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NUCLEOSIDE 3'-*N,N*-DIALKYLPHOSPHONAMIDATES: NOVEL BUILDING BLOCKS FOR OLIGONUCLEOTIDE SYNTHESIS

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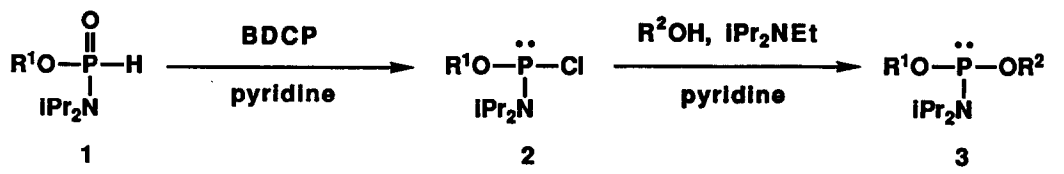
SUMMARY: Nucleoside 3'-*N,N*-diisopropylphosphonamidates reacted with tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP) to generate the corresponding aminophosphorochloridites without cleavage of the P-N bond. The reaction was applied to internucleotidic bond formation.

Recently, nucleoside 3'-phosphorodiamidites[1] have been employed as building blocks for the synthesis of oligonucleotides and their analogues. However, the nucleoside 3'-phosphorodiamidites are very sensitive toward moisture compared with the ordinary phosphoramidite units. It has been reported by van Boom[1a] that the nucleoside 3'-phosphorodiamidites are readily hydrolyzed to the 3'-phosphonamidates in the presence of 1*H*-tetrazole. Recently, we have reported that non-oxidative chlorination of dialkyl phosphonates to the dialkyl phosphorochloridites by use of tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP) as a chlorinating reagent.[2] As an extention of our study, we have focused *O*-alkyl *N,N*-dialkylphosphonamidates.

In the first place, we examined the reaction of methyl *N,N*-diisopropyl-phosphonamidate[3] (**1a**) with BDCP as a model reaction for oligonucleotide synthesis. Compound **1a** (34 mg, 0.2 mmol) was treated with BDCP (327 mg, 0.3 mmol) in the presence of diisopropylethylamine (90 µl, 0.7 mmol) in pyridine (3 ml) and the reaction was monitored by ^{31}P-NMR. After 5 min, signals of **1a** (12.98 ppm) and BDCP (-66.17 ppm) were completely disappeared and new signals were observed at 182.23 ppm and -24.41 ppm.[4]



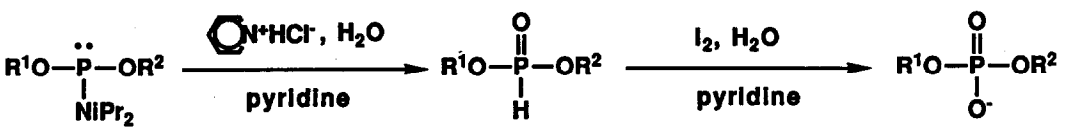
1 **2** **3**

BDCP = (Br-C6H2-O)3PCl2 (Br)

a; $R^1 = R^2 = CH_3$

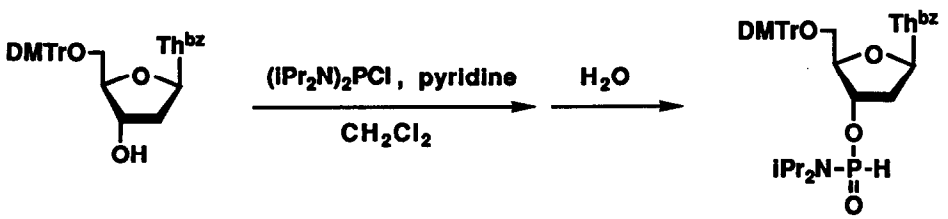
b; R^1 = 5'-*O*-dimethoxytrityl-N^3-benzoylthymidine-3'-yl,
R^2 = 3'-*O*-benzoyl-N^3-benzoylthymidine-5'-yl

This result indicates that **1a** was transformed to the methyl *N,N*-diisopropylamino-phosphorochloridite (**2a**) quantitatively along with the formation of tris(2,4,6-tribromophenyl) phosphate. To the above mixture, dry methanol (12 μl, 0.3 mmol) was added and a spectrum was further recorded. After 5 min, quantitative formation of dimethyl *N,N*-diisopropylphosphoramidite **3a** (148.13 ppm) was observed. To obtain **3a**, the addition of diisopropylethylamine was essential, otherwise, P-N bond of **3a** was easily activated by pyridinium hydrochloride in pyridine to form dimethyl phosphorochloridite (169.44 ppm). In fact, **3a** (39 mg, 0.2 mmol) was treated with pyridinium hydrochloride (116 mg, 1.0 mmol) in pyridine-water (9:1, v/v) for 5 min, **3a** was readily converted into dimethyl phosphonate **4a** (10.27 ppm). In contrast, P-N bond of **2a** could not be activated by pyridinium hydrochloride in pyridine even in the absence of a strong tertiary amine. The resulting dimethyl phosphonate (**4a**) was oxidized *in situ* by iodine (152 mg, 0.6 mmol) in aqueous pyridine for 10 min to give dimethyl phosphate **5a** (2.23 ppm). ^{31}P-NMR spectra of each step in the above reactions are shown in Fig. 1.



