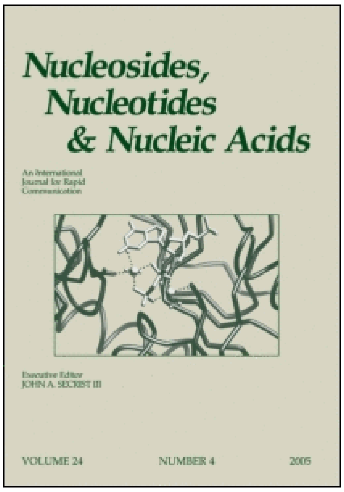

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A NEW PHOSPHORYLATING AGENT, DI(2,2,2-TRIFLUOROETHYL) TRIMETHYLSILYL PHOSPHITE. ITS APPLICATION IN DNA SYNTHESIS BY THE PHOSPHOTRIESTER APPROACH

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Abstract: A new phosphorylating agent, di(2,2,2-trifluoroethyl) trimethylsilyl phosphite, has been prepared and is proved to be a useful agent for the phosphorylation of the 3'-hydroxyl group of deoxyribonucleosides in the absence of coupling agents. The resulting deoxyribonucleoside 3'-(2,2,2-trifluoroethyl) phosphates are key intermediates for the synthesis of deoxyribooligonucleotides by the phosphotriester approach.

The phosphotriester approach is by far the most convenient and popular method for the synthesis of oligonucleotides.[1] In this approach, a large number of phosphorylating agents have been proposed and applied to the phosphorylation of 3'-hydroxyl group of nucleosides or oligonucleotides.[2] However, these agents are liable to cause the formation of by-products such as 3'-3' dinucleoside monophosphates, sulfonylated nucleotides, and phosphorylated bases, which make the isolation of the desired oligonucleotides more difficalt and lower yields.[3]

Recently, we developed a new type phosphorylating agent, di(2,2,2-trifluoroethyl) phosphonate (1), which does not need a coupling agent for the phosphorylation of the 3'-hydroxyl

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group of nucleosides.[4] However, the yields of phosphorylation of nucleosides using this agent depended on the molar ratios of 1 to the nucleoside and the reaction temperature (59 °C). We have now found that di(2,2,2-trifluoroethyl) trimethylsilyl phosphite (2) is much more effective for the phosphorylation of the 3'-hydroxyl group of deoxyribonucleosides than 1.

The new phosphorylating agent, di(2,2,2-trifluoroethyl) trimethylsilyl phosphite (2), was prepared as follows: Trimethylsilyl chloride was added to a solution of 1 in the presence of triethylamine in dry ether at 0 °C and the reaction mixture was gradually warmed to room temperature and refluxed for an additional 2.5 h. The desired phosphorylating agent 2 was obtained in 80% yield as a colorless liquid. The phosphorylating agent 2 can be kept stable at room temperature for one month.

The utility of the new phosphorylating agent 2 can be demonstrated for the phosphorylation of the 3'-hydroxyl group of deoxyribonucleosides (3). The phosphorylating agent 2 (1.5 mmol) was treated with 5'-O-dimethoxytritylthymidine (3a) (1.0 mmol) in dry pyridine at room temperature. After 5 h, TLC analysis showed complete conversion of starting material 3a into 4a, R_f 0.82 (solvent A). The phosphite intermediate 4a was oxidized directly to the phosphate 5a using m-chloroperbenzoic acid (MCPBA)[5] at 0 °C for 20 min. After the usual workup, the corresponding 5'-O-dimethoxytritylthymidine 3'-O-(2,2,2-trifluoroethyl) phosphate (5a) was isolated in 91% yield.

In a similar manner, 5'-O-dimethoxytrityl-N-protected deoxyribonucleoside 3'-O-(2,2,2-trifluoroethyl) phosphates (5) were obtained in good yields as shown in Table 1.

