CHEMISTRY LETTERS, pp. 949-952, 1985.

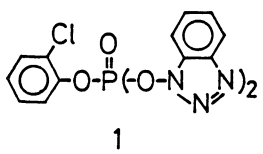
A CONVENIENT PROCEDURE FOR THE PREPARATION OF DINUCLEOSIDE PHOSPHATES BY THE USE OF A BIFUNCTIONAL PHOSPHORYLATING REAGENTS, 2-CHLOROPHENYLPHOSPHOROBIS(NITROBENZOTRIAZOLIDE)

Oyo MITSUNOBU,* Miyako TAKAHASHI, Hiroshi SEKI, Makoto ARAI, Fumiko YASUMOTO, Hideaki IWAMI, Naoto KAWAKAMI, and Junko KAMISHIRO
Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, Chitosedai, Setagaya-ku, Tokyo 157

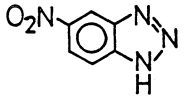
The reagent formed by the reaction of 2-chlorophenyl phosphorodichloridate with 2 molar amounts of 5-nitrobenzotriazole was allowed to react successively with nucleosides having free 3'-hydroxyl groups and nucleosides having free 5'-hydroxyl groups affording the corresponding dinucleoside phosphates in 63-97% yields.

Although many phosphorylating systems have been reported and successfully utilized in oligonucleotide synthesis,[1] there still exist ongoing needs to develop simple procedures for internucleotidic phosphate linkage formation. One possible approach to simplify the oligonucleotide synthesis is the use of a bifunctional phosphorylating reagent where (3'-5')-internucleoside phosphate linkage could be formed by a one-pot procedure. However, many bifunctional phosphorylating reagents reported so far are not so reactive as to be utilized in di- and oligo-nucleotide synthesis unless activating catalysts such as N-methylimidazole are used.[1,2]

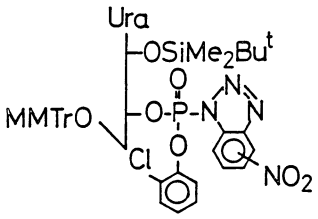
Recently, van Boom and his coworkers have reported the internucleotidic phosphotriester linkage formation from 5'-protected nucleosides and 3'-protected nucleosides by the use of 2-chlorophenyl bis(1-benzotriazolyl) phosphate (1) in the absence of an activating reagent.[3] They also demonstrated that the bifunctional phosphorylating reagent could be utilized in block coupling of oligonucleotides.[3]



1



2



3

In this communication, we wish to report one-pot synthesis of dinucleoside phosphates by the use of a reagent formed by the reaction of 2-chlorophenyl phosphorodichloridate and 5-nitrobenzotriazole (**2**).[4,5] Thus, 2-chlorophenyl phosphorodichloridate (0.96 mmol) was allowed to react with **2** (1.92 mmol) in pyridine for 15 min at room temperature.[6] 5'-O-Tritylthymidine (TrT; 0.8 mmol)[7] was added and the mixture was stirred for 40 min at 0 °C (first step).[8] Then, 3'-O-acetylthymidine (TAc; 0.8 mmol)[9] was added and the reaction mixture was stirred for 3.75 h at room temperature (second step) giving 5'-O-tritylthymidylyl-(3'-5')-3'-O-acetylthymidine 2-chlorophenyl ester. The product was isolated by silica gel column chromatography ($CHCl_3$-MeOH = 22 : 1 v/v; 93% isolated yield) and characterized, after deprotection, by enzymatic degradation. When the second step was carried out for 24 h, the fully protected thymidylyl-(3'-5')-thymidine was obtained in 95% yield.

By a similar way, 5'-O-monomethoxytrityl-N^6-benzoyldeoxyadenosine (d-MMTr-A^{Bz}),[10] 5'-O-monomethoxytrityl-N^4-benzoyldeoxycytidine (d-MMTrC^{Bz}),[10] or 5'-O-monomethoxytrityl-N-isobutyryldeoxyguanosine (d-MMTrG^{ib}),[10,11] was successively treated with 2-chlorophenylphosphorobis(nitrobenzotriazolide) and TAc to afford the corresponding fully protected dideoxyribonucleoside phosphates as summarized in Table 1.

