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THE METHYL GROUP AS PHOSPHATE PROTECTING GROUP IN NUCLEOTIDE SYNTHESES

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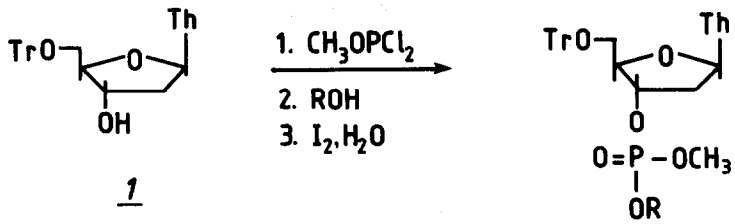
A novel method is described for the removal of a methyl protecting group from phosphotriesters in oligonucleotides.

The critical factor in the successful synthesis of oligonucleotides by the modern triester method[1] remains the phosphate protecting group. Numerous protecting groups have been proposed and these have been collected in the excellent review by Kössel and Seliger.[2] The two protecting groups most widely used in recent triester syntheses are the substituted phenyls[3] and the trichloroethyl group.[4] Both of these types of protecting groups have serious disadvantages. The removal of substituted phenyl protecting groups leads to concomitant cleavage of the internucleotide chain[3] and removal of trichloroethyl groups from cytidine and guanosine nucleotides often leads to low recovery of products.[5]

It is our opinion that the procedures described in this communication show that the methyl group possesses the necessary characteristics to be a very useful protecting group in oligonucleotide synthesis. Van Tamelen[6] has employed the methyl group in adapting the Letsinger dichloridite procedure[7] to methylphosphoro-dichloridite in the synthesis of some di- and triribonucleotides. The methyl group was easily removed from triesters using thiophenol and triethylamine. Miller and Ts'O had earlier used methyl protection of phosphate[8] in the synthesis of some deoxynucleotides.

Here we report a convenient method[9] for the removal of methyl groups from the phosphate triesters of protected oligonucleotides. Phosphate triesters are dissolved in t-butylamine, resulting in the quantitative removal of one methyl group. In the case of nucleotides we find that the fastest results are obtained when the nucleotide is dissolved in t-butylamine and the resulting solution is heated at reflux (46°C) for 15 h. Methyl groups are quantitatively removed within this period of time. The solvent is then evaporated to leave the pure nucleotide as the salt. In our experience this is the cleanest and easiest procedure for the removal of a triester protecting group since the use of cyanoethyl group in 1967.[1] The stability of the methyl group to the general conditions employed during synthesis, work-up and chromatography makes it superior to the cyanoethyl group in many situations.



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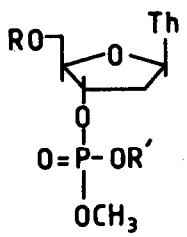

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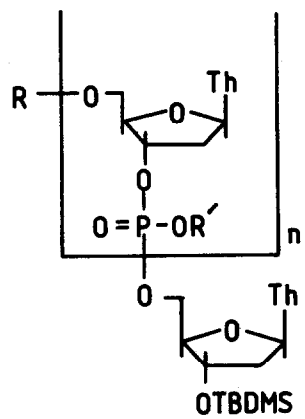

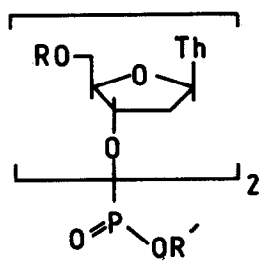

5

3 n=1

4 n=2

a R=Tr R′=CH_3

b R=H R′=CH_3

c R=H R′=H

While removal of the methyl group does require an overnight treatment, the conditions (46°C) are so mild, the solvent is so easily removed, and the product is so clean that the time used is a very minor consideration.

The procedures for the synthesis and demethylation of the triesters 2b-5b will illustrate the practicality of this method. Thus 5'-O-tritylthymidine (Tr-T, 1, 1.0 mmole) in THF (0.5 ml) was added over a 10 min period to a solution of $MeOPCl_2$ (1 eq) in THF (1 ml) containing collidine (4 eq) at -78°C. After an additional 15 min, methanol (1 mmole) was added and the solution was stirred at -78° for 30 min. The solution was then treated with I_2, H_2O as previously described.[7] The trityl group was removed directly with 80% HOAc (90°, 20 min) and the reaction mixture was applied directly to TLC plates (developed in $CHCl_3$:EtOH(9:1)). The yield of 2b was 40% along with a 25% yield of 5b. Compound 2b was heated under gentle reflux (46°C) in t-butylamine for 15 h to give, after evaporation of solvent, a quantitative yield of the monomethyl ester of thymidine 3'-phosphate (2c).

The dinucleotide 3b was obtained in 69% yield when TrT (0.5 mmole), $MeOPCl_2$ (1 eq) and 3'-t-butyldimethylsilylthymidine[10] (T-TBDMS, 0.25 mmole) were condensed as above followed successively by the iodine/water and acetic acid treatments. Compound 3b was extended to the trinucleotide 4a in 54% yield by a repetition of the phosphorylation and iodine steps. Compound 4a yielded 4b on detritylation. The nucleotides 3b and 4b were each treated with t-butylamine at reflux for 15 h to give quantitative conversions in to the nucleotides 3c and 4c respectively. It is of interest to note that the alkylsilyl group was unaffected by t-butylamine.

The 3'-3'-linked nucleotides 5b (or 5a, depending on work-up) were obtained as by-products in each of these syntheses in yields ranging from 10 to 20% (based on TrT). Compound 5b was quantitatively converted in to 5c on treatment with t-butylamine.

We are currently using the methyl protecting group in our attempt to synthesize a transfer RNA. The combination of alkylsilyl protecting groups, the Letsinger dichloridite procedures, and the methyl protecting group constitute a remarkably efficient total procedure for the synthesis of oligonucleotides.

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References:

1. R.L. Letsinger and K.K. Ogilvie, J. Amer. Chem. Soc., 89, 4801 (1967).
2. H. Kossel and H. Seleger, Prog. in the Chem. of Org. Nat. Prod., 32, 297 (1975).
3. J.H. van Boom, P.M.H. Burgers, P.H. van Deursen, R. Arentzen and C.B. Reese, Tetrahedron Letters, 3785 (1974); K. Itakura, N. Katagiri and S.A. Narang, Canad. J. Chem., 52, 3689 (1974).
4. F. Eckstein, Chem. Ber., 100, 2228 (1967); T. Neilson and E.S. Werstiuk, Canad. J. Chem., 49, 3004 (1971).
5. J.C. Catlin and F. Cramer, J. Org. Chem., 38, 245 (1973).
6. G.W. Daub and E.E. van Tamelen, J. Amer. Chem. Soc., 99, 3526 (1977).
7. R.L. Letsinger and W.B. Lunsford, ibid, 98, 3655 (1976).
8. P.S. Miller, K.N. Fang, N_S. Kondo and P.O.P. Ts'O, ibid., 6657 (1971).
9. M.D.M. Gray and D.J.H. Smith, preceding communication.
10. K.K. Ogilvie, Canad. J. Chem., 51, 3799 (1973).

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