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PROTECTION OF 5'-TERMINAL PHOSPHATE OF DEOXYRIBOOLIGONUCLEOTIDES BY USE OF o,o'-DIAMINOBIPHENYL

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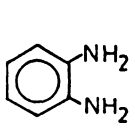
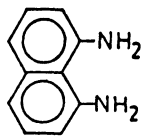
Cyclic phosphorodianilidate derivatives of nucleoside 5'-phosphates were prepared by use of triphenylphosphine and 2,2'-dipyridyl disulfide as condensing agent and used for the synthesis of oligodeoxyribonucleotides. Removal of the diamino group was remarkably accelerated by coaddition of AgOAc and acid anhydrides to the conventional system, iAmONO-pyridine-AcOH.

Among several methods for protection of phosphates,the protection mode using phosphoroanilidates is unique since the P-N bond of the anilidates can be cleaved by treatment with isoamyl nitrite under mild conditions.[1] Hashimoto[2] reported the synthesis of deoxyribonucleoside 5'-phosphorodianilidates by the reaction of deoxyribonucleoside 5'-phosphates with aniline in the presence of triphenylphosphine (Ph_3P) and 2,2'-dipyridyl disulfide $(PyS)_2$] (Oxidation-Reduction Condensation). In this paper, we report the study of cyclic phosphorodianilidate structure as a new protective skeleton of nucleoside 5'-phosphates and also a new efficient method for the deprotection of cyclic phosphorodianilidates.

We have tested three kinds of diamines: o-Phenylenediamine (1a), 1,8-diaminonaphthalene (1b), and o,o'-diaminobiphenyl (1c).


1a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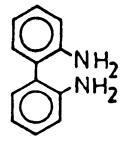
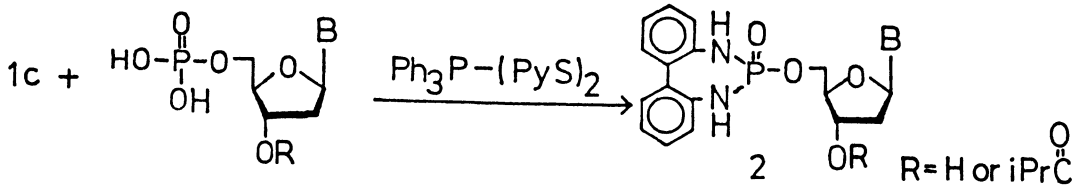

1c

First, the use of 1a and 1b was examined. However, the former did not afford the corresponding cyclic phosphorodianilidate. The latter gave the stable cyclic derivative but its isoamyl nitrite treatment resulted in very complicated unidentified substances. Finally, we found that 1c (NN) had suitable properties as the amine component: When the reaction of thymidine 5'-phosphate (pT) with 1c was carried out in the presence of Ph_3P-$(PyS)_2$, the corresponding seven-membered phosphorodianilidate (2a) was obtained.



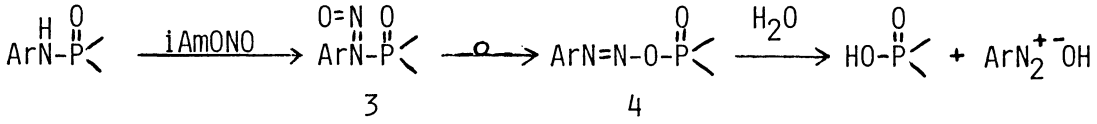
In order to increase the yield of 2a, the reaction conditions were evaluated by several experiments. The best result was obtained by the reaction of pT (1 equiv.) with 1c (2 equiv.) in the presence of Ph_3P-$(PyS)_2$ (6 equiv.) in dry pyridine at room temperature for 3 h.

According to this method, the dianilidates of thymidine 5'-phosphate (2a), N^6-benzoyldeoxyadenosine 5'-phosphate (2b), N^4-anisoyldeoxycytidine 5'-phosphate (2c), and 3'-O-isobutyryl-N^2-isobutyryldeoxyguanosine 5'-phosphate (2d) were obtained in 74%, 70%, 53%, and 80% yields, respectively.

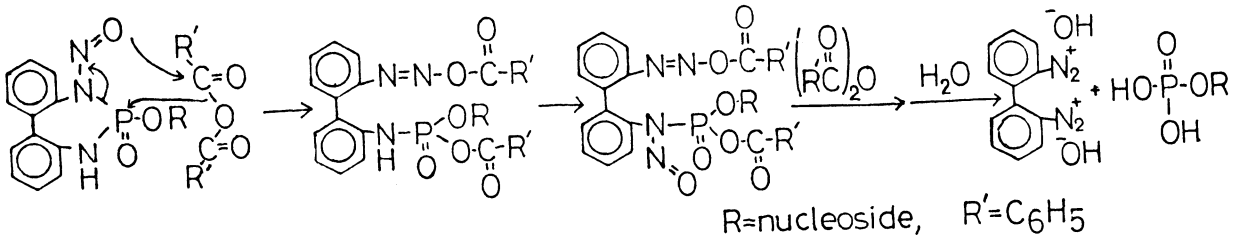
Compound 2 was separated by preparative TLC on silica gel using a mixture of chloroform and methanol (9:1, v/v) as eluent.

