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Oxidation of 2′-Deoxycytidine to Four Interconverting Diastereomers of N^1-Carbamoyl-4,5-dihydroxy-2-oxoimidazolidine Nucleosides

Sébastien Tremblay,[†] Tsvetan Gantchev,[†] Luc Tremblay,[‡] Pierre Lavigne,[‡] Jean Cadet,[†,§] and J. Richard Wagner*,[†]

Department of Nuclear Medicine and Radiobiology, Faculty of Medicine and Health Science, University of Sherbrooke, Quebec, Canada J1H 5N4, Pharmacology Institute, Faculty of Medicine and Health Science, University of Sherbrooke, Quebec, Canada J1H 5N4, and Département de Recherche Fondamentale sur la Matière Condensée, SCIB/LAN, UMR-E n°3 (CEA-UJF), CEA/Grenoble, F-38054 Grenoble Cedex 9, France

richard.wagner@usherbrooke.ca

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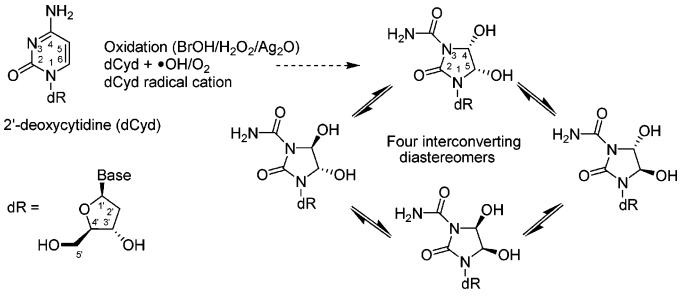

Modification of 2′-deoxycytidine (dCyd) by hydroxyl radicals and direct ionization leads to the formation of various oxidation products, including dCyd 5,6-glycols, 5-hydroxy-2′-deoxycytidine, and ring fragmentation products. The mechanism of oxidation is complex and poorly understood. In the present work, we have prepared four *cis*- and *trans*-diastereomers of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine by bromination of dCyd followed by peroxidation of the resulting dCyd bromohydrins. The structure and stereochemistry of each product were determined by ^{1}H NMR, ^{13}C NMR, and 2D NOE analyses. The formation of imidazolidine products involves rearrangement of initial 5(6)-hydroxy-6(5)-hydroperoxides to C6−C2 endoperoxides, which subsequently decompose by a concerted pathway to imidazolidine products. A remarkable feature of the four diastereomers was their ability to interconvert via single and successive cycles of ring-chain tautomerism at N1−C5 and N3−C4, leading to epimerization of C5 and C4, respectively. The rate of isomerization was greater for *cis*-diastereomers compared to *trans*-diastereomers, and the rate sharply increased with pH (pH 9.0 > 7.0 > 5.5).

Introduction

The constant generation of reactive oxygen species (ROS) by biological processes, such as oxidative phosphorylation and inflammation, together with exposure to ionizing radiation, ultraviolet radiation, and xenobiotics, is believed to contribute to aging and age-related diseases.[1−3] Oxidative modification of C residues by ROS induces hydrolysis of the exocyclic amino group, which converts this group to a carbonyl group. [4] This process may be responsible for the bias of GC→AT transitions in the spectrum of spontaneous mutations and the spectra of mutations induced by Fenton reactions and ionizing radiation.[5−7]

* Corresponding author. Phone: 819-820-6868, ext. 12717. Fax: 819-564-5442.
[†] Department of Nuclear Medicine and Radiobiology, University of Sherbrooke.
[‡] Pharmacology Institute, University of Sherbrooke.
[§] Département de Recherche Fondamentale sur la Matière Condensée, SCIB/LAN, UMR-E n°3 (CEA-UJF), CEA/Grenoble.

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The mechanism of mutagenesis likely involves oxidation-induced loss of the exocyclic amino group, which changes the base-pairing properties of C causing C to T transitions during replication.[8−10] Despite the biological importance, the mechanism of oxidation of cytosine derivatives remains poorly understood. This is due to the formation of polar products, which are difficult to separate by reversed-phase chromatography, as well as to the formation of labile products, including cytosine glycols, dialuric acid, and hydantoin derivatives.[11−13]

In general, one of the most efficient methods to induce DNA damage involves hydroxyl radicals (•OH), which are generated by either ionizing radiation or the Fenton reaction (e.g., $Fe^{2+} + H_2O_2$).[4,8,14] Another efficient pathway to DNA damage involves agents that induce single-electron oxidation, including ionizing radiation, photosensitization, high-intensity laser photolysis, or vacuum UV photolysis.[9,15] Once base radical cations are generated in DNA, however, the radical cations of T, C, and A have a tendency to migrate to G residues because G and G combinations have a significantly lower oxidation potential.[15,16] From pulsed radiolysis studies, the reaction of •OH with DNA components is fairly well-established.[4,17] In the case of cytosine derivatives, •OH mainly adds to the C5 position of the cytosine 5,6-double bond (90%) giving rise to intermediate 5-hydroxy-5,6-dihydrocytos-6-yl radicals.[18] In contrast to the reaction of •OH, cytosine radical cations incorporate H_2O at the C6 position giving rise to intermediate 6-hydroxy-5,6-dihyro-2′-deoxycytos-5-yl radicals.[9,10] Thus, •OH addition and single-electron oxidation of cytosine give intermediates that differ in the position of the OH group (either C5 or C6): 5-hydroxy-5,6-dihydrocytos-6-yl and 6-hydroxy-5,6-dihyro-2′-deoxycytos-5-yl radicals. In the presence of oxygen, the latter radicals likely incorporate O_2 at the radical site to give the corresponding 5(6)-hydroxy-6(5)-hydroperoxyl radicals. Peroxyl radicals may then transform to stable products by bimolecular decay routes that involve tetroxides and/or alkoxyl radicals. Alternatively, peroxyl radicals may react with reducing agents, such as superoxide radical anions, to give intermediate 5(6)-hydroxy-6(5)-hydroperoxides that subsequently decompose to stable products.[19]