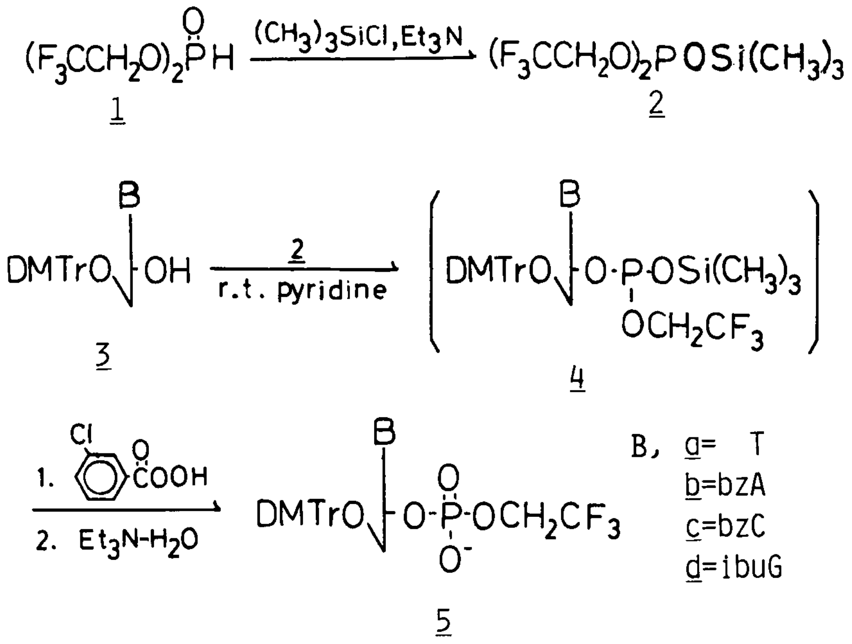
As shown in Table 1, the new phosphorylating agent 2 has advantages for the phosphorylation of nucleosides as compared with 1; i) the reaction was carried out at room temperature; ii) the molar ratios of the phosphorylating agent 2 to the nucleosides was decreased; iii) the desired phosphodiesters 5 were obtained in good yields

TABLE 1

SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-N-PROTECTED NUCLEOSIDE 3'-O-(2,2,2-TRIFLUOROETHYL) PHOSPHATES (5).

B	Molar ratio of phosphite/ nucleoside	Temp (°C)	Molar ratio of MCPBA/2	Yield of 5 (%)
T	1.5	r.t.	2.0	91
T	1.0	r.t.	2.0	70
T	3.0[a]	50	2.0	88
T	3.0[a]	r.t.	2.0	45
d-bzA	1.5	r.t.	2.0	87
d-bzC	1.5	r.t.	2.0	85
d-ibuG	1.5	r.t.	2.0	88

