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A Convenient Approach to the Synthesis of Deoxyribonucleoside-3'-hydrogen Phosphonates via Bis(1,1,1,3,3,3-hexafluoro-2-propyl) Phosphonate Intermediate

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Transesterification of a new reagent, bis(1,1,1,3,3,3-hexafluoro-2-propyl)phosphonates was found to be very effective for the preparation of deoxyribonucleoside-3'-hydrogen phosphonates. The yields of deoxyribooligonucleotides by the H-phosphonate method on a solid support depended on the molar concentrations of the reacting species.

The use of nucleoside-3'-hydrogen 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. For instance, Ogilvie[2] and Hata[3] have reported the synthesis of 3'-5'-internucleotidic H-phosphonate bonds via the phosphite triester intermediates and aroylphos-phonate protected nucleosides. More recently, Stawinski[4] and Matteucci[5] have reported an efficient activator for the formation of 3'-5'-internucleotidic H-phosphonate bonds which involves the reaction of nucleoside-3'-hydrogen phosphonates with nucleoside components in the presence of pivaloyl chloride as the activator. The deoxyribooligonucleotide H-phosphonate is easily oxidized to the corresponding deoxyribooligonucleotides by aqueous iodine oxidation.[2] The deoxyribonucleoside-3'-hydrogen phosphonates are key intermediate for the synthesis of deoxyribooligonucleotide H-phosphonates. However, only a few examples are known of the synthesis of nucleoside-3'-hydrogen phosphonates.[5—8]

In this communication, we wish to report a general and simple method for the synthesis of deoxyribonucleoside-3'-hydrogen phosphonates using the transesterification of a new reagent, bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphonate (1).

We examined the possibility of phosphonylation of the 3'-hydroxyl group of 5'-O-dimethoxytrityl-N-protected deoxyribonucleosides using the phosphonylating agent 1. The phosphonylating agent 1 was prepared as follows: A solution of t-butyl alcohol (18.8 ml, 200 mmol) in dry CH_2Cl_2 (40 ml) was added dropwise to a stirred solution of phosphorus trichloride (17.5 ml, 200 mmol) in dry CH_2Cl_2 (40 ml) over a period of 45 min. The reaction mixture was maintained at 0-5 °C under nitrogen atmosphere. A solution of 1,1,1,3,3,3-hexafluoro-2-propanol (42.1 ml, 400 mmol) in dry CH_2Cl_2 (40 ml) was added to the mixture at 0 °C over a period of 30 min. Stirring was continued under a stream of nitrogen for 16 h to remove hydrogen chloride. The solvent was removed by evaporation and the residue oil was dis-

tilled under reduced pressure. The main fraction (57 g, 75%) was obtained as colorless liquid: bp 35 °C/1 mmHg; ^{31}P-NMR ($CDCl_3$) 8.534 ppm; ^{1}H-NMR ($CDCl_3$) 5.25 (m, 2H, CH); IR (film) 2960 (P-H), 1260 (P=O) and 1110 cm^{-1} (CF_3). Compound 1 can be stored unchanged in a screw-cap vial in a desiccator for 10 months. The phosphonylating agent 1 (0.89 ml, 4.0 mmol) thus obtained was treated with 5'-O-dimethoxytritylthymidine (2a) (544 mg, 1.0 mmol) in dry pyridine (7 ml) at room temperature for 9 h. A mixture of 1 M triethylammonium bicarbonate (TEAB) (60 ml) and triethylamine (3 ml) was added to the reaction mixture. After 30 min, the product was extracted with CH_2Cl_2 (50 ml X 2), washed with 1 M TEAB and dried over $MgSO_4$. The CH_2Cl_2 layer was evaporated and the residue was applied to a column of silica gel and eluted with a stepwise gradient of MeOH (0-5%) in CH_2Cl_2 containing triethylamine (2%). The appropriate fractions were pooled, washed with 1 M TEAB and dried over $MgSO_4$. The CH_2Cl_2 layer was evaporated to give 5'-O-dimethoxytritylthymidine-3'-hydrogen phosphonate (3a) 648 mg (91%).[9] The yield of 3 depended on the molar ratios of the phosphonylating agent 1 to the nucleosides 2, and the better results were obtained by use of 4 molar equiv. of 1 in dry pyridine. In a similar manner, 5'-O-dimethoxytrityl-N-protected deoxyribonucleoside-3'-hydrogen phosphonates (3) were obtained in good yields as shown in Table 1.

