Tetrahedron Letters,Vol.29,No.33,pp 4143-4146,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

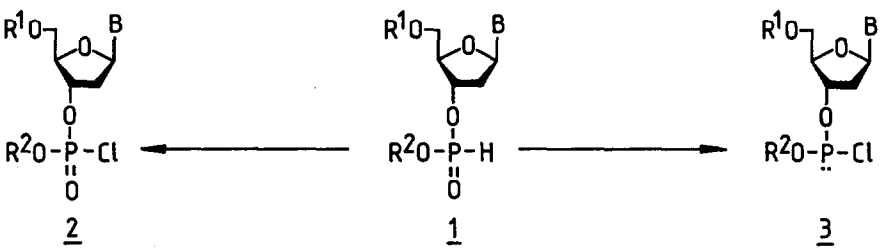
2-CYANOETHYL NUCLEOSIDE 3'-PHOSPHONATES AS NOVEL STARTING MATERIALS FOR OLIGONUCLEOTIDE SYNTHESIS

Takeshi Wada, Hitoshi Hotoda, Mitsuo Sekine, and Tsujiaki Hata*

Department of Life Chemistry, Tokyo Institute of Technology,
Nagatsuta, Midoriku, Yokohama 227, Japan

Abstract: Various disubstituted phosphonates including alkylnucleoside 3'-phosphonates were converted rapidly into the corresponding phosphorochloridites by use of tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP) as a chlorinating reagent. The reaction was found to be applicable to the rapid and practical synthesis of oligonucleotides.

In the chemical synthesis of oligonucleotides, alkyl nucleoside 3'-phosphonates have not been used directly as starting nucleotide units except for the first synthesis of TpT achieved by Todd[1]. He employed NCS as an oxidative chlorinating reagent for conversion of benzyl 5'-O-acetylthymidine 3'-phosphonate (1a) to the corresponding phosphorochloridate (2a). It is also well known that certain "positive halogen" compounds such as chlorine[2], bromine[3], iodine[4], carbon tetrahalides[5], and sulfuryl chloride[6] react with dialkyl phosphonates in a similar manner to generate the phosphoryl halides with oxidation of the phosphorus atom. However, non-oxidative halogenation of dialkyl phosphonate (e.g. conversion of 1 to 3) has not been reported.



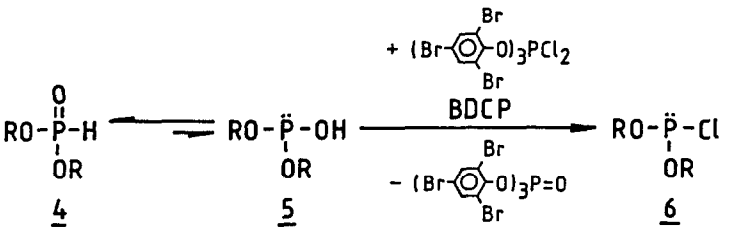
2 1 3

a, B = Th, R^1 = Ac, R^2 = Bn

b, B = $A^{bz}d$, R^1 = DMTr, R^2 = CH_2CH_2CN

Recently, we have reported that new types of condensing reagents, i.e., bis(2,4,6-trihalophenoxy)trichlorophosphorans[7] and tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP)[8] were found to be effective for the phosphotriester approach in oligonucleotide synthesis. These unique reagents were applied to the transformation of nucleoside 3'-phosphodiesters into the corresponding phosphorochloridates, namely, the P(O)OH function of phosphodiesters was converted into P(O)Cl. It is known that dialkyl phosphonate (4)

tautomerizes with dialkyl phosphite (5). First, we examined whether BDCP could convert the ÖPOH group of (5) into ÖPCl without oxidation of phosphorus atom. It is apparent that the ÖPCl function is more reactive than P(O)Cl, and the former is advantageous for the rapid and quantitative phosphorylation.

