CHEMISTRY LETTERS, pp. 1267 - 1270, 1984.

DI(2,2,2-TRIFLUOROETHYL) PHOSPHONATE, A NEW PHOSPHORYLATING AGENT, ITS APPLICATION IN THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES BY THE PHOSPHOTRIESTER APPROACH

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A new phosphorylating agent, di(2,2,2-trifluoroethyl) phosphonate (1) was found to be a useful agent for the phosphorylation of 3'-hydroxyl group of deoxyribonucleosides. When 5'-O-protected deoxyribonucleosides are treated with 1 in the absence of coupling agents and the products subjected to oxidation, the corresponding 3'-phosphodiesters are obtained in good yields. They are key intermediates for the synthesis of oligodeoxyribonucleotides by the phosphotriester approach.

Recently, the phosphotriester approach is widely used in the synthesis of various biologically important polynucleotides. In this approach, two separate phosphorylation steps are required. The first step includes the phosphorylation of a free 3'-hydroxyl group of nucleoside or oligonucleotide to form a phosphodiester linkage. The second step involves the condensation of a partially protected nucleoside or oligonucleotide with a free 5'-hydroxyl group with a 3'-phosphodiester to form a phosphotriester. Therefore, the nucleoside 3'-phosphodiester derivative is a key intermediate for the synthesis of oligonucleotide by the phosphotriester approach. A large number of phosphorylating agents have been proposed and applied to the phosphorylation of 3'-hydroxyl group of nucleoside or oligonucleotide.[1] However, a few of these agents cause various side reactions in the phosphorylation of 3'-hydroxyl group of nucleoside or oligonucleotide.[2] In order to overcome this problem, we have therefore undertaken the development of a new type of phosphorylating agent for this phosphorylation step in the oligonucleotide synthesis by the phosphotriester approach.

We examined the posibility of the phosphorylation of 3'-hydroxyl group of 5'-O-monomethoxytrityl-N-protected deoxyribonucleosides using di(2,2,2-trifluoroethyl) phosphonate (1) in the absence of coupling agents. The phosphorylation agent 1 was prepared as follows: A solution of t-butyl alcohol (10.6 ml, 125 mmol) in dry CH_2Cl_2 (25 ml) was added dropwise to a stirred solution of phosphorus

trichloride (10.9 ml, 125 mmol) in dry CH_2Cl_2 (25 ml) over a period of 45 min. The reaction mixture was maintained at 0-5 °C under a nitrogen atmosphere. A solution of 2,2,2-trifluoroethanol (25 g, 250 mmol) in dry CH_2Cl_2 (25 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 was distilled under reduced pressure. After some amount of forerun, the main fraction (26 g, 81%) was obtained as colorless liquid, bp 66-69 °C/8 mmHg.[3] The phosphorylating agent 1 (3 mmol) thus obtained was treated with 5'-O-monomethoxytritylthymidine (2a) (1 mmol) in dry pyridine (7 ml) at 50 °C for 3 h. TLC analysis showed complete conversion of the starting product 2a into Rf 0.80 (CH_2Cl_2-MeOH, 9:1, v/v) material. To the reaction mixture was added m-chloroperbenzonic acid[4] (6 mmol) at 0 °C. After 10 min, the mixture was extracted with CH_2Cl_2 (10 ml X 2). The combined organic extracts were washed with 5% $NaHCO_3$ solution (15 ml X 3), dried over Na_2SO_4, and coevaporated with pyridine twice. The residual glass was dissolved in CH_2Cl_2 (4 ml) and it was added dropwise to stirred hexane (150 ml). A white precipitate was collected and dried over P_2O_5 in vacuo to give pyridinium salt (92%) of 5'-O-monomethoxytritylthymidine 3'-O-(2,2,2-trifluoroethyl) phosphate (4a). The yields of 4 depended on the reaction temperature and the molar ratios of the phosphorylating agent 1 to the nucleosides 2, and better results were obtained by use of a slight excess of 1 at a reaction temperature of 50 °C as shown in Table.