Table 1. Yields and other relevant data on the synthesis of 3

Nucleoside (B)	Yield/% of 3	^{31}P-NMR[a]	Rf values[b]
T	91	2.810	0.23
bz_2A	83	2.713	0.37
ibuG	80	2.886	0.35
bzC	86	2.710	0.38

a) Chemical shifts are given in ppm relative to the H_3PO_4 in $CDCl_3$ as an external standard. b) Solvent: CH_2Cl_2-MeOH (9:1, v/v).

Next, we examined the synthesis of d-$(Tp)_{14}$T on a polymer support according to the reaction conditions described by Matteucci.[5] The reaction was carried out on controlled pore glass[10] (50 mg, 45 μmol, R^1=DMTr, B=T) in a similar column to that previously described.[11] The synthetic cycle was consisted of the following steps: (1) 5'-unblocking [2.5% $Cl_2CHCOOH$ in CH_2Cl_2, 2 min], (2) washing [CH_2Cl_2, 2 min], (3) washing [CH_3CN, 3 min], (4) washing [CH_3CN-pyridine (1:1, v/v), 3 min], (5) coupling [3a (30 molar equiv.), pivaloyl chloride (150 molar equiv.)/CH_3CN-pyridine (1:1, v/v), 5 min], (6) washing [CH_3CN, 3 min], (7) washing [CH_2Cl_2, 2 min]. The extent of coupling in each cycle was monitored by the spectrophotometric assay of DMTr cation. However, when the coupling reaction was carried out in the molarity [3a (30 molar equiv.); pivaloyl chloride (150 molar equiv.); CH_3CN-pyridine (1:1, v/v) 1.6 ml/1 μmol-resin] described by Matteucci,[5] each average yield was ca. 60%. In order to overcome this problem, we tested the synthesis of d-$(Tp)_{14}$T under various conditions and have found that the coupling reaction was carried out effectively at very high concentration of the reacting species than the conditions described above. Thus, the nucleoside resin (1 μmol) was treated

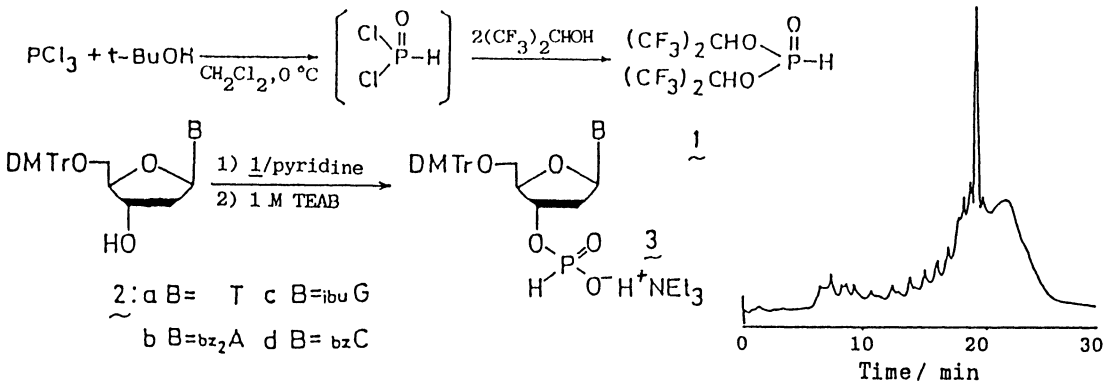
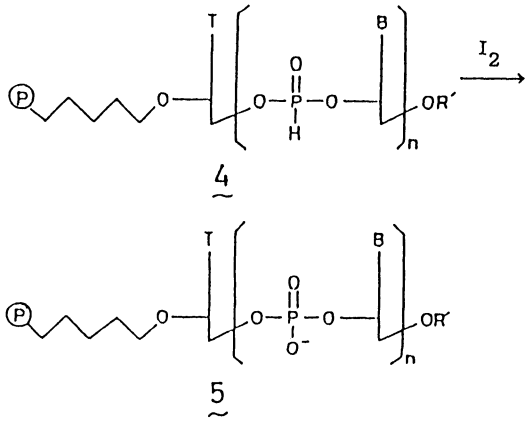


