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A SIMPLE PROTECTING GROUP PROTECTION-PURIFICATION "HANDLE" FOR POLYNUCLEOTIDE SYNTHESIS. IV. SYNTHESIS OF DEOXYRIBOTRINUCLEOTIDES.

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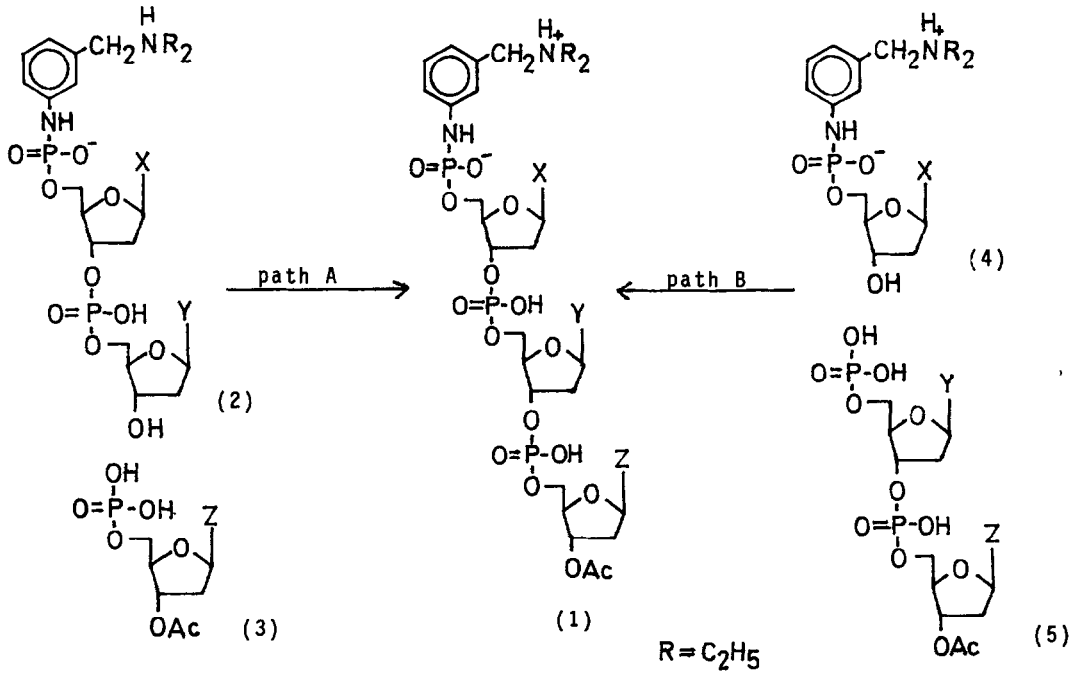
It is well recognized that the synthesis of deoxyribotrinucleotides bearing 5'-phosphate end group is of considerable importance in the synthesis of deoxyribopolynucleotides having definite sequences. Several protecting groups have been offered in attempts to synthesize oligonucleotides bearing 5'-phosphate end group.[1] In a previous paper,[2] it was shown that the synthesis of deoxyribodinucleotides was achieved by use of a basic protecting group which acts as a role of purification handle for separation of desired dinucleotides.

In this communication, we describe an extended study on the synthetic reaction and the separation procedure of deoxyribotrinucleotides by employing 3-(N,N-diethylaminomethyl)anilino group (N), proposed previously, attached to 5'-phosphate end.

Our first experiment was designed to test the yields of coupling reactions with the object to choice the synthetic route of deoxyribotrinucleotides

such as type 1. Two possible routes were examined; first, coupling of 3'-hydroxyl group of 5'-protected dinucleotide (2) with 5'-phosphate group of 3'-protected nucleotide (3)(path A); second, coupling of 3'-hydroxyl group of 5'-protected nucleotide (4) with 5'-phosphate group of 3'-protected dinucleotide (5)(path B).