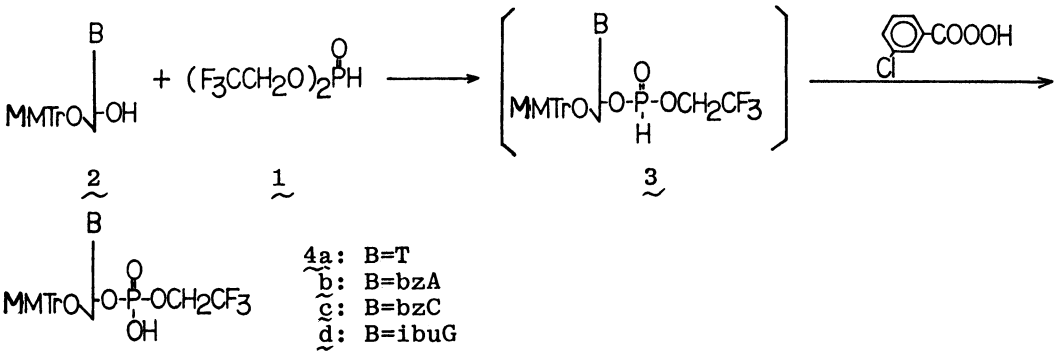
In a similar manner, 5'-O-monomethoxytrityl-N-protected deoxyribonucleoside 3'-O-(2,2,2-trifluoroethyl) phosphates (4) were obtained in good yields as shown in Table.[5] The phosphodiester products 4 were isolated as stable, colorless solid, uncontaminated with di(2,2,2-trifluoroethyl) phosphate as indicated by ^{31}P NMR.[6]

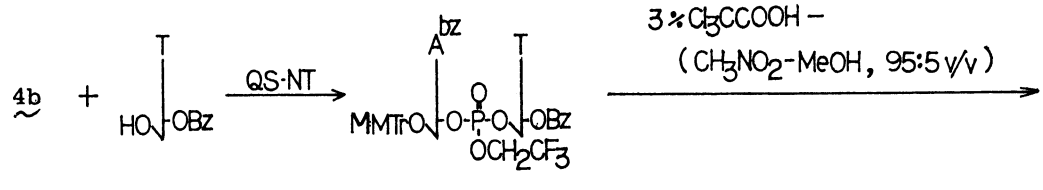
Next, the synthesis of trinucleotide, d-MMTribuGp(TFE)bzAp(TFE)TOBz (7), was tried by use of 4 prepared in the above experiment as starting materials. The phosphodiester derivative 4b (688 mg, 0.8 mmol) was treated with 3'-O-benzoylthymidine (184 mg, 0.54 mmmol) in the presence of 8-quinolinesulfonyl-3-nitro-1H-1,2,4-triazole (QS-NT)[7] (623 mg, 2.01 mmol) in dry pyridine (2.4 ml) at room temperature for 3 h. The reaction mixture was quenched with ice-water (1 ml) and extracted with CH_2Cl_2 (10 ml X 3). The combined organic extracts were washed with 0.1M TEAB (pH 7.5) solution, dried over Na_2SO_4, concentrated in vacuo, coevaporated with toluene twice, and chromatographed on silica gel column. The fully protected dinucleotide, d-MMTrbzAp(TFE)TOBz (5) was isolated in 96% (582 mg) yield by eluting the column with a stepwise gradient of MeOH (0-7%) in CH_2Cl_2. The dimer 5 (582 mg, 0.52 mmol) was treated with 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5, v/v) at room temperature for 3 min.[8] The mixture was quenched with pyridine and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel to give the corresponding detritylated product (6) in 87% yield. The 5'-hydroxyl component 6 (198 mg, 0.23 mmol) was coupled with 4d (287 mg, 0.35 mmol) in the presence of QS-NT (216 mg, 0.87 mmol) in dry pyridine (1.2 ml) for 4 h. After usual workup, chromatography on silica gel (CH_2Cl_2-MeOH, 95:5, v/v) afforded the fully protected trinucleotide 7 (297 mg, 80%).