Fig. 1. Analysis of the crude mixture containing d-$(Tp)_{14}T$ on a column of TSKgel DEAE-2SW: buffer A: 0.1 M ammonium formate (pH 6.8), 20% CH_3CN; buffer B: 1.5 M ammonium formate (pH 6.8) 20% CH_3CN; gradient 0-100% buffer B in 50 min; flow 1.0 ml/min.



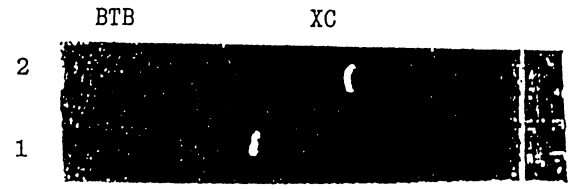


Fig. 3. Electrophoresis on 20% polyacrylamide gel of d-$(Tp)_{14}$ T (track 1) and d-TT$(ATTT)_7$ (track 2), synthesized by the H-phosphonate method on solid support.

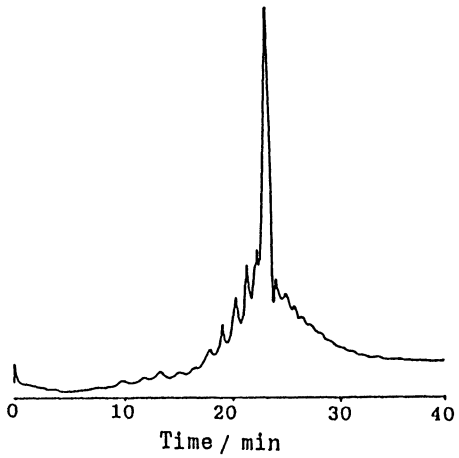


Fig. 2. Analysis of the crude mixture containing d-TT$(ATTT)_7$ on a column of TSK-gel DEAE-NPR: buffer A: 0.1 M ammonium formate (pH 6.8), 20% CH_3CN; buffer B: 2.0 M ammonium formate (pH 6.8), 20% CH_3CN; gradient 25-45% buffer B in 40 min; flow 1.0 ml/min.

with 3a (30 molar equiv.) and pivaloyl chloride (150 molar equiv.) in CH_3CN-pyridine (1:1, v/v, 300 µl) to give the d-$(Tp)_{14}T$ in each average yield of 93%. After the synthetic cycles were over, the polythymidine H-phosphonate product (4) was oxidized to polythymidylic acid (5) with 0.1 M I_2 in THF-pyridine-H_2O (44:3:3, v/v, 3 ml). Further, we examined the use of automatic synthesizer to prepare of d-TT$(ATTT)_7$.[12] The synthesis was performed very smoothly to give the d-TT$(ATTT)_7$ in each average yield of 94%. The solid supports were treated with conc. ammonia at 55 °C for 5-12 h. The tritylated products were separated by reversed phase C-18 silica gel and unblocked with 80% AcOH. The unblocked oligomers, d-$(Tp)_{14}T$ and d-TT$(ATTT)_7$ were further purified by TSKgel DEAE-2SW and -NPR (Figs. 1 and 2).[13] The main peak was found to be homogeneous by TSKgel DEAE-NPR HPLC and by electrophoresis (Fig. 3). The nucleosides and nucleoside 5'-phosphates were analyzed by the reversed phase C-18 HPLC after hydrolysis of the unblocked

oligomer with snake venom phosphodiesterase and found to be agree with the calculated value.

This result and those shown above clearly demonstrate that the transesterification of a new phosphonylating agent 1 would prove to be very effective for the preparation of deoxyribonucleoside-3'-hydrogen phosphonates (3) without any side reactions. They can be used for the synthesis of deoxyribooligonucleotides by the H-phosphonate approach, both manually and with an automatic synthesizer (Biosearch-SOME ONE). Further, the coupling reaction is carried out effectively at very high concentration of the reacting species.

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