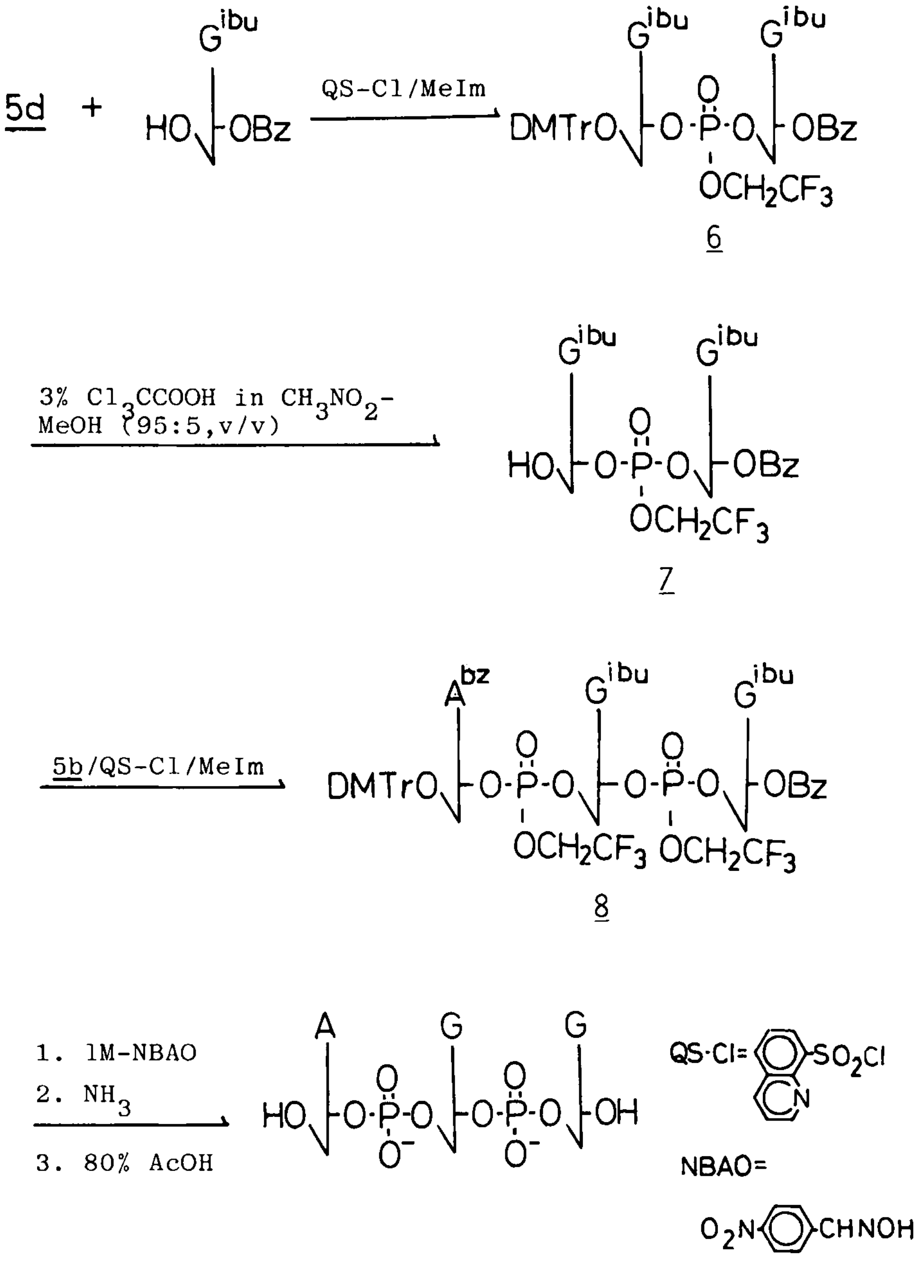
a) This reaction was performed by use of di(2,2,2-trifluoroethyl) phosphonate (1) in place of 2.



accompanied with high selectivity; and iv) no side reactions on guanine and thymine were detected in the phosphorylation step.

The phosphodiester derivatives 5 were applied to the synthesis of a trinucleotide, d-DMTrbzAp(TFE)ibuGp(TFE)-ibuG(OBz) (8). The phosphodiester 5d (1.28 mmol) was treated with 3'-O-benzoyl-N^2-isobutyryldeoxyguanosine (0.80 mmol) in the presence of 8-quinolinesulfonyl chloride (QS-Cl)[6] (3.83 mmol) and 1-methylimidazole[7] (3.83 mmol) in dry pyridine at room temperature for 2 h. Thus, the usual workup gave the fully protected dinucleotide, d-DMTribuGp-(TFE)ibuG(OBz) (6) in 70% yield. Removal of the dimethoxytrityl group of 6 was performed by treatment with 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5, v/v) at room temperature for 3 min.[8] The 5'-hydroxyl dinucleotide 7 was isolated in 80% yield by precipitation from hexane-ether (95:5, v/v) and was used for the next coupling reaction without further purification. A solution of the phosphodiester 5b (0.64 mmol) and 7 (0.45 mmol) in dry pyridine was then condensed in the presence of QS-Cl and 1-methylimdazole for 2 h. The usual workup including separation and purification by chromatography on silica gel gave a 78% yield of the fully protected trinucleotide 8. In the above reaction, the use of QS-Cl and 1-methylimidazole as coupling agents shortened the condensation time as compared with 8-quinolinesulfonyl-3-nitro-1,2,4-triazolide (QS-NT)[9] and suppressived the side reactions.