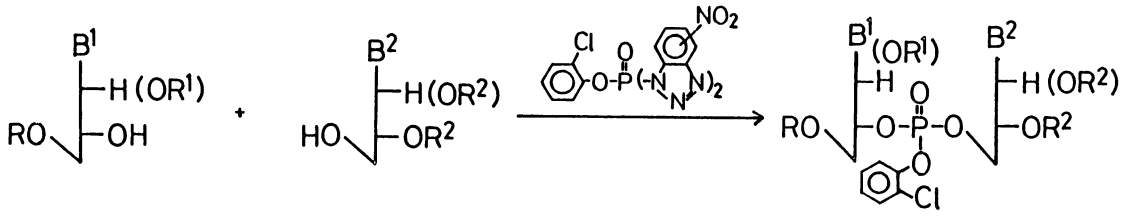
Table 1. Preparation of dinucleoside phosphates[a]

3'-OH Component (0.5 mmol)	5'-OH Component (0.5 mmol)	Product[b]	Yield %
TrT	TAc	TrTpTAc	95
d-MMTrC^{Bz}	TAc	d-MMTrC^{Bz}pTAc	86
d-MMTrA^{Bz}	TAc	d-MMTrA^{Bz}pTAc	97
d-MMTrG^{ib}	TAc	d-MMTrG^{ib}pTAc	70
MMTrU(2'-O-TBDMS)	$U(OBz)_2$	MMTrU(2'-O-TBDMS)p$U(OBz)_2$	70
MMTrU(2'-O-TBDMS)	$C^{Bz}(OBz)_2$	MMTrU(2'-O-TBDMS)p$C^{Bz}(OBz)_2$	63
MMTrU(2'-O-TBDMS)	U(2'-O-TBDMS)	MMTrU(2'-O-TBDMS)pU(2'-O-TBDMS)	73

a) The first step was carried out at 0 °C for 40 min. For deoxyribonucleosides, the second step was carried out at room temperature for 24 h. For ribonucleosides, the second step was carried out at room temperature for 20 h.

b) p = 2-Chlorophenyl phosphate. TBDMS = t-Butyldimethylsilyl.

The present procedure could also be utilized for the preparation of diribonucleoside phosphates. Thus, phosphorylation (first step) of 5'-O-monomethoxytrityl-2'-O-t-butyldimethylsilyluridine [MMTrU(2'-O-TBDMS)][12] at 0 °C for 40 min, followed by the reaction with 2',3'-di-O-benzoyluridine [$U(OBz)_2$][13] or N^4,2',3'-



O-tribenzoylcytidine [$C^{Bz}(OBz)_2$][13] at room temperature for 20 h (second step) afforded the expected fully protected uridylyl-(3'-5')-uridine or uridylyl-(3'-5')-cytidine in 70% [recovered $U(OBz)_2$: 29%] or 63% [recovered $C^{Bz}(OBz)_2$: 36%] yield, respectively (Table 1).

The reactions shown in Table 1 were carried out by the use of 0.5 mmol each of 3'-hydroxy component and 5'-hydroxy component, while a small excess of 2-chlorophenyl phosphorodichloridate (0.6 mmol) and 2 (1.2 mmol) was used. TLC-analysis ($CHCl_3$-MeOH system) of the reaction mixture revealed that nucleosides having 3'-hydroxyl group were converted into products with zero mobility in a period of 40 min at 0 °C (first step). After completion of the second step and subsequent workup, no detectable amount of side product was isolated by silica gel column chromatography expect for the case of d-$MMTrG^{ib}$ in which a small amount of product with higher mobility than d-$MMTrG^{ib}$pTAc was formed. The structure of the side product, however, has not yet been assigned.

When 2-chlorophenylphosphorobis(nitrobenzotriazolide) prepared as described above was allowed to react with 2 molar amounts of MMTrU(2'-O-TBDMS) at room temperature for 20 h, no fully protected uridylyl-(3'-3')-uridine was obtained but 49% of the starting nucleoside was recovered. The result suggests that uridine 3'-phosphoronitrobenzotriazolide (3) formed at the first stage of the reaction would selectively react with the 5'-hydroxyl group of ribonucleosides having a bulky 2'-protecting group.[14] Thus, MMTrU(2'-O-TBDMS) was successively reacted with 2-chlorophenylphosphorobis(nitrobenzotriazolide) and 2'-O-t-butyldimethylsilyluridine [U(2'-O-TBDMS)][12] under the same conditions described above giving protected uridylyl-(3'-5')-uridine having a free 3'-hydroxyl group in 73% yield with 18% recovery of U(2'-O-TBDMS).

