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USE OF NEW PHOSPHONYLATING AND COUPLING AGENTS IN THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES VIA THE H-PHOSPHONATE APPROACH

Osamu Sakatsume, Hiroshi Yamane, Hiroshi Takaku*, and Naoki Yamamoto+

Laboratory of Bioorganic Chemistry, Department of Industrial Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275, Japan, and Department of Virology and Parasitology, Yamaguchi University School of Medicine, Ube 755, Japan

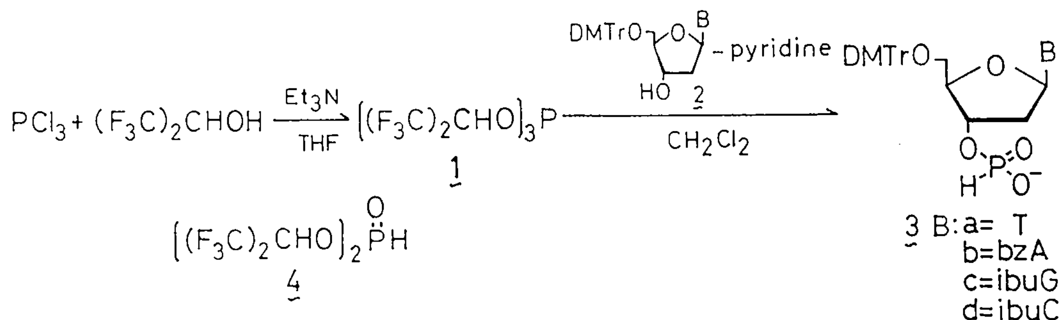
Abstract---*Tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite was used as a phosphonylating reagent for the preparation of nucleoside 3'-H-phosphonate units. The use of a new coupling reagent, 1,3-dimethyl-2-chloro-imidazolinium chloride (DMCI) for the internucleotidic H-phosphonate bond formation via the H-phosphonate approach is also discussed in detail.*

The use of nucleoside 3'-H-phosphonates was introduced for the first time by Todd et.al.[1] to prepare 3'-5'-internucleotidic bonds. The synthesis of 3'-5'-H-phosphonate bonds was explored further in more recent studies by several groups.[2-5] The H-phosphonate approach shows the following advantages: protection of phosphate is not required; the oxidation reaction is performed by I_2 solution at end of the synthetic cycles; the coupling reaction is much faster than the phosphoramidite approach; the H-phosphonate units are more stable than the phosphoramidite units, and capping step after each coupling reaction can be omitted.[3,6] However, this approach introduces some problems, such as the need for a convenient procedure for the preparation of H-phosphonate units and the stability of pivaloyl chloride as a coupling reagent which still remain to be solved.[3,7,8]

In this paper, we wish describe two developments that tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite (**1**) is one of promising phosphonylating reagents for the preparation of deoxyribonucleoside 3'-H-phosphonate units (**3**) and DMCI as a new coupling reagent can be used for the internucleotidic H-phosphonate bonds formation via the H-phosphonate approach.

The deoxyribonucleoside 3'-H-phosphonates are key intermediates in the synthesis of oligodeoxyribonucleotides by the H-phosphonate approach. However, only a few methods have been reported for the synthesis of H-phosphonate units.[3,9-12] One method, reported by Garegg et al.[3], involves the use of tris-(imidazol-1-yl)phosphine, an unstable reagent that is generated in situ. In a previous paper[13], we reported that bis(1,1,1,3,3,3-hexafluoro-2-propyl)phosphonate (**4**) was a useful reagent for the preparation of H-phosphonate units. We now found that tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite (**1**) is more effective for the preparation of H-phosphonate units (**3**) than the phosphonylating reagent **4**. The latter reagent **1** is easily prepared in 73% yield by treatment with PCl_3 and 1,1,1,3,3,3-hexafluoro-2-propanol in the presence of triethylamine using a modification of the procedure reported by Denney et al.[14]. The required deoxyribonucleoside 3'-H-phosphonate building blocks (**3**) were readily prepared by allowing the corresponding N-acyl-5'-O-(dimethoxytrityl)-deoxyribonucleosides (**2**) to react

with 1.1 molar equiv. of **1** in the presence of a catalytic amount of triethylamine in CH_2Cl_2 at room temperature for 5 min, followed by a hydrolytic work-up and chromatography of the products. Yields of H-phosphonate units **(3)** ranged from 87-95%. The ^{31}P-NMR spectra data ($CDCl_3$/85% H_3PO_4) were as follows: B=T, 2.81; B=bzA, 2.71; B=ibuG, 2.89; B=ibuC, 2.71ppm. This results indicate that the use of **1** as a phosphonylating reagent considerably shortens the reaction time for the preparation of protected deoxyribonucleoside 3'-H-phosphonates **(3)**.