The trimer 8 was completely deblocked by treatment with (i) 1M-N^1,N^1,N^3,N^3-tetramethylguanidium salt of p-nitrobenzaldoxime[10] in dioxane-H_2O (2:1, v/v) at room temperature for 24 h;[4] (ii) concentrated ammonia at 60 °C for 6 h;[11] (iii) 80% AcOH at room temperature for 15 min. The deblocked trimer, d-ApGpG was isolated in 79% (363 A_{260} OD units from 10 µmol) yield after chromatographic separation using Whatmann 3 MM paper. The purity of d-ApGpG was checked by HPLC on µBondapak C_{18} as well as hydrolysis with nuclease P1 to d-A and d-pG in aratio of 1.00:1.93.



EXPERIMENTAL SECTION

Ultraviolet spectra were recorded on a Shimazu UV-200 spectrometer. ^{1}H-NMR spectra were recorded on a Hitachi R-24B spectrometer. Thin layer chromatography (TLC) was performed on precoated TLC plates of silica gel 60 F-254 (Merck, Art. No. 5715) and the R_f values of the protected nucleoside derivatives were measured after development with solvent A (CH_2Cl_2-MeOH, 9:1, v/v). Column chromatography was performed with Silica gel C-200 purchased from Wako Co. Ltd.. Paper chromatography was performed by descending techinique with Whatman 3 MM paper using solvent B (1-propylalcohol-conc. ammonia-water, 55:10:35, v/v). HPLC was performed on a µBondapack C_{18} reverse phase column and elution was performed by a linear gradient of 5-20% CH_3CN in 0.1M ammonium acetate (pH 7) over 32 min at flow rate of 2.0 ml/min. Pyridine was distilled twice from p-toluenesulfonyl chloride and from CaH_2 and then stored over molecular sieves 4A. The protected nucleosides, DMTrT, d-DMTrbzA, d-DMTrbzC, and d-DMTribuG were prepared according to published procedures.[12,13] 8-Quinolinesulfonyl chloride (QS-Cl) was purchased from Aldrich Chemical Co. Nuclease P1 was purchased from Yamasa Co.

Di(2,2,2-trifluoroethyl) trimethylsilyl phosphite (2). Trimethylsilyl chloride (3.7 ml, 33 mmol) was added dropwise to a stirred solution of di(2,2,2-trifluoroethyl) phosphonate (1) (4.6 ml, 30 mmol) in the presence of triethylamine (4.2 ml, 33 mmol) in dry ether (150 ml) at 0 °C and the reaction mixture was gradually warmed to room temperature and refluxed with stirring for an additional 2.5 h. The triethylammonium chloride was removed by filtration and ether was removed by distillation at atmospheric pressure The product was distilled through a Vigreux column. The yield of 2 was 6.26 g (80%): bp 37-40 °C/9 mm Hg; ^{1}H-NMR [$(CH_3)_2CO$] δ 4.50-3.95 (m, 4H, CH_2), 0.12 (s, 9H, CH_3: IR (film) cm^{-1}, 2970 (CH_2), 1270 [$(CH_3)_3Si$], 1160 (CF_3), 1050 (CF_3), 960 (C-O-P). Anal. Calcd for $C_{17}H_{13}O_3F_6PSi$: C, 26.42; H. 4.11. Found: C, 26.13; H, 3.85.

