Tetrahedron Letters,Vol.25,No.38,pp 4237-4240,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

AMINOPHOSPHORDICHLORIDITE : A NEW PHOSPHORYLATING REAGENT FOR ONE-POT SYNTHESIS OF 3'-5'- OR 2'-5'-LINKED DIRIBONUCLEOTIDE HAVING DEFINED SEQUENCE

Takeo Shimidzu*, Kazushige Yamana, and Seiichiro Maikuma
Division of Molecular Engineering, Graduate School of Engineering,
Kyoto University, Yoshida, Sakyo-ku, Kyoto 606, Japan

SUMMARY : 3'-5'- or 2'-5'-linked diribonucleotides having definite sequences have been synthesized by an one-flask procedure utilizing the cyclic phosphorylation of the *cis*-glycol in the ribonucleosides with aminophosphordichloridites.

The phosphorylation of a nucleoside hydroxyl group is the key step in the synthesis of oligonucleotides. Much efforts have been directed toward the developments of the highly reactive phosphorylating reagents. Many active phosphites, *e.g.* phosphorchloridites,[1] phosphorazolides,[2] and phosphoramidites[3] have been shown to be very effective in the preparation of oligonucleotide chains. On the contrary, we have recently developed the new sort of phosphorylating reagents such as phosphorus trisazoles, including tri-(imidazol-1-yl)phosphine, having the potential reactivity and selectivity in the reaction with the *cis*-glycol of ribonucleosides.[4] The utility of these reagents have been demonstrated in the synthesis of 3'-5'- or 2'-5'-linked homooligoribonucleotides by polymerization of unprotected ribonucleosides.[5]

The next object of our research is to synthesize defined sequence oligoribonucleotides utilizing the selective phosphorylation to the *cis*-glycol of ribonucleosides. We have tried to join the two different nucleosides with the inter-ribonucleotidic linkage using tri-(imidazol-1-yl)phosphine. The phosphorylation of the *cis*-glycol in a 5'-protected ribonucleoside with an equivalent of tri-(imidazol-1-yl)phopshine gave almost quantitatively the cyclic phosphorimidazolide derivative. In its reaction with a ribonucleoside, however, the coupling product was obtained in a moderate yield. To overcome this problem, we have adopted the 5'-protected ribonucleoside 2',3'-cyclic phosphoramidite in place of the imidazolide, because of its stability and availability to produce the phosphite linkage with the other ribonucleoside.[3] A new sort of phosphorylating reagents, aminophosphordichloridites, have been developed for the formation of the cyclic phosphoramidite.[6] We describe herein an effective cyclic phosphorylation of the *cis*-glycol in ribonucleosides and its application to one-pot synthesis of diribonucleotides having definite sequences.

Aminophosphordichloridites(I a-d) were prepared by the reaction of phosphorus trichloride with dialkylamine. A solution of dialkylamine(1.0 mol) in 200 ml of dry ether was added dropwise at -10°C over 2 h to a vigorously stirred solution of phosphorus trichloride(0.5 mol) in 200 ml of dry ether. After the standing for 1 h,

$$PCl_3 + HNR_2 \rightarrow Cl_2PNR_2$$

Ia-d

Ia:-N(◯)$_2$ Ib:-N O Ic:-N(<)$_2$ Id:-N

the aminehydrochloride was removed and then the desired material was isolated in a pure form[7](Yields range: 56-75 %) by a vacuum distilation.

We first examined the cyclic phosphorylation reaction with aminophosphordichloridites(I). 5'-O-monomethoxytrityluridine(II) was allowed to react at 0°C in THF with an equivalent of I in the presence of 2,6-lutidine(2 equiv.). After the appropriate time intervals, the aliquot of the reaction mixture was treated with iodine and water followed by $ZnBr_2$. The phosphorylated product thus obtained was uridine 2',3'-cyclic phosphate,[8] clearly indicating that these phosphorylating reagents selectively attack the 2',3'-hydroxyl groups in the 5'-protected ribonucleoside to give the cyclic phosphoramidite compound(III). As shown in Figure 1, diphenylamino and morpholino derivatives(Ia and Ib) gave the phosphorylated product in an almost quantitative yield within a few minutes. By using diisopropylamino and lupetidino-phosphines(Ic and Id), the rate of the phosphorylation was rather slow under the present reaction conditions and unreacted uridine was remained.

