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SELECTIVE N-DEBENZOYLATION OF N,O-POLYBENZOYLNUCLEOSIDES[1]

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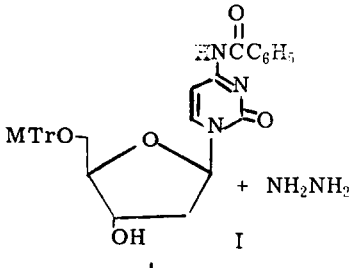
Amino groups on the purine and pyrimidine bases of nucleosides and nucleotides may be protected by benzoyl or acetyl groups[3] during the chemical synthesis of oligonucleotides. These blocking groups are removed by extended treatment with concentrated ammonium hydroxide or aqueous sodium hydroxide.[4]

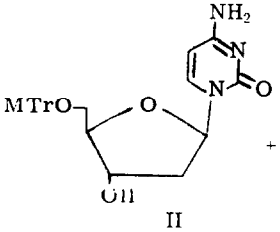
We wish to report a technique for selectively removing an N-benzoyl group from polybenzoylated deoxycytidine or deoxyadenosine derivatives under exceedingly mild conditions. The reagent is 0.5 M hydrazine hydrate in a mixture of pyridine and acetic acid (4:1 v/v). Neither p - methoxytrityl ether derivatives of nucleosides, which are readily cleaved by aqueous acetic acid, nor β -cyanoethyl phosphotriesters, which are very sensitive to ammonium hydroxide, are affected by this reagent.[5] In addition, benzoic acid esters are stable to hydrazine in the pyridine-acetic acid medium. As a consequence one can remove N-benzoyl groups without disturbing O-benzoyl groups in the same molecule.

An example of the cleavage is the conversion of N-benzoyl-5'-O-p-monomethoxytrityldeoxycytidine (I) to 5'-O-p-monomethoxytrityldeoxycytidine (II) and benzhydrazide (III). Thin layer chromatography on Eastman Chromagram sheets, 6060, with ethyl acetate showed that the reaction was complete after 14 hours at room temperature (R_f values: I, 0.68; II, 0.03; III, 0.27). From a reaction of 250 mg (0.4 mmole) of I with 0.1 ml (1.6 mmole) of hydrazine hydrate in 3ml of the pyridine-acetic acid solution was isolated 157 mg (76%) of II (mp 120-124 ; Found: C, 69.01; H, 5.91, N, 8.28. Calcd. for $C_{29}H_{29}N_3O_5$: C, 69.72; H, 5.85 ; N, 8.41), identical with the product obtained by hydrolysis of I with ammonium hydroxide.



+ NH_2NH_2

I



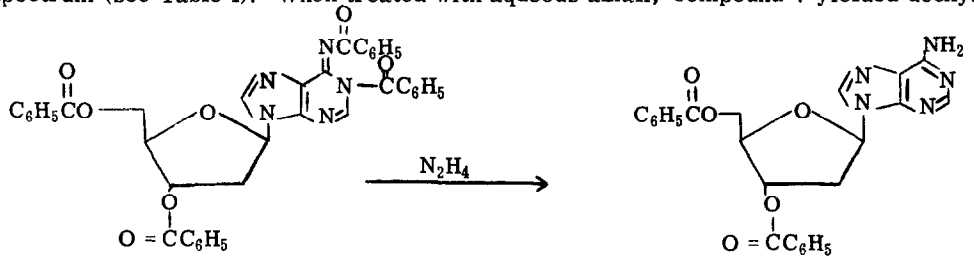
II

+ $C_6H_5CNHNH_2$

III

MTr = $CH_3OC_6H_4C(C_6H_5)_2$

The selectivity achievable in removing benzoyl groups from nucleoside derivatives is demostrated by the reactions of tetrabenzoyldeoxyadenosine (IV). Schaller et al.[3] have shown that this compound is converted to N-benzoyldeoxyadenosine (65% isolated) by reaction with 0.6 M sodium hydroxide for 5 min. at room temperature. We find, on the other hand, that the N-benzoyl groups are preferentially removed when IV is treated with hydrazine hydrate in pyridine-acetic acid (room temperature, 16 hours). From 200 mg of tetrabenzoyldeoxyadenosine was obtained 122 mg (88%) of 3'-O, 5'-O-dibenzoyldeoxyadenosine (V), mp 116-117.5. This compound was recovered by pouring the reaction mixture into water, extracting the solution with chloroform, and chromatographing the extract, after concentration, on silica gel with tetrahydrofuran-ethyl acetate (1:1). The product was then recrystallized from benzene-hexane. It was characterized by elemental analysis (Found:[6] C, 62.73; H, 4.61; N, 15.25. Calcd. for $C_{24}H_{21}N_5O_5$: C, 62.74; H, 4.61; N, 15.24) and by its chromatographic behavior and ultraviolet spectrum (see Table I). When treated with aqueous alkali, compound V yielded deoxyadenosine.



