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Chemical carcinogens in non-enzymatic cytosine deamination: 3-isocyanatoacrylonitrile

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Abstract Uracil has long been known as the main product of nitrosative cytosine deamination in aqueous solution. Recent mechanistic studies of cytosinediazonium ion suggest that the cation formed by its dediazoniation can ring-open to N-protonated (*Z*,*s-cis*)-3-isocyanatoacrylonitrile **7**. Stereochemical preferences are discussed of the 3-isocyanatoacrylonitriles (*Z*,*s-cis*)-**10**, (*E*,*s-cis*)-**11**, (*Z*,*s-trans*)-**12**, and (*E*,*s-trans*)-**13**. The electronic structures of **7** and **10**–**13** have been analyzed and a rationale is provided for the thermodynamic preference for (*Z*,*s-cis*)-**10**. It is shown that *s-cis*/*s-trans*-interconversion occurs *via* C–N rotation–inversion paths with barriers below 3 kcal mol^{-1}. The proton affinities of 3-isocyanatoacrylonitrile **10** and water are nearly identical and, thus, 3-isocyanatoacrylonitriles can and should be formed in aqueous media from **7** along with 3-aminoacrylonitriles **9**. The results highlight the relevance of the chemistry of 3-isocyanatoacrylonitriles for the understanding of the chemical toxicology of nitrosation of the nucleobase cytosine.

Keywords Isocyanate · Acrylonitrile · Rotational barrier · Inversion · Ab initio · Non-covalent bonding · Electronic structure · Chemical toxicology · Carcinogenesis

Introduction

DNA cytosine methyltransferases [1] deaminate cytosine (C, **1**) to uracil (U, **3**), and cytosine deamination also can be effected by nitrosation [2, 3]. C-to-U damage can be repaired *via* enzymatic base excision [4] and, if left unrepaired, causes the G:C→A:T mutation [5, 6]. The nitrosative C-to-U process had been thought to occur *via* the transient cytosinediazonium ion **2** and its hydrolysis by direct nucleophilic aromatic substitution (Fig. 1), which is very much S_N1Ar-like [7]. However, theoretical study of the unimolecular dediazonation of cytosinediazonium ion [8] showed that the classical diazonium ion **2** (**5**←N≡N) is merely a shallow minimum and *less stable* than free **5** and N_2 and that an electrostatic complex **4** (**5**···N≡N) is bound by only 5.4 kcal mol^{-1}. The HO-tautomer of cytosinediazonium ion **2′** (not shown in Fig. 1) is 3.7 kcal mol^{-1} more stable than **2** itself. **2′** has a classical diazonium-ion structure and it is bound by 10.8 kcal mol^{-1} relative to **5′** and N_2. Hence, cytosine deamination essentially produces a free cation, **5** or **5′**. More recently, it has been shown that the ions **5** or **5′** are best described as cyclic nitrilium ions **6** and **6′** and that the dative bond easily breaks to form the more stable ring-opened cations **7** and **7′**. These results seriously put in question whether uracil is the only product [9] of nitrosative cytosine deamination.

If the *R*-group is ribose or 2′-deoxyribose, **6** and **7** are the appropriate models for the deamination of cytidine, its nucleotides CMP, CDP, and CTP, and their 2′-deoxy derivatives. Ubiquitous water may react with **7** by addition, substitution (*R*≠H), or deprotonation (*R*=H). Water addition forms of carbamic acid [10] and subsequent decarboxylation [11] leads to (*Z*)-3-aminoacrylonitrile **9**. (*Z*)-3-aminoacrylonitrile is susceptible to base- [12, 13] and acid-catalyzed [14] nucleophilic addition to the C=C and C≡N bonds and this chemistry also might lead to DNA adducts. Alternatively, deglycation by substitution (*R*≠H) or deprotonation (*R*=H) would lead to (*Z*)-3-isocyanatoacrylonitrile **10**. Unsaturated isocyanates are toxic [15] and 3-isocyanatoacrylonitrile can form adducts and cross-links [16, 17].

Here, we report the results of a study of the formation of **10** by deprotonation of **7** and **7′**, of the *E*/*Z*-preferences of 3-isocyanatoacrylonitrile **10** and **11** and of their conformers **12** and **13**, and of C–N rotation–inversion in both *E*/*Z*-isomers *via* **TS1** and **TS3** (Fig. 2).

