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A NEW METHOD FOR THE SYNTHESIS OF DINUCLEOTIDES[1)]

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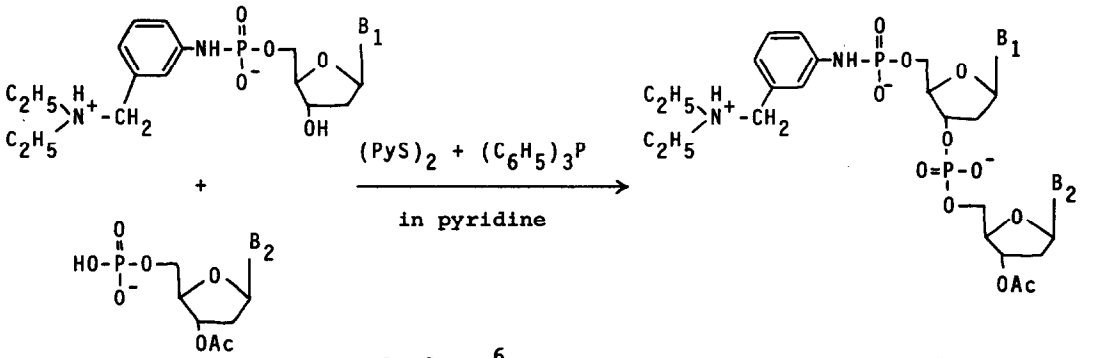
An attempt to prepare oligonucleotides by the use of a protecting group which acts as a purification handle for separation has been investigated in our laboratory.[2)]

We now wish to report a new synthetic method for dinucleotides containing 2'-deoxyadenosine and thymidine nucleotides.

3-(N,N-Diethylaminomethyl)anilino group (N) was chosen as a protecting group on the 2'-deoxyribonucleoside 5'-phosphate end. This group was introduced to form a phosphoroanilidate linkage starting from N,N-diethyl-3-aminobenzyl amine and the corresponding nucleoside 5'-phosphate by means of Mukaiyama's reagent.[3)] This phosphoroanilidate linkage was found to be cleaved easily by treatment with isoamylnitrite according to Ikehara's method.[4)] Both of the 5'-phosphoroanilidate derivatives of N^6-benzoyl 2'-deoxyadenosine (d-NpA^{Bz}) and thymidine (d-NpT) were obtained in 80% and 86% yields, respectively.[5)] The phosphoroanilidates in the form of their inner salts are neutral and very stable substances and were easily isolated and purified from the reaction mixture by washing with ether and further by using DEAE cellulose (carbonate form) column chromatography.

When a solution of d-NpA^{Bz} (0.1 mmole) and 3'-O-acetylthymidine 5'-phosphate (pTOAc) (0.15 mmole) in dry pyridine (0.8 ml) was treated with 2,2'-dipyridyl disulfide (0.5 mmole) and triphenylphosphine (0.5 mmole) at room temperature for two days, the corresponding dinucleotide derivative, d-NpA^{Bz}pTOAc, was obtained in 49% yield along with P^1,P^2-di-3'-O-acetylthymidine 5'-pyrophosphate, 2-pyridone and triphenylphosphine oxide.



B_1=B_2= N^6-benzoyladenine or thymine residue

$(PyS)_2$= 2,2'-dipyridyl disulfide

The mixture was concentrated and dissolved in pyridine (2 ml) and this solution was added dropwise to dry ether under vigorous stirring. The precipitate was collected by centrifuging and washed three times with dry ether. It was dissolved in water and chromatographed on DEAE cellulose. First d-NpA^{Bz} was eluted with water and then a linear gradient of triethylammonium bicarbonate solution from 0 to 0.05 M was used. The desired dinucleotide, d-NpA^{Bz}pTOAc, was the first to be eluted at about 0.01 M. The eluate was concentrated and treated with isoamylnitrite and then with methanolic ammonia for removal of the protecting groups. Thymidylyl(5'-3') 2'-deoxyadenosine 5'-phosphate (d-pApT) was obtained in 47% yield (1107 $O.D._{261}$ units at pH 7) based on d-NpA^{Bz}.

In a similar manner, the dinucleotide derivatives, d-NpA^{Bz}pA^{Bz}OAc, d-NpTpA^{Bz}OAc and d-NpTpTOAc were obtained. The data on yields, paper electrophoretic mobilities and spectral properties of the compounds which

have been prepared are summarized in Table 1.

According to this method, the complete experimental procedure for their preparation takes only five days, since in the present method the dinucleotide derivatives are eluted first on column chromatography.

Table 1. Yield and Paper Electrophoretic Mobilities and Spectral Properties of the Reported Compounds

Compound	Yield (%)	P.E.* Mobility relative to d-pT (pH 8)	Spectral data (in H_2O pH 7) λ_{max} ($10^{-3}\varepsilon$)	λ_{min} (mμ)
d-pT	-	1.00	267 (9.6)	236
d-pA	-	0.90	259 (15.3)	230
d-pTOAc	-	0.96	267 (9.6)	236
d-pA^{Bz}	-	0.76	280 (18.3)	245
d-pA^{Bz}OAc**	-	0.70	280 (18.3)	245
d-NpA^{Bz}	80	-0.07	280 (18.3), 236	259, 224
d-NpT	86	-0.07	267 (9.6), 236	253, 224
d-NpA^{Bz}pA^{Bz}OAc	51	0.16	280 (36.6), 236	250, 226
d-NpA^{Bz}pTOAc	47	0.18	275 (24.6)	256
d-NpTpA^{Bz}OAc	48	0.19	275 (24.8)	256
d-NpTpTOAc	50	0.25	267 (18.5), 236	251, 225

* P.E.= Paper Electrophoresis

** The compound was prepared by the procedure of Khorana and coworkers (G.Weimann,H.Schaller and H.G.Khorana, J.Amer.Chem.Soc.,85,3835(1963) .

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References

1) Part III in a series on A Simple Protecting Group Protection-Purification "Handle" for Polynucleotide Synthesis; for Part II, see K.Tajima and T.Hata, Bull.Chem.Soc.Japan, in press.

2) T.Hata, K.Tajima and T.Mukaiyama, J.Amer.Chem.Soc.,93,4928(1970).

3) An addition compound of 2,2'-dipyridyl disulfide and triphenylphosphine is a very effective condensating reagent for the syntheses of peptides and nucleotides; T.Mukaiyama, R.Matsueda and M.Suzuki, Tetrahedron Lett., 1901(1970); T.Mukaiyama and M.Hashimoto, Bull.Chem.Soc.Japan,44,2284(1971).

4) M.Ikehara, S.Uesugi and T.Fukui, Chem.Pharm.Bull.(Tokyo),15,440(1967); E.Ohtsuka, K.Murao, M.Ubasawa and M.Ikehara, J.Amer.Chem.Soc.,92,3441 (1970).

5) A solution of nucleoside 5'-phosphate in pyridine must be added to a mixture of N,N-diethylamino-3-aminobenzyl amine and Mukaiyama's reagent. If the solution is not mixed in this manner, the yield of the phosphoroanilidate is remarkably decreased.