridine to that of deprotection of (6) to (11) and the reaction was complete within 3h at 20°C. An excess of (14) was then coupled to (15) in a usual fashion to obtain the fully protected octamer (16), R_f=0.47[11],in 74.0% yields(powder) which was then depixylated[7,8] to obtain the 5'-hydroxy octamer (17) quantitatively. Finally, a condensation of (14) with (17), again under a standard condition[7], gave the desired dodecathymidine undecaphosphate (18), R_f=0.38[11], in 69.0% yield (powder) after work-up and chromatography (8% EtOH-$CHCl_3$)[5,6,7]. The (18) was then deprotected following a literature procedure[8,10]. The HPLC elution pattern of the crude dodecathymidylic acid through a Permaphase AAX column at 60°C (linear gradient: 0.01M KH_2PO_4, 0.0M KCl to 0.05M KH_2PO_4 and 0.7M KCl, pH 4.45) is shown in Figure 1 and the main peak was found to contain more than 95% of the desired material. An aliquot of this material was ^{32}P-labelled with γ-^{32}P-ATP and kinase[12]. This ^{32}P-labelled thymidylic acid was partially digested with Crottalus adamantus snake venom phosphodiesterase and then it was electrophoretically shown to contain the desired number of fragments of differently charged species expected from dodecathymidylic acid as shown in Figure 2.

Thus it is clearly demonstrated that 2-phenylsulfonylethyl group can be used for the protection of 3'-phosphodiester function and We are currently exploiting this observation to synthesize oligodeoxyribonucleotide of mixed sequences in our laboratory.

Acknowledgements: The authors thank Professor L. Philipson for encouragements and Swedish Board for Technical Development for generous financial assistance. One of us (J.B.C) specially thank Professor C.B. Reese for many serious discussions during J.B.C's postdoctoral years in C.B.R's laboratory.

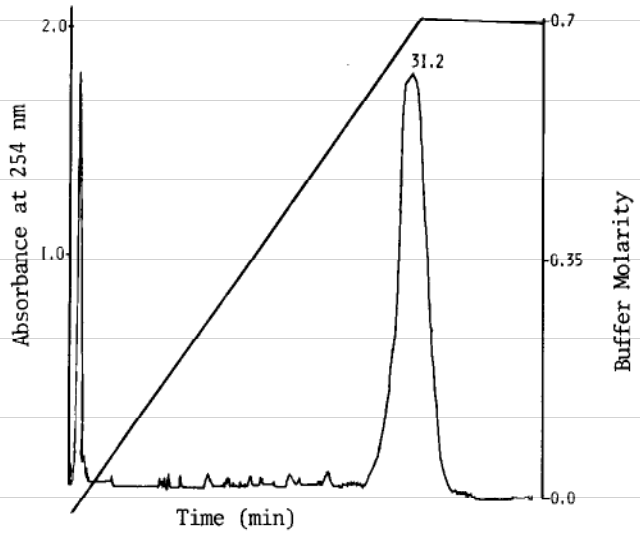


Figure 1

Figure 2

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(Received in UK 26 February 1981)