Tetrahedron Letters Vol. 22, pp 991 - 994
0040-4039/81/0308-0991$02.00/0

DEBENZOYLATION OF N-BENZOYLNUCLEOSIDE DERIVATIVES WITH ETHYLENEDIAMINE-PHENOL[1]

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Abstract: Ethylenediamine-phenol (1:4 v/v) serves as a selective reagent for removing N-benzoyl protecting groups on purine and pyrimidine bases without disturbing trichloroethyl phosphotriester functions present in the same system.

In connection with experiments aimed at the synthesis of oligonucleotide phosphotriester derivatives possessing free NH_2 groups on the purine and pyrimidine rings, a procedure was needed for selectively removing N-benzoyl protecting groups without affecting sensitive trichloroethyl phosphotriesters. Ammonium hydroxide (the standard reagent for deprotecting N-acylnucleosides)[2], butylamine in methanol (which deprotects N-benzoyldeoxyguanosine but yields an extraneous product on reaction with N-benzoyl or N-anisoyldeoxycytidine)[2], and aqueous sodium hydroxide were precluded since these reagents attack the trichloroethyl phosphotriester link, $ROP(O)(OR')OCH_2CCl_3$, faster than the N-benzoyl groups. We report here a reagent, ethylenediamine in phenol, which exhibits the desired selectivity.

Initial experiments revealed that N-aroyldeoxyribonucleosides react readily with neat ethylenediamine, yielding the nucleosides and N-aroylethylenediamine ($t_{1/2}$ at 40°C for disappearance of N-benzoyldeoxycytidine, N-anisoyldeoxycytidine, and N-benzoyldeoxyguanosine approximately 15 min, 1.5 h, and 3 h, respectively). However, a side product[3] was formed in the reaction of the deoxycytidine derivatives, and ethylenediamine also readily attacked the trichloroethyl phosphotriester of thymidylyl(3'-5')-3'-monomethoxytritylthymidine (d-T(tce)Tmmtr), yielding preponderately an aminoethyl phosphoramidate derivative.[4]

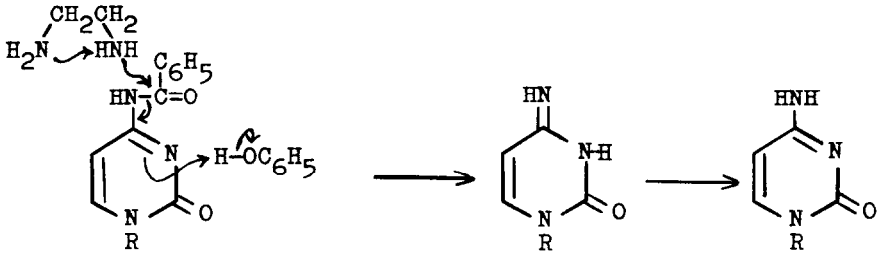
We then found that phenol has a remarkable effect on the reactions of the nucleoside derivatives with ethylenediamine. In a mixture of ethylenediamine and phenol (1:4 v/v), attack at the trichloroethyl phosphotriester function is

effectively eliminated (e.g. d-T(tce)Tmmtr is stable in this mixture for 24 h at 40°C or 1 h at 100°C) and formation of side products from the aroyldeoxycytidines is reduced below the detectable limit. On the other hand phenol only slightly retards the reaction of ethylenediamine at the carbonyl group of the N-acylnucleosides. In the 1:4 mixture at 40°C N-benzoyldeoxycytidine, N-anisoyldeoxycytidine, N-benzoyldeoxyadenosine, and N-benzoyldeoxyguanosine were each cleanly converted to the corresponding nucleosides ($t_{1/2}$ approximately 15 min, 2 h, 1.5 h, and 3.5 h, respectively) as shown by analytical TLC. Preparative reactions were carried out with the aroylnucleoside (50 mg) in freshly prepared ethylenediamine-phenol (1.5 mL; 1:4 v/v) at 40°C for 20 h. Dilution with CH_2Cl_2 (1 mL), development on Analtech silica plates with CH_3OH-CH_2Cl_2 (1:4 v/v) and elution of the bands with C_2H_5OH-$CHCl_3$ (1:4 v/v) afforded the nucleosides (deoxyadenosine 83%, deoxycytidine 67%, and deoxyguanosine 50%).[5] Comparable recoveries were obtained when samples of the pure nucleosides were chromatographed and eluted from the silica by the same procedure.

