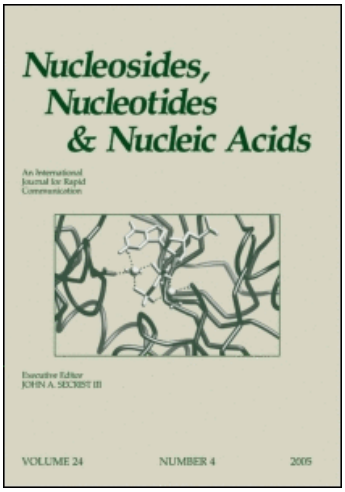

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Phosphotriester Synthesis of Oligonucleotides with the Use of N- and O-Nucleophilic Intramolecular Catalysis

V. A. Efimov[a]; A. A. Buryakova[a]; N. N. Polushin[a]; I. Y. Dubey[a]; O. G. Chakhmakhcheva[a]; Yu. A. Ovchinnikov[a]
[a] Shemyakin Institute of Bioorganic Chemistry USSR Academy of Sciences, Moscow, USSR

To cite this Article Efimov, V. A. , Buryakova, A. A. , Polushin, N. N. , Dubey, I. Y. , Chakhmakhcheva, O. G. and Ovchinnikov, Yu. A.(1987) 'Phosphotriester Synthesis of Oligonucleotides with the Use of N- and O-Nucleophilic Intramolecular Catalysis', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 279 — 282
To link to this Article: DOI: 10.1080/07328318708056204
URL: http://dx.doi.org/10.1080/07328318708056204

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 279-282 (1987)

PHOSPHOTRIESTER SYNTHESIS OF OLIGONUCLEOTIDES WITH THE USE OF N- AND O-NUCLEOPHILIC INTRAMOLECULAR CATALYSIS

V.A.Efimov, A.A.Buryakova, N.N.Polushin, I.Y.Dubey, O.G.Chakhmakhcheva and Yu.A.Ovchinnikov

Shemyakin Institute of Bioorganic Chemistry USSR Academy of Sciences, ul.Miklukho-Maklaya 16/10, Moscow 117871,USSR

Abstract. An effective method for the synthesis of oligonucleotides by the phosphotriester approach has been developed. It is based on the use of phosphate protecting groups enabling O-nucleophilic intramolecular catalysis.

Earlier we reported the improved rapid phosphotriester method based on the use of oxygen-nucleophilic catalysts - 4-substituted derivatives of pyridine N-oxide in conjunction with condensing and phosphorylating reagents for the internucleotide bond formation [1]. The application of these catalysts allowed to reduce the coupling time in solution to 1-2 min and on solid-phase to 4-5 min. It was shown that O-nucleophilic catalysts are effective with the use of alkyl phosphate blocking groups [2].

Recently, 2-(1-methylimidazole-2-yl)phenyl group has been reported as catalytic phosphate protecting group enhancing the rate of internucleotide condensation due to an active cyclic intermediate formation [3,4]. We have shown that the similar effect was observed with the use of several other N- and O-nucleophilic catalytic blocking groups, such as 1-oxido-4-alkoxy-2-picolyl, 4-alkoxy-2-picolyl and 1-oxido-2-picolyl groups, for the internucleotide phosphate protection [5].

The monomeric units carrying these protecting groups were obtained from the corresponding fully blocked nucleo-

side phosphotriesters by the selective cleavage of the non-catalytic aryl, or alkyl, phosphate blocking group.The synthesis of phosphotriesters (II, III) was performed by several ways shown in the Scheme. The arylphosphotriesters (II) were quantitatively converted into the desired phosphodiesters (IV) without any isolation by the action of bases, such as an oximate reagent or LiOH, whereas the cyanoethyl blocking group was removed from the crude phosphotriester (III) by the action of triethylamine. The resulting monomeric nucleotide components (IV) were isolated by a silica gel colunm chromatography.

The results on the comparison of the reaction rates in the synthesis of a dinucleotide using different protective groups are shown in Fig.1. The highest coupling rate was provided by 1-oxido-4-alkoxy-2-picolyl derivatives. The condensations with the use of this intramolecular catalysts and TPSCl were complete less than in 1 min in pyridine and less than in 0.5 min in acetonitrile containing 10% of pyridine, 2,6-lutidine or 2,4,6-collidine. The application of MSCl and MSNT gave the coupling two times faster. On a polymer support the use of the same protecting groups provided 98-99% yields in coupling reactions within 1-2 min. The reaction rate was not practically dependent on the structure of the substituent in the fourth position of the pyridine ring of the 1-oxido-4-alkoxy-2-picolyl group. It should be noted that the use of catalytic prote ting groups provided a minimum of 5'-sulfonated by-product (<0.2%), that is apparently due to differential catalysis of the phosphorylation and sulfonation reactions.

The examination of the stability of new catalytic protecting groups in various conditions have revealed that they are stable during internucleotide condensation. These groups are stable to the action of acidic reagents, which are used for the removal of 5'-trityl protecting group,and to the action of bases. At the same time, they can be easily removed by the action of nucleophilic reagents, such as triethylammonium thiophenate or piperidine.