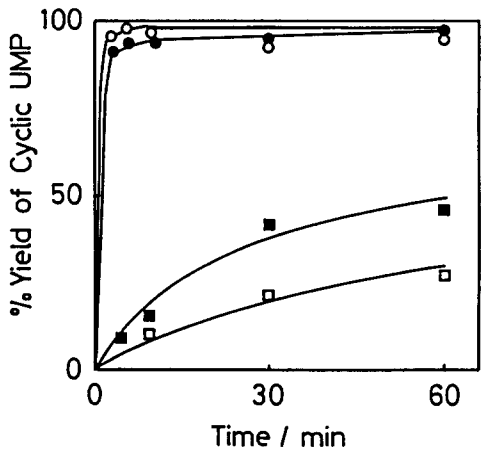
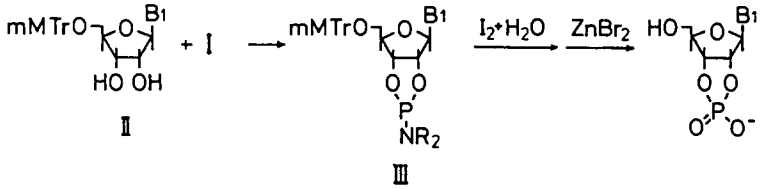
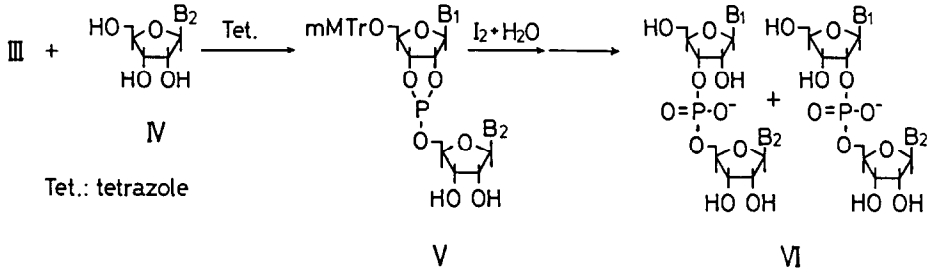


Figure 1. Formation of uridine 2',3'-cyclic phosphate(Cyclic UMP) in the reactions of 5'-O-monomethoxytrityluridine with aminophosphordichloridites(○, Ia; ●, Ib; □, Ic, ■, Id).

It is expected that the 5'-protected ribonucleoside 2',3'-cyclic phosphoramidite(III) could be coupled with a ribonucleoside(IV) by using tetrazole as a catalyst.[3] An ideal



II III



procedure for the synthesis of difinite sequence oligoribonucleotides involves the cylcic phosphorylation of a 5'-protected ribonucleoside(II) with I, the coupling reaction of the resulting cyclic phosphoramidite(III) with a 5'-OH of a ribonucleoside(IV) as a second component, the oxidation employed iodine and water, and the final deprotection of the protecting group in V to produce the 3'-5'- or 2'-5'-linked diribonucleotide(VI), which are carried out in one-flask. Typically, 5'-O-monomethoxytrityluridine(141.3 mg, 0.28 mmol) was allowed to react at 0°C for 5 min in THF with diphenylaminophosphor-dichloridite, Ia,(60 µl, 0.28 mmol) in the presence of 2,6-lutidine(70 µl, 0.56 mmol). To the mixture, a solution of N^6-benzoyladenosine(97.1 mg, 0.28 mmol) and tetrazole(196 mg, 2.8 mmol) in 1.0 ml of DMF was added and stirred for 15 min. After the usual oxidation with iodine and water,[5] the solution was treated with aq. $NaHSO_3$(5 %). The solvent was removed in vacuo and then the residue was treated with conc. NH_3 in

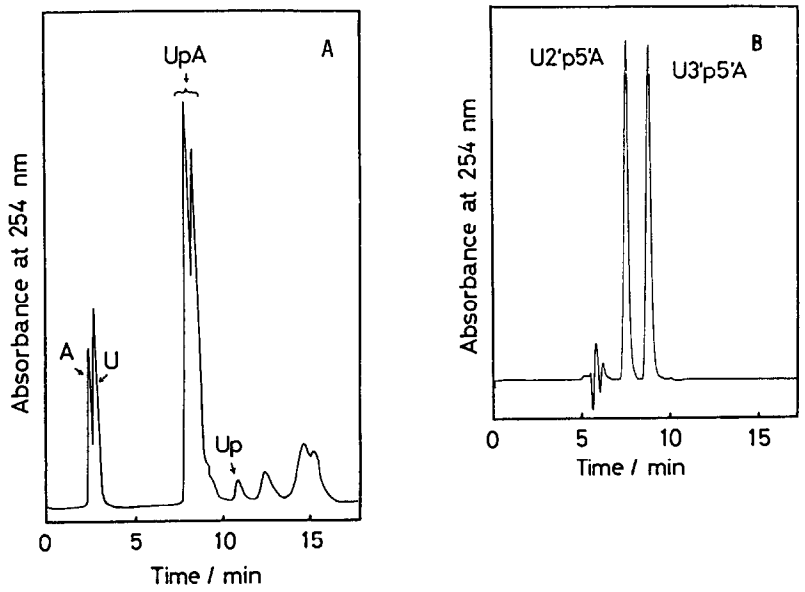


Figure 2. H.p.l.c.(Partisil 10 SAX column[5]) of the reaction mixture obtained from the reaction of 5'-O-monomethoxytrityl-uridine and N^6-benzoyladenosine with diphenylaminophosphor-dichloridite(Panel A) and reverse phase h.p.l.c.(Nucleosil 7 C_{18} column[5]) of the obtained UpA from the ion exchange column chromatography(Panel B).