The work described in this paper provides a convenient one-pot procedure for the preparation of dinucleoside phosphates. The reactivity and selectivity of phosphorobis(benzotriazolide) derivatives would be controlled by changing the substituent group on the aromatic ring.[15]

References

1) V. Amarnath and A. D. Broom, Chem. Rev., 77, 183 (1977); C. B. Reese, Tetrahedron, 34, 3143 (1978); S. A. Narang, Tetrahedron, 39, 3 (1983).

2) See for example, P. Cashion, K. Porter, T. Cadger, G. Sathe, T. Tranquilla, H. Notman, and E. Jay, Tetrahedron Lett., 1976, 3769, and refs. therein.
3) J. E. Marugg, M. Tromp, P. Jhurani, C. F. Hoyng, G. A. van der Marel, and J. H. van Boom, Tetrahedron,40, 73 (1984), and refs. therein. See also, N. Katagiri, K. Itakura, and S. A. Narang, J. Am. Chem. Soc., 97, 7332 (1975); K. Itakura, N. Katagiri, and S. A. Narang, J. Biol. Chem., 250, 4592 (1975).
4) The reaction was carried out under nitrogen atmosphere.
5) Jager and Engels have reported the use of benzotriazole as an activating agent in their phosphoramidite approach. A. Jager and J. Engels, Tetrahedron Lett., 25, 1437 (1984).
6) Although no attempt has been made to elucidate the structure, it would be assumed that the bifunctional phosphorylating reagent formed is 2-chlorophenylphosphorobis(nitrobenzotriazolide).
7) G. Weimann and H. G. Khorana, J. Am. Chem. Soc., 84, 419 (1962).
8) When the first step was carried out at room temperature, there was formed a considerable amount of 5'-O-tritylthymidylyl-(3'-3')-5'-O-tritylthymidine 2-chlorophenyl ester which was also obtained in 77% isolated yield by the reaction of 2-chlorophenylphosphorobis(nitrobenzotriazolide) with 2 molar amounts of TrT at room temperature for 20 h.
9) A. M. Michelson and A. R. Todd, J. Chem. Soc., 1953, 951.
10) G. S. Ti, B. L. Gaffney, and R. A. Jones, J. Am. Chem. Soc., 104, 1316 (1982).
11) H. Buchi and H. G. Khorana, J. Mol. Biol., 72, 251 (1972).
12) D. Flockerzi, G. Silver, R. Charubara, W. Schlossed, R. S. Varma, F. Creegan, and W. Pfleiderer, Justus Liebigs Ann. Chem., 1981, 1968.
13) D. H. Rammler and H. G. Khorana, J. Am. Chem. Soc., 84, 3112 (1962).
14) For the selective phosphorylation of the 5'-hydroxyl group of nucleosides, see for example, J. Kimura, Y. Fujisawa, T. Yoshizawa, K. Fukuda, and O. Mitsunobu, Bull. Chem. Soc. Jpn., 52, 1191 (1979); E. Ohtsuka, A. Yamane, T. Doi, and M. Ikehara, Tetrahedron, 40, 47 (1984); Reference 3 of this communication.
15) There are many precidents for controlling the reactivity by the change of the nature and position of substituents on aromatic ring. See for example, E. Uhlmann and W. Pfleiderer, Helv. Chim. Acta, 64, 1688 (1981); M. J. Gait and S. G. Popov, Tetrahedron Lett., 21, 2841 (1980), and refs. therein. 1-Hydroxybenzotriazoles having a variety of substituents have been utilized in the preparation of peptides and carboxylic esters. See for example, B. Castro, J. R. Dormy, G. Evin, and C. Selve, Tetrahedron Lett., 1975, 1219: M. Itoh, D. Hagiwara, and J. Notani, Synthesis, 1975, 456: M. Furukawa, N. Hokama, and T. Okawara, Synthesis, 1983, 42, and refs. therein.

(Received April 11, 1985)