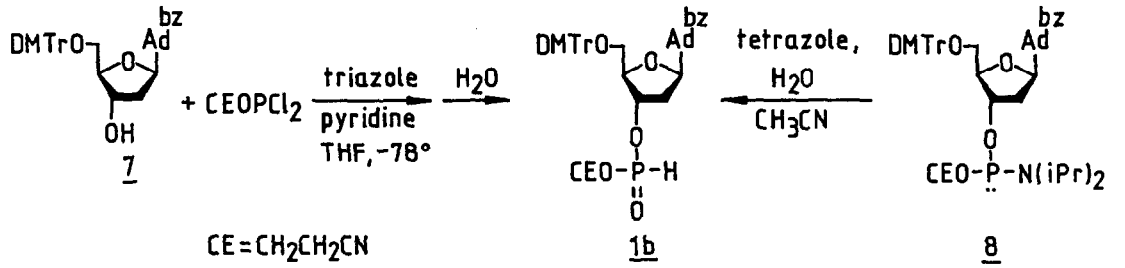


An appropriate dialkyl or diphenyl phosphonate (4, 0.2 mmol) was mixed with BDCP (327 mg, 0.3 mmol) in pyridine (4 ml) and the reaction was monitored by FT-^{31}P-NMR. In each case, the spectrum of the reaction mixture showed that a signal of the phosphonate (4) completely disappeared within 5 min and a new signal was observed in the low-field region around 160-170 ppm. The chemical shift suggested that 4 was converted into the corresponding highly reactive phosphorochloridite (6) without oxidation. On the other hand, BDCP (-64.62 ppm) was completely converted into an inert species, tris(2,4,6-tribromophenyl) phosphate (-23.01 ppm)[8]. The results are listed in Table 1.

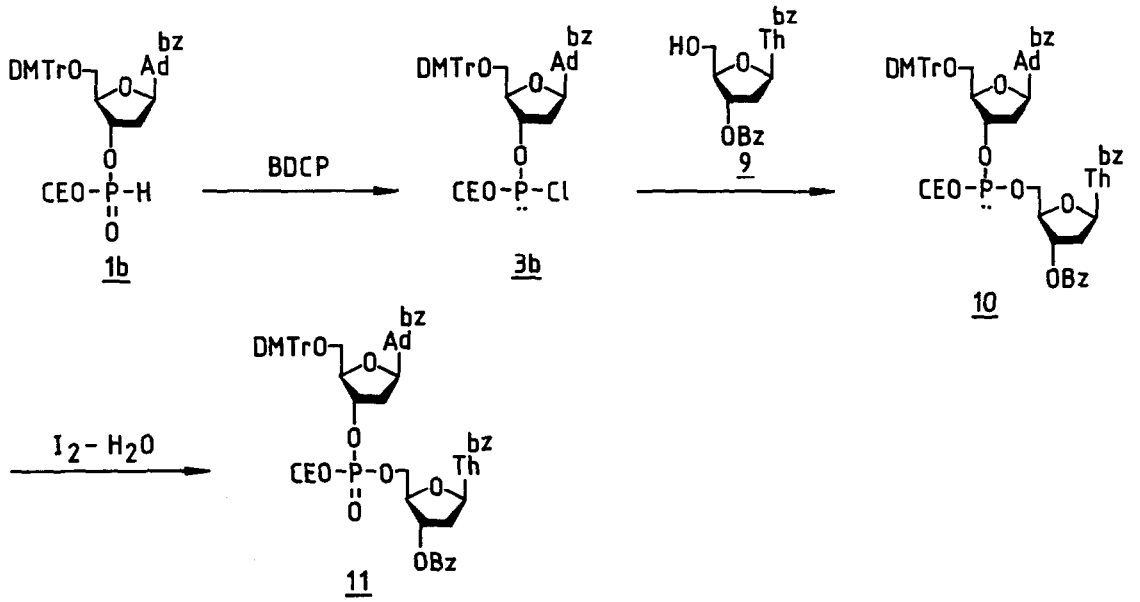
Table 1 ^{31}P-NMR chemical shifts of compounds 4 and 6 (C_5D_5N)

	R	δ (ppm) of 4	δ (ppm) of 6
a	Me	10.95	169.44
b	Et	7.27	166.14
c	iPr	4.36	166.82
d	Bu	8.12	166.92
e	Ph	1.26	158.59

Next, the reaction was applied to the synthesis of oligonucleotides. 2-Cyanoethyl 5'-O-dimethoxytrityl-N^6-benzoyldeoxyadenosine 3'-phosphonate (1b) was prepared as follows. To a well-stirred solution of 2-cyanoethyl phosphorodichloridite (285 mg, 1.5 mmol) in THF (0.5 ml) was added dropwise a solution of 5'-O-dimethoxytrityl-N^6-benzoyldeoxyadenosine (7, 658 mg, 1 mmol), 1,2,4-triazole (207 mg, 3 mmol), and pyridine (0.5 ml) in THF (1.5 ml) under cooling (-78 °C) for 10 min. After the mixture was stirred for 30 min, successive hydrolysis and extraction gave pure 1b (765 mg, δ, 7.85 ppm in C_5D_5N, $^1J_{PH}$ 718.7 Hz) in 98% yield. It was noteworthy that contamination of the 3'-3' dinucleoside phosphite and the decyanoethylated derivative was not detected in the products by means of ^{31}P-NMR. Independently, the authentic sample of 1b was prepared from the corresponding 2-cyanoethyl 5'-O-dimethoxytrityl-N^6-benzoyldeoxyadenosine 3'-phosphoramidite (8)[9] by the hydrolysis in the presence of 1H-tetrazole in acetonitrile.