From studies of radical-induced oxidation of 2′-deoxycytidine (dCyd), we have identified 17 stable nucleoside products.[9,10] The final products may be divided into common and unique pyrimidine oxidation products. The group of common pyrimidine oxidation products includes 5,6-glycols, 5-hydroxyhydantoin, and formamide derivatives of dCyd. These products are analogous to those of other pyrimidines (i.e., 2′-deoxyuridine, thymidine) except that the 5,6-glycols of 2′-deoxycytidine are not stable and undergo either deamination to the 5,6-glycols of 2′-deoxyuridine or dehydration to 5-hydroxy-2′-deoxycytidine.[11] The formation of these products may be attributed to either the bimolecular decay of intermediate peroxyl radicals or decomposition of hydroperoxides. In contrast, the second group of products is unique to cytosine derivatives. The unique modifications of 2′-deoxycytidine include imidazolidine, biuret, and carbamic acid derivatives.[9,10,20] These products are likely formed by a novel pathway, which involves intramolecular addition of the peroxyl radical or hydroperoxide to the N3−C4 double bond of cytosine hydroperoxides, followed by cleavage of the C4−C5 bond.[10,21] Interestingly, the ratio of common to unique products of dCyd is 2.4 for •OH-induced decomposition compared to 3.8 for single-electron oxidation.[10] This difference may be related to the structure of intermediate peroxyl radicals and peroxides. The addition of •OH to the 5,6-double bond of dCyd mainly produces intermediates with a hydroxyl group at C5 and a peroxyl (or hydroperoxide) group at C6, whereas the addition of H_2O to the radical cation of dCyd predominantly leads to intermediates with the reverse orientation of hydroxyl and peroxyl (or hydroperoxide) groups. In free radical reactions of the monomer, however, it is not possible to distinguish between these two pathways of damage because intermediate peroxyl radicals undergo bimolecular decay, which appears to divert the pathway of decomposition toward common pyrimidine products.

A large fraction of peroxyl radicals likely convert to hydroperoxides in DNA. For example, the yield of intact hydroperoxides is nearly equal to the total yield of •OH-induced thymine and cytosine damage in DNA when exposed to γ-irradiation in oxygenated aqueous solutions.[22−24]The reaction of peroxyl radicals with neighboring base and sugar moieties may also contribute to the formation of alternative types of damage, including strand breaks, tandem lesions, and interstrand cross-links in dinucleotides and oligonucleotides.[25,26] In the present work, the hydroperoxides of dCyd were prepared in situ by treatment of the bromohydrin with H_2O_2, a well-known procedure for the preparation of thymidine hydroperoxides.[19] This route bypasses the involvement of peroxyl radicals and the formation of products by bimolecular decay pathways. In contrast to the 5(6)-hydroxy-6(5)-hydroperoxides of thymidine, which decompose with half-lives in the range of 7−550 h at 25 °C, the corresponding hydroperoxides of cytosine and dCyd are not at all stable in aqueous solution (estimated half-life of 16 min at 25 °C).[22] Here, we show that treatment of dCyd bromohydrins with H_2O_2 leads to the formation of four *cis*- and *trans*-diastereomers of N^1-carbamoyl-5,6-dihydroxy-2-oxoimidazolidine nucleosides. The structure of each diastereomer was examined by ^{1}H NMR, ^{13}C NMR, and 2D NOE analyses. Using

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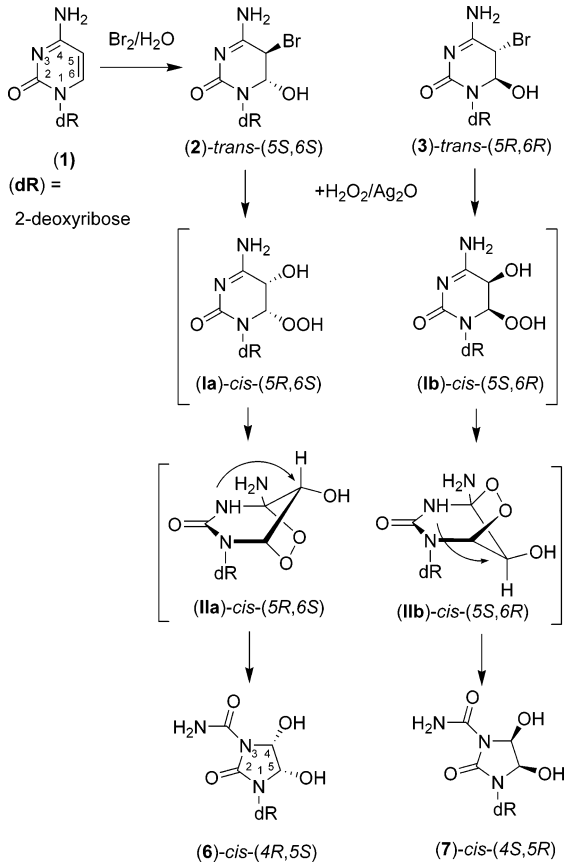
SCHEME 1. Synthesis of dCyd Bromohydrins (2, 3) and Imidazolidine Products (6, 7)



purified compounds, the ability of each diastereomer to interconvert between another diastereomer by ring-chain tautomerism at two sites of the imidazolidine ring was examined.

Results and Discussion

Preparation of dCyd Bromohydrins (2 and 3). The nucleoside derivatives of N^1-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (referred to as imidazolidine products) were prepared in two steps: (1) conversion of dCyd (**1**) to 5-bromo-6-hydroxy-5,6-dihydro-2′-deoxycytidine (referred to as dCyd bromohydrins; **2** and **3**) and (2) conversion of dCyd bromohydrins (**2** and **3**) to imidazolidine products (**4**−**7**; Scheme 1). The bromination of pyrimidine nucleoside in aqueous solution leads to the formation of two *trans*-diastereomers with Br and OH substituents at C5 and C6, respectively.[27−29] MS analysis of products **2** and **3** depicted molecular ion peaks at *m*/*z* 326 and 324 (ratio of ^{81}Br and ^{79}Br stable isotopes was 1:2) accompanied with ion peaks at *m*/*z* 308 and 306, indicating the initial loss of H_2O from the molecular ions. The configuration of dCyd bromohydrins (either *trans*-(5*S*,6*S*) or *trans*-(5*R*,6*R*)) was determined by comparison of the NMR features of the base and sugar moieties to analogous compounds derived from thymidine, 2′-deoxyuridine, and 5-methyl-2′-deoxycytidine.[10,19,30,31] Thereby,

TABLE 1. Cross-Peaks in 2D NOE Analysis[a]

cross-peak	*trans*-(4*S*,5*S*) (**4**)	*trans*-(4*R*,5*R*) (**5**)	*cis*-(4*R*,5*S*) (**6**)	*cis*-(4*S*,5*R*) (**7**)
H4−H5	−[b]	−4.8	−	−4.7
H4−OH5	−18.5	−8.6	−	−
H5−OH5	−36.9	−30.0	−30.7	−30.0
OH4−H4	−43.4	−19.3	−57.5	−29.7
OH4−H5	−6.8	−3.2	−	−
OH4−OH5	−	+40.7	−	+71.8
OH5−H2′	−8.2	−	−	−
H5−H2′	−10.3	−16.4	−	−

