ORGANIC LETTERS

2005
Vol. 7, No. 22
4903−4905

Hydrothermal Deamidation of 4-*N*-Acylcytosine Nucleoside Derivatives: Efficient Synthesis of Uracil Nucleoside Esters†

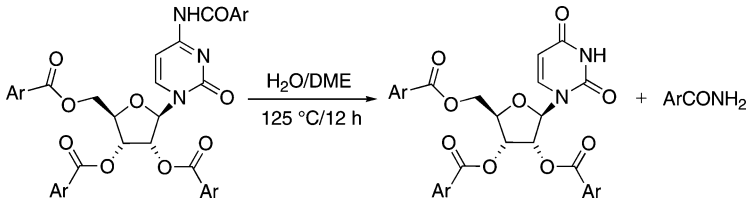
Ireneusz Nowak and Morris J. Robins*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602-5700

morris_robins@byu.edu

Received August 1, 2005

ABSTRACT



N,O-Peracylated cytidine and 2′-deoxycytidine derivatives in superheated water/DME solutions (oil bath at 125 °C) undergo hydrolytic deamidation (and/or N-deacylation). Acylated starting materials derived from arylcarboxylic acids give the corresponding uridine esters cleanly, and such derivatives crystallize selectively from the cooled reaction mixtures in high yields.

Genetic information is altered by chemical or enzymatic modification of nucleic acid sequences. Deamination of cytosine in DNA is the most common promutagenic change. This can result in generation of misfolded polypeptides and dominant-negative proteins and can be the cause of other mutations. "DNA cytidine deaminases" provide mechanisms for the beneficial diversification of Ig genes and inhibition of retroviral infections.[1,2]

Cytidine deaminases (CDAs) are metalloenzymes that employ zinc at the active site to enhance the nucleophilicity of a sequestered water molecule. Addition of the water oxygen at C4 of the cytosine ring followed by enzyme-mediated departure of ammonia and concomitant tautomerization give the uracil products.[3] Various zinc-dependent multisubunit CDA enzymes have been found in all organisms.[2] Different families catalyze deamination of substrates including the cytosine base, nucleosides, nucleotides, oligonucleotides, DNA, and RNA.

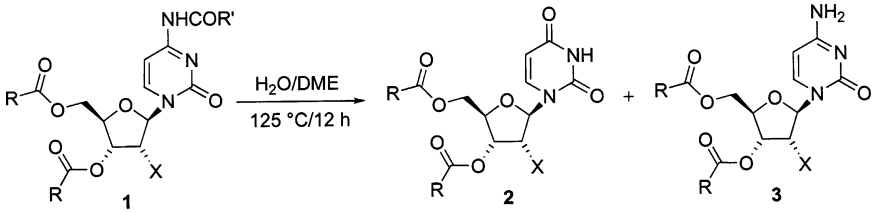
A limited number of chemical conversions of cytosine to uracil compounds have been described. Diazotive deamination of cytosine with nitrous acid gave uracil, but yields were poor because of decomposition(s) of the cytosine ring.[4] Nitric oxide and its progenitors also cause DNA damage and mutations.[5] Holy noted that treatment of 4-*N*-2′,3′,5′-tri-*O*-tetraacetyl(or tetrabenzoyl)cytidine with 80% aqueous acetic acid at reflux resulted in rearrangement of the acyl group from N4 to N3 of the cytosine ring. Uridine was obtained upon treatment of the N3 isomers with NaOMe/MeOH.[6] Hydrolysis pathways for 3-methyl-2′-deoxycytidine were dependent on pH, and fairly clean amino-hydrolysis was

† Nucleic Acid Related Compounds. 129. Paper 128: Zhong, M.; Nowak, I.; Robins, M. J. *Org. Lett.* **2005**, *7*, 4601−4604.