5'-O-Dimethoxytrityl-N-protected deoxyribonucleoside 3'-O-(2,2,2-trifluoroethyl) phosphates (5a-d). To a solution of 5'-O-dimethoxytrityl-N-protected deoxyribonucleosides (3) (1.0 mmol), dried by repeated coevaporation with dry pyridine, in dry pyridine (3 ml) was added di(2,2,2-trifluoroethyl) trimethylsilyl phosphite (2) (0.26 ml, 1.5 mmol). After 5 h, MCPBA (518 mg, 3.0 mmol) was added and the reaction mixture was stirred at 0 °C for 20 min. The mixture was quenched with ice-water containing 5% triethylamine and extracted with CH_2Cl_2 (10 ml X 2). The combined organic extracts were washed with 5% $NaHCO_3$ solution (5 ml X 3) and water (5 ml X 2). The solution was dried with Na_2SO_4 and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 and it was added dropwise to stirred hexane-ether (95:5, v/v). The resulting colorless precipitate was collected and dried over P_2O_5 in vacuo to give triethylammonium salts of 5a-d in yields as shown in Table 1.

(a) 5'-O-Dimethoxytritylthymidine 3'-O-(2,2,2-trifluoroethyl) phosphate. RPTLC [$(CH_3)_2CO-H_2O$, 7:3, v/v] R_f 0.88; UV (MeOH) λ max 265, 233 nm, λ min 253 nm, ^{31}P-NMR (C_6D_6, 85%H_3PO_4) δ -2.41.

(b) 5'-O-Dimethoxytrityl-N^6-benzoyldeoxyadenosine 3'-O-(2,2,2-trifluoroethyl) phosphate. RPTLC [$(CH_3)_2CO-H_2O$, 7:3, v/v] R_f 0.89; UV (MeOH)λ280, 233 nm,λmin 256 nm; ^{31}P-NMR (C_6D_6, 85%H_3PO_4) δ -1.49.

(c) 5'-O-Dimethoxytrityl-N^4-benzoyldeoxycytidine 3'-O-(2,2,2-trifluoroethyl) phosphate. RPTLC [$(CH_3)_2CO-H_2O$, 7:3, v/v] R_f 0.74; UV (MeOH)λ302, 255, 233 nm, λmin 287, 243 nm; ^{31}P-NMR (C_6D_6, 85%H_3PO_4) δ -1.96.

(d) 5'-O-Dimethoxytrityl-N^2-isobutyryldeoxyguanosine 3'-O-(2,2,2-trifluoroethyl) phosphate. RPTLC [$(CH_3)_2CO-H_2O$, 7:3, v/v] R_f 0.81; UV (MeOH)λmax 280, 256, 237 nm, λmin 270, 240 nm; ^{31}P-NMR (C_6D_6, 85%H_3PO_4) δ -2.00.

Dinucleotide derivative, d-DMTribuGp(TFE)ibuG(OBz) (6). The phosphodiester 5d (1.55 g, 1.28 mmol) was combined with 3'-O-benzoyl-N^2-isobutyryldeoxyguanosine (0.37 g, 0.80 mmol) rendered anhydrous by repeated coevaporation with dry pyridine, and then treated with 8-quinolinesulfonyl chloride (QS-Cl) (0.87 g, 3.83 mmol) and 1-methylimidazole (0.61 ml, 7.65 mmol) in dry pyridine (4.0 ml) at room temperature for

2 h. The mixture was quenched with ice-water (1 ml), followed by extraction with CH_2Cl_2 (10 ml X 3). The organic layer was washed with 0.1M triethylammonium bicarbonate (TEAB) (pH 7.5, 15 ml X 2) and then water (10 ml X 2), dried with Na_2SO_4, filtered, and evaporated to a gum. The gum was dissolved in CH_2Cl_2 and subjected to silica gel column chromatography. The column was eluted with a stepwise gradient of MeOH (0-4%) in CH_2Cl_2. The appropriate fractions were evaporated to give 6 which was isolated (0.72 g, 70%) by precipitation from hexane-ether (95:5, v/v); TLC (solvent A) R_f 0.42; UV (MeOH) λ min 280. 257, 230 nm, λ min 270, 240 nm.

