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5-Cyanoamino-4-imidazolecarboxamide and Nitrosative Guanine Deamination: Experimental Evidence for Pyrimidine Ring-Opening during Deamination

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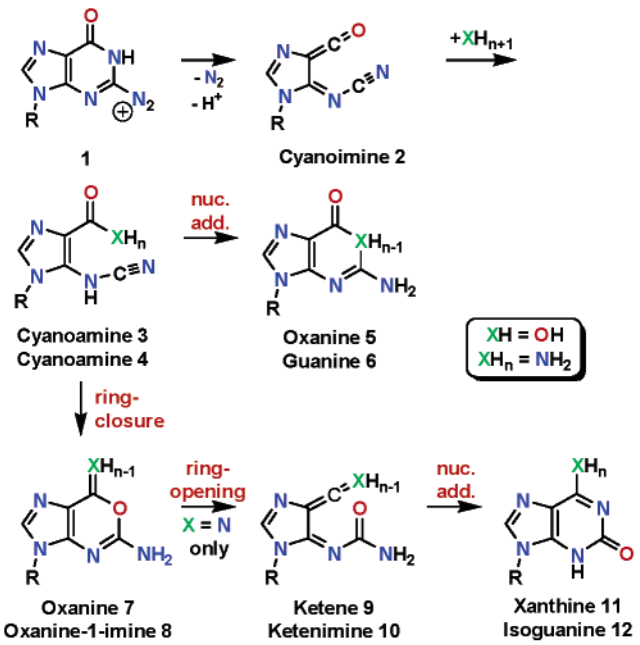
Nitrosative guanine deamination via guanine diazonium ion **1** to xanthine was discovered by Strecker in 1861[1] before Kossel identified guanine as a DNA base in 1884.[2] A century later this chemistry became a central topic in toxicology,[3] and it is now well understood that endogenous nitrosation[4] occurs and that dG-to-dG and dG-to-dA cross-links also are formed.[5] Guanine deamination chemistry became interesting once again with the 1996 discovery by Suzuki, et al. that deamination may form oxanine.[6−8] We proposed a reaction mechanism that involves dediazoniation of **1** with concomitant pyrimidine ring-opening[9] and deprotonation[10] leading to intermediate **2**[11] (Scheme 1). It is our hypothesis that the highly reactive dihydroimidazole **2** adds water to form the cyanoamine **3** which then cyclizes to oxanine **5**. The cyanoimine **2** also may add water to cyanoimine instead of the ketene moiety, and the resulting ketene might then ring-close to xanthine. Our published theoretical predictions are now being tested experimentally, and as a first step, we targeted the amide **4**[12] for one practical and one conceptual reason (vide infra).

Here, we report the synthesis of **4a** and its recyclization to guanine **6a** and isoguanine **12a**. Importantly, we were able to purify **4a** so that we can firmly establish that **4a** is the precursor for the formations of **6a** and **12a**. We employed the R-group $CH_2-O-CH_2-CH_2-OH$ as a sugar model. This (2-hydroxyethoxy)methyl group occurs in acyclovir, and we indicate this R-group by adding "**a**" to the compound numbers.

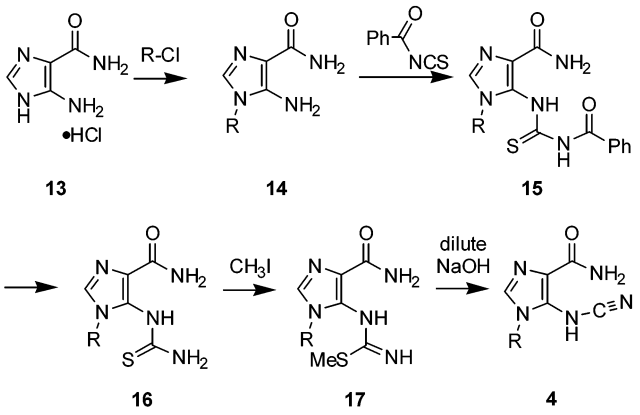
The synthesis of **4a** is outlined in Scheme 2. Compound **16** was synthesized with the efficient method by Clausen[13] via alkylation of **13**, condensation of **14** with benzoyl isothiocyanate, and hydrolysis of **15**. The methylation of **16** in basic aqueous solution gave low yields[14] even though this procedure works well for the related ester.[15] We found the methylation of **16** in the mixed solvent 5/1 acetone/methanol to give **17** in good yield.

Cyanoamine **4a** was obtained by methylthioether elimination from **17a**. Yamazaki, Okutsu, and Yamada[16] studied the base-catalyzed methylthioether elimination from **17** (R = H, **17h**) leading to guanine **6** and isoguanine **12** via cyanoamine **4** (R = H, **4h**). The unstable cyanoamine **4h** was not isolated and was characterized only by its IR spectrum (ν(C≡N) = 2190 cm^{-1}). We have now succeeded in the preparation of the cyanoamine **4a** and the isolation of this reactive intermediate. Reactions to form **4a** were quenched in liquid nitrogen after 3 min, and after HPLC separation and lyophilization, **4a** was stored at −18 °C to prevent cyclization. The cyanoamine **4a** was characterized by mass spectrometry (MS, MS/MS, HRMS), by IR spectroscopy (ν(C≡N) = 2138.6 cm^{-1}), and by ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum (Figure 1) demonstrates the purity of the synthetic **4a**. The cyanoamine **4a** may exist in equilibrium with the carbodiimide. Ab initio studies of the parent cyanamide and carbodiimide show a preference of about 7 kcal/mol for the cyanamide,[17] and our IR data also show that the observed tautomer is the cyanoamine **4a**. The practical reason for the choice of **4** is now clear. While **3** cannot be synthesized starting with the acid analogue of **13**, with the experience of the synthesis of **4** it appears possible to prepare **3** starting with an ester of **13**.

