Communications to the Editor

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AN EFFICIENT PHOSPHORYLATING AGENT FOR THE SYNTHESIS OF NUCLEOSIDE 3'-PHOSPHODIESTER INTERMEDIATES IN OLIGONUCLEOTIDE SYNTHESIS

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By using 3-chloro-1,2-benzoisothiazole 1,1-doxide (BIDCl), 5-chloro-8-quinolyl phosphate can be converted into an efficient phosphorylating agent (4), which can be used to synthesze nucleoside 3'-phosphodiester intermediates (2).

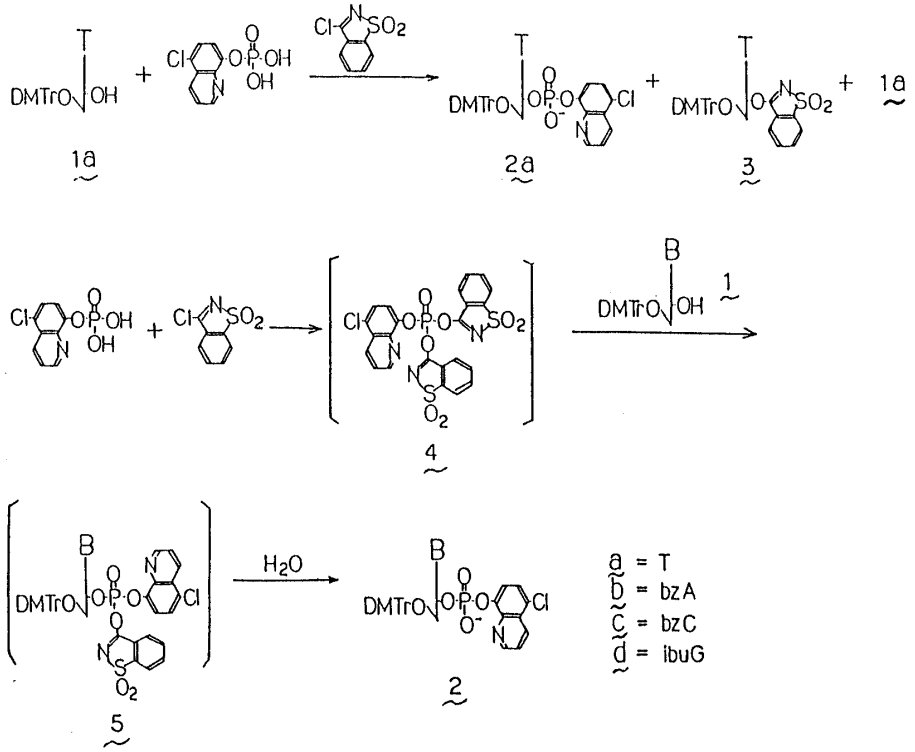
KEYWORDS —— phosphorylation; phosphorylating agent; 3-chloro-1,2-benzoisothiazole 1,1-dioxide; nucleoside 3'-phosphodiester; nucleotide

Oligonucleotide synthesis by the phosphotriester approach necessitates two separate phosphorylation steps. 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.[1] Therefore, the nucleoside 3'-phosphodiester derivative is a key intermediate for the synthesis of oligonucleotide by the phosphotriester approach. In our studies[2] on the synthesis of oligonucleotides by the phosphotriester approach, we used the 5-chloro-8-quinolyl phosphate-coupling agent, 8-quinolinesulfonyl chloride (QS) or 4-chlorophenyl 5-chloro-8-quinolyl phosphorotetrazolide as phosphorylating agents for the synthesis of nucleoside 3'-phosphodiester intermediates. A number of phosphorylating agents have been proposed and applied to the phosphorylation of 3'-hydroxyl group of nucleosides or oligonucleotides.[3] However, aryl dihydrogen phosphates-coupling agents and aryl phosphorodichloridates (or ditriazolides) are liable to cause the formation of by-products such as 3'-3' dinucleoside monophoshates or sulfonylated nucleotides.[4] In order to overcome this problem, we developed a new phosphorylating agent (4) prepared simply from 3-chloro-1,2-benzoisothiazole 1,1-dioxide (BIDCl)[5] and 5-chloro-8-quinolyl phosphate.[6]