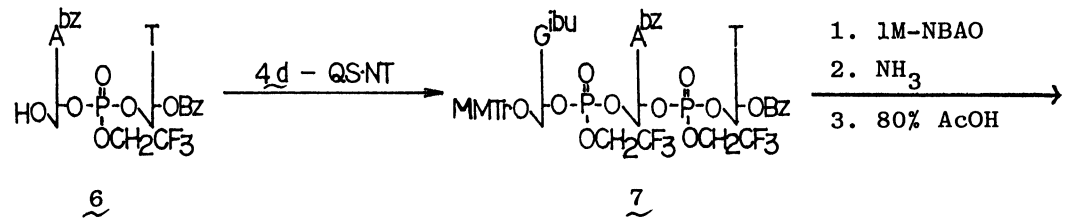
Table 1. Synthesis of 5'-O-Monomethoxytrityl-N-Protected Nucleoside 3'-Phosphodiesters (4)[a]

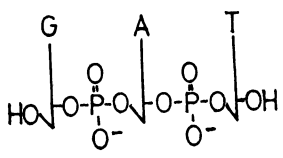
	Step I		Step II	
B	Molar ratio of phosphite/nucleoside	Temp °C	Molar ratio of m-ClC_6H_4COOH/phosphite	Yield of 4 %
T	3.0	50	2.0	92
T	1.5	50	2.0	65
T	1.0	50	2.0	50
T	3.0	37	2.0	45
T	3.0	25	2.0	21
d-bzA	3.0	50	2.0	90
d-bzC	3.0	50	2.0	90
d-ibuG	3.0	50	2.0	87

a) All reactions were performed by the same procedure described in the text.









QS-NT=8-quinolinesulfonyl-3-nitro-1H-1,2,4-triazole

NBAO =p-nitrobenzaldoxime

Finally, we examined complete deblocking for the fully protected trinucleotide 7. We could not obtain satisfactory results when activated zinc was used for removal of 2,2,2-trifluoroethyl group from internucleotidic bonds. We now found that p-nitrobenzaldoxime[9] was much more effective for removal of 2,2,2-trifluoroethyl moiety than activated zinc powder. The trimer 7 (8.4 mg, 5 μmol) was treated with 1M N^1,N^1,N^3,N^3-tetramethylguanidium salt of p-nitrobenzaldoxime in a mixture of dioxane and water (2:1, v/v, 1.5 ml). After 24 h at room temperature, TLC analysis showed complete conversion of 7 into base line material (CH_2Cl_2-MeOH, 9:1, v/v). The mixture was treated with Dowex 50W-X2 (pyridinium form) and the resin was removed by filtration. The filtrate was evaporated in vacuo and the residue was dissolved in conc. ammonia. The mixture was kept at 60 °C for 6 h. The solution was concentrated and 80% AcOH was added. After 15 min, the solution was evaporated in vacuo and the residue was dissolved in water and washed with ether. The solution was concentrated to an oil. The deblocked trimer d-GpApT was obtained in 81% (64 OD) yield after chromatographic separation using Toyo Roshi No. 514 paper with iso-PrOH-conc. NH_4OH-H_2O (7:1:2, v/v). The purity of d-GpApT was checked by HPLC on μBondapak C_{18} as well as hydrolysis with nuclease P1 to d-G, d-pA, and d-pT in the ratio of 1.00:0.96:1.09.

We thank Professor Tsujiaki Hata, Tokyo Institute of Technology, for measurement of ^{31}P NMR spectra.

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3) IR(film), 2484 (P-H), 1250 (P=O), 1150 and 1090 cm^{-1} (CF_3); Anal. Calcd for $C_4H_5F_6O_3P$: C, 19.52; H, 2.05. Found: C, 19.48: H, 1.95.
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5) An important observation we made in the preparation of 3'-phosphodiester was that no side reactions were detected in the phosphorylation step.
6) ^{31}P NMR spectra of 4a-d are shown below. 4a: δ -2.44 (C_6D_6, 85% H_3PO_4), R_f=0.95; 4b: δ -1.47 (pyridine-d_5, 85% H_3PO_4), R_f=0.90; 4c: δ -1.96 (C_6D_6, 85% H_3PO_4), R_f=0.90; 4d: δ -1.99 (C_6D_6, 85% H_3PO_4), R_f=0.88. RPTLC was performed on plates of silanized silica gel (Merck $60F_{254}$) by using acetone-water (7:3, v/v)
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(Received January 21, 1984)