Tetrahedron Letters,Vol.25,No.40,pp 4511-4514,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

IMPROVED PROCEDURE FOR THE PREPARATION OF DEOXYNUCLEOSIDE PHOSPHORAMIDITES: ARYLPHOSPHORAMIDITES AS NEW CONVENIENT INTERMEDIATES FOR OLIGODEOXYNUCLEOTIDE SYNTHESIS

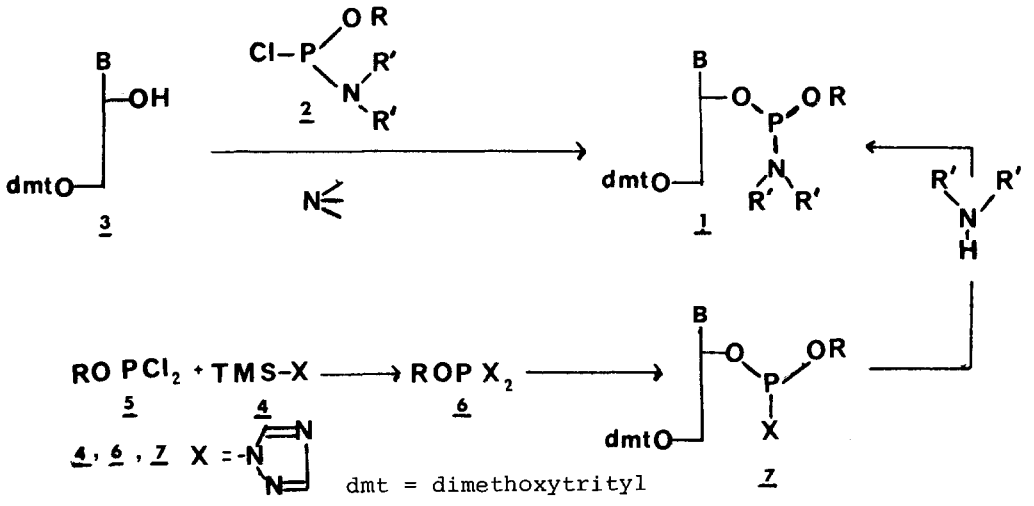
Jean-Louis FOURREY and Jeannette VARENNE

Institut de Chimie des Substances Naturelles , C.N.R.S., 91190-Gif sur Yvette, France

A simplified procedure for the preparation of deoxynucleoside methyl- and arylphosphoramidites is described. Both types of phosphoramidites can be conveniently activated by N-methylaniline trifluoracetate for their use in oligodeoxynucleotide synthesis.

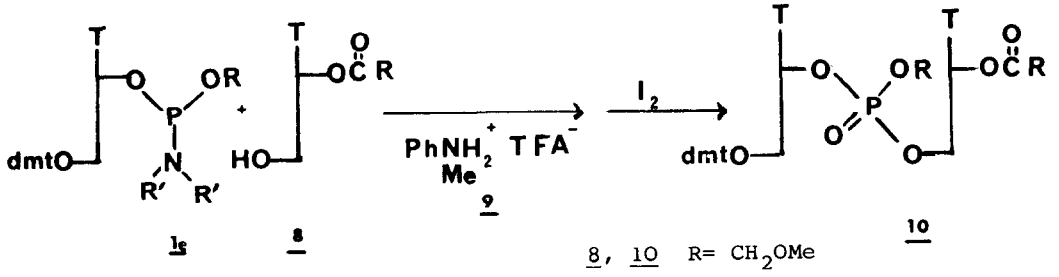
The phosphite triester method originally proposed by Letsinger [1] for the synthesis of oligonucleotides has greatly developped since the introduction of nucleoside methylphosphoramidites by Caruthers[2]. However, several recent studies indicate that there is still a need for easily accessible and reasonably stable nucleoside phosphoramidites which can be used by personnel without exceptional chemical skill. In the methylphosphoramidite series the morpholino- and the diisopropylaminophosphoramidites 1a [2] and 1b[2,3], respectively are considered as being the best derivatives . But the methyl group is not fully accepted as the most suitable protecting group for the phosphate moiety. Instead, the 2-cyanoethyl [4] and the 2-methylsulfonyl [5] groups have been recently proposed as in 1c and 1d .