Trinucleotide, d-DMTrbzAp(TFE)ibuGp(TFE)ibuG(OBz) (8). The fully protected dinucleotide 6 (627 mg, 0.51 mmol) was treated with 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5, v/v, 0.07 mmol of 6/ml) at room temperature for 3 min. The mixture was quenched with pyridine and extracted with CH_2Cl_2. The extract CH_2Cl_2 was washed with water, dried with Na_2SO_4, and evaporated in vacuo. The residue was dissolved in a small amount of CH_2Cl_2 and poured into hexane-ether (95:5, v/v, 200 ml). A white precipitate was collected to give the 5'-hydroxyl dinucleotide 7 (421 mg, 80%). The 5'-hydroxyl dinucleotide 7 (415 mg, 0,45 mmol) thus obtained was combined with the phosphodiester 5b (583 mg, 0.64 mmol) rendered anhydrous by repeated coevaporation with dry pyridine, and then QS-Cl (441 mg, 1.94 mmol) and 1-methylimidazole (0.31 ml, 3.88 mmol) in dry pyridine (2 ml) for 2 h. The mixture was then worked up as described for the preparation of 6 and purified by silica gel column chromatography. The appropriate fractions [eluted with a stepwise gradient of MeOH (0-3%) in CH_2Cl_2] were evaporated to *ca.* 3-4 ml and poured into hexane-ether (9:1, v/v, 150 ml). A white precipitate was collected to give the fully protected trinucleotide 8 in 78% (613 mg) yield; TLC (solvent A) R_f 0.40; UV (MeOH) λ max 278, 255, 225 nm, λ min 269, 248 nm.

Deprotection of the fully protected trinucleotide 8. The trimer 8 (78 mg, 10 μmol) was treated with p-nitrobenzaldoxime (83 mg, 0.5 mmol) and 1,1,3,3-tetramethylguanidine (0.09 ml, 0.75 mmol) in dioxane-water (1:1,v/v, 1 ml) at room temperature for 24 h. Then the solution was passed slowly through a column Dowex 50W-X2 (pyridinium form, 10 ml), and the column was washed with pyridine-water (7:3, v/v, 50 ml). The eluant and washings were combined and evaporated in vacuo. The residue was dissolved in conc. ammonia-pyridine (9:1, v/v, 10 ml). The solution was sealed and kept at 60 °C for 6 h. The solution was evaporated in vacuo. The residue was treated with 80% AcOH at room temperature for 15 min. The solvent was removed in vacuo, and the residue was chromatographed on Whatman 3MM papers with solvent B to give 363 OD (79%) of d-ApGpG; R_f 0.43 (solvent B), UV (H_2O, pH 7.0) λmax 255 nm.

To a solution of d-ApGpG (10 OD) in 50 mM acetate buffer (0.5 ml, pH 5.4) was added nuclease P1 solution (10 μL, 1mg/ml). The mixture was incubated at 37 °C for 12 h.[14] The HPLC pattern showed complete digestion of the trinucleotide to d-A and d-pG in a ratio of 1.00:1.93.

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REFERENCES

1. H. Kössel and H. Seliger, Fortschr. Chem. Org. Naturstoffe, 32, 297 (1975); V. Amarnath and A. D. Broom, Chem. Rev., 77, 183 (1977); C. B. Reese, Tetrahedron, 34, 3143 (1978); M. Ikehara, E. Ohtsuka, and A. F. Markham, Adv. Carbohydr. Chem. Biochem., 36, 135 (1979); E. Ohtsuka, M. Ikehara, and D. Söll, Nucleic Acids Res., 10, 6553 (1982); S. A. Narang, Tetrahedron, 39, 3 (1983).

