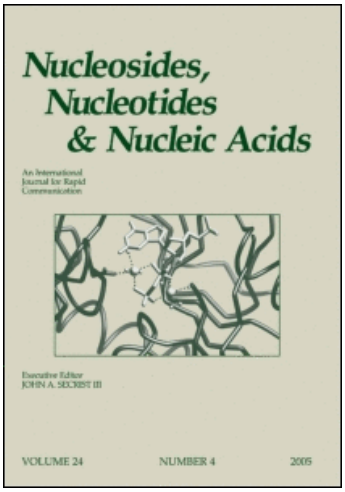

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Use of New Phosphonylating and Condensing Agents in The Synthesis of Oligonucleotides Via The H-Phosphonate Approach

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USE OF NEW PHOSPHONYLATING AND CONDENSING AGENTS IN THE SYNTHESIS OF OLIGONUCLEOTIDES VIA THE H-PHOSPHONATE APPROACH

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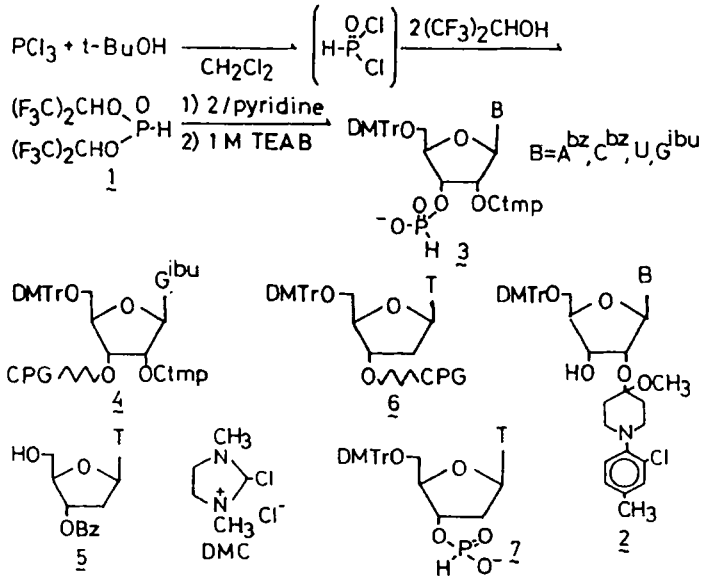
Abstract

Bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphonate was most promising as a phosphonylating agent for the preparation of nucleoside-3'-phosphonate units. 1,3-Dimethyl-2-chloro-imidazolinium chloride (DMC) as a coupling agent has successfully been used for the internucleotidic H-phosphonate bonds formation via the H-phosphonate approach on a solid support.

The use of nucleoside-3'-H-phosphonates was introduced for the first time by Todd et al. to prepare 3'-5'-internucleotidic bonds.[1] The synthesis of 3'-5'-H-phosphonate bonds was explored further in more recent studies by several groups.[2] 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 much more stable than the phosphoramidite units, and capping step after each coupling reaction can be omitted. However, this approach has still remain some problems of the preparation of H-phosphonate units and the lifetime of pivaloyl chloride as a coupling agent.[3,4]

In this paper, we wish describe two developments that bis-(1,1,1,3,3,3-hexafluoro-2-propyl) phosphonate agent (1) is most promising as a phosphonylating agent for the preparation of nucleoside H-phosphonate units (3) and DMC as a coupling agent can be used for the internucleotidic H-phosphonate bonds formation via the H-phosphonate approach.



The nucleoside H-phosphonate units (3) are key intermediates for the oligonucleotide synthesis by the H-phosphonate approach. One of the ordinary phosphitylating agent is tri(1,2,4-triazole)-phosphite reported by Garegg[5]. However, this agent has some disadvantages; instability of the phosphitylating agent and a necessity of use of a large excess of the phosphitylating agent for the preparation of H-phosphonate units. We have found that the transesterification of a new phosphonylating agent (1) is much more effective for the preparation of 3 than other phosphitylating agents[5,6]. The phosphonylating agent 1 was prepared in good yield by a modification of the procedure reported by Imai et al.[7,8]. The 5',2'-O-N-protected ribonucleosides (2) (1.0 molar equiv.) were treated with 1 (3.0 molar equiv.) in dry pyridine. After 6 h, the reaction mixture was quenched with 0.1M TEAB solution and the product was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and dried over $MgSO_4$. The organic layer was concentrated to give the corresponding H-phosphonate units (3) in 90-97% yields. We observed ^{31}P-NMR spectroscopically pure materials which can be used coupling reaction without further purification. In above reaction, the use of tetrazole shortened dramatically the time for the preparation of the H-phosphonate units (3). The reaction could be achieved by using tetrazole for 15 min to give the H-phosphonate units (3) in good yields.

Next, we examined the solid phase synthesis of oligoribonucleotides using the H-phosphonate approach and the 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (Ctmp)[9] and DMTr groups for the protection of the 2'- and 5'-hydroxyl functions, respectively. The succinate ester of 2' or 3'-acetylated guanosine derivative was attached to fanctionalized CPG in usual way[10] to give the loaded support (4). The nucleoside loading of 4 was estimated to be 24 μmol/g. The synthesis of $(Up)_7G$ was performed by manual method. After the synthetic cycles were over, the H-phosphonate product was oxidized to the phosphate with 0.1 M I_2 in THF-pyridine-H_2O (44:3:3, V/V). The solid support was treated with conc. ammonia at 55 °C for 8 h. The support was removed by filtration. The filtrate was evaporated and dissolved in 0.01 N HCl (pH 2.0) (12 h). The unblocked oligomer was purifited by TSKgel DEAE-2SW.

Further, we examined the possibility of coupling agent for the internucleotidic H-phosphonate bonds formation using DMC. Recently, pivaloyl chloride (PV-Cl) proved to be a most suitable coupling agent for the synthesis of oligonucleotides by the H-phosphonate approach on solid support.[2c] However, PV-Cl must be carefully distilled prior to the coupling reaction, and in pyridine-CH_3CN is unstable. In contract, DMC was very stable in the solvent and the side reactions to the 5'-hydroxyl function of nucleosides during the coupling reactions were not observed at all. In order to explore the side reactions of the 5'-hydroxyl function of nucleosides with DMC, we tried the reaction of DMC with 3'-O-benzoylthymidine (5) in pyridine-CH_3CN. In this reaction, 99% of 5 was recovered unchanged from the reaction mixture after 8 h.

Finally, we examined the solid phase synthesis of d-$(Tp)_{14}T$ using DMC as a coupling agent via the H-phosphonate intermediates. The nucleoside resin (6) (1 μmol) was treated with 7 (30 molar equiv.) and DMC (150 molar equiv.) in pyridine-CH_3CN for 1 min to give the d-$(Tp)_{14}T$ in the each average yield of 92%. It is reasonable to conclude from the data presented here that the new phosphonylating and condensing agents are likely to be suitable for the synthesis of oligonucleotides by H-phosphonate approach on a solid support.

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