Usually , nucleoside alkylphosphoramidites of general structure 1a-d are prepared by adding to a 3'-OH free nucleoside derivative 3 the corresponding alkyloxydialkylamino-chlorophosphine 2 in the presence of an organic base [2-5] . Such a procedure presents at least two drawbacks . First , chlorophosphines 2 are in general difficult to obtain fully pure when the R(and R') moiety is a higher homologue of methyl; this is due to thermal decomposition, even under very high vacuum distillation . Second , the amine hydrochloride formed during the preparation of 1 might be particularly harmful. Great care must be taken at the work up stage since phosphoramidites 1 undergo immediate hydrolysis (or alcoholysis) upon activation by amine hydrochlorides [6] . Recently , to circumvert such inconveniences, two different methods for *in situ* preparation of phosphoramidites have been reported[7,8] . Our own procedure[7] has now been improved by avoiding the side formation of amine hydrochloride. Moreover, we have established that arylphosphoramites 1e are the intermediates of choice for solid phase synthesis of oligodeoxynucleotides according to Caruthers' procedure[9].



^{31}P nmr data of compounds 1e

B	δ ppm (PO_4H_3 external standard)		R	N R' R'
T	140.93	140.46	1a Me	$N(CH_2CH_2)_2O$
C^{Bz}	141.42	141.18	1b "	$N(C_3H_7)_2$
A^{Bz}	141.87	140.46	1c CH_2CH_2CN	$N(CH_2CH_2)O$
G^{Ibu}	141.27	140.16	1d $CH_2CH_2SO_2Ph$	"
			1e o-ClC_6H_4	"



The general method for the preparation of nucleoside phosphoramidites 1 is as follows : Trimethylsilyltriazole 4 [10] (2.2 equivalents) was added to a THF solution of 5 R=Me or o-C_6H_4Cl (1 equivalent) maintained at 0°C. The solution was evaporated to dryness to eliminate trimethylsilyl chloride providing 6 as an oil. Into a THF solution of the latter maintained at -78°C was succesively syringed a THF solution of a 3'-OH free

nucleoside derivative 3 (0.5 equivalent) followed by neat morpholine (5 equivalents). The reaction mixture was allowed to reach room temperature and diluted with ethyl acetate. The resulting solution was washed with aqueous sodium bicarbonate and water and evaporated . The residue obtained after evaporation was purified either by precipitation in cold hexane when R=CH_3 or by chromatography (short column of silica gel, elution with a hexane/ethyl acetate gradient) when R=o-ClC_6H_4.

The methylphosphoramidites 1a were obtained in excellent yields and exhibited the same physical data (^1H and ^{31}P NMR) as those previously described[7] . They could be used for oligodeoxynucleotide synthesis on silica gel support by following the literature methodology[9] except that tetrazole could be advantageously replaced by N-methylaniline trifluoroacetate 9[11] as the activating agent (see below) .

The corresponding o-chlorophenylphosphoramidites 1e were isolated in yields over 75% of chromatographically pure products thus indicating that our previous procedure [7] has been significantly improved. Their NMR data (^1H and ^{31}P) are fully consistent with structure 1e. These chlorophenyl derivatives are much more stable than their methyl analogues. In the presence of methanol (100 equivalents) and tetrazole (4 equivalents) in acetonitrile solution they were transformed into the methoxy derivative 7 (X=OMe) but at a rate which was too slow to suggest such activation conditions for their use in solid support synthesis of oligodeoxynucleotides . However, activation with N-methylaniline trifluoroacetate 9[11] appeared very promising as illustrated by the synthesis in solution of a dinucleoside phosphate 10.

Thus , an acetonitrile solution of phosphoramidite 1e (B=T) (1.2 equivalent) was added to an acetonitrile solution containing 3'-methoxyacetylthymidine 8 (1 equivalent) and 9 (2.2 equivalent). Immediate disappearance of 1e was observed by TLC. The reaction product was oxidized with aqueous iodine to the dinucleoside phosphate 10 which was isolated in 70% yield. It is noteworthy that methylphophoramidites 1a have not been used so far to phosphorylate preparatively a 5' -OH free nucleoside derivative in solution .