IV V

TABLE I

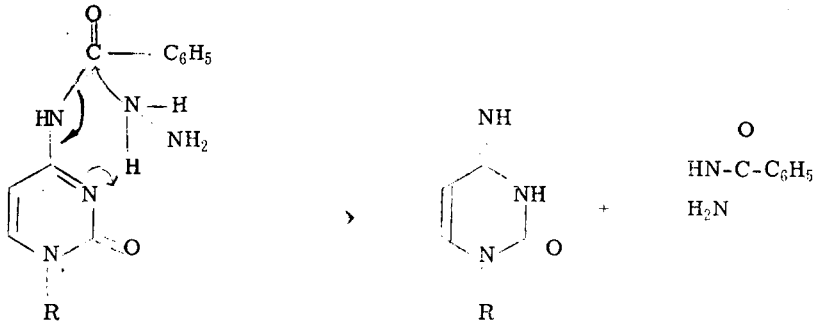
	R_f (EtOAc, Silica slides	$\lambda^a_{max} \times 10^{-4}$	$\lambda^b_{max} \times 10^{-4}$
Tetrabenzoyldeoxyadenosine	0.89		
3'-O, 5'-O-Dibenzoyldeoxyadenosine	0.14	259(1.4)	257(1.4)
N-Benzoyldeoxyadenosine	0.00	280(2.0)	280(2.0)
Deoxyadenosine	0.00	260(1.3)	257(1.3)

(a) 50% aqueous ethanol
(b) 0.01 M HCl in 50% aqueous ethanol

N, 5'-O, 3'-O-Tribenzoyldeoxycytidine[3] (VI), mp 182-183, similarly yielded 3'-O, 5'-O-dibenzoyldeoxycytidine (VII) when treated with hydrazine hydrate under the standard conditions. Compound VII, mp 206-208, gave a satisfactory analysis (Found:[6] C, 63.56; H, 4.96; N, 9.66. Caldc. for $C_{23}H_{21}N_3O_6$: C, 63.44; H, 4.86; N, 9.65) and yielded deoxycytidine on alkaline hydrolysis. N-Acetyldeoxyguanosine did not react with hydrazine in pyridine-acetic

acid under these conditions.

The remarkable preference for cleavage of amide bonds relative to ester bonds in these reactions is consistent with the assumption that (a) a significant part of the activation free energy in the hydrazinolysis of amides and esters in organic solvents is associated with the process of transferring a proton from the attacking nucleophile (hydrazine) to the departing group and (b) the heterocyclic rings in deoxycytidine and deoxyadenosine derivatives provide a low energy pathway for this proton transfer. As shown below, the 1-nitrogen in the cytosine ring is favorably situated to accept a proton from hydrazine concomitantly with cleavage of the C-N bond. The tautomeric cytosine ring thereby generated should be a good departing group and subsequent isomerization would produce the normal ring structure. A similar pathway can be formulated for the N-benzoyldeoxyadenosine derivative that would result on removal of one of the two N-benzoyl groups (a reaction that occurs readily in the presence of active nucleophiles). These reactions complement the reaction of benzamidine with p-nitrophenyl acetate in chlorobenzene, an unusually rapid ester cleavage which involves a proton transfer via a bifunctional nucleophile.[7]



Since hydrazine hydrate is known to attack the cytosine and uracil rings[8] several experiments were carried out to determine the conditions under which hydrazine hydrate in mixtures of pyridine and acetic acid can be used without interference from such a reaction. In Table II are tabulated the times for appearance of a new spot (R_f 0.29 for silica slides with methanol) from mixtures containing deoxycytidine and hydrazine. It is clear from these data that acetic acid inhibits the undesired reaction, and that the 4:1 pyridine-acetic acid mixture can be used safely at room temperature over the time period required for removal of an N-benzoyl group. Other deoxyribonucleosides reacted with hydrazine hydrate even more slowly than deoxycytidine.

TABLE II

REACTION OF DEOXYCYTIDINE WITH HYDRAZINE HYDRATE (0.5 M)

Vol % HOAc in C_5H_4N-HOAc Solvent[a]	Temperature 0 °C	Approximate Time for Appearance of Reaction Product (Hours)
3	25	17
	37	1.5
	100	0.08
5	25	23
	37	3
20	25	no reaction in 50 hrs.
	37	7
	100	0.3

[a] On dilution of the reaction mixtures with 4 parts water, the pH's of the solutions were 7, 6.4, 5.6 for vol. % HOAc of 3, 5, and 20 respectively.

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