In our continuing study to develop the coupling reagents for the internucleotidic H-phosphonate bond formation, we have found that a new coupling reagent, 1,3-dimethyl-2-chloroimidazolinium chloride (DMCI) which is a stable, crystalline compound, and has good solubility in most organic solvents. The coupling properties of DMCI (2.0 molar equiv.) have first been studied with the H-phosphonate unit **(3a)** (1.0 molar equiv.) which reacted with 3'-O-benzoylthymidine (1.1 molar equiv.) in CH_3CN-pyridine (1:1) at room temperature for 10 min. The reaction was monitored by ^{31}P-NMR (Fig. 1). The spectrum of the reaction mixture showed that a signal of the H-phosphonate **(3a)** completely disappeared and new signals were observed at 8.21 and 7.20 ppm. The chemical shift suggested that **3a** was converted into the corresponding H-phosphonate diester **(5)**.

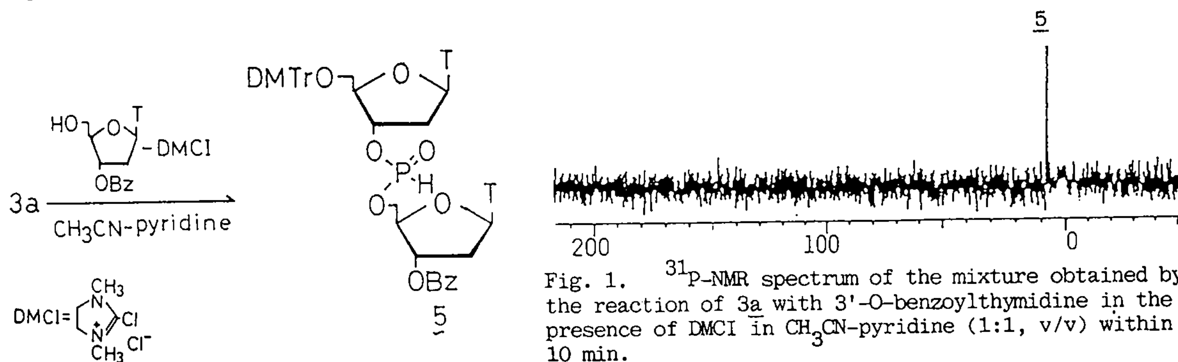


Fig. 1. ^{31}P-NMR spectrum of the mixture obtained by the reaction of 3a with 3'-O-benzoylthymidine in the presence of DMCI in CH_3CN-pyridine (1:1, v/v) within 10 min.

To ascertain the coupling efficiency of DMCI, we tried to a comparison with pivaloyl chloride (PVC) in the solid phase synthesis of d-$(Tp)_{14}$T. The reaction was carried out on deoxythymidine-CPG (19.5 mg, 39 µmol/g) with a Applied Biosyatems Model 381A DNA synthesizer. We showed the following elongation cycle to be effective: treatment with (1) washing [CH_3CN, 20 sec], (2) 5'-unblocking [3% Cl_3C-COOH in CH_2Cl_2, 90 sec], (3) washing [CH_3CN, 30 sec], (4) coupling [17.5 µmol H-

phosphonate unit (**3a**), 87.5 μmol PVC (a), 4.38 μmol DMCI (b) in CH_3CN-pyridine (1:1, v/v), 5 min], (5) washing [DMF, 30 sec]. When the assembly of the oligo-

