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THE N^4-BENZOYLATION OF DEOXYCYTIDYLIC AND CYTIDYLIC ACIDS BY MEANS OF 2-CHLOROMETHYL-4-NITROPHENYL BENZOATE[1)]

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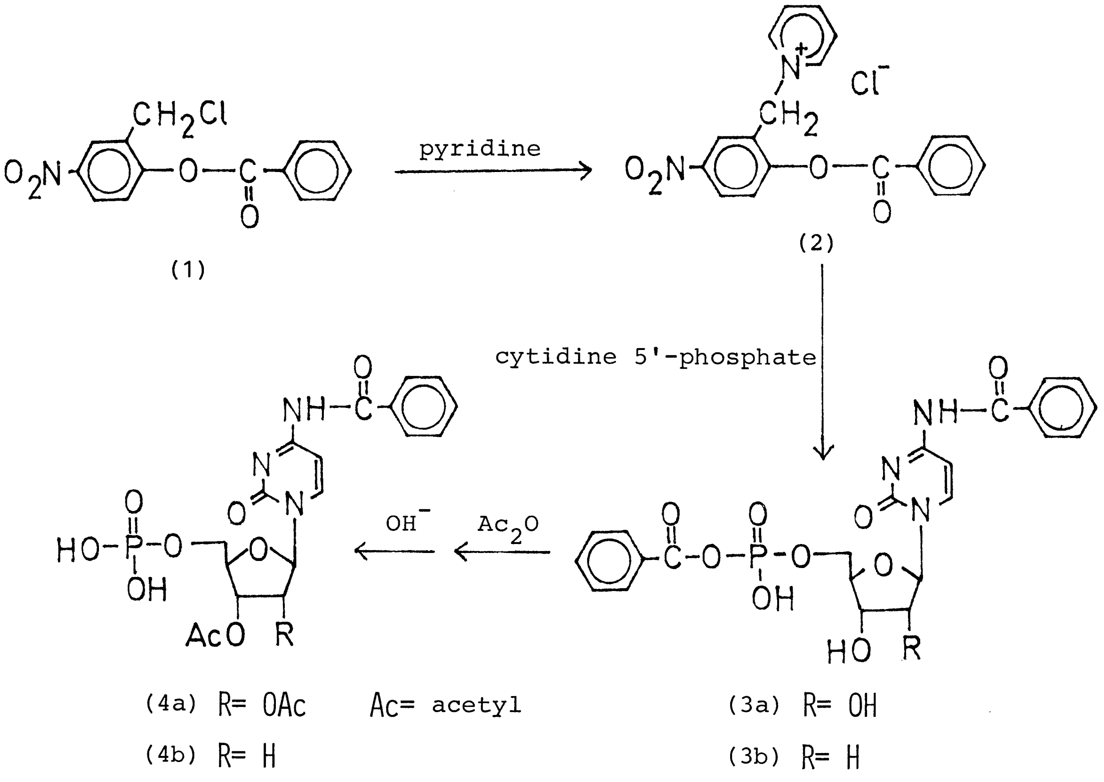
Useful intermediates in the syntheses of polynucleotides, such as 3'-O-acetyl N^4-benzoyldeoxycytidine 5'-phosphate, 2',3'-O-diacetyl N^4-benzoylcytidine 5'-phosphate, N^4-benzoyldeoxycytidine and N^4-benzoylcytidine, were prepared conveniently in high yields by the use of a new mild benzoylating reagent, 2-chloromethyl-4-nitrophenyl benzoate.

N^4-Acylated derivatives of deoxycytidylic and cytidylic acids are of importance in the syntheses of polynucleotides. N^4-Acylated cytidine 5'-phosphate was prepared ordinarily by partial hydrolysis of the corresponding fully acylated cytidylic acid.[2)] Several methods for the selective N^4-acylation of cytidine were proposed,[3)] however, there is no applicable method for the selective acylation of cytidylic and deoxycytidylic acids because of their lower solubility caused by the formation of inner salt between phosphate and 4-amino group.