[a] Cross-peak intensity was normalized to the signal for H2′ and H2″ of the 2-deoxyribose moiety. [b] Not detected.

the features of dCyd bromohydrins were nearly identical to those of other pyrimidine bromohydrins. In particular, the 6*S* diastereomer displayed a doublet of a doublet ($J_{1'2'}$ = 8.8 Hz); the 6*R* diastereomer showed a pseudotriplet ($J_{1'2'}$ = 7.3 Hz); the 6*S* diastereomer eluted before the 6*R* diastereomer on reversed-phase chromatography; and the yield of the 6*S* diastereomer was approximately half of that of the 6*R* diastereomer.[10,19,30,31]

Structural Analysis of Imidazolidine Products (4−7). The structure of imidazolidine products (**4**−**7**) was determined by NMR analyses (^{1}H NMR, ^{13}C NMR, and 2D NOE; see Supporting Information). ^{1}H NMR spectra depicted nine nonexchangeable protons of which two protons may be attributed to the imidazolidine moiety and seven protons may be attributed to an intact sugar moiety. In addition, there were six exchangeable protons attributable to the carbamoyl and 2-deoxyribose moieties. Assignment of protons was confirmed by 2D-COSY analysis. Interestingly, the amino protons of the carbamoyl group were nonequivalent, pointing to an interaction between H of the exocyclic amino group and C═O of the adjacent carbon. This is consistent with X-ray crystallography analysis of the modified nucleobase without the 2-deoxyribose moiety: *trans*-1-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine.[32] A salient feature of the ^{1}H NMR spectra of **4**−**7** was the presence of two proton signals between 5.0 and 5.6 ppm, which may be attributed to two nonexchangeable methine protons of the imidazolidine ring. Moreover, coupling between H4 and H5 protons was absent for products **4** and **5** (J_{H4-H5} = ∼0 ppm), whereas it was fairly strong for products **6** and **7** (J_{H4-H5} = 6 ppm). According to the Karplus equation, the absence of coupling indicates that the dihedral angle between vicinal protons approaches 90° within a half-chair conformation. The difference in coupling between H4 and H5 clearly distinguishes **4** and **5** as *trans*-diastereomers and **6** and **7** as the corresponding *cis*-diastereomers. A similar difference was reported between *trans*- and *cis*-diastereomers of the modified nucleobase without the 2-deoxyribose moiety.[21,32−34] In addition, ^{13}C NMR and 2D NOE analyses revealed a number of interesting differences between *trans*- and *cis*-diastereomers. From ^{13}C NMR analysis, the chemical shift of methine carbons was shifted downfield by about 5 ppm in *trans*- compared to *cis*-diastereomers; e.g., C4 was 81 ppm for *trans*-diastereomers (**4** and **5**) and 75 ppm for *cis*-diastereomers (**6** and **7**). From 2D NOE analysis (Table 1), the H4 and OH5 protons, and the corresponding OH4 and H5 protons, gave cross-peaks for *trans*-diastereomers but not

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TABLE 2. Conformational Analysis of Imidazolidine Products

population	% 2′-*endo*[a]	% $g^+(\psi)$[b]	% $t(\psi)$[b]	% $g^-(\psi)$[b]
trans-(4*S*,5*S*) (**4**)	73	48	17	37
trans-(4*R*,5*R*) (**5**)	68	53	26	21
cis-(4*R*,5*S*) (**6**)	74	30	40	30
cis-(4*S*,5*R*) (**7**)	62	30	43	27

[a] % 2′-*endo*-3′-*exo* (2′-*endo*) = $100 \times J_{1'2'}/(J_{1'2'} + J_{3'4'})$.[30] [b] Orientation of the 5-hydroxymethyl group with respect to the C4′−O4′ and C4′−C3′ bonds. % *gauche*−*gauche* ($g^+(\psi)$) = $100 \times (13.8 - (J_{4'5'} + J_{4'5'\epsilon}))/10.7$; % *gauche*−*trans* ($t(\psi)$) = $100 \times (J_{4'5''} - 1.5)/10$; % *trans*−*gauche* ($g^-(\psi)$) = % $g^+(\psi)$ − % $t(\psi)$.

for *cis*-diastereomers, indicating the shorter distance between vicinal H and OH substituents within *trans*-diastereomers.

The configuration of *trans*-diastereomers (5*S* or 5*R*) was determined by ^{1}H NMR and 2D NOE analyses (see spectra in Supporting Information). For *trans*-diastereomers, the conformation of the nucleobase moiety is *anti* with respect to the 2-deoxyribose moiety (χ(O4′−C1′−N1−C2) = 180−245°) in view of the presence of cross-peaks in the 2D NOE spectra for H5−H2′ and OH5−H2′ of the imidazolidine and 2-deoxyribose moieties (Table 1).[35] Furthermore, the conformation of the 2-deoxyribose ring is preferentially 2′-*endo*-3′-*exo* (2′-*endo*) on the basis of the H−H proton scalar coupling constants of the 2-deoxyribose moiety (73 and 68%; Table 2). The crystal structure of *trans*-1-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (without the 2-deoxyribose moiety) depicts the five-membered ring as nearly planar, with the C5 atom tilted about 13° out of the plane.[32] It should be noted that these conformations (*anti* and 2′-*endo*) are observed for the majority of pyrimidine oxidation products with both six-membered (e.g., thymidine 5,6-glycols, thymidine 5(6)-hydroxy-6(5)-hydroperoxides) and five-membered rings (5-hydroxyhydantoin derivatives).[12,19,30,36,37] Thus, the same features likely apply to imidazolidine products. Accordingly, the above preferences in conformation allow one to predict the approximate orientation of C5 substituents of the imidazolidine ring within *trans*-diastereomers. For 5*S* diastereomers, the OH5 group will be in front (near H2′) and the H5 group will be in back (near O4′) of the 2-deoxyribose ring, whereas for 5*R* diastereomers, the reverse orientation will be observed with H5 in front and OH5 in back of the 2-deoxyribose ring. To determine the configuration of **4** and **5**, we need to consider differences in H−H distances from 2D NOE analysis (Table 1). The 2D NOE spectra of **4** displayed cross-peaks for OH5 of the imidazolidine moiety and H2′ of the 2-deoxyribose moiety as well as between the corresponding H5 and H2′. The presence of both cross-peaks indicates that OH5 and H5 are above the plane of the 2-deoxyribose ring without any indication about their relative orientation. In contrast, the spectra of **5** showed a cross-peak between H5 and H2′ but *not* between OH5 and H2′. The presence of a cross-peak between H5 and H2′ confirms that the imidazolidine and 2-deoxyribose moieties of **5** remain in a preferential *anti* conformation, similar to its diastereomer **4**. The absence of a cross-link between OH5 and H2′ however indicates that the OH5 group is orientated in back of the 2-deoxyribose ring (near O4′) that is a 5*R* configuration. The other orientation in which the OH5 group is in front of the 2-deoxyribose, which is a 5*S* configuration, may be ruled out because the imidazolidine moiety must rotate outside a preferential *anti* conformation to avoid contact between OH5 and H2′ of the 2-deoxyribose moiety.[35] Thus, we conclude that **4** has a 5*S* configuration whereas **5** has a 5*R* configuration. This assignment is also consistent with H−H coupling constants of the 2-deoxyribose moiety (Table 2). In particular, $J_{H1'-H2'}$ was higher for **4** (8.3 ppm) than for **5** (7.1 ppm), indicating a higher population of 2′-*endo* for **4** (73%) compared to **5** (68%). The higher population of 2′-*endo* for **4** may be attributed to an increase in steric hindrance between OH5 of the imidazolidine moiety and H2′ of the 2-deoxyribose moiety within a 5*S* configuration. The configuration of **4** and **5** is also supported by changes in $J_{H4'-H5'}$ and $J_{H4'-H5'}$, which are associated with the population of low-energy rotamers of the 5-hydroxymethyl group.[37] In particular, the high population of $g^+(\psi)$ for **4** (48%) and for **5** (53%) suggests that the OH5 group of the imidazolidine moiety interacts to some extent with the 5-hydroxymethyl group of the 2-deoxyribose moiety (Table 1).