Dedicated to professor Dr. Paul von Ragué Schleyer on the occasion of his 75th birthday

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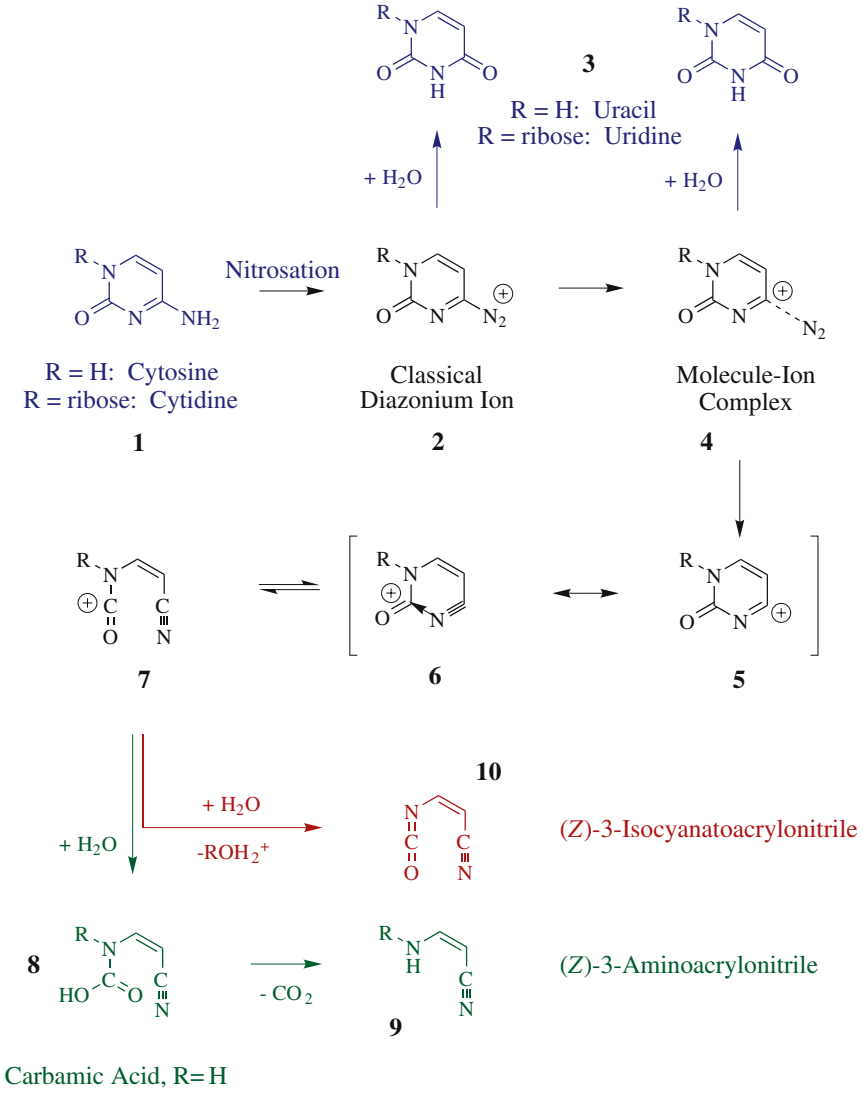