The NN group can be removed from 2 by means of isoamyl nitrite. However the deprotection proceeded incompletely under the usual conditions.[1)]

The isoamyl nitrite treatment generates nitrosonium ion (NO^+) derived from isoamyl nitrite, which attacks on the nitrogen atom of the phosphorodianilidate to form a N-nitroso compound (3). At this time silver salts may assist the N-nitrosonation by coordination to two nitrogen atoms of the dianilidate. The intermediate 3 migrates intramolecularly to compound (4) having a N=N-O-P bond. Compound 4 decomposes into the phosphate in the presence of water.



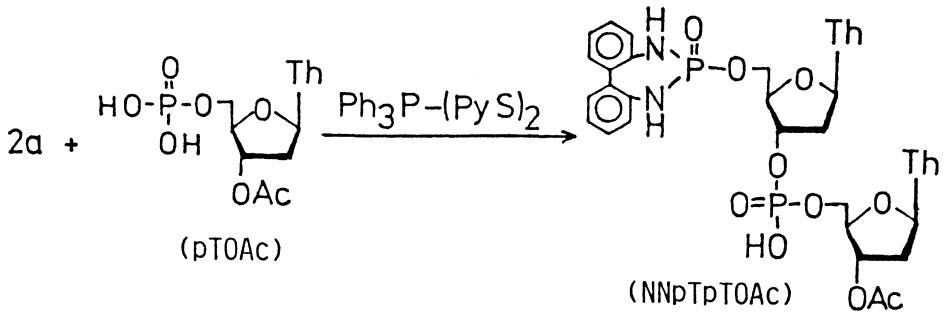
In cosideration of the above mechanism, the rate-determining step may be the migration of phosphoryl group from nitrogen to oxygen because the stable seven-membered ring compound (3) should be converted to the nine-membered ring compound (4). This migration can be regarded as an acylation of the oxygen by the phosphoryl group. The rate-determining step may be accelerated by the external acylation using an excess amount of acetic anhydride since the acetyl group can attack more smoothly the oxygen instead of the intramolecular phosphoryl migration without ring expansion. Consequently, the deprotection reaction was carried out by use of isoamyl nitrite in the presence of acetic anhydride and silver acetate (AgOAc). Expectedly, the deprotection reaction was improved considerably. Furthermore, the reaction proceeded more smoothly and completely when benzoic anhydride was employed in place of acetic anhydride except in the case of 2d:



To a solution of 2 (1 equiv.) in a mixture of pyridine-AcOH (1:1, v/v) was added benzoic anhydride (50 equiv.) and AgOAc (5 equiv.). After the silver salt was dissolved completely, isoamyl nitrite (50 equiv.) was further added. The mixture was allowed to stand at room temperature for 6 h and hydrogen sulfide gas

was bubbled into the solution. After filtration of silver sulfide, the filtrate was concentrated to dryness. The residue was treated with methanolic ammonia to afford pT (97%), pA (100%), pC (96%), and pG (53%).

On the basis of the above experiments, dinucleotides were synthesized starting from 2: For example, a mixture of 2a (0.3 mmol) and pTOAc (0.39 mmol) was dissolved in dry pyridine. The moisture was removed by repeating coevaporation with dry pyridine and then the residue was dissolved in dry pyridine (0.25 ml). Finally, Ph_3P (1.5 mmol) and $(PyS)_2$ (1.5 mmol) were added and the mixture was stirred at room temperature for 1 d in the dark.



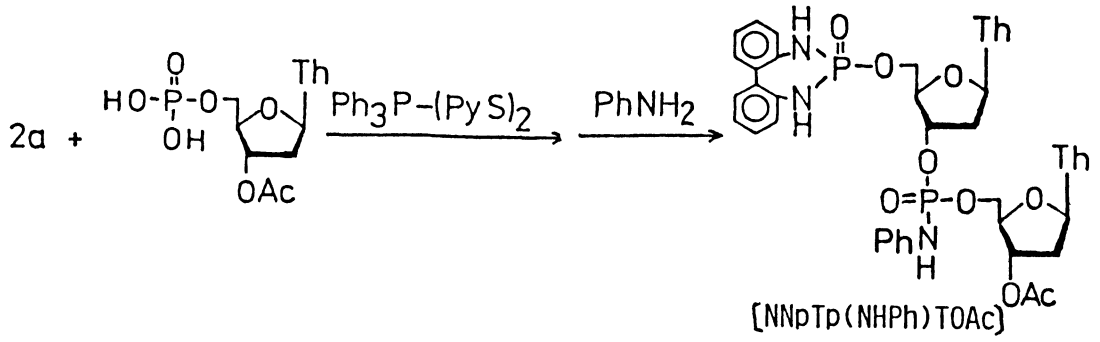
Separation of the dinucleotide derivative was performed by extraction: The reaction mixture was concentrated to dryness in vacuo and water was added. The aqueous solution was washed with ether to remove triphenylphosphine oxide and a major part of 2-mercaptopyridine (PySH). From the aqueous layer containing NNpTpTOAc and PySH, NNpTpTOAc was extracted with CH_2Cl_2-nBuOH (6:4, v/v). The solvent was removed in vacuo and the residue was applied to a DEAE cellulose column. Pure NNpTpTOAc was eluted by using 0.05 M triethylammonium bicarbonate in 30% EtOH and obtained in 82% yield; Rf_{712}[3] 0.48, Rm[4] 0.32, $\lambda_{max}^{H_2O}$ 257 nm, $\lambda_{min}^{H_2O}$ 238 nm, $\varepsilon_{267}=2.2 \times 10^4$.