2-Cyanoethyl N⁶-benzoyldeoxyadenosine 3'-phosphonate (1b, 116 mg, 0.15 mmol) was mixed with BDCP (246 mg, 0.225 mmol) in pyridine (1.4 ml) at room temperature. After 1 min, the ^{31}P-NMR analysis suggested that the reaction was almost completed (Fig. 1). After additional 4 min, 1b and BDCP were completely converted into the highly reactive nucleoside 3'-phosphorochloridite (3b, 165.27 ppm) and tris(2,4,6-tribromophenyl) phosphate, respectively. To the resulting solution was added 3'-O,N³-dibenzoylthymidine (9, 45 mg, 0.1 mmol) in pyridine (0.2 ml). Immediately, formation of the 3'-5' dinucleoside phosphite (10, 139.31 ppm) was observed. The phosphite intermediate 10 was oxidized in situ with I_2-H_2O to give the dinucleoside phosphate (11, -1.84 ppm).



In contrast with the above results, it is known that alkyl nucleoside 3'-phosphonates of type 1 can not be activated by ordinary condensing reagents such as arenesulfonyl chlorides or diphenyl phosphorochloridate in the presence or absence of azoles[16]. Letsinger[11] reported that nucleoside 3'-phosphorochloridites could be employed as active intermediates in oligonucleotide synthesis. However, these compounds are unstable under the usual conditions. Our approach enabled us to generate these intermediates rapidly from stable alkyl nucleoside 3'-phosphonates by simple treatment with BDCP.

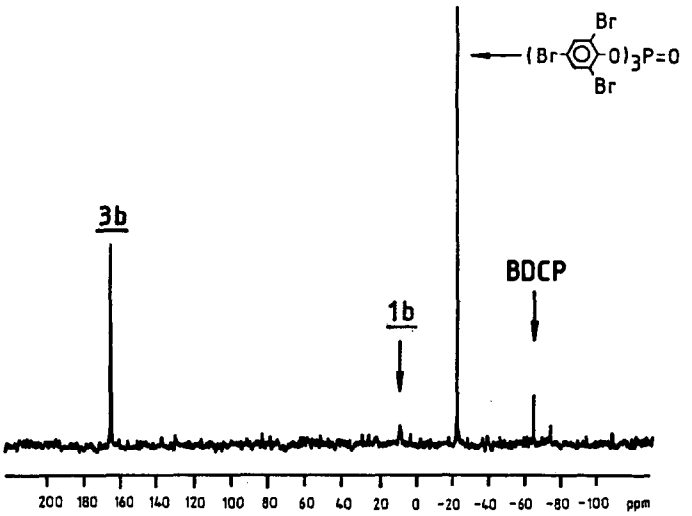


Fig. 1 ^{31}P-NMR spectrum of the mixture obtained by the reaction of 1b with BDCP (1.5 equiv) in C_5D_5N within 1 min.

References

1) A. M. Michelson, and A. R. Todd, J. Chem. Soc., 2632 (1955).
2) F. R. Atherton, H. T. Oppenshaw, and A. R. Todd, ibid., 382 (1945).
3) W. Gerrard and Jeacocke, ibid., 3647 (1954).
4) H. McCombie, B. C. Saunders, and J. Stacey, ibid., 921 (1945).
5) F. R. Atherton, and A. R. Todd, ibid., 647 (1947).
6) F. R. Atherton, H. T. Howard, and A. R. Todd, ibid., 1106 (1948).
7) J. Matsuzaki, H. Hotoda, M. Sekine, and T. Hata, Tetrahedron Lett., 27, 5645 (1986).
8) H. Hotoda, T. Wada, M. Sekine, and T. Hata, ibid., 28, 1681 (1987).
9) Compound 8 was purchased from Applied Biosystem Inc.
10) P. J. Garreg, T. Regberg, J. Stawinski, and R. Strömberg, Chemica Scripta, 25, 280 (1985).
11) R. L. Letsinger, Finnan, G. A. Heavner, and W. B. Lunsford, J. Am. Chem. Soc., 97, 278 (1975).

(Received in Japan 23 May 1988)