The assignment of *cis*-diastereomers was based on comparison of the ^{1}H NMR and 2D NOE spectra with *trans*-diastereomers (see spectra in Supporting Information). In contrast to *trans*-diastereomers, we did not observe cross-peaks between substituents of the imidazolidine ring (OH5 and H5) and H2′of the 2-deoxyribose moiety in the 2D NOE spectra of *cis*-diastereomers. The absence of these cross-peaks for *cis*-diastereomers indicates that OH5 and H5 of the imidazolidine ring do not lie directly above the 2-deoxyribose ring as was the case for *trans*-diastereomers. Despite this difference, however, there were remarkable similarities in the ^{1}H NMR and 2D NOE spectra between 5*S* and 5*R* diastereomers. For example, $J_{H1'-H2'}$ for **4** (8.3 ppm) was similar to that for **6** (8.0 ppm) and likewise for **5** (7.1 ppm) and **7** (6.8 ppm). This suggests that the conformation of 2-deoxyribose depends on the configuration at C5 of the imidazolidine ring with similar effects for 5*S* and 5*R* diastereomers (Table 2). Furthermore, the pattern of cross-peaks between C4 and C5 substituents of the imidazolidine ring was very similar for 5*S* and 5*R* diastereomers (Table 1). For example, cross-peaks for H4−H5 and OH4−OH5 appeared in the spectra for **5** and **7** but not in that for **4** and **6**. In addition, the intensity of cross-peaks for OH4−H4 was about 2-fold greater for 5*S* compared to 5*R* (Table 1). These findings strongly suggest that products **4** and **6** have the same configuration at C5, and likewise, products **5** and **7** have the same configuration. The proposed configuration of *cis*-diastereomers is also supported by the mechanism of conversion of dCyd bromohydrins to imidazolidine products (see below).

Interconversion of Imidazolidine Products (4−7). The isomerization of imidazolidine products was studied by monitoring each diastereomer as a function of time of incubation at 37 °C, starting with purified diastereomers. The four diastereomers are well separated by reversed-phase HPLC (Figure 1). The disappearance and formation of individual diastereomers followed first-order kinetics (Figure 2). The rate of isomerization

(35) The structure of imidazolidine products was examined by molecular mechanic simulation with the crystal structure coordinates of the 2-deoxyribose moiety of thymidine and N^1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine (ref 32). Using a torsional space grid search, the O4′−C1′−N1−C2 torsional angle (χ) of imidazolidine nucleosides was sterically restricted to the syn (40−60°) and anti (160−240°) conformations. The other possible conformations were not allowed because of bad contacts between the 2-deoxyribose and imidazolidine units including the following atoms: C2O2−H2′C2′ (60−180°); O5−H2′ (240−300°); and C2O2−O4′ (300−60°).

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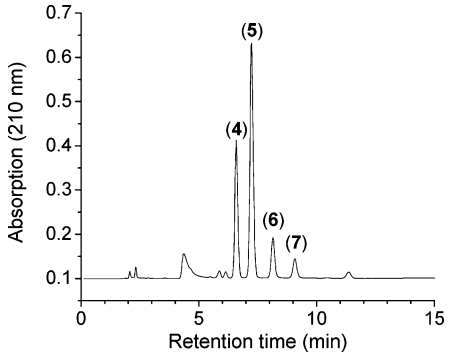
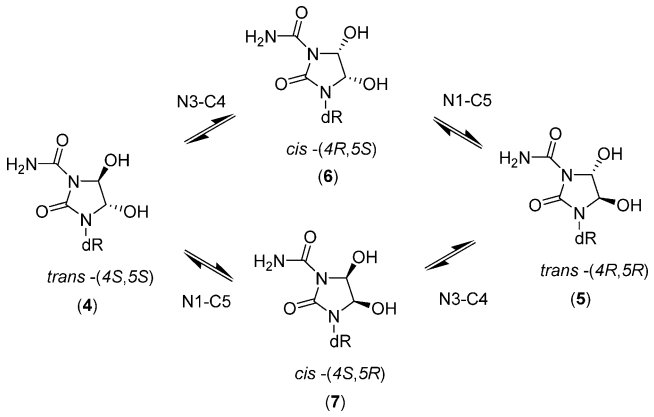


FIGURE 1. HPLC analysis of the mixture resulting from reaction of H_2O_2 with dCyd bromohydrins in the presence of Ag_2O. The four major peaks (**4**−**7**) correspond to the four diastereomers of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (**4**−**7**): product **4**, *trans*-(4*S*,5*S*); product **5**, *trans*-(4*R*,5*R*); product **6**, *cis*-(4*R*,5*S*); product **7**, *cis*-(4*S*,5*R*).

