Tetrahedron Letters,Vol.25,No.12,pp 1307-1310,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

USE OF 2-METHYLSULFONYLETHYL AS A PHOSPHORUS PROTECTING GROUP IN OLIGONUCLEOTIDE SYNTHESIS VIA A PHOSPHITE TRIESTER APPROACH

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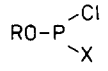
ABSTRACT: 2-Methylsulfonylethoxy dichlorophosphine has been converted into the mono-N-morpholino derivative and applied for the preparation of 5'-O,N-protected d-nucleoside-3'-phosphoramidites. The latter intermediates could be used in the presence of 1-hydroxybenzotriazole for the formation of 3'-5'-phosphotriester linkages. The MSE protecting group can be removed selectively and fast under mild basic conditions.

The introduction of the phosphite triester method by Letsinger[1] stimulated several groups to study in detail the application of different alkyl/aryl phosphorodichloridites[2] and, especially so, methyl phosphorodichloridite[3] towards the synthesis of DNA. The results of these studies indicated that methyl phosphorodichloridite (i.e. 1a) could, despite its high reactivity, be used for the synthesis of DNA on a solid support. In order to decrease the reactivity of agent 1a, Caruthers[4] and also Adams[5] converted 1a into the relatively more stable chloro methyl N,N-dialkylamino/N-morpholino phosphoramidites (e.g. 1b). The latter agents, and particularly so 1b, proved to be suitable for the synthesis of 5'-O,N-protected d-nucleoside phosphoramidites (e.g. 2c; X=N-morpholino; R=Me), which served as key intermediates for the preparation of immobilized DNA fragments (e.g. 4; R=Me; R^1=4,4'-dimethoxytrityl). The removal of the methyl P-protecting groups from 4 (R=Me) can be effected by treatment with a mixture of thiophenol/Et_3N/dioxane[6] or t-butylamine/methanol[7].

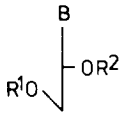
We now wish to report that: (i) the easily accessible agent 2-methylsulfonylethyl N-morpholino phosphoromonochloridite (Id) has the same phosphitylating properties as the methyl analogue 1b; (ii) the 2-methylsulfonylethyl (MSE) group for protecting P-O can be removed selectively under mild basic conditions.

The favourable properties of MSE as a protecting group has been demonstrated before in peptide chemistry[8]. In order to find out if these properties could be exploited in nucleic acid chemistry, we firstly examined the feasibility of preparing agent 1c. It turned out that agent 1c could be obtained as a stable crystalline solid by the following procedure. To a sevenfold molar excess of phosphorus trichloride in acetonitrile was added dropwise, during 20 min at 20°C, methylsulfonylethanol[9] in aceton-itrile. After 4 h at 20°C, the solution was concentrated to a small volume and the residue was distilled to afford pure 1c [yield 90%; b.p. 120°C, 0.05 mm Hg; ^{31}P-NMR, δ 178.71 which crystallized on standing. Agent 1c was now converted into the N-morpholino derivative 1d by treatment with N-trimethylsilylmorpholine[4b] in THF. After 1 h at 20°C, the reaction mixture was concentrated to afford crude 1d as a gum. Analysis of 1d thus obtained by ^{31}P-NMR[11] showed the presence of mainly one resonance (δ 167.5). Phosphitylation of the 5'-O,N-protected d-nucleosides 2a (1 mmol), according to the method of Dörper et al.[10], with 1d (1.7 mmol) in dichloromethane afforded, after work-up and purification by column-chromatography, the phosphoramidites 2c (X=N-morpholino; R=MSE) as homogeneous[11] solids (yields 88-97%; based on 2a). The applicability of 2c was demonstrated by the synthesis

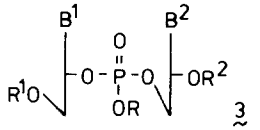
of a fully-protected dimer 3 and a hexadecamer fragment 4 (n=15) on a solid support. Thus, 1-hydroxybenzotriazole[12] (HOBT; 1.6 mmol) in THF was added to a solution of 2c (0.65 mmol; B=T) and 2b (0.5 mmol)[13] in acetonitrile. After 5 min at 20°C, the mixture was concentrated and the remaining residue was oxidized with I_2-H_2O during 2 min. Work-up and purification gave pure 3 (B^1=T; B^2=G^{DPA}) as a homogeneous solid in 65% yield (based on 2b).