(1) Cascalho, M. *J. Immunology* **2004**, *172*, 6513−6518.
(2) Pham, P.; Bransteitter, R.; Goodman, M. F. *Biochemistry* **2005**, *44*, 2703−2715.
(3) Xiang, S.; Short, S. A.; Wolfenden, R.; Carter, C. W., Jr. *Biochemistry* **1997**, *36*, 4768−4774.
(4) Wempen, I.; Brown, G. B.; Ueda, T.; Fox, J. J. *Biochemistry* **1965**, *4*, 54−56.
(5) Wink, D. A.; Kasprzak, K. S.; Maragos, C. M.; Elespuru, R. K.; Misra, M.; Dunams, T. M.; Cebula, T. A.; Koch, W. H.; Andrews, A. W.; Allen, J. S.; Keefer, L. K. *Science*, **1991**, *254*, 1001−1003.
(6) Holy, A. *Collect. Czech. Chem. Commun.* **1979**, *44*, 1819−1827.

10.1021/ol0518378 CCC: $30.25
Published on Web 10/07/2005

Scheme 1. Hydrolysis of the *N*-Acylcytosine Derivatives **1**



observed only in basic solution (pH ~12). Amino-hydrolysis of cytosine to uracil derivatives proceeds slowly at most pH values,[7,8] and a patent report[9] of hydrolytic deamination of cytidine in 1 M NaOH/H_2O at 100 °C attests to the severity of the conditions required.

Bisulfite-mediated hydrolytic deaminations are accelerated by prior addition of the sulfite nucleophile at C6 of the 5,6-double bond.[10] The latter method is useful for small-scale deaminations but impractical for gram-scale synthesis.

The need arose in our laboratory to convert multigram quantities of 2′-deoxycytidine into 2′-deoxyuridine. Attempted application of Holy's method to 2′-deoxy-4-*N*-3′,5′-di-*O*-tri(4-methylbenzoyl)cytidine (**1b**) resulted in formation of complex mixtures. We then substituted a neutral organic cosolvent (1,4-dioxane or DME) for Holy's acetic acid component and heated the solution in a pressure vessel at 125 °C. We were delighted that 2′-deoxy-3′,5′-di-*O*-(4-methylbenzoyl)uridine (**2b**) was produced in high yield and that **2b** crystallized from the reaction mixture upon cooling. We then investigated the generality of this hydrothermal deamination with other cytosine nucleoside derivatives **1** (Scheme 1, Table 1).

Table 1. Hydrolysis of **1** (H_2O/DME/Δ)[a] To Give **2** and **3**

entry[b]	R	R′	X	h	**2**[c]	**3**[c]
1 (**a**)	Ph	Ph	H	12	82	*d*
2 (**b**)	MePh	MePh	H	12	84	*d*
3 (**c**)	Ph	Ph	$PhCO_2$	12	*e*	
4 (**d**)	MePh	MePh	$MePhCO_2$	12	87	*d*
5 (**e**)	ClPh	ClPh	$ClPhCO_2$	12	85	*d*
6 (**f**)	Ph	*t*-Bu	$PhCO_2$	12	53	25
7 (**g**)	^{i}Pr	^{i}Pr	$^i PrCO_2$	12	54	38
8 (**h**)	Et	Et	$EtCO_2$	9	49	30
9 (**i**)	Me	Me	$MeCO_2$	9	55	25
10 (**j**)	Ph	CF_3	$PhCO_2$	1	16	66

[a] Reactions were performed by the general procedure (see Supporting Information). [b] Starting materials in parentheses. [c] Isolated % yield relative to starting nucleoside (acylated and then subjected to hydrolysis). [d] Traces of **3** were detected in the polar residue from the mother liquor. [e] Reaction was complete (^{1}H NMR), but **2c** and benzamide were not separated by chromatography or recrystallization.

In almost all cases, reactions were complete (or near complete) within 12 h. Starting material (18%) remained after heating the 4-*N*-(trimethylacetyl) compound (entry 6) for 12 h. This presumably resulted from the increased steric hindrance and/or hydrophobic effect of the 4-*N*-pivalyl group. The starting material and product pairs were soluble in H_2O/DME (2:3) solutions at 125 °C. However, the product esters derived from the arylcarboxylic acids, **2a**,**b**,**d**,**e**, precipitated upon cooling and were isolated directly from the reaction mixtures in high yields (entries 1, 2, 4, and 5). Both the starting materials **1** and products **2** derived from the aliphatic acids were soluble in H_2O/DME solutions at ambient temperature. The latter products **2** were isolated by evaporation of volatiles and chromatography of the residues.