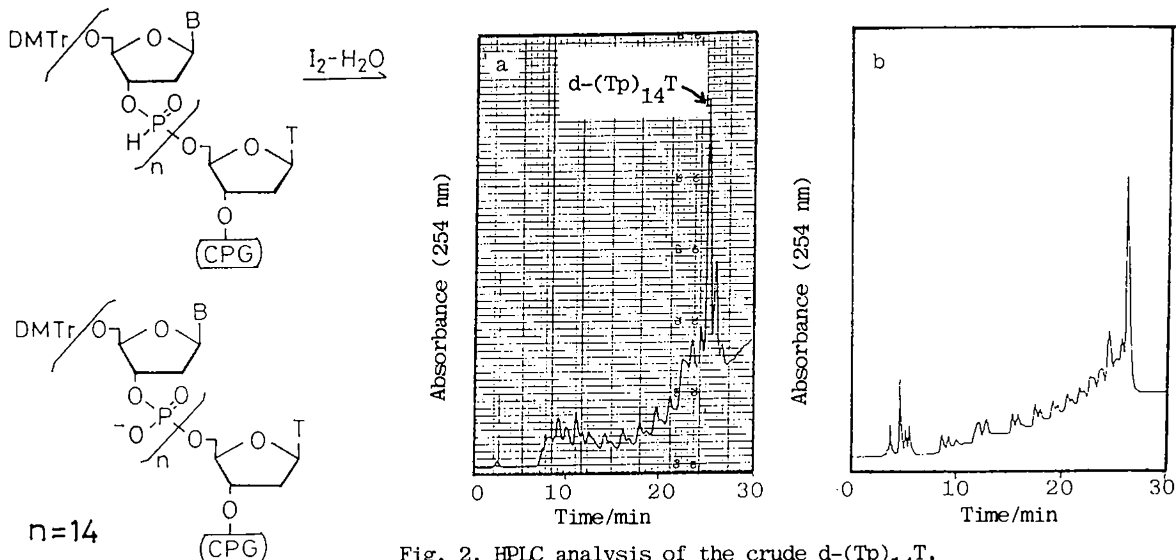


Fig. 2. HPLC analysis of the crude d-$(Tp)_{14}$T.
A TSK gel DEAE 2SW column was used with a linear gradient of ammonium formate (0.17-0.94 M) in 20% CH_3CN during 30 min. The molar quantities of the coupling reagents [PVC (a) and DMCI (b)] based on the H-phosphonate unit 3a were 5.0 equivalent and 2.5 equivalent.

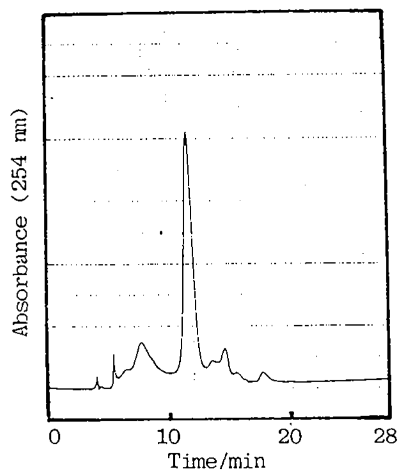


Fig. 3. HPLC analysis of the crude mixture containing d-CCCAATTCTGAAAAT after deprotection using a TSK gel oligo-DNA RP column with a linear gradient of CH_3CN in 0.1 M TEAA (pH 7.0) during 30 min at the flow rate of 1 min/min.

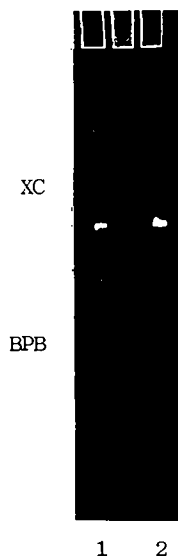


Fig. 4. 20% Polyacrylamide gel elctrophoresis in 7 M urea of $d(Tp)_{14}T$ (Lane 1) and dCCCAATTCTGAAAAT (Lane 2).

nucleotide chain was completed, the solid support was treated with 0.1 M I_2 in THF-pyridine-H_2O (4:3:3, v/v) for 15 min. After usual deprotection, isolation of the desired oligomer, d-$(Tp)_{14}$T was performed by TSKgel DEAE-2SW (Fig. 2a,b). The

profiles showed that the pentadecamers were synthesized with the same efficiency in each coupling reagent. However, the coupling reaction using DMCI was carried out effectively at a half coupling reagent compared with pivaloyl chloride[3]. Further, it is a stable, crystalline compound, and has good solubility in most organic solvents. On the other hand, the condensation using excess (5 molar equiv.) DMCI did not proceed smoothly and the yield became lower.

Finally, we applied to the synthesis of 5'-dCCCAATTCTGAAAAT-3', complementaly sequence to the splice aceptor site in HTLV-III[15]. The synthesis was performed smoothly to give the the dCCCAATTCTGAAAAT in each average yield of 94%. The solid support was treated with conc. ammonia at 55°C for 6 h. The tritylated product was separated by reversed phase C-18 silica gel and unblocked with 80% AcOH. The unblocked oligomer, dCCCAATTCTGAAAAT was further purified by reverse phase C-18 HPLC (Fig. 3). The main peak was found to be homogeneous by reverse phase C-18 HPLC and by gel electrophoresis (Fig. 4). The proportions of four nucleosides were analyzed by reverse phase C-18 HPLC after hydrolysis with snake venom phosphodiesterase and alkaline phosphatase. The overall yield from the deoxythymidine-CPG was ca. 21%.

In conclusion, tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite (**1**) and 1,3-dimethyl-2-chloroimidazolinium chloride (DMCI) could be utilized as promising reagents for the synthesis of oligodeoxyribonucleotides by the H-phosphonate approach on a solid support.

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