Selectivity was further demonstrated by reaction of the trichloroethyl phosphotriester of N-benzoyldeoxycytidylyl-(3'-5')-3'-mono-p-methoxytrityl-thymidine (d-bzC(tce)Tmmtr) (23 mg, 22.4 μmol; Rf 0.80 in 15% CH_3OH-CH_2Cl_2) with 1:4 ethylenediamine-phenol (1.25 mL) at 40°C. After 1 h, separation by TLC (Merck silica plate; 15% CH_3OH-$CHCl_3$) and elution, concentration, and precipitation into hexane gave d-C(tce)Tmmtr as a white solid; Rf 0.41 in 15% CH_3OH-CH_2Cl_2; λ_{max} (95% C_2H_5OH) 268.5 nm, λ_{min} 252 nm; 276 $A_{268.5}$ units[6] (16.4 μmol, 73% yield). That the triester function was intact was shown by the ^{31}P NMR spectrum (single peak at -14.94 ppm relative to triphenyl phosphate, characteristic of a trichloroethyl 3'-5' internucleotide link). That the nucleoside moieties were intact was shown by removal of the trichloroethyl group (zinc in pyridine and acetylacetone)[7] and the monomethoxytrityl group (80% aq. acetic acid) to give d-CpT (RfF 0.58[8]; λ_{max} in C_2H_5OH, 271 nm) which in turn was completely hydrolyzed to deoxycytidine and thymidine 5'-phosphate by snake venom phosphodiesterase. A similar reaction (40°C, 5 h) conducted with d-bzG(tce)Tmmtr (14.7 mg, 13.6 μmol; Rf 0.67 in 15% CH_3OH-$CHCl_3$) in 1:4 ethylenediamine-phenol (0.9 mL) afforded on isolation and precipation into hexane 12.2 mg (12.2 μmol, 92%) of d-G(tce)Tmmtr (Rf 0.19 in 15% CH_3OH-$CHCl_3$). On deprotection by treatment with zinc[7] followed by 80% aq. acetic acid, this ester was converted to d-GpT (RfF, 0.42[8]; λ_{max} in 95% C_2H_5OH, 264 mm), which hydrolyzed completely to deoxyguanosine and thymidine 5'-phosphate on treatment with snake venom phosphodiesterase.

Ethylenediamine seems particularly effective as a nucleophile for the deprotection reactions. Solutions of butylamine in phenol and of ethanolamine in phenol react with the benzoylnucleosides too slowly to be useful. Phenol is the most effective hydroxylic component we have investigated for controlling the reactions of ethylenediamine. Sufficient phenol must be present to form hydrogen bonds at both amino groups; otherwise, some attack at the phosphotriester will occur. Acetic acid, n-butyl alcohol, and water are not satisfactory as proton donors. Both 2,2,2-trichloroethanol and 2,2,2-trifluoroethanol bias the reactions of ethylenediamine in favor of N-deacylation, but neither leads to the selectivity realized with phenol. We ascribe the effectiveness of phenol to an acidity appropriate for moderating the nucleophilicity of the amino groups (thereby reducing the rate of attack at both phosphorus and the acyl function), repressing proton loss at the acylated purine (thereby facilitating acyl cleavage), and catalyzing the deacylation reaction.

Neither N-benzoylaniline nor N-benzoyl-p-nitroaniline is cleaved by the 1:4 ethylenediamine-phenol mixture within 20 h at 40°C. Since aniline (pK_b 9.4) is a stronger base and p-nitroanaline (pK_b 13) is a weaker base than deoxycytidine (pK_b 9.7), it is apparent that some factor other than basicity of the amine component determines the rate of cleavage. An attractive possibility for the catalytic role of phenol is represented in scheme 1, in which phenol serves as a proton donor in a reaction leading to a tautomer of deoxycytidine. It may be noted that a similar pathway is available for debenzoylation of N-benzoyldeoxyadenosine and N-benzoyldeoxyguanosine but not for reaction of N-benzoylaniline and N-benzoyl-p-nitroaniline.



Scheme 1

References and Notes.

1. This work was supported by the Division of General Medical Sciences of the National Institutes of Health, Grant GM10245.

2. H. Weber and H. G. Khorana, J. Mol. Biol. 72, 219 (1972). The isobutyryl group is commonly used to protect the N^2 nitrogen of the guanine residue. Concentrated ammonium hydroxide fails to cleave the benzoyl group from N-benzoyldeoxyguanosine over a period of two days at room temperature.

3. By analogy to the butylamine reaction (ref. 2), it seems likely that this product (Rf 0.07 in 25% CH_3OH-$CHCl_3$ on silica; gray on heating with aq. perchloric acid) is N-(2-aminoethyl)deoxycytidine. Deoxycytidine itself does not react with ethylenediamine.

4. Unpublished work, Steven A. Bach.

5. In addition to spots corresponding to the nucleoside, phenol, and ethylenediamine, a spot was observed at Rf 0.3 in 25% CH_3OH-$CHCl_3$ on silica (positive ninhydrin test; negative for nucleoside after spraying with 10% aq. $HClO_4$ and heating). Although this material was not isolated, it may be noted that the properties correspond to those of N-(2-aminoethyl)benzamide, an expected product of the reaction.

6. The term $A_{268.5}$ specifies the absorption at 268.5 nm for 1 mL of a solution in a quartz cell with a 1 cm light path.

7. R. W. Adamiak, E. Biala, K. Grozeskowiak, R. Kierzek, A. Kraszewski, W. T. Markiewicz, J. Stawinski, and M. Wiewiorowski, Nucleic Acids Res. 4, 2321 (1977).

8. Paper chromatography with solvent F (n-C_3H_7OH: concd NH_4OH: H_2O 55:10:35 v/v/v).

(Received in USA 19 August 1980)