Fig. 1 Nitrosative deamination of cytosine and cytidine

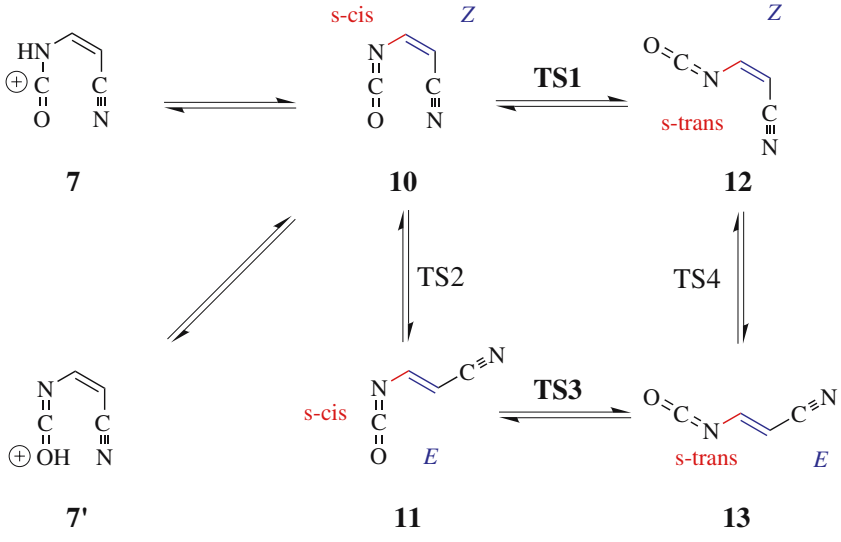

Fig. 2 Scope of the present study of 3-isocyanatoacrylonitrile

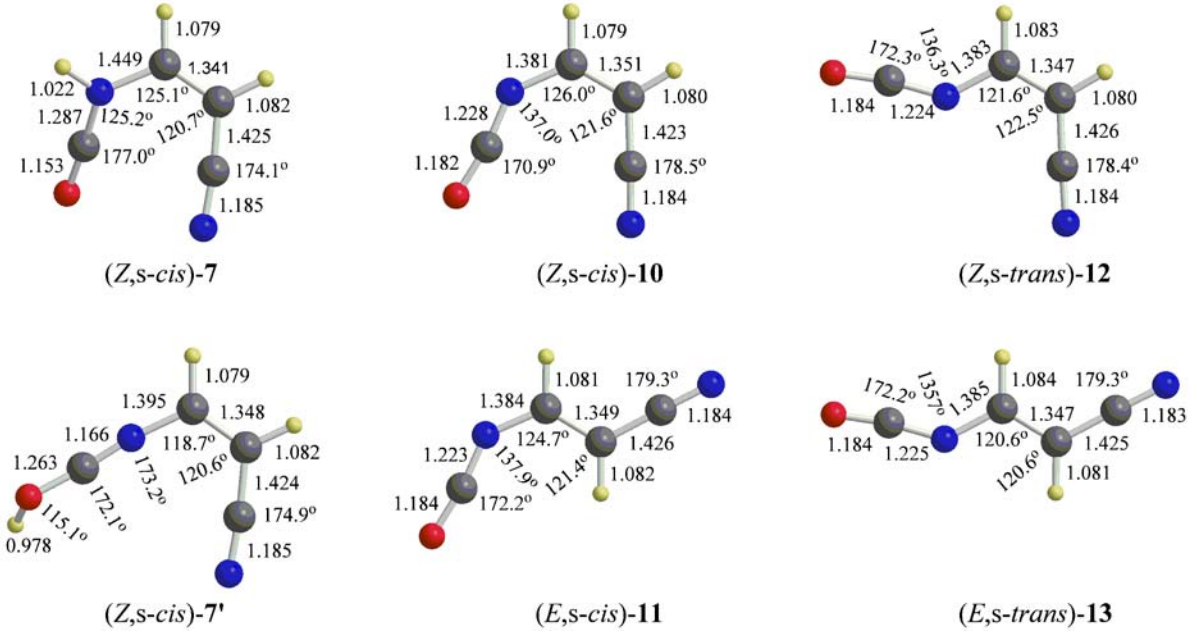

Fig. 3 MP2(full)/6-31G** structures of **7**–**13**

Computational methods

Geometry optimizations and vibrational analyses were carried out at the MP2(full)/6-31G** level [18] with the program *Gaussian03* [19]. We employed perturbation theory deliberately because it is preferable to (hybrid) density functional theory for the present purpose. Structure (*Z,s-cis*)-**10** benefits from through-space interaction and such dispersion interactions are not properly accounted for by the standard DFT models [20]. Atomic charges were calculated with the natural bond orbital (NBO) method [21, 22] at the same level. Molecular models of the minima and major structural data and atom and fragment charges are reported in Figs. 3 and 4, respectively. Molecular models of the transition-state structures are shown in Fig. 5. Coordinates of all stationary structures are available as Supporting Information.