Phosphorylation of 5'-O-dimethoxytrityl-N-protected nucleosides (1) with 5-chloro-8-quinolyl phosphate using BIDCl was performed as follows: Addition of BIDCl (4 mol eq) to a dry pyridine solution of 5'-O-dimethoxytritylthymidine (1a) (1 mol eq) and 5-chloro-8-quinolyl phosphate (2 mol eq) afforded 5'-O-dimethoxytritylthymidine 3'-(5-chloro-8-quinolyl) phosphate (2a) in 80% yield along with 3[7] (7%), and 6% of 1a was recovered. In order to suppress this by-product 3, 5-chloro-8-quinolyl phosphate was treated with BIDCl at first and 1 was added, and the products were subjected to hydrolysis to give the corresponding phosphodiesters 2 in good yields without formation of 3. A typical procedure is as follows: 5-chlor-8-quinolyl phosphate (519 mg, 2.0 mmol) was added to a stirred

solution of BIDCl (850 mg, 4.0 mmol) in dry pyridine (12 ml), and kept for 15 min at room temperature. The ^{31}P-NMR spectra of the reaction mixture indicated that complete formation of the phosphorylating agent 4 had occurred.[8] The nucleoside 1a (544 mg, 1.0 mmol) was then added to a stirred solution of 4. After 30 min at room temperature, TLC analysis showed complete conversion of the starting material 1a into the intermediate 5a (Rf 0.61 → 0.02, CH_2Cl_2-MeOH, 9:1, v/v). It is noted that no side reaction products such as 3'-3' dinucleoside monophoshates and 3 were observed. The reaction mixture was quenched with aqueous triethylamine, and kept for 30 min at room temperature. The solution was poured into a separating funnel with CH_2Cl_2 and washed with 0.1M-triethylammonium bicarbonate (TEAB) and with water. The organic layer was dried over Na_2SO_4, concentrated to 5 ml, and added by drops to hexane-ether (9:1, v/v, 200 ml). A white precipitate was collected and dried over P_2O_5 in vacuo to give triethylammonium salt (842 mg, 95%) of 2a.

In a similar manner, the 3'-phosphodiesters of N^6-benzoyldeoxyadenosine (2b), N^4-benzoyldeoxycytidine (2c), and N^2-isobutyryldeoxyguanosine (2d) were obtained in 94%, 95%, and 92% yields, respectively. The nucleoside 3'-phosphodiesters 2 were isolated as white solids, uncontaminated with 5-chloro-8-quinolyl phosphate as indicated by ^{31}P-NMR.[9] In addition, no side reactions[4] on the guanine nucleus was detected in the phosphorylation of 1d. The use of 4-chlorophenyl phosphate[10] in place of 5-chloro-8-quinolyl phosphate also gave 5'-O-dimethoxytrityl-N-protected deoxyribonucleoside 3'-(4-chlorophenyl) phosphates in 93-95% yields.



Next, we examined whether the formation of phosphotriester linkage was possible by using the intermediate 5. The intermediate 5 was treated with nucleoside component in dry pyridine, but the phosphotriester linkage formation did not proceed.

The results indicate that the phosphorylating agent 4 can be prepared readily from 5-chloro-8-quinolyl phosphate and BIDCl which is a useful phosphorylating agent for the formation of selective phosphodiester linkages. The nucleoside 3'-phosphodiesters are key intermediates for the liquid phase and solid phase synthesis by the phosphotriester approach.