We now wish to report a selective N^4-benzoylation of cytidine 5'-phosphate and deoxycytidine 5'-phosphate, leaving the hydroxyl groups of ribose and deoxyribose unaffected. A benzoylating reagent, 2-chloromethyl-4-nitrophenyl benzoate (1)[4)], was prepared by treating 1 equiv. of 2-chloromethyl-4-nitrophenol[5)] with 1 equiv. of benzoyl chloride at 100°C for 1 hour. The reagent (1) was purified by recrystallization from ligroin (bp 75-125°C) and obtained in 69% yield, mp 84-86°C.

Found: C,57.92; H,3.28; N,4.97%. Calcd for $C_{14}H_{10}ClNO_4$: C,57.64; H,3.45; N,4.80%.

When a solution of 1 (0.7 mmole) in dry pyridine (3.4 ml) was heated at 85°C for 30 minutes, the corresponding pyridinium salt (2) appeared. After cooling, this suspension was added to a suspension of 4-morpholino-N,N'-dicyclohexylcarboxyamidinium salt of cytidine 5'-phosphate (0.2 mmole) in dry dimethylformamide (3 ml) and warmed at 45°C for 3.5 hours. Cytidine 5'-phosphate was converted completely by this treatment into N^4-benzoyl cytidine 5'-phosphoric benzoic anhydride (3a). After removal of solvent, the oily product was further treated with large excess amount of acetic anhydride (1 ml) in dry pyridine (6 ml) at room temperature overnight. The reaction mixture was concentrated and further diluted with water and then passed through a column of Dowex 50W-X2 (pyridinium form). The eluate was washed with ether and concentrated to dryness. The product, 2',3'-O-diacetyl N^4-benzoylcytidine 5'-phosphate (4a) was obtained in 82% yield based on cytidine 5'-phosphate and was homogeneous on paper chromatography.



In a similar manner, 3'-O-acetyl N^4-benzoyldeoxycytidine 5'-phosphate (4b) was obtained in 76% yield.

On the other hand, when the reaction of 1 with cytidine 5'-phosphate was carried out at a higher temperature (85-90°C), N^4-benzoylcytidine 5'-phosphate and P^1,P^2-di-N^4-benzoylcytidine 5'-pyrophosphate were formed instead of 3a.

Further, benzoylation of the corresponding nucleosides, i.e., cytidine and deoxycytidine by means of 1 was tried. When cytidine (3 mmole) was treated with 1 (4.2 mmole) in dry pyridine (30 ml) at room temperature for 2 days, N^4-benzoylcytidine (5a) was obtained in 86% yield. In the same manner, N^4-benzoyldeoxycytidine (5b) was obtained in 84% yield.

Data on the yields and chromatographic and spectral properties of the reported compounds are listed in Table 1.

According to this method, no benzoylation was observed in the cases of adenosine and guanosine derivatives under the same condition.

Table 1. N^4-Benzoyl Derivatives of Cytidine and Deoxycytidine.

Compound	Yield (%)	Rf value* (solvent)	Spectral Data λ_{max} (εx10^{-3})	λ_{min}	(mμ)
4a	82	0.51(A)	302, 258(18.8)	284, 233	(in water at pH=7)
4b	76	0.61(A)	302, 258(18.8)	284, 233	(in water at pH=7)
5a	86	0.65(B)	303, 259(22.3)	285, 232	(in ethanol)
5b	84	0.75(B)	304, 259(20.9)	285, 232	(in ethanol)

* Paper chromatography was performed by descending technique using Toyo Roshi No.51 paper. Solvent system used were ethanol, 1M ammonium acetate (7:3 v/v)(Solvent A) and n-butanol, water (84:16 v/v)(Solvent B).

References and Footnotes

1) This paper is part II in the series of Preparation of Intermediates in the Synthesis of Polynucleotides. A preliminary report by T.Hata and I.Nakagawa, Bull.Chem.Soc.Japan,43,3619(1970) is part I in this series.

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