In solid phase synthesis of oligonucleotides the arylphosphoramidites 1e offer some real advantages over their methyl analogues 1a . Good and reproducible coupling yields could be observed by reacting only 5 to 10 equivalents of arylphophoramidite 1e per equivalent of 5'-OH free nucleoside at the terminal position of a silica gel bound oligonucleotide chain. To obtain similar results with methylphosphoramidites 1a it is recommended to utilize a 20 times equivalent excess. This improvement is attributed to the high purity and to the enhanced stability of the new phosphitylating agent.

The experimental conditions are the following : To a suspension of silica gel (Fractosil 200) functionalized according to Caruthers[9] (50 mg, 2.5 μM of thymidine) in acetonitrile (0.6 ml) containing N-methylaniline trifluoroacetate 9 (50 μM) was added a solution of phosphoramidite 1e in acetonitrile (0.25 ml, 25 μM) . The reaction mixture was

shaken for 5 min. at room temperature . Then the reagents were removed by washing with acetonitrile . Oxidation to the phosphate triester was effected by a solution of iodine containing water and pyridine. Subsequent spectrophotometric measurements of the dimethoxytrityl cation released after acid treatment indicated reproductive yields over 95% for each condensation . The oligonucleotides prepared in this manner were fully characterized after purification; the full corresponding analytical data will be reported elsewhere.

In conclusion, the herein reported procedure provides a simple and efficient access to nucleoside phosphoramidites and in particular to the o-chlorophenylphosphoramidites 1e. These derivatives , compared with their methyl (or presumably any other alkyl) analogues, exhibit an enhanced stability. They can be conveniently activated by N-methylaniline trifluoroacetate 9 either to prepare dinucleoside phosphates in solution or to be used for solid phase synthesis of oligonucleotides whose phosphate functions are protected by o-chlorophenyl groups. The deprotection of such oligonucleotides can be achieved by the oximate method which is the most selective regarding the cleavage of P-O bonds[12] . This advantage as well as those given above clearly favours an extended use of o-chlorophenylphosphoramidites 1e in oligonucleotides synthesis.

1- R.L. LETSINGER and W.B. LUNSFORD, J. Am. Chem. Soc.(1976) 98, 3655.

2- S.L. BEAUCAGE and M. H. CARUTHERS, Tetrahedron Letters, (1981) 22, 1859; L.J. Mc BRIDE and M. H. CARUTHERS, Ibid., (1983) 24, 245; T. DORPER and E.L. WINNACKER Nucleic Acids Res., (1983) 11, 2575.

3- S.P. ADAMS. K.S. KAVKA, E.J. WYKES, S.B. HOLDER and G.R. GALLUPPI, J. Am. Chem. Soc., (1983) 105, 661.

4- N.D. SINHA , J. BIERNAT and H. KOSTER, Tetrahedron Letters, (1984) 24, 5843.

5- C. CLAESEN , G.I. TESSER, J.E. MARUGG, G.A. van der MAREL and J.H. Van BOOM, Ibid (1984) 25, 1307.

6- E.E. NIFANT'EV and N.L. IVANOVNA, Vestn. Mosk. Univ. Khim., (1968) 23, 104 (Chem. Abstracts (1969) 70, 2935c).

7- J.L. FOURREY and J. VARENNE, Tetrahedron Letters,(1983) 24, 1963.

8- S.L. BEAUCAGE , Ibid., (1984) 25, 375.

9- M.D. MATTEUCI and M.H. CARUTHERS, J. Am. Chem. Soc.,(1981) 103, 3185

10-L. BIRKOFER, P. RICHTER and A. RITTER, Chem. Ber., (1960) 93, 2804.

11-J.L. J.L. GRAS, Tetrahedron Letters,(1978) 2111. This non hygroscopic salt 10 can be purified by sublimation , it is also very soluble in acetonitrile.

12-C.B. REESE , R.C. TITMAS and L. YAU, Ibid., (1978) , 2727. C.B. REESE and L. ZARD, Nucleic Acids Res. , (1981) 9, 46111.

(Received in France 25 June 1984)