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REFERENCES AND NOTES

1) R. L. Letsinger and K. K. Ogilvie, J. Am. Chem. Soc., 89, 4801 (1967); F. Eckstein and I. Rizk, Chem. Ber., 102, 2362 (1969); F. Cramer and J. C. Catlin, J. Org. Chem., 38, 245 (1974); K. Itakura, N. Katagiri, and S. A. Narang, Can. J. Chem., 52, 3689 (1974).
2) H. Takaku, T. Nomoto, R. Yamaguchi, and T. Hata, Tetrahedron Lett., 1979, 3357; H. Takaku, M. Kato, M. Yoshida, and R. Yamaguchi, J. Org. Chem., 45, 3347 (1980); H. Takaku, T. Nomoto, and K. Kamaike, Chem. Lett., 1981, 543; H. Takaku and M. Yoshida, J. Org. Chem., 46, 589 (1981).
3) For recent reviews, see R. I. Zhadanov and S. M. Zhenodarova, Synthesis, 1975, 222; H. Kössel and H. Seliger, Prog. Chem. Org. Prod., 32, 297 (1975); V. Amarnath and A. D. Boom, Chem. Rev., 77, 183 (1977); C. B. Reese, Tetrahedron, 34, 3143 (1978); M. Ikehara, E. Ohtsuka, and A. F. Markham, Ad. Carbohydr. Chem. Biochem., 36, 135 (1978); E. Ohtsuka, M. Ikehara, and D. Söll, Nucleic Acids Res., 10, 6553 (1982).
4) C. B. Reese and R. Safhill, J. Chem. Soc., Chem. Commun., 1968, 767; K. Itakura, N. Katagiri, C. P. Bahl, R. H. Wightmann, and S. A. Narang, J. Am. Chem. Soc., 97, 7327 (1975); J. B. Chattopadhaya and C. B. Reese, Tetrahedron, Lett., 1979, 5059; J. H. M. de Rooij, G. Welle-Hazelgers, P. H. van Deursen, J. Serdijin, 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. Rayer, C. B. Reese, and A. Ubasawa, J. Chem. Soc., Chem. Commun., 1980, 972; P H. Daskalov, M. Sekine, and T. Hata, Tetrahedron Lett., 21, 3899 (1980); 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 (1982).
5) J. A. Jesurn, Chem. Ber., 26, 2286 (1893); I. R. Meadoe and E. E. Reid, J. Am. Chem. Soc., 65, 457 (1943); H. Hettler, Tetrahedron Lett., 1966, 1265; H. Somer and F. Cramer, Chem. Ber., 107, 24 (1974); K. Inomata, H. Kinoshita, H. Fukuda, O. Miyano, Y. Yamashiro, and H. Kotake, Chem. Lett., 1979, 1265.
6) H. Takaku, T. Nomoto, Y. Sakamoto, and T. Hata, Chem. Lett., 1979, 1255.

7) 3: mp 146-148°C, Rf 0.41 (CH_2Cl_2-MeOH, 9:1, v/v), UVλmax (MeOH) 266, 230 nm, λmin (MeOH) 252 nm. ^{1}H-NMR ($CDCl_3$) δ : 1.51 (3H, s, CH_3), 2.75 (2H, m, H-2'), 3.22 (2H, br s, H-5'), 3.59 (6H, s, OCH_3), 4.85 (1H, m, H-4'), 5.85 (1H, m, H-3'), 6.50 (1H, d, $J_{1',2'}$=6Hz, H-1'), 6.68-7.98 (18H, m, ArH and H-6), 9.50 (1H, br s, NH). Anal. Calcd for $C_{38}H_{35}N_3O_9S$: C, 64.31; H, 4.98; N, 5.92. Found: C, 63.93; H, %.11; N, 5.66.

8) ^{31}P-NMR spwctroscopy (pyridine-$CDCl_3$) δ = +5.71 p.p.m. relative to 85% H_3PO_4.

9) ^{31}P-NMR [(pyridine-C_6D_6), 85% H_3PO_4] spectra of 2a-d are shown below. 2a= δ+6.03; 2b= δ +7.31; 2c= δ +6.43; 2d= δ +7.35.

10) W. Shelver, M. I. Blake, and C. E. Miller, J. Am. Pharm. Assoc., 47, 72 (1958).

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