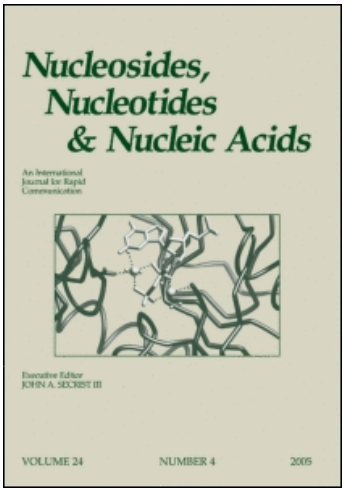


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Studies on the Synthesis of Oligonucleotides *via* the Hydrogenphosphonate Approach

Per J. Garegg[a]; Tor Regberg[a]; Jacek Stawinski[a]; Roger Stromberg[a]
[a] Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden

To cite this Article Garegg, Per J. , Regberg, Tor , Stawinski, Jacek and Stromberg, Roger(1987) 'Studies on the Synthesis of Oligonucleotides *via* the Hydrogenphosphonate Approach', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 283 — 286
To link to this Article: DOI: 10.1080/07328318708056205
URL: http://dx.doi.org/10.1080/07328318708056205

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 283-286 (1987)

STUDIES ON THE SYNTHESIS OF OLIGONUCLEOTIDES VIA THE HYDROGENPHOSPHONATE APPROACH

Per J. Garegg, Tor Regberg, Jacek Stawinski*, Roger Strömberg

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden.

Abstract. ^{31}P NMR studies on the activation and coupling of nucleoside 3'-hydrogenphosphonates with nucleosides in the presence of various condensing agents, are discussed.

Recently we have reported on a new type of nucleotidic units[1], namely nucleoside 3'-H-phosphonates, which can be useful in chemical synthesis of DNA and RNA fragments[2].

Nucleoside 3'-H-phosphonates were found to be stable compounds, but became very reactive upon activation by various condensing reagents, affording in ca 1 min, the corresponding H-phosphonate diesters in the reaction with nucleosides[1,2].

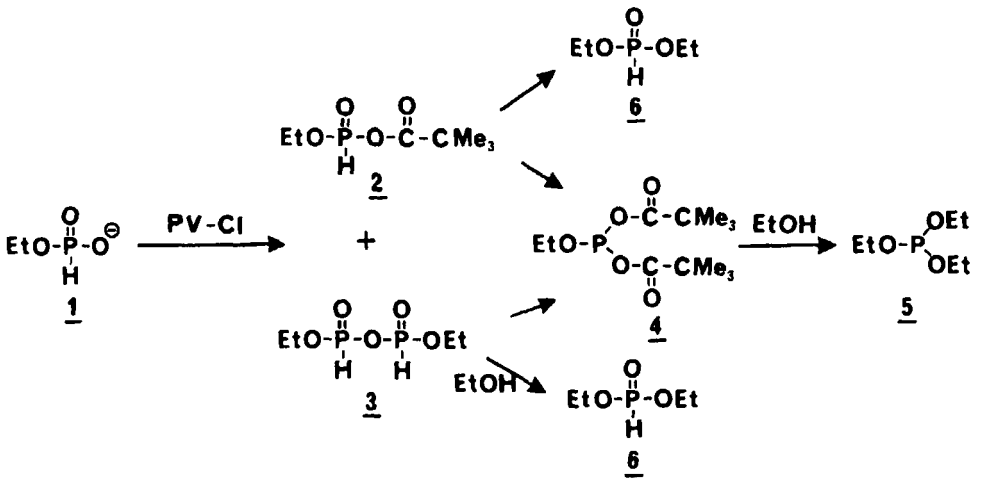
A rapid coupling reaction, which was even faster than that in the phosphoroamidite approach to oligonucleotide synthesis, promped us to investigate, what kind of intermediates are involved in the hydrogenphosphonate diester formation.

^{31}P NMR studies on the activation of H-phosphonate monoesters.

A characteristic feature of the reaction of nucleoside 3'-H-phosphonates with nucleosides is an extremly rapid H-phosphonate diester formation, when H-phosphonate monoester is activated by condensing reagent in the presence of nucleoside. Preactivation of nucleoside 3'-H-phosphonate, followed by addition of a nucleosidic component, resulted in substantial lower yields of the desired H-phosphonate diesters. This was invariably observed for all coupling reagents investigated, e. g. 2,4,6-triisopropylbenzenesulfonyl chloride and tetrazole (TPS-Cl and TPS-Te), diphenyl chlorophosphate (DPCP), N,N-bis(2-oxo-3-oxazolidinyl)-phosphorodiamidic chloride (OXP), pivaloyl chloride (PV-Cl), and anisoyl chloride (An-Cl), under different reaction conditions.