3 **4** **5**

The reactions were applied to internucleotidic bond formation: Nucleoside 3'-*N,N*-diisopropylphosphonamidates (**1b**) were synthesized according to the modified procedure of van Boom.[1a] 5'-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidine (649 mg, 1.0 mmol) was treated with bis(*N,N*-diisopropylamino)phosphorochloridite (320 mg, 1.2 mmol) in the presence of pyridine (0.137 ml, 1.7 mmol) in CH_2Cl_2 (10 ml) at room temperature for 10 min. Then, the reaction was quenched with water. After usual work up, and precipitated from *n*-hexane[5] at -30°C, 5'-*O*-dimethoxytrityl-*N*^{3}-benzoylthymidine 3'-*N,N*-diisopropylphosphonoamidate **1b** (634 mg, 0.80 mmol) was obtained in 80% yield.[6]



1b

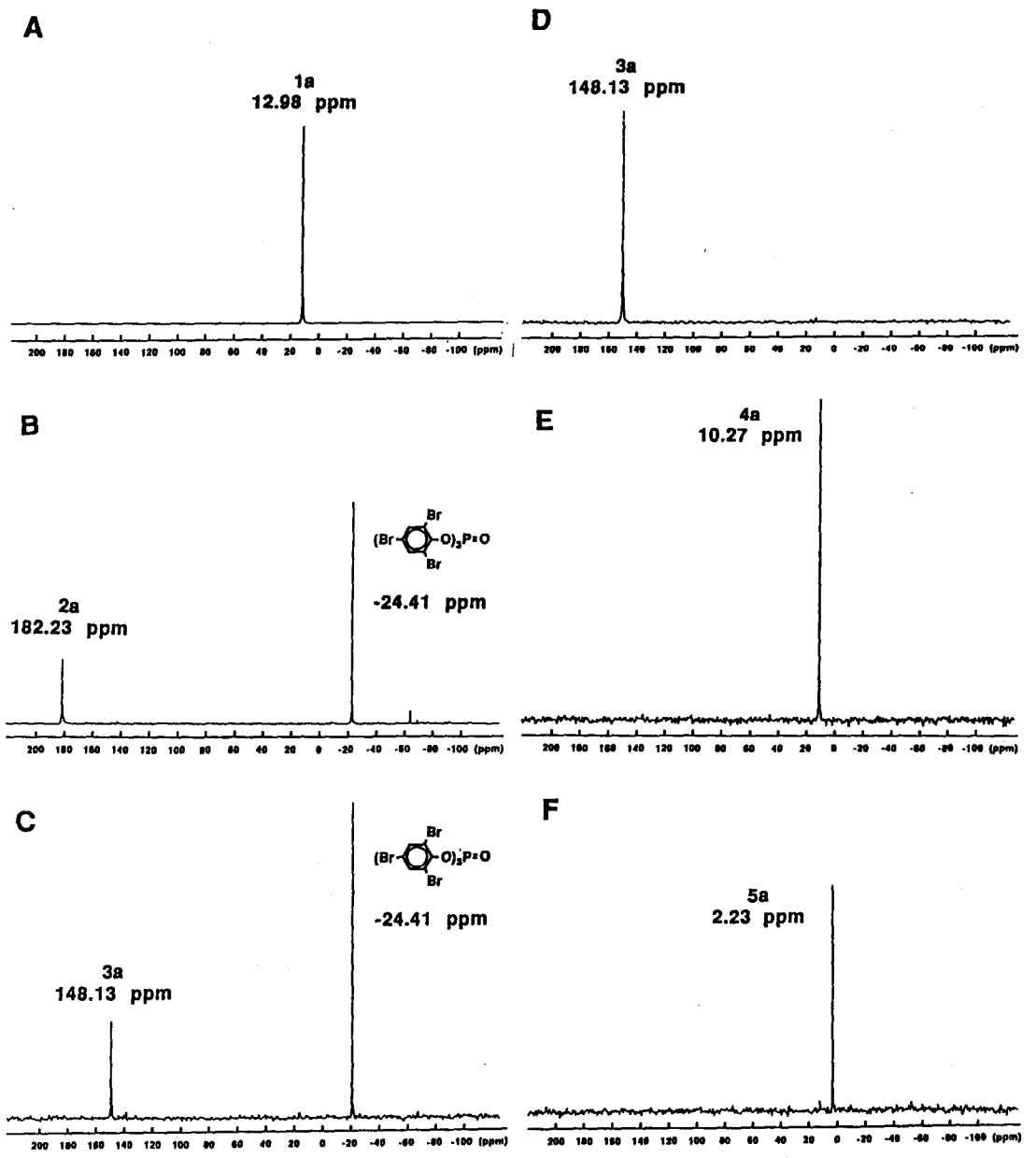


Fig. 1 ^{31}P-NMR spectra. **A**: **1a** in pyridine. **B**: **1a** with BDCP (1.5 equiv) in pyridine for 5 min. **C**: **2a** with CH_3OH (1.5 equiv) in pyridine in the presence of iPr_2NEt for 5 min. **D**: **3a** in pyridine. **E**: **3a** with pyridinium hydrochloride (5 equiv) in pyridine-H_2O (9:1, v/v) for 5 min. **F**: **4a** with I_2 (3 equiv) in aqueous pyridine for 10 min.

Compound **1b** (96 mg, 0.12 mmol) was treated with BDCP (196 mg, 0.18 mmol) in the presence of diisopropylethylamine (63 μl) in pyridine for 5 min, quantitative conversion of **1b** (12.01 ppm) into **2b** (177.87 ppm) was observed by ^{31}P-NMR. Compound **2b** (0.15 mmol) prepared *in situ* was treated with 3'-*O*-benzoyl N^3-benzoylthymidine (45 mg, 0.1 mmol) in pyridine for 5 min to give the 3',5'-*O*-dithymidine phosphoramidite **3b** (148.22, 148.71 ppm) quantitatively.[7] It was hydrolyzed in the presence of pyridinium hydrochloride (5 equiv) in pyridine-water (98:2, v/v) for 5 min to give the 3',5'-*O*-dithymidine phosphonate **4b** (6.88, 8.53 ppm). The mixture was treated with iodine (76 mg) in aqueous pyridine for 10 min and purified by silica gel column chromatography to give the 3',5'-*O*-dithymidine phosphate **5b** (-2.81 ppm, 104 mg, triethyl ammonium salt) in 83% yield.