2. R. L. Letsinger and K. K. Ogilvie, J. Am. Chem. Soc., 89, 4801 (1967); C. B. Reese and R. Saffhill, J. Chem. Soc., Chem. Comunn., 1968, 767; F. Eckstein and I. Rizk, Chem. Ber., 102, 2362 (1969); F. Cramer and J. C. Catlin, J. Org. Chem., 38, 245 (1973); K. Itakura, N. Katagiri, C. P. Bahl, R. H. Wightman, and S. A. Narang, J. Am. Chem. Soc., 97, 7327 (1975); P. T. Cashion, K. Porter, T. Cadger, G. Sathe, T. Tranquilla, H. Notman, and E. Jay, Tetrahedron lett., 1976, 3769; J. H. van Boom, P. M. J. Burgers, and P. H. van Deursen, ibid., 1976, 869; K. Itakura, T. Hirose, R. Crea, A. D. Riggs, H. L. Heyneker, F. Bolivar, and H. W. Boyer, Science, 198, 1056 (1977); G. van der Marel, C. C. A. van Boeckel, Mrs G. Wille, and J.H. van Boom, Tetrahedron Lett., 22, 3887 (1981); E. Ohtsuka, S. Shibahara, and M. Ikehara, Chem. Pharm. Bull., 29, 3440 (1981); S. Ueda and H. Takaku, ibid., 32, 1650 (1984).

3. K. Itakura, N. Katagiri, and S. A. Narang, Can. J. Chem., 52, 3689 (1974); P. K. Bridson, W. T. Markiewicz, and C. B. Reese, J. Chem.Chem., Chem. Commun., 1977, 791; J. B. Chattopadhyaya and C. B. Reese, Tetrahedron Lett., 1979, 5059; J. F. M. de Rooij, G. Wille-Hazeleger, P. H. van Deursen, J. Serdijn, and J. H. van Boom, Recl. Trav. Chim. Pays-Bas, 98, 537 (1979); C. B. Reese and A. Ubasawa, Tetrahedron Lett., 21, 2265 (1980); B. Rayner, C. B. Reese, and A Ubasawa, J. Chem. Soc., Chem. Commun., 1980, 972; H. P. Daskalov, M. Sekine, and T. Hata, Bull. Chem. Soc. Jpn., 54, 3076 (1981); W. L. Sung, Nucleic Acids Res., 9, 6139 (1981); H. Takaku, K. Kamaike, and K. Kasuga, Chem. Lett., 1982, 197; E. Ohtsuka, A. Yamane, and M. Ikehara, Nucleic Acids res., 11, 1325 (1983).

4. H. Takaku, H. Tsuchiya, K. Imai, and D. E. Gibbs, Chem. Lett., 1984, 1267.

5. K. K. Ogilvie and M. J. Nemer, Tetrahedron Lett., 22, 2531 (1981).

6. H. Takaku, M. Kato, M. Yoshida, and T. Hata, Chem. Lett., 1979, 811; H. Takaku, M. Kato, M. Yoshida, and R. Yamaguchi, J. Org. Chem., 45, 3347 (1980).

7. V. A. Efimov, S. V. Reverdatto, and O. G. Chakhmakhcheva, Tetrahderon Lett., 23, 961 (1982); idem., Nucleic Acids Res., 10, 6675 (1982).
8. H. Takaku, K. Morita, and T. Sumiuchi, Chem. Lett., 1983, 1661.
9. H. Takaku, K. Kamaike, and K, Kasuga, Chem. Lett., 1982, 197.
10. C. B. Reese, R. C. Titmas, and L. Yau, Tetrahedron Lett., 1978, 2727; C. B. Reese and L. Yau, ibid., 1978, 4443; C. B. Reese and L.Zard, Nucleic Acids Res., 9, 4611 (1981).
11. H. Takaku, K. Kamaike, and M. Suetake, Chem. Lett., 1983, 111.
12. H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, J. Am. Chem. soc., 85, 3821 (1963).
13. H. Büchi and H. G. Khorana, J. Mol. Biol., 72, 251 (1972).
14. H. Takaku and M. Yoshida, J. Org. Chem., 46, 589 (1981).

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