SCHEME 2. Isomerization of Imidazolidine Products by Ring-Chain Tautomerism at N1−C5 and N3−C4



was about 100-fold greater for *cis*- compared to *trans*-diastereomers, and for each pair of diastereomers, the rate was slightly higher for 5*R* diastereomers compared to 5*S* diastereomers, except at pH 9 (Table 3). The rate of isomerization was also dependent on pH. Under acidic conditions (pH 5.5), isomerization was governed by ring-chain tautomerism at N1−C5 (**4** ↔ **7**; **5** ↔ **6**). In contrast, under neutral and alkaline conditions (pH 7.0 and 9.0), there was evidence for an additional pathway of isomerization involving tautomerism at N3−C4. This pathway explains the ability of purified *trans*-(4*S*,5*S*) (**4**) to give *trans*-(4*R*,5*R*) (**5**) in the final decomposition mixture and vice-versa for the conversion of **5** to **4** (Figure 2, Table 4). The same phenomenon was also observed between *cis*-diastereomers at neutral and alkaline pH (Table 4). Thus, imidazolidine products (**4**−**7**) undergo successive cycles of isomerization involving ring-chain tautomerism at N1−C5 and in addition at N3−C4, leading to interconversion of all four diastereomers (Scheme 2). It is noteworthy that the exponential loss of **4** and the concomitant growth of **5** divided into fast (initial) and slow (later) exponential components (Figure 1, upper panel). In the case of the growth of **5**, the rates of the fast and slow components were +4.2 and +0.9 s^{-1}, respectively. Assuming that isomerization from *trans*- to *cis*-diastereomers is the rate-limiting step, the difference between fast and slow components (4.7-fold) likely corresponds to the difference in ring-chain tautomerism at N3−C4 and N1−C5 (Scheme 2).

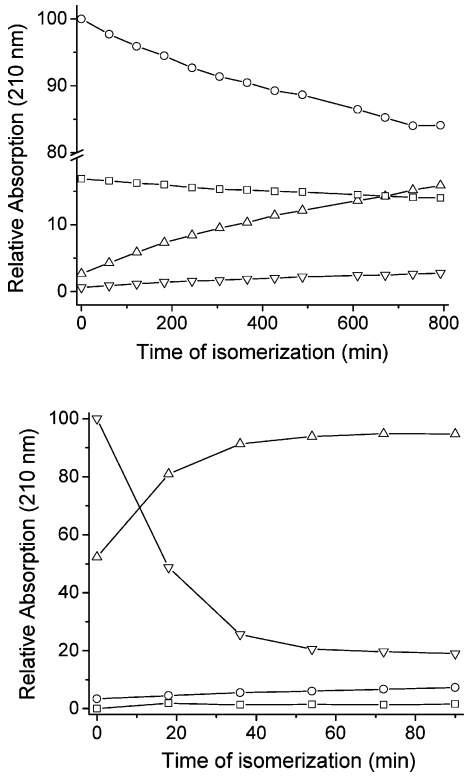


FIGURE 2. Kinetics of isomerization of imidazolidine products. Upper panel: the purified *trans*-(4*S*,5*S*) diastereomer (**4**) was incubated in 50 mM sodium phosphate buffer (pH 7.0) at 37 °C. Lower panel: the same except with the purified *cis*-(4*R*,5*S*) diastereomer (**6**). Symbols: ○, *trans*-(4*S*,5*S*) (**4**); △, *trans*-(4*R*,5*R*) (**5**); ▽, *cis*-(4*R*,5*S*) (**6**); □, *cis*-(4*S*,5*R*) (**7**).

TABLE 3. Rates of Isomerization of Imidazolidine Products[a]

rate × 10^{-6} s^{-1}	pH 5.5	pH 7.0	pH 9.0
trans-(4*S*,5*S*) (**4**)	−1.1 ± 0.4	−5.1 ± 0.3	−47.5 ± 4.2
trans-(4*R*,5*R*) (**5**)	−1.4 ± 0.2	−5.8 ± 0.2	−41.0 ± 0.9
cis-(4*R*,5*S*) (**6**)	−95 ± 3	−845 ± 57	−[b]
cis-(4*S*,5*R*) (**7**)	−106 ± 7	−[b]	−[b]

[a] Rates of conversion were the estimated linear regression of $\ln(A_t/A_o)$ vs time, where *A* represents the HPLC peak area at initial (o) and time (*t*) of incubation at 37 °C ($n > 6$; $r^2 > 0.98$). [b] The rate of conversion was too rapid for determination. The amount of *trans*- was comparable to that of *cis*-diastereomers at the beginning of incubation.

The isomerization of imidazolidine products by N1−C5 ring-chain tautomerism is analogous to the isomerization of 5,6-saturated pyrimidines bearing a hydroxyl group at C6, as reported for the 5,6-glycols of thymine, uracil, and cytosine derivatives.[10,29,38] Ring-chain tautomerism of pyrimidine 5,6-glycols involves formation of an open-chain intermediate containing either a carbonyl or hemiacetal group. The existence of an open-chain intermediate is supported by the incorporation of ^{18}OH at C6 of pyrimidine 5,6-glycols in the presence of $H_2{}^{18}O$.[27,29] In the case of imidazolidine products, it should be noted that a second ring-chain tautomerism does not occur from the open-chain intermediate, which may lead to the liberation of the biuret nucleoside and glyoxal. Even at pH 9, fragmentation of the imidazolidine ring was minor as inferred by the low yield of the biuret nucleoside (<10%). Thus, the open-chain

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TABLE 4. Distribution of Imidazolidine Diastereomers at Equilibrium[a]

		pH 5.5	pH 7.0	pH 9.0
trans-(4*S*,5*S*) (**4**)	*trans* (**4**)	81	72	46
	→ *trans* (**5**)	5	15	37
	→ *cis* (**6**)	1	2	7
	→ *cis* (**7**)	13	12	10
trans-(4*R*,5*R*) (**5**)				
	→ *trans* (**4**)	8	28	47
	trans (**5**)	78	56	38
	→ *cis* (**6**)	14	10	7
	→ *cis* (**7**)	1	5	9
cis-(4*R*,5*S*) (**6**)				
	→ *trans* (**4**)	3	5	27
	→ *trans* (**5**)	58	74	25[b]
	cis (**6**)	39	21	32
	→ *cis* (**7**)	1	1	15
cis-(4*S*,5*R*) (**7**)				
	→ *trans* (**4**)	69	63[b]	39[b]
	→ *trans* (**5**)	3	21	29
	→ *cis* (**6**)	0	4	8
	cis (**7**)	28	13	24

[a] % of one diastereomer with respect to the total amount of all four diastereomers assuming equal absorption at 210 nm in HPLC analysis. [b] Amount of *trans*- was equal to that of *cis*-diastereomers at the beginning of incubation.

ureid resulting from opening of the N1−C5 bond of imidazolidine products is refractory to subsequent ring-chain tautomerism at N3−C4.