Scheme 1



Scheme 2



The cyclization of **4a** was studied at room temperature in 0.2M K_2HPO_4/KH_2PO_4 buffer solution (pH = 6.0, 7.0, 8.0, 9.0). Figure 2 shows the HPLC chromatograms recorded at pH = 7.0. There are two products, the guanine **6a** and the isoguanine **12a**, and they were identified by comparison to authentic samples. **6a** is commercially available, and **12a** was synthesized with an adaptation

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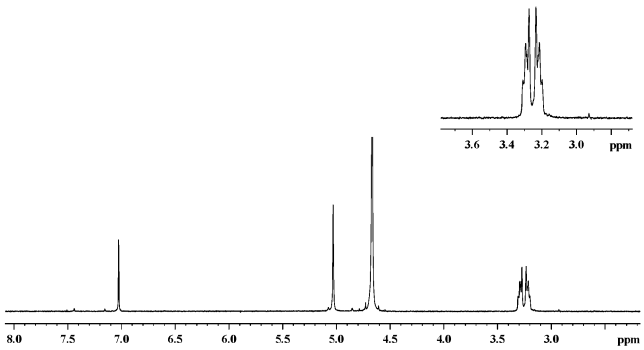


Figure 1. ^{1}H NMR spectrum (250 MHz, D_2O, 0.05 *N* NaOD at 4 °C) of **4a** shows signals at δ 7.0 (s, 1H, C_2-H), 5.0 (s, 2H, N1-CH_2), 3.1−3.3 (m, 4H, -CH_2-CH_2-) and establishes the purity of **4a**.

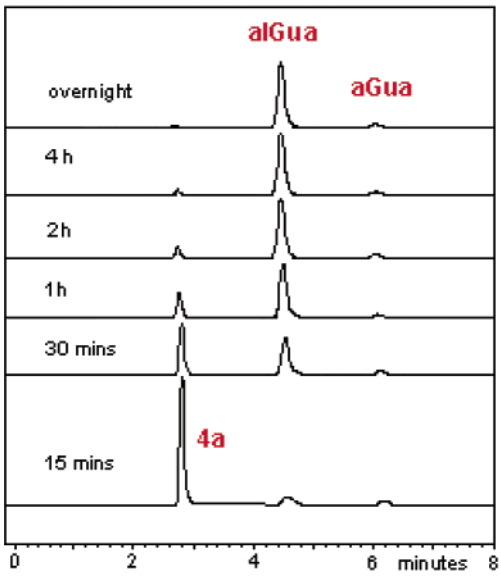
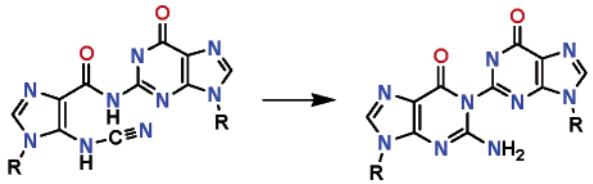


Figure 2. HPLC chromatograms of the cyclization of cyanoamine **4a** in buffer solution at pH 7 show formation of **6a** (**aGua**) and **12a** (**aIGua**). Final yields are 74.2% **12a** and 5.8% **6a** with 4.3% **4a** remaining.

of the synthesis by Chern, et al.[18] The formation of **6a** from **4a** is a nucleophilic addition of the weakly nucleophilic amide-amino group to the cyanoamine, and this reaction most likely is acid-catalyzed. The formation of **12a** is less obvious and does *not* involve the hydrolysis of **4** with subsequent condensation of the urea-amino group with the amide. Instead, Yamazaki, et al.[16] proposed a mechanism (see SI for details) that involves initial cyclization of the conjugate base of **4**. This mechanism accounts for the fact that the amide-O of **4** becomes the carbonyl-O of **12**.[19] We found that **12a** forms faster at lower pH, and we propose a mechanism (see SI) for its formation that does not require the initial deprotonation and that retains all other essential features to proceed via **8** and **10** (Scheme 1). This mechanism involves electrocyclic cyclization, tautomerization, and electrocyclic ring-opening to achieve the O-transfer, and this mechanism also might operate (in addition) at higher pH values.

We have achieved the synthesis of the pure cyanoamine **4a**. With the availability of pure **4a**, we were able to study its cyclization chemistry. The results of this study have several important implications for nitrosative guanine deamination. (1) The demonstrated formation of **6a** from **4a** is the model reaction for the

Scheme 3



formation of oxanine **5** from **3**. (2) Interestingly, the formation of **12a** and the explanation of its formation suggests that there is a second pathway to oxanine via an electrocyclic reaction (**3** to **7**, carbonyl-O as nucleophile), and for X = O, oxanine is the final product of that path because it is known that xanthine **11** does not form from oxanine **7** via rearrangement.[6] (3) Finally, the conceptual reason for the choice of **4** is as follows: Any potentially formed **2** in nitrosative DNA deamination might add to the amino group of a proximate DNA base, and this addition would lead to *N*-substituted derivatives of **4**, as exemplified in Scheme 3. We have synthesized the classical G-to-G cross-link *and its structure-isomer* **19** by addition of **4a** to guanosine.[20] The reaction of **4a** to **6a** strongly suggests that **18** would cyclize to **19** and provide a second path to this cross-link.

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Supporting Information Available: Details for the syntheses of **17**, **4a**, and **12a**; ^{1}H and ^{13}C NMR spectra of **17**, **4a**, and **12a**; the IR spectrum of **4a**; and HPLC chromatograms of the pH-dependent cyclization of **4a** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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