1 a: R=Me; X=Cl
b: R=Me; X=-N(CH$_2$)$_4$O
c: R=-CH$_2$CH$_2$SO$_2$Me; X=Cl
d: R=-CH$_2$CH$_2$SO$_2$Me; X=-N(CH$_2$)$_4$O



2 a: R^1=DMTr; R^2=H
b: R^1=H; R^2=Lev
c: R^1=DMTr; R^2=-P(X)-OR



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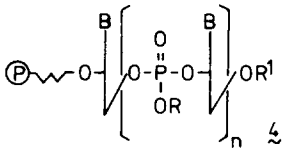

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Table: Steps involved in one complete elongation cycle.

Steps	Solvents and reagents	Time (min)
1.	5% trichloroacetic acid in 1,2-dichloroethane	3
2.	CH_3CN	4
3.	2c (20 equ) in CH_3CN + HOBT (50 equ) in THF	3
4.	THF	1
5.	I_2 (0.1 M in THF/2,6-lutidine/H_2O: 95:4:1; v/v)	1
6.	THF	1
7.	CH_3CN	1
8.	N,N-dimethylaminopyridine (0.1 M in THF/Ac_2O/2,6-lutidine; 6:1.4:3; v/v)	2
9.	THF	1
10.	CH_3CN	2
11.	1,2-dichloroethane	1

Dimer 3 was deblocked in two different ways. Firstly, the MSE-group was removed by treatment with 0.2 N NaOH in dioxane/methanol (14:5; v/v). TLC-analysis showed that the MSE group was removed quantitatively within 1 min at 20°C. The diphenylacetyl (DPA) and levulinoyl (Lev) groups were now removed with aq. ammonia at 50°C for 48 h. The DMTR-group was then deblocked with HOAc. In the second process, we firstly removed the DMTR-group and treated 3 (R^1=H) thus obtained with NaOH followed by aq. NH_3. Purification of both samples by DEAE-Sephadex column-chromatography and analysis of the compounds by HPLC as well as ^{31}P-NMR revealed that they were identical with d-TpG which was prepared independently by a recently developed phosphotri-

ester approach[14]. The above results indicate that the removal of the MSE group is a fast and selective process which is not accompanied by neighbouring group participation[15]. The synthesis of the hexadecamer d-GGTCGACGTTTTTATT was accomplished starting from the solid support 4 (n=0; R^1=DMTR) in which the d-nucleoside thymidine is immobilized at the 3'-end by a succinate linkage to a controlled pore glass support[5]. The solid support (100 mg; capacity: 32 µmol/g) was packed into a column which is part of a continuous flow-bench synthesizer[16]. The several steps involved in one elongation process [i.e. conversion of 4 (n=0; B=T) into 4 (n=1; B=T)] are summarized in the Table. It can be seen that for the performance of one complete cycle seven steps (i.e. 2, 4, 6, 7 and 9-11) are required for the washing of the polymer after executing four different chemical steps: deblocking (step 1); coupling (step 3); oxidation (step 5) and capping (step 8). The effectiveness of the coupling step 3, which was estimated by measuring the release of the DMTR-cation by UV-spectroscopy at 498 nm, proceeded with an efficiency higher than 95%. In applying the above elongation procedure we prepared a fully-protected hexadecamer, which was completely deblocked under the conditions (base followed by acid) as described earlier for the deblocking of dimer 3 (B^1=T; B^2=G^{DPA}). The purified[17] (Sephadex G50) hexadecamer was completely digested, as judged from HPLC-analysis[18], by venom and spleen phosphodiesterase to give the expected d-nucleot(s)ides in the correct ratios. Furthermore, the hexadecamer was in every aspect - HPLC-analysis and gel electrophoresis - identical with the same hexadecamer prepared independently by a recently developed phosphotriester approach[19].

In conclusion, agent 1d has the same favourable phosphitylation properties (i.e. fast coupling) as the methyl derivative 1b, however, the MSE group, which can be removed selectively and fast under mild basic conditions, is more suitable, with respect to the basic conditions necessary to remove the other base labile groups [i.e. the N-protecting DPA and 2-methylbenzoyl (TOL) as well as hydrolysis of the succinate linkage] from 4, than the methyl group[20] for protecting P-O. Furthermore, preliminary experiments indicated that the crystalline agent 1c was very convenient for the introduction of phosphate functions at the anomeric centre of sugars.

Acknowledgements

We wish to thank Dr. J.F. Bol (Biochemistry Department) for performing the gel electrophoresis, and M. Lefeber for recording the ^{31}P-NMR spectra. One of us (J.E.M.) thanks SON for financial support.

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(Received in UK 9 January 1984)