Mechanism of Formation of Imidazolidine Products (4−7). The four diastereomers of imidazolidine products were prepared by reaction of H_2O_2 with dCyd bromohydrins in the presence of Ag_2O. The *cis*-diastereomers (**2** and **3**) were formed in large excess of the *trans*-diastereomers (∼10% of *cis*-diastereomers) as observed by direct HPLC analysis of the reaction mixture. In contrast, the *trans*-diastereomers (**3** and **4**) were the major products after workup under neutral conditions because of isomerization from *cis*- to *trans*-diastereomers. Thus, we conclude that the peroxidation (H_2O_2/Ag_2O) of *trans*-(5*S*,6*S*)-dCyd bromohydrin initially gives *cis*-(4*R*,5*S*)-imidazolidine (**2** → **6**) and *trans*-(5*R*,6*R*)-dCyd bromohydrin gives *cis*-(4*S*,5*R*)-imidazolidine (**3** → **7**). In other words, the transformation of dCyd bromohydrins (**2** and **3**) to imidazolidine products (**6** and **7**) is stereospecific with inversion of the configuration at C4 (C5 of dCyd bromohydrins) together with retention of the configuration at C5 (C6 of dCyd bromohydrins) (Scheme 1).

The reaction of pyrimidine bromohydrins with H_2O_2 in the presence of Ag_2O likely involves nucleophilic addition of H_2O_2 to an intermediate open-chain zwitterion with the positive charge at C6 and the negative charge at C5−OH (−H+).[27,28] The zwitterion intermediate likely results from migration of the C6−OH group to the C5 position of pyrimidine bromohydrins with concomitant release of bromide. Previous studies with thymine bromohydrins demonstrated the migration of C6−OH during the conversion of bromohydrins to 5,6-glycols in the presence of $H_2{}^{18}O$.[27] Similarly, H_2O_2 is incorporated at C6 rather than at C5 in the preparation of thymidine hydroperoxides from the corresponding bromohydrins.[19] Furthermore, the addition of H_2O, H_2O_2, and other nucleophiles to the C6 position of thymidine bromohydrins predominantly gives *cis* addition products because of achimeric assistance from the C5 hydroxyl group.[19,27,28] Although the hydroperoxides of dCyd are too unstable to be identified as intermediates in the reaction of H_2O_2 with dCyd bromohydrins, it is nevertheless reasonable to propose these intermediates in view of the similarity in structure and chemistry with other pyrimidine bromohydrins. Thereby, *trans*-(5*S*,6*S*)-bromohydrin (**2**) and *trans*-(5*R*,6*R*)-bromohydrin (**3**) should give *cis*-(5*R*,6*S*)-5-hydroxy-6-hydroperoxide (**Ia**) and *cis*-(5*S*,6*R*)-5-hydroxy-6-hydroperoxide (**Ib**) as intermediates in the reaction pathway, respectively (Scheme 1). In the next step, we propose that the intermediate 5-hydroxy-6-hydroperoxides of dCyd (**Ia**,**b**) rearrange into the corresponding 4,6-endoperoxides (**IIa**,**b**). Again, 4,6-endoperoxides have not been identified as intermediates in the formation of imidazolidine products. However, the involvement of 4,6-endoperoxides is strongly supported by the incorporation of two atoms of ^{18}O into the structure of imidazolidine products from the •OH-induced decomposition of dCyd in the presence of $^{18}O_2$.[10] Because each bromohydrin (**2** and **3**) initially gives a single imidazolidine product (**2** → **6** and **3** → **7**), we propose that the conversion of 4,6-endoperoxides (**IIa**,**b**) to imidazolidine products (**6** and **7**) takes place in a single step by concerted cleavage of the peroxide and pyrimidine C4−C5 bonds (Scheme 1).

Conclusions

In this work, we have characterized the *cis*-diastereomers of imidazolidine products. The *trans*-diastereomers were previously characterized as modified nucleobase enantiomers and modified nucleoside diastereomers.[10,21,32−34] The study of all four diastereomers reveals a fascinating chemical reaction involving the interconversion of four diastereomers by successive cycles of ring-chain tautomerism at N1−C5 and N3−C4 of the imidazolidine ring. In addition, our results suggest that imidazolidine nucleosides are formed from the decomposition of 5-hydroxy-6-hydroperoxides of dCyd. The formation of imidazolidine products is likely a major pathway of •OH-induced decomposition of cytosine in DNA because the addition of •OH takes place mainly at C5 of cytosine derivatives leading to putative 5-hydroxy-6-hydroperoxides; in contrast, 6-hydroxy-5-hydroperoxides with OOH at C5 and OH at C6 probably decompose to 5,6-glycols. Although *trans*-imidazolidine nucleosides have been detected in γ-irradiated dGpdC, dCpdG, d(TpApCpG), and calf-thymus DNA under oxygenated conditions,[39−41] further studies are necessary to determine the yield of these products in relation to other products in DNA. In view of the short half-life of *cis*-diastereomers in aqueous solution (τ = 14 min), however, *trans*-diastereomers probably prevail in DNA and ultimately dictate the biological consequences. This work reports novel aspects about the chemistry of imidazolidine nucleosides that will help us understand the biological effects of oxidative damage to cytosine in duplex DNA.

Experimental Section

Synthesis of dCyd Hydroperoxides. The bromohydrins of dCyd were prepared by slow addition of Br_2 to a solution of dCyd (500 mg in 500 μL of water) at 4 °C until development of a pale yellow color. The ice-cold solution was bubbled with air to remove unreacted Br_2. The resulting bromohydrins were purified by HPLC using an octadecylsilyl silica gel column with 100% H_2O as the mobile phase at a flow rate of 1.4 mL/min. The eluent containing purified bromohydrins (1−2 mL) was immediately mixed with 5

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(41) Paul, C. R.; Wallace, J. C.; Alderfer, J. L.; Box, H. C. *Int. J. Radiat. Biol.* **1988**, *54*, 403−415.

mL of 30% H_2O_2 at 4 °C. Small portions (20 mg) of powdered Ag_2O were added until the reaction was complete over a period of 1 h. After an additional 1 h at 4 °C, the reaction mixture was diluted with 10 volumes of H_2O and the entire mixture was frozen and lyophilized to dryness under a vacuum. The resulting black residue was extracted with 10 mL of cold CH_3OH at −20 °C and filtered using a 0.22 μm nylon membrane. Methanol was removed by evaporation on a rotory evaporator at room temperature. The residue was dissolved in 2 mL of water, and the diastereomers of imidazolidine products were separated by HPLC using an octadecylsilyl silica gel column with 50 mM NaH_2PO_4 buffer at pH 5.5 at 1.4 mL/min as the mobile phase. For the preparation of large (mg) quantities of product, the mixture of bromohydrins was treated directly with H_2O_2 and Ag_2O, and the four imidazolidine products were purified by HPLC using the same chromatographic conditions as those described above.