Because the *O*-acetyl and *O*-propionyl esters underwent slow hydrolysis under our standard conditions, the reaction time was shortened from 12 to 9 h (entries 8 and 9). The amides derived from aliphatic acids, **1f**−**i**, underwent hydrolytic attack at both C4 and the amide carbonyl carbon to give uridine **2** and cytidine **3** derivatives (**2**/**3**, 3:2−2:1) (entries 6, 7, 8, and 9). Compound **1j** (entry 10) with the strongly electron-withdrawing trifluoroacetyl group at N4 underwent rapid hydrolysis of the amide bond. A minor amount of uridine derivative **2c** (16%) was formed plus the cytidine compound **3j** (66%, as a trifluoroacetate salt).

Two sites are susceptible to nucleophilic attack by water. Addition at C4 followed by elimination of an amide results in formation of uridine derivatives **2** (Scheme 2, path 1). Attack at the amide carbonyl carbon results in hydrolysis of the amide linkage and formation of a carboxylic acid and cytidine products **3** (path 2). The preference for path 1 or 2 depends primarily on the electronic effects of the group attached to the carbonyl carbon. However, steric and/or hydrophobic effects might also be involved. Conjugation of the carbonyl group with an aromatic ring results in clean elimination of an aryl amide from C4. By contrast, amides derived from aliphatic acids undergo partitioning between paths 1 and 2, and in the case of the trifluoroacetamide **1j**, pathway 2 is clearly preferred.

We also considered the possibility that compounds **3** might undergo hydrolytic deamination under our reaction condi-

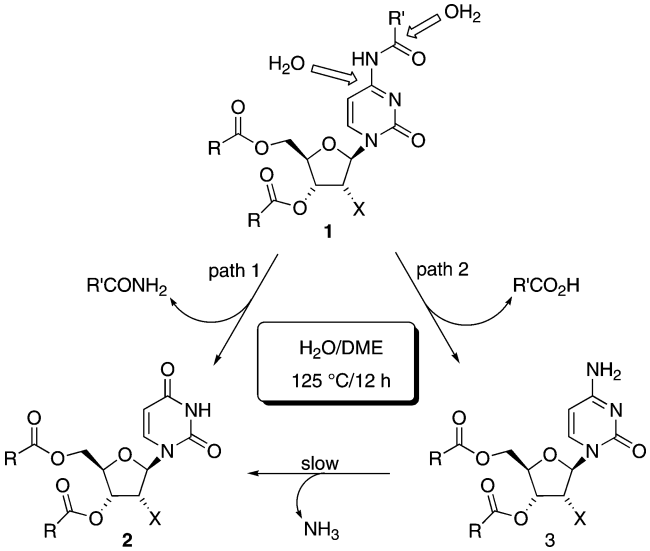
(7) Garret, E. R.; Tsau, J. *J. Pharm. Sci.* **1972**, *61*, 1052−1061.

(8) (a) Frick, L.; Mac Neela, J. P.; Wolfenden, R. *Bioorg. Chem.* **1987**, *15*, 100−108. (b) Snider, M. J.; Gaunitz, S.; Ridgway, C.; Short, S. A.; Wolfenden, R. *Biochemistry* **2000**, *39*, 9746−9753.

(9) Fujishima, T.; Uchida, K.; Yoshino, H. *Jpn. Kokai Tokkyo Koho* **1973**; *Chem. Abst.* **1974**, *80*, 3772.

(10) (a) Shapiro, R.; Servis, R. E.; Welcher, M. *J. Am. Chem. Soc.* **1970**, *92*, 422−424. (b) Hayatsu, H.; Wataya, Y.; Kai, K. *J. Am. Chem. Soc.* **1970**, *92*, 724−726. (c) Shapiro, R.; DiFate, V.; Welcher, M. *J. Am. Chem. Soc.* **1974**, *96*, 906−912.

Scheme 2. Possible Pathways for Hydrolysis of **1**



tions. Byproducts in the mother liquor after filtration of **2b** were analyzed. The major component was *p*-toluamide, and traces of *p*-toluic acid were present. Subjection of purified **3d** to our standard hydrolysis conditions resulted in recovery of almost all of the unchanged cytidine derivative **3d**. Only trace amounts of **2d** (<10%) were detected by ^{1}H NMR and TLC. This confirmed the expectation[8] that an unactivated amino group at C4 is resistant to hydrolysis in H_2O/DME at 125 °C.