In a similar manner, $NNpA^{bz}pTOAc$ was obtained in 79% yield; Rf_{712}[3] 0.76, Rm[4] 0.23, $\lambda_{max}^{H_2O}$ 255, 277 nm, $\lambda_{min}^{H_2O}$ 239, 265 nm, $\varepsilon_{280}=2.7 \times 10^4$.

The deprotection of NNpTpTOAc and $NNpA^{bz}pTOAc$ was performed according to the same procedure as described in the case of 2. Dinucleotides, pTpT and pApT, were obtained in 74% and 84% yields, respectively.

Enzymatic degradation of NNpTpT was examined with snake venom phosphodiesterase: NNpTpTOAc was treated with methanolic ammonia at room temperature overnight. NNpTpT was obtained almost quantitatively. NNpTpT (0.007 mmol, 153 OD_{267}) was dissolved in 1.5 ml of a mixture of 0.01 M ammonium acetate, 0.3 M magnesium acetate, 0.1 M Tris-acetate, and water (10:3:10:6, v/v). It was incubated with snake venom phosphodiesterase at 25 °C for 5.5 h. NNpT and pT was obtained (NNpT:pT=1:1.04).

On the other hand, the reaction mixture obtained by the oxidation-reduction condensation of 2a (0.2 mmol) with pTOAc (0.3 mmol) was further treated with aniline (3.0 mmol) for 2 d. Consequently, we found the internucleotidic bond was protected as the anilidate to give NNpTp(NHPh)TOAc in 80% yield by silica gel column chromatography: mp 176-178 °C, Rf 0.27 ($CHCl_3$-MeOH, 9:1 v/v), λ_{max}^{MeOH} 255 nm, λ_{min}^{MeOH} 240 nm, $\varepsilon_{267}=1.8 \times 10^4$.



From NNpTp(NHPh)TOAc, the 3'-acetyl group was selectively removed by use of 2 M NaOH-pyridine (1:1 v/v) at 0 °C for 20 min. After neutralization with Dowex 50Wx2, the resin was washed with MeOH-H_2O (1:1 v/v). The solvent was evaporated and the residue was applied to a silica gel column. Elution was performed by $CHCl_3$-MeOH (9:1 v/v). NNpTp(NHPh)T was obtained almost quantitatively. NNpTp(NHPh)T (0.05 mmol) was allowed to react with pTOAc (0.075 mmol) in the presence of Ph_3P-$(PyS)_2$ (0.25 mmol). NNpTp(NHPh)TpTOAc was formed in 93% yield. After one day the same reaction mixture was in situ treated with aniline (0.5 mmol) at room temperature for 3 d. The fully protected trimer, NNpTp(NHPh)Tp(NHPh)TOAc, was obtained in 57% yield: Rf 0.12 ($CHCl_3$-MeOH, 9:1 v/v); λ_{max} 257 nm, λ_{min} 239 nm (MeOH-H_2O,1:1 v/v), $\varepsilon_{267} = 2.5 \times 10^4$.

Finally, deprotection of the di- and tri-nucleotides, NNpTp(NHPh)TOAc and NNpTp(NHPh)Tp(NHPh)TOAc, was performed by successive treatment first with 2 M NaOH for removal of the acetyl group and then with isoamyl nitrite (2.5 mmol) in the presence of AgOAc (0.25 mmol) and benzoic anhydride (2.5 mmol) in AcOH-pyridine (1:1 v/v) at room temperature for 6 h. Further treatments were the same as described previously. Thus, pTpT and pTpTpT were obtained in 76% and 59% yields, respectively.

In addition it can be said that the solubility and the reactivity of the cyclic phosphoranilidates are higher than that of reported phosphoranilidates, and the coupling agent, Ph_3P-$(PyS)_2$, was the most useful for the formation of phosphoranilidate and internucleotidic bonds. Bu_3P can not be used in place of Ph_3P since no cyclic phosphoranilidate was obtained by use of Bu_3P-$(PyS)_2$. A popular condensing agent, 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) was not effective for the above reactions because the yields of P-N and internucleotidic bond formations decreased remarkably.

References

1) E. Ohtsuka, M. Ubasawa, and M. Ikehara, J. Am. Chem. Soc., 92, 3445 (1970).
2) M. Hashimoto and T. Mukaiyama, Chem. Lett., 1973, 513
3) Rf_{712} refers to the solvent system; 2-propanol-concentrated ammonia-water (7:1:2, v/v).
4) Rm value shows the mobility relative to pTOAc at pH 8.0 (0.2 M phosphate buffer).

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