Tetrahedron Letters, Vol.22, No.27, pp 2531 - 2532
Printed in Great Britain

0040-4039/81/272531-02$02.00/0

NONAQUEOUS OXIDATION OF PHOSPHITES TO PHOSPHATES IN NUCLEOTIDE SYNTHESIS

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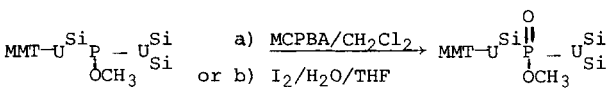
Summary - m-Chloroperbenzoic acid in methylene chloride represents a rapid non-aqueous method for the conversion of phosphites to phosphates during oligonucleotide synthesis both in solution and on a solid support.

The phosphite triester procedure, originally introduced by Letsinger (1), has proven to be a rapid and general procedure for the synthesis of oligonucleotides (1-4). The method involves the initial condensation of an activated phosphite with a nucleoside to provide an intermediate phosphite (e.g. 1, 5). This intermediate can be isolated (5)

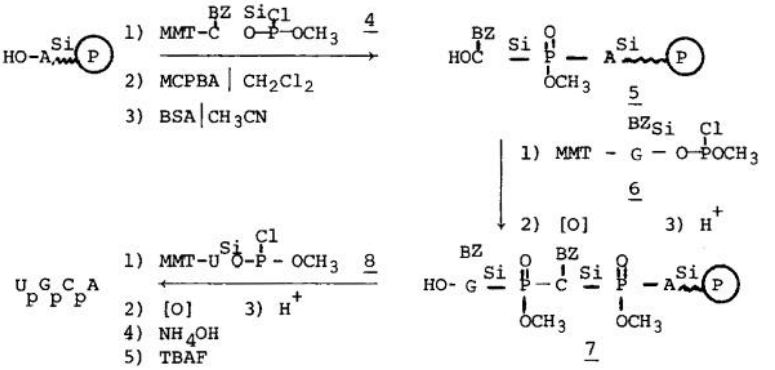


but is usually oxidized directly to the phosphate 2 using iodine in aqueous solution (1-5). There are situations, particularly during polymer support syntheses (3,4) where it is desirable to avoid the introduction of water at any stage. This is particularly true in attempts to minimize the number of steps (i.e. time) in each cycle of an automated procedure. The use of aqueous solvents at any stage necessitates a drying step thereafter.

We wish to report that m-chloroperbenzoic acid (MCPBA) in methylene chloride (CH_2Cl_2) represents a rapid, non-aqueous oxidizing agent for the conversion of phosphites to phosphates during nucleotide synthesis. For example, to compound 1 (40 mg) in CH_2Cl_2 (1 ml) was added 0.1 ml of a 0.5 N solution of MCPBA in CH_2Cl_2. After 5 min the solution was extracted successively with sodium bisulfite (10% aq) and sodium bicarbonate (5% aq). The organic layer was concentrated to reveal a quantitative yield of 2 which was identical to an authentic sample prepared previously (6) using iodine oxidation.

To determine the applicability of this reagent to solid phase synthesis, the tetramer $U_pG_pC_pA$ was prepared in the following manner. Polymer bound adenosine (3, 1 g, 0.03 mmole of A/g, prepared as previously described (4)), was suspended in THF (1.5 ml). A solution containing the 3'-methylchlorophosphite of 5'-monomethoxytrityl-2'-TBDMS-N-benzoylcytidine (4, 0.13 mmole, ref. 4) in 0.35 ml of THF containing 0.1 ml of collidine was added dropwise at room temperature. The mixture was stirred for 30 min and the polymer was collected by filtration and washed with CH_2Cl_2. The polymer was then stirred with CH_2Cl_2 (1 ml) containing 0.08 ml of a 0.5 N solution of MCPBA in CH_2Cl_2. After 5 min the gel was collected by

filtration, washed with CH_2Cl_2 and ether, and treated for 5 min with a 10% solution of benzenesulfonic acid (BSA) in acetonitrile. The polymer was collected by filtration, washed with CH_2Cl_2 and after removal of a small sample for analysis, the remainder was used immediately in the next step.



The cycle was repeated except that the quanosine derivative 6 (0.13 mmole, 4) was used. Again, a small sample of 7 was removed and the remainder was treated to a cycle using the uridine derivative 8 (0.13 mmole, 4). At the end of the acid hydrolysis (step 3), the product was cleaved from the polymer using NH_4OH:EtOH(4:1) during 14 h at 20° C. The product was treated with TBAF (4) followed by passage through a DOWEX 50W x 8 Na^+ column (4) and was then isolated pure by paper chromatography in solvent F (4). The isolated yield of $U_pG_pC_pA$ was 80% based on the amount of A attached to the original polymer.

The product $U_pG_pC_pA$ was completely degraded by RNAseT$_2$ to U_p, G_p, C_p and A in the correct ratio. The intermediate nucleotides C_pA and G_pC_pA were identical to authentic samples and were completely degraded by RNAseT$_2$.

The results described in this report demonstrate that m-chloroperbenzoic acid provides an excellent non-aqueous oxidizing agent for use in the phosphite triester procedure, both in solution and on a polymer support.

Acknowledgement

We are grateful to the Natural Sciences and Engineering Research Council of Canada, The Quebec Ministry of Education (FCAC), and Bio Logicals Inc. for financial support.

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(Received in USA 13 April 1981)