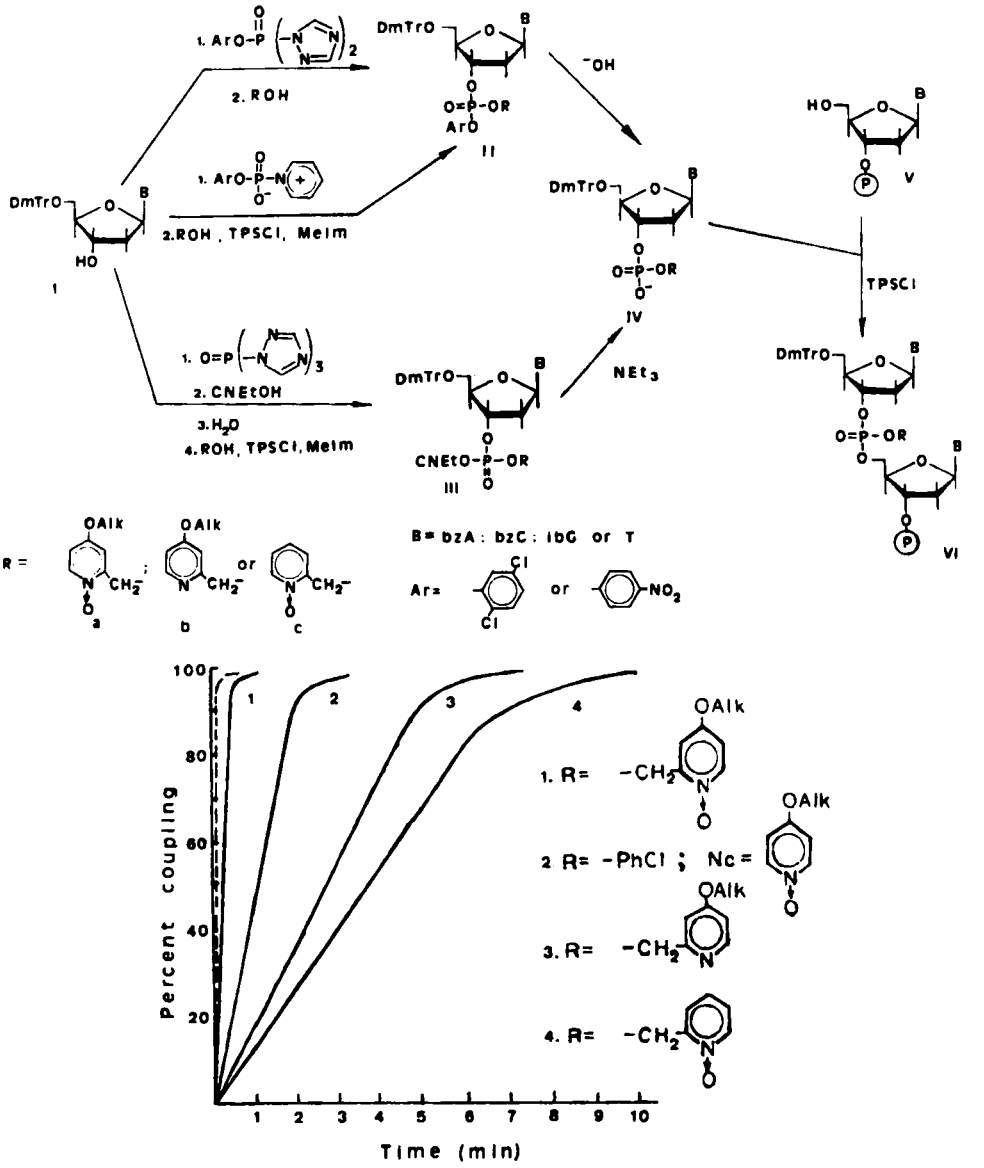

FIGURE 1. Coupling rates in the synthesis of dinucleotide [$(MeO)_2$ Tr] Tp(R)T(Ac). Reactions were carried out in 1 ml of pyridine (solid line) or acetonitrile-pyridine (9:1) (dashed line) using 0.05 mmol of T(Ac) and 0.05 mmol of [$(MeO)_2$Tr] Tp(R) in the presence of 0.1 mmol of TPSCl. In the case of the o-chlorophenyl derivative, 0.2 mmol of 4-methoxypyridine N-oxide was added.

The monomers carrying O-nucleophilic catalytic blocking groups were successfully employed for the synthesis of various oligodeoxyribonucleotides on solid phase. The coupling reactions were performed using the 10-fold excess of a P-component over the resin capacity in the presence of 3-fold excess of a condensing agent with respect to a P-component. Pyridine, its mixture with acetonitrile, mixtures of acetonitrile and 2,6-lutidine,or 2,4,6-collidine, were used as solvents in condensation reactions. The time needed to perform one cycle of chain elongation was less than 7 min. The average yield per step about 98% [5].

The removal of the protecting groups from the final oligonucleotides after completion of the synthesis was carried out in three steps: a) treatment with piperidine (or with triethylammonium thiophenate) to remove phosphate protecting groups; b) treatment with concentrated ammonia to remove acyl protecting groups; c) treatment with 80% acetic acid to remove 5'-dimethoxytrityl group. The deprotected oligonucleotides were isolated by preparative electrophoresis on denaturing polyacrylamide gels and/or reversed-phase chromatography.

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