***trans*-(5*S*,6*S*)-5-Bromo-6-hydroxy-5,6-dihydro-2′-deoxycytidine (2).** HPLC (octadecylsilyl silica gel with H_2O): k′ = (retention time − void volume)/void volume, $k' = 1.3$. ^{1}H NMR (300 MHz, 99.99% D_2O, reference to 3-(trimethylsilyl)-propionate-2,2,3,3-d_4): δ 6.13 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8,8$ Hz, $J_{1',2''} = 6.0$ Hz), δ 5.31 (d, 1H, H_6, $J_{5,6} = 2.3$ Hz), δ 4.45 (d, 1H, H_5), δ 4.27(m, 1H, $H_{3'}$, $J_{2',3'} = 6.1$ Hz, $J_{2'',3'} = 2.7$ Hz), δ 3.80 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.0$ Hz, $J_{4',5'} = 4.4$ Hz, $J_{4',5''} = 4.5$ Hz), δ 3.58 (d, 2H, H5′−H5″), δ 2.21 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.0$ Hz), δ 2.04 (m, 1H, $H_{2''}$). ESI-MS (positive mode): *m*/*z* 326 and 324 (M + H), 308 and 306 (M + H − H_2O), 192 and 190 (5-bromocytosine).

***trans*-(5*R*,6*R*)-5-Bromo-6-hydroxy-5,6-dihydro-2′-deoxycytidine (3).** HPLC (octadecylsilyl silica gel with H_2O): $k' = 4.2$. ^{1}H NMR (600 MHz, 99.99% D_2O): δ 6.12 (pst, 1, $H_{1'}$, $J_{1',2'} = 7.3$ Hz, $J_{1',2''} = 6.3$ Hz), δ 5.47 (d, 1, H_6, $J_{5,6} = 2.4$ Hz), δ 4.67 (d, 1, H_5), δ 4.29 (m, 1, $H_{3'}$, $J_{2',3'} = 6.8$ Hz, $J_{2'',3'} = 3.9$ Hz), δ 3.84 (m, 1, $H_{4'}$, $J_{3',4'} = 3.9$ Hz), δ 3.69 (dd, 1, $H_{5'}$, $J_{5',5''} = -12.6$ Hz), δ 3.62 (dd, 1, $H_{5''}$), δ 2.24 (m, 1, $H_{2'}$, $J_{2',2''} = -13.7$ Hz), δ 2.15 (m, 1, $H_{2''}$). ESI-MS (positive mode): *m*/*z* 326 and 324 (M + H), 308 and 306 (M + H − H_2O), 192 and 190 (5-bromocytosine).

***trans*-(4*S*,5*S*)-N^1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (4).** HPLC (octadecylsilyl silica gel with H_2O): k′= 2.2. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.10 (s, 1H, NH), 7.40 (s, 1H, NH), δ 6.62 (d, 1H, OH_4, $J_{OH4,H4} = 5.6$ Hz), δ 6.25 (d, 1H, OH_5, $J_{OH5,H5} = 8.5$ Hz), δ 5.57 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.3$ Hz, $J_{1',2''} = 6.3$ Hz), , δ 5.05 (d, 1H, H_4), δ 4.94 (d, 1H, $OH_{3'}$, $J_{OH3',H3'} = 4.1$ Hz), δ 4.66 (d, 1H, H_5), δ 4.64 (t, 1H, $OH_{5'}$, $J_{OH5',H5'} = J_{OH5',H5''} = 5.5$ Hz), δ 4.01 (m, 1H, H3′, $J_{2',3'} = 6.1$ Hz, $J_{2'',3'} = 3.0$ Hz), δ 3.51 (m, $H_{4'}$, $J_{3',4'} = 3.1$ Hz, $J_{4',5'} = 3.7$ Hz, $J_{4',5''} = 5.2$ Hz), δ 3.28 (m, 2H, $H_{5'}$), δ 2.21 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.4$ Hz), δ 1.73 (m, 1H, $H_{2''}$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 154.9 (C_2═O), δ 152.8 (C_6═O), δ 82.4 ($C_{1'}$), δ 86.3 ($C_{4'}$), δ 82.0 (C_5), δ 80.9 (C_4), δ 70.8 ($C_{3'}$), δ 62.4 ($C_{5'}$), δ 37.6 ($C_{2'}$). ESI/MS/MS (50% CH_3CN, 1 mM NaCl) *m*/*z* (relative intensity): 300 (M + Na, 100), 282 (M + Na − H_2O, 25), 242 (N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-biuret) (30), 239 (42), 225 (65), 156 (1-amino-2-deoxy-β-D-*erythro*-pentofuranose + Na, 70). HRMS (ES, positive mode): calcd for $C_9H_{15}N_3O_7Na$ ([M + Na]$^+$) 300.0808, found 300.0808.