aq. pyridine followed by 80 % acetic acid. H.p.l.c.[5](ion exchange) of the product is shown in Figure 2(Panel A). Diribonucleotide, UpA, was obtained in a yield of over 80 %. The obtained UpA was subjected on a reverse phase h.p.l.c. As shown in Panel B in Figure 2, U3'p5'A was easily separated from U2'p5'A (molar ratio of the 3'-5'- to the 2'-5'- = 53:47), and there exists no trace amount of the 5'-5'- and 3'-3'-linked UpA, since separated portions were completely hydrolyzed by alkaline and snake venom. It is obviously indicated that the 5'-protected ribonucleoside 2',3'-cyclic phosphoramidite(III) is coupled only with the 5'-OH of the second component(IV). Accordingly, the present approach does not require any protection of the hydroxyl groups in the second component. Similar experiment was performed using morpholinophosphordichloridite(Ib). The product, UpA, was obtained in yield of over 75 %. In this case, U3'p5'A(27.3 %) and U2'p5'A(42.5 %) together with small amount of 5'-5'- or 3'-3'-linked UpA was observed in the reverse phase h.p.l.c.

The present approach worked equally with all common ribonucleosides and gave definite sequence diribonucleotides in satisfactory yields. The products contains both 3'-5'- and 2'-5'-linkages which can be separated by a reverse phase column chromatography.

We anticipate that the procedure described here can be used by repeating the synthetic cycle for the synthesis of longer oligoribonucleotides having defined sequences. Although the separation of the linkage isomers becomes a serious problem, our present approach has appreciable merits in the preparation of short-chain defined sequence oligoribonucleotides.

REFERENCES AND NOTES

1. R.L. Letsinger and W.B. Lunsford, J. Am. Chem. Soc., 98, 3655(1976); K.K. Ogilvie, N. Therioult, and K.L. Sadana, J. Am. Chem. Soc., 99, 7741(1977).
2. M.D. Matteucci and M.H. Caruthers, J. Am. Chem. Soc., 103, 3185(1981); T.M. Cao, S.E. Bingham, and M.T. Sung, Tetrahedron Lett., 1091(1983).
3. S.L. Beaucage and M.H. Caruthers, Tetrahedron Lett., 1859(1981); L.J. McBride and M.H. Caruthers, Tetrahedron Lett., 245(1983).
4. T. Shimidzu, K. Yamana, N. Kanda, and S. Kitagawa, Bull. Chem. Soc. Jpn., 56, 3483 (1983).
5. T. Shimidzu, K. Yamana, A. Murakami, and K. Nakamichi, Tetrahedron Lett., 2717 (1980); T. Shimidzu, K. Yamana, K. Nakamichi, and A. Murakami, J. Chem Soc. Perkin Trans.I, 2294(1981); T. Shimidzu and K. Yamana, Polymer J., 15, 727(1983); T. Shimidzu, K. Yamana, N. Kanda, and S. Maikuma, Nucleic Acids Res., 12, 3257(1984).
6. T. Shimidzu, K. Yamana, S. Maikuma, and Y. Oikawa, Nucleic Acids Res. Symposium Series, No.12, 55(1983).
7. Satisfactory ^{31}P nmr and elementary analysis data for the aminophosphordichloridites were obtained. Ia, δ-^{31}P = 149.7 ppm(from external 85 % H_3PO_4), Found(Calcd.): C:53.92(53.36), H:3.70(3.73), N:5.29(5.19), P:11.38(11.47), Cl:25.75(26.25); Ib, δ-^{31}P = 156.5 ppm, Found(Calcd.): C:25.48(25.55), H:4.33(4.29), N:7.66(7.45), P:16.33(16.47), Cl:37.45(37.71); Ic, δ-^{31}P = 168.1 ppm, Found(Calcd.): C:35.86(35.66), H:7.18(6.98), N:7.09(6.93), P:15.17(15.32), Cl:34.70(35.11); Id, δ-^{31}P = 156.9 ppm, Found(Calcd.): C:39.24(39.27), H:6.69(6.54), N:6.64(6.54), P:14.30(14.47), Cl:32.92 (33.12). We are grateful to Dr. Shiro Kobayashi and Mr. Masato Suzuki(Kyoto University) for their ^{31}P nmr measurements.
8. Analogous reaction of a 5'-protected ribonucleoside with morpholinophosphorodichloridate gave the corresponding cyclic phosphate; E. Ohtsuka, M. Ubasawa, and M. Ikehara, J. Am. Chem. Soc., 92, 3445(1970).

(Received in Japan 4 June 1984)