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A FACILE METHOD FOR THE REMOVAL OF PHOSPHATE PROTECTING GROUPS IN NUCLEOTIDE SYNTHESIS

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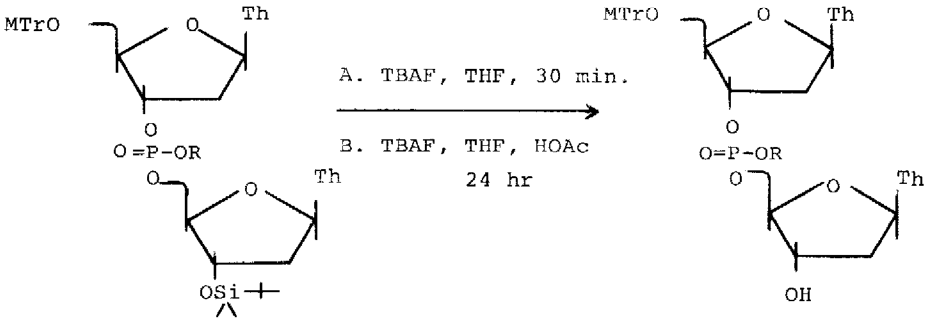
We have recently demonstrated the use of the alkylsilyl groups for protection of hydroxyl groups in nucleosides (1). A major advantage of these groups lies in their ready removal by tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF). We have found that this latter reagent also selectively removes one group from a triester of phosphoric acid. In nucleotide triesters (I) TBAF rapidly removes the phosphate protecting group (R) without affecting the internucleotide linkage. On workup quantitative yields of the nucleotide IIa are obtained. Among the protecting groups evaluated are the three most commonly used in nucleotide triester syntheses, the cyanoethyl (Ia,2), trichloroethyl (Ib,3) and the phenyl group (Ic,4). All of these groups are removed within 30 min. by two equivalents of TBAF in THF at room temperature. We have not detected any internucleotide cleavage under these conditions. At least for Ib and Ic fluoride ion attacks the phosphate displacing the alcohol since trichloroethanol and phenol are produced quantitatively (GC analysis).



I a, R = $-CH_2CH_2CN$
b, R = $-CH_2CCl_3$
c, R = $-C_6H_5$

II a, R = H
b, R = $-CH_2CCl_3$
c, R = $-C_6H_5$

We have not isolated the phosphorofluoridate which is presumably the other initial product. However, if formed, the phosphorofluoridate would be rapidly hydrolysed under the work-up conditions which involve either silica gel chromatography (large scale) or paper chromatography using aqueous solvents (small scale).

These conditions (A) represent a marked improvement in the removal of the trichloroethyl group where yields from the standard procedures employing zinc are often quite low (5). The phenyl protecting groups have been extensively used but present methods for their removal often result in cleavage of the nucleotide chain (6).

The above conditions (A) also result in the removal of the alkyl silyl group. However we have found that it is possible to completely remove the silyl group without any effect on the trichloroethyl or phenyl groups. Thus treating I with 10 equivalents of TBAF in THF containing 400 equivalents of glacial acetic acid for 24 hr. gives IIb and IIc in quantitative yields (Conditions B). Indeed with IIb only 12% of the trichloroethyl group is lost after six days. For Ia there is a 10% loss of the cyanoethyl group after 24 hrs. Acetic acid strongly solvates fluoride ions (7) and thus apparently reduces the rate of nucleophilic attack at phosphorus sufficiently to permit selective attack at silicon.

These procedures lead to a highly efficient synthesis of nucleotides. For example, the diphenyl derivative of 5'-O-monomethoxytritylthymidine 3'-phosphate is first treated with two equivalents of TBAF in THF for 30 min. Condensation of the product with 1.5 equivalents of 3'-*t*-butyldimethylsilylthymidine using two equivalents of trisopropylbenzenesulfonyl chloride (TPS) for 15 hr. and removal of protecting groups using first TBAF in THF followed by 80% acetic acid (to remove the methoxytrityl group) gives a 95% (isolated) yield of the dinucleotide TpT.

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References

+ Upjohn graduate fellowship awarded (1974-).

1. K.K. Ogilvie, E.A. Thompson, M.A. Quilliam and J.B. Westmore, Tetrahedron Letters 2865 (1974).
2. R.L. Letsinger and K.K. Ogilvie, J. Amer. Chem. Soc. 89, 4801 (1967).
3. F. Eckstein and I. Rizk, Angew. Chemie., 79, 939 (1967).
4. C.B. Reese and R. Saffhill, Chem. Comm., 767 (1968).
5. J.C. Catlin and F. Cramer, J. Org. Chem., 38, 245 (1973).
6. J.H. van Boom, P.M.J. Burgers, P.H. Van Deursen, R. Arentzen and C.B. Reese, Tetrahedron Letters, 3785 (1974).
7. M.K. Wong and A.I. Popov, J. Inorg. Nucl. Chem., 33, 1203 (1971).