When the reaction of d-NpA^{Bz}pT[3] (0.1 mmole) with d-pTOAc (0.15 mmole) was carried out in the presence of triphenylphosphine (0.5 mmole) and 2,2'-dipyridyl disulfide (0.5 mmole) in dry pyridine (1 ml) at room temperature for 2 days, the corresponding trinucleotide derivative, d-NpA^{Bz}pTpTOAc, was obtained in 68% yield. On the other hand, the same trinucleotide was obtained through path B in 40% yield from the reaction of d-NpA^{Bz} (0.2 mmole) and d-pTpTOAc (0.1 mmole) under the same condition. From the facts, we have chosen the former (path A) for the synthesis of deoxyribotrinucleotides.

The protected deoxyribotrinucleotides prepared by the present method are listed in Table 1.

Separation of the trinucleotides were performed as shown in the following:

The reaction mixture was concentrated to dryness and the residue was dissolved in water (30 ml). The aqueous solution was washed with two portions of ether (2 X 60 ml) and was concentrated to small volume. This was applied to a column of trityl-cellulose[4] (1.5 X 20 cm). A predicted by-product, P^1,P^2-dithymidine 5'-pyrophosphate [d-(pTOAc)$_2$], and unreacted d-pTOAc were removed simply by washing the column with 0.05 M TEAB solution[5] (1 l). The column then was washed with a mixture of ethanol and 0.05 M TEAB solution (9:1 v/v; 1 l). A mixture of trinucleotide, d-NpA^{Bz}pTpTOAc, and unreacted dinucleotide, d-NpA^{Bz}pT was eluted. The eluate was concentrated carefully[6] with adding pyridine. It was dissolved in water and chromatographed on DEAE cellulose column (1.5 X 20 cm). The elution was performed by a linear gradient of TEAB solution from 0 to 0.2 M. First, d-NpA^{Bz}pT was eluted and then the desired d-NpA^{Bz}pTpTOAc was separated. The second fraction was concentrated and was treated with isoamyl nitrite[7] and then with methanolic ammonia for removal of the protecting groups. The trinucleotide, d-pApTpT, was isolated and it was homogeneous on paper chromatogram and on paper electrophoresis. The structure was confirmed by degradation to the corresponding mononucleotides with snake venom phosphodiesterase.

Table 1. The Synthesis of Deoxyribotrinucleotides

Starting Material		Trinucleotide	Yield*	P.E.**	Spectral Data (at pH 7)	
NpXpY	pZOAc	(NpXpYpZOAc)	(%)		$\lambda_{max}^{H_2O}$ ($\varepsilon \times 10^{-3}$)***	$\lambda_{min}^{H_2O}$
NpA^{Bz}pT	pTOAc	NpA^{Bz}pTpTOAc	68	0.46	274(30.0), 240	248, 234
NpA^{Bz}pT	pC^{An}OAc	NpA^{Bz}pTpC^{An}OAc	61	0.49	282(40.6)	241
NpTpT	pC^{An}OAc	NpTpTpC^{An}OAc	56	0.42	273(22.4)	245
NpA^{Bz}pT	pG^{iBu}OAc	NpA^{Bz}pTpG^{iBu}OAc	21	0.48	276(36.2), 261	271, 230

* Yields were determined spectrophotometrically. ** P.E. refers to paper electrophoretic mobility relative to pT. The buffer used was phosphate (0.2 M, pH 8). *** ε Value listed were given at 280 nm except in the case of NpTpTpC^{An}OAc (In this case the measurement was made at 300 nm.).

From the above experiments, it is noted that the protecting group (N) attached to nucleotide acts effectively as hydrophobic and basic function and results in the perfect separation of the desired trinucleotide during the treatments with trityl-cellulose and DEAE cellulose.

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References and Notes

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2) T.Hata, I.Nakagawa, and N.Takebayashi, Tetrahedron Lett.,2931(1972).

3) $NpA^{Bz}pTOAc$ refers to 3'-O-acetylthymidylyl(5'→3')N^6-benzoyladenosine 5'-(3-N,N-diethylaminomethyl)phosphoroanilidate.

4) Trityl-cellulose was prepared by literature procedure. P.J.Cashion, M.Fridkin, K.L.Agarwal, E.Jay, and H.G.Khorana, Biochemistry, 12,1985(1973).

5) TEAB refers to triethylammonium bicarbonate.

6) The temperature was kept below 35°.

7) The N group was easily removed from d-$NpA^{Bz}pTpTOAc$ by treatment with isoamyl nitrite by a modification of the procedure of Ikehara. 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.,82,3441(1960).