The nucleoside 3'-*N,N*-dialkylphosphonamidates are favorable building blocks in oligonucleotide synthesis because they are neutral and stable.

This paper is dedicated to Professor Jan Michalski on the occasion of his 70 th birthday.

REFERENCES AND NOTES

(1) (a) Marugg, J. E.; Burik, A.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2271. (b) Uzanski, B.; Wilk, A.; Stec, W. J. *ibid.* **1987**, *28*, 3401. (c) Yamana, K.; Nishijima, Y.; Oka, A.; Nakano, H.; Sangen, O.; Ozaki, H.; Shimidzu, T. *Tetrahedron* **1989**, *45*, 4140.

(2) (a) Wada, T.; Hotoda, H.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1988**, *29*, 4143. (b) Wada, T.; Kato, R.; Hata, T. *J. Org. Chem.*, submitted for publication.

(3) Wada, T.; Ishikawa, K.; Hata, T., in preparation.

(4) A signal of tris(2,4,6-tribromophenyl) phosphate.

(5) *N,N,N'N'*-Tetraisopropylphosphonodiamidate (δ_P 7.07 ppm, J_{PH} = 550.8 Hz, $CDCl_3$) formed by hydrolysis of excess bis(*N,N*-diisopropylamino)phosphorochloridite could not be separated from **1b** by silica gel column chromatography but it was effectively removed by precipitation of the crude **1b** from *n*-hexane. However, loss of **1b** was observed due to solubility in *n*-hexane even at -30°C.

(6) ^{31}P-NMR ($CDCl_3$) δ 13.18, 13.47, J_{PH} = 632.8 Hz, ^{1}H-NMR (100 MHz, $CDCl_3$) δ 1.18, 1.21 (12H, 2d, *J* = 6.3, 6.7 Hz, CH_3 of isopropyl, diastereomers), 1.39, 1.41 (3H, 2s, 5-CH_3, diastereomers), 2.56 (2H, m, 2'-H), 3.05-3.71 (4H, m, 5'-H and CH of isopropyl), 3.79 (6H, s, OCH_3 of DMTr), 4.30 (1H, m, 4'-H), 5.12 (1H, m, 3'-H), 6.42 (1H, m, 1'-H), 6.86 (4H, d, *J* = 8.6 Hz, 3,5-H of DMTr), 6.88, 6.95 (1H, 2d, J_{PH} = 630.3, 630.4 Hz, PH, diastereomers), 7.26-7.98 (15H, m, ArH).

(7) When the reaction of **2b** with 3'-*O*-benzoyl N^3-benzoylthymidine was carried out in the absence of diisopropylethylamine, **3b** expected was not detected at all and signals of the 3',5'-*O*-dithymidine phosphorochloridite (164.79 ppm) and 3',5',5'-*O*-trithymidine phosphite (138.44 ppm) were detected.

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