***trans*-(4*R*,5*R*)-N^1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (5).** HPLC (octadecylsilyl silica gel with H_2O): $k' = 2.5$. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.22 (d, 2H, NH_2), δ 6.60 (d, 1H, OH_4, $J_{OH4,H4} = 6.0$ Hz), δ 6.23 (d, 1H, OH_5, $J_{OH5,H5} = 6.2$ Hz), δ 5.47 (pst, 1H, $H_{1'}$, $J_{1',2'} = 7.1$ Hz, $J_{1',2''} = 6.4$ Hz), δ 5.24 (d, 1H, H_4), δ 5.18 (d, 1H, $OH_{3'}$, $J_{OH3',H3'} = 3.6$ Hz), δ 4.66 (d, 1H, H_5), δ 4.82 (t, 1H, $OH_{5'}$, $J_{OH5',H5'} = J_{OH5',H5''} = 5.2$ Hz), δ 4.07 (m, 1H, H3′, $J_{2',3'} = 5.9$ Hz, $J_{2'',3'} = 3.4$ Hz), δ 3.55 (m, $H_{4'}$, $J_{3',4'} = 3.3$ Hz, $J_{4',5'} = 4.0$ Hz, $J_{4',5''} = 4.1$ Hz), δ 3.32 (m, 2H, $H_{5'}$), δ 2.20 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.2$ Hz), δ 1.80 (m, 1H, $H_{2''}$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 154.8 (C_2═O), δ 152.7 (C_6═O), δ 82.9 ($C_{1'}$), δ 86.5 ($C_{4'}$), δ 82.1 (C_5), δ 81.5 (C_4), δ 71.1 ($C_{3'}$), δ 61.8 ($C_{5'}$), δ 36.5 ($C_{2'}$). ESI/MS/MS (50% CH_3CN, 1 mM NaCl) *m*/*z* (relative intensity): 300 (M + Na, 100), 282 (M + Na − H_2O, 25), 242 (N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-biuret) (30), 239 (42), 225 (65), 156 (1-amino-2-deoxy-β-D-*erythro*-pentofuranose + Na, 70). HRMS (ES, positive mode): calcd for $C_9H_{15}N_3O_7Na$ ([M + Na]$^+$) 300.0808, found 300.0808.

***cis*-(4*R*,5*S*)-N^1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (6).** HPLC (octadecylsilyl silica gel with H_2O): $k' = 2.9$. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.15 (d, 2H, NH_2), δ 6.63 (d, 1H, OH_4, $J_{OH4,H4} = 6.8$ Hz), δ 5.81 (d, 1H, OH_5, $J_{OH5,H5} = 10.4$ Hz), δ 5.43 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.0$ Hz, $J_{1',2''} = 6.5$ Hz), δ 5.37 (dd, 1H, H_4, $J_{4,5} = 5.4$ Hz), δ 5.14 (d, 1H, $OH_{3'}$, $J_{OH3',H3'} = 4.1$ Hz), δ 5.1 (dd, 1H, H_5), δ 4.59 (t, 1H, $OH_{5'}$, $J_{OH5',H5'} = 5.7$ Hz, $J_{OH5',H5''} = 6.0$ Hz), δ 4.17 (m, 1H, H3′, $J_{2',3'} = 6.2$ Hz, $J_{2'',3'} = 2.9$ Hz), δ 3.59 (m, $H_{4'}$, $J_{3',4'} = 2.8$ Hz, $J_{4',5'} = 5.1$ Hz, $J_{4',5''} = 5.5$ Hz), δ 3.37 (m, 2H, $H_{5'}$), δ 2.53 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.2$ Hz), δ 1.75 (m, 1H, $H_{2''}$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 154.6 (C_2═O), δ 152.9 (C_6═O), δ 82.6 ($C_{1'}$), δ 86.6 ($C_{4'}$), δ 77.4 (C_5), δ 74.7 (C_4), δ 71.2 ($C_{3'}$), δ 62.5 ($C_{5'}$), δ 35.2 ($C_{2'}$). ESI/MS/MS (50% CH_3CN, 1 mM NaCl) *m*/*z* (relative intensity): 300 (M + Na, 100), 282 (M + Na − H_2O, 12), 242 (N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-biuret) (25), 239 (33), 225 (30), 156 (1-amino-2-deoxy-β-D-*erythro*-pentofuranose + Na, 32). Exact mass (ESI-MS/MS, positive mode): calcd for $C_9H_{15}N_3O_7$-Na ([M + Na]$^+$) 300.0808, found 300.0821 (Δ = 4.3 ppm).

***cis*-(4*S*,5*R*)-N^1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-carbamoyl-4,5-dihydroxy-2-oxoimidazolidine (7).** HPLC (octadecylsilyl silica gel with H_2O): $k' = 3.4$. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.22 (d, 2H, NH_2), δ 6.67 (d, 1H, OH_4, $J_{OH4,H4} = 5.7$ Hz), δ 5.60 (pst, 1H, $H_{1'}$, $J_{1',2'} = 6.8$ Hz, $J_{1',2''} = 6.4$ Hz), δ 5.59 (d, 1H, OH_5, $J_{OH5,H5} = 10.7$ Hz), δ 5.25 (dd, 1H, H_4, $J_{4,5} = 5.8$ Hz), δ 5.09 (dd, 1H, H_5), δ 4.89 (d, 1H, $OH_{3'}$, $J_{OH3',H3'} = 4.2$ Hz), δ 4.56 (t, 1H, $OH_{5'}$, $J_{OH5',H5'} = 5.6$ Hz, $J_{OH5',H5''} = 5.8$ Hz), δ 4.01 (m, 1H, H3′, $J_{2',3'} = 6.4$ Hz, $J_{2'',3'} = 4.3$ Hz), δ 3.59 (m, $H_{4'}$, $J_{3',4'} = 3.9$ Hz, $J_{4',5'} = 4.8$ Hz, $J_{4',5''} = 5.8$ Hz), δ 3.27 (m, 2H, $H_{5'}$), δ 2.44 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.2$ Hz), δ 1.71 (m, 1H, $H_{2''}$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 154.9 (C_2═O), δ 152.8 (C_6═O), δ 82.7 ($C_{1'}$), δ 86.3 ($C_{4'}$), δ 75.6 (C_5), δ 74.5 (C_4), δ 70.4 ($C_{3'}$), δ 62.2 ($C_{5'}$), δ 36.8 ($C_{2'}$). ESI/MS/MS (50% CH_3CN, 1 mM NaCl) *m*/*z* (relative intensity): 300 (M + Na, 100), 282 (M + Na − H_2O, 28), 242 (N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-biuret) (28), 239 (43), 225 (61), 156 (1-amino-2-deoxy-β-D-*erythro*-pentofuranose + Na, 62). Exact mass (ESI-MS/MS, positive mode): calcd for $C_9H_{15}N_3O_7$-Na ([M + Na]$^+$) 300.0808, found 300.0824 (Δ = 5.3 ppm).

Interconversion of 1-Carbamoyl-4,5-dihydroxy-2-oxoimidazolidine Products (4−7). Purified diastereomers were dissolved in 25 mM phosphate buffer at pH 5.5, 7.0, and 9.0, and their transformation to other diastereomers was monitored by HPLC as a function of time of incubation at 37 °C. The separation of diastereomers was achieved by HPLC with 50 mM phosphate buffer (pH 5.5) as the mobile phase at a flow rate of 1.4 mL/min.

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Supporting Information Available: General experimental methods and ^{1}H NMR and 2D NOE spectra of the four diastereomers of imidazolidine products (**4**−**7**). This material is available free of charge via the Internet at http://pubs.acs.org.

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