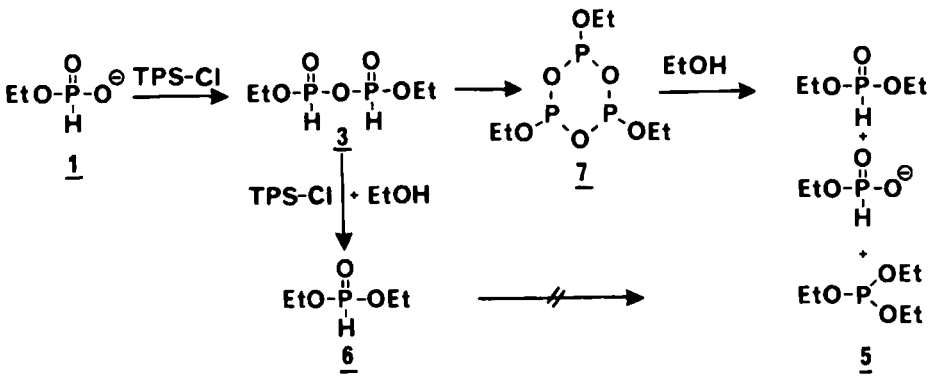
The ^{31}P NMR spectra recorded during regular coupling reaction (activation of a H-phosphonate monoester in the presence of a nucleoside), showed no accumulation of any intermediate, which indicated that reactive species formed from the H-phosphonate monoester and a coupling reagent are immediately trapped by the nucleoside, forming the H-phosphonate diester.

Model studies on the reaction of ethyl H-phosphonate 1 with PV-Cl revealed the formation of two intermediates, acyl-hydrogenphosphonate ? (main product, ca 90%) and H-pyrophosphonate 3 (minor product, ca 10%), which were converted with an excess of acyl chloride into the bisacyl phosphite 4. The latter intermediate, upon addition of ethanol



was converted into phosphite triester 5, while the two former ones (in a separate experiment), under these conditions afforded the H-phosphonate diester 6. Because we did not observe the formation of phosphite triester 5during the regular coupling reaction, and since the H-phosphonate 6 could not be converted into 5 even with an excess of PV-Cl and ethanol, 2 and 3 should be considered as main intermediates involved in H-phosphonate diester formations, when acyl chlorides are used as condensing reagents.

When ethyl H-phosphonate 1 was subjected to the activation in pyridine with aryl sulfonyl derivatives (TPS-Cl, TPS-Te, BS-Cl) or chlorophosphates (DPCP, OXP), the formation of another intermediate, containing trivalent phosphorus, was observed. This intermediate has been identified as a trimetaphosphite 7. Since the subsequent reaction of 7 with ethanol



afforded a mixure of the starting material 1, diethyl H-phosphonate 6 and triethyl phosphite 5, compound 7 can not be considerd as intermediate involved in the H-phosphonate diester formation during regular coupling reaction. However, when activation of 1 has been carried out in acetonitrile, we did not observe formation of trimetaphosphite 7, but instead, H-pyrophosphonate 3. Because the latter species afforded a H-phosphonate diester in the reaction with ethanol, we may assume that H-pyrophosphonate 3 and/or mixed anhydrides, (which are supposed to be the primary products of the activation of H-phosphonate monoesters, but which we could not detect in the ^{31}P NMR spectra), are the reactive intermediates involved in the H-phosphonate diester formation.

It should be added that during preactivation of H-phosphonate monoesters with aryl sulfonyl derivatives, also an additional reaction, namely, oxidation of H-phosphonate monoester, occurs.

Possible side-reaction during H-phosphonate diester synthesis

It might be expected that the formation of H-phosphonate diester bonds via activation of nucleoside 3'-H-phosphonates by condensing reagents, be accompanied by several side-reactions. However, due to the very short time of condensation, many of these reactions occur to rather small extent and can be practically neglected. For example, we have demonstrated previously[1] that synthesis of H-phosphonate diester may be accomplished even with benzenesulfonyl chloride, without noticeable sulfonation of a nucleosidic component.

Thus, the only serious sources of side-reactions, which may occur during the assemble of a oligonucleotidic chain, are phosphorus centers with the P-H bonds, and guanine residues, having a rather reactive heterocyclic lactam system.

^{31}P NMR studies on the synthesis of dinucleoside H-phosphonates in the presence of aryl sulfonyl derivatives showed, that formation of H-phosphonate diester bond is faster than the oxidation of the starting material. However, when the reaction mixtures were kept for a prolonged time with an excess of the condensing reagent, oxidation of H-phosphonate diesters was observed.

Chlorophosphates (DPCP, OXP) and acyl chlorides (PV-Cl, AnCl), were found to promote fast and clean H-phosphonate diester formation. However, with an excess of condensing reagent (10 equiv.) and prolonged condensation time (up to 1 h), a subsequent O^6-phosphorylation or O^6-acylation of guanine residues in the dinucleoside H-phosphonates, occurred. Phosphorylation of guanine residues under these conditions was almost quantitative in the case of DPCP, while with OXP, this side-reaction occurred only to small extent (less than 5%). Generally, the reaction of condensing reagents with heterocyclic lactam system of guanine can be alleviated by O^6- protection of guanosine[3]. However, we have found, that both kind of modifications can be reversed by treatment with aq. pyridine or 2% aq. ammonia in pyridine, during a few minutes.

We could not detect, using ^{31}P NMR spectroscopy, any reaction of PV-Cl with the P-H bonds, and it seems that a strong base (e. g. triethylamine) or a nucleophilic catalyst (e. g. N-methyl imidazole) is necessary to promote the acylphosphonate formation.

REFERENCES

1. P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, Chem. Scr., 25, 280 (1985); ibid., 26, 59 (1986).
2. P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, C. Henrichson, Tet. Lett., 27, 4051 (1986); ibid., 27, 4055 (1986).
3. F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, Tetrahedron, 40, 59 (1984).