The essentially exclusive attack by water at C4 of the aryl amides is in marked contrast with our recent findings on solvolysis of *N*,*O*-peracylated cytidine derivatives in superheated methanol.[11] In those cases, the exocyclic amide bond was cleaved. A minor amount (8%) of a 4-methoxy compound (formed by attack of methanol at C4) was observed only with the 4-*N*-toluyl derivative **1d**. Attack by water at C4 of the aryl amide derivatives **1a**−**e** might result from a combination of conjugated aromatic ring-carbonyl group and hydrophobic effects in the aqueous DME solutions. Another significant factor is the favorable equilibration of the initial 4-hydroxypyrimidin-2-one intermediates into their more thermodynamically stable (in aqueous environments) 4-keto tautomers, which is impossible with methanolysis to 4-methoxypyrimidin-2-one derivatives.

It is noteworthy that perbenzoylated 2′-deoxycytidine undergoes selective O-debenzoylation in a basic aqueous solution (NaOH/H_2O/MeOH/THF at 0 °C) without alteration of the amide group on the nucleobase.[12] Thus, our neutral hydrothermal conditions provide a new mode of highly selective reactivity at C4 of cytosine nucleosides that are activated by N,O-peracylation with arylcarboxylic acids. Spontaneous crystallization of the product uracil nucleoside 4-chlorobenzoyl and 4-methylbenzoyl esters upon cooling the hydrolysis reaction mixtures provides very convenient access to these compounds **2**.

Idoxuridine (2′-deoxy-5-iodouridine) has been in clinical practice as an ophthalmic antiviral agent for decades.[13] We had demonstrated that iodination of 2′-deoxy-3′,5′-di-*O*-(4-methylbenzoyl)uridine (**2b**) (ICl/CH_2Cl_2) gave the 5-iodo compoud in 98% isolated yield.[14] The present methodology produces the crystalline intermediate **2b** in 84% overall yield from 2′-deoxycytidine (without optimization).[15]

In conclusion, we have demonstrated that *N*,*O*-peracyl derivatives of cytosine nucleosides **1** undergo hydrolysis in superheated H_2O/DME to produce the corresponding *O*-acyluracil nucleoside derivatives **2** (and/or *O*-acylcytosine analogues **3**). Almost exclusive selectivity for hydrolytic attack at C4 to give **2** is observed with derivatives of arylcarboxylic acids, whereas attack at the amide carbonyl carbon to give **3** is competitive with aliphatic acid derivatives. As expected, hydrolytic deamidation is much faster than deamination under hydrothermal conditions. Precipitation of arylcarboxylate esters of the product uracil nucleosides from the hydrolytic reaction mixtures improves the convenience and practicality of this method. Cytidine deaminases employ coordination of zinc to strengthen the nucleophilicity of water and proton transfer to promote the leaving ability of the 4-amino group. Enhancing the nucleophilicity of water with a hydrogen bond-accepting cosolvent and increasing the leaving ability of the amino group as a conjugated amide are analogous to such strategies employed in enzyme active sites.

Acknowledgment. We gratefully acknowledge pharmaceutical company gift funds (M.J.R.) and Brigham Young University for support of this research.

Supporting Information Available: Experimental procedures, spectral data, and ^{13}C NMR spectra for compounds **1f**, **1j**, **2a**−**e**, **2g**−**i**, and **3g**−**j**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0518378

(11) Nowak, I.; Conda-Sheridan, M.; Robins, M. J. *J. Org. Chem.* **2005**, *70*, 7455−7458.

(12) (a) Rammler, D. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 3112−3122. (b) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.

(13) Fischer, P. H.; Lee, J. J.; Chen, M. S.; Lin, T.-S.; Prusoff, W. H. *Biochem. Pharmacol.* **1979**, *28*, 3483−3486.

(14) Robins, M. J.; Barr, P. J.; Giziewicz, J. *Can. J. Chem.* **1982**, *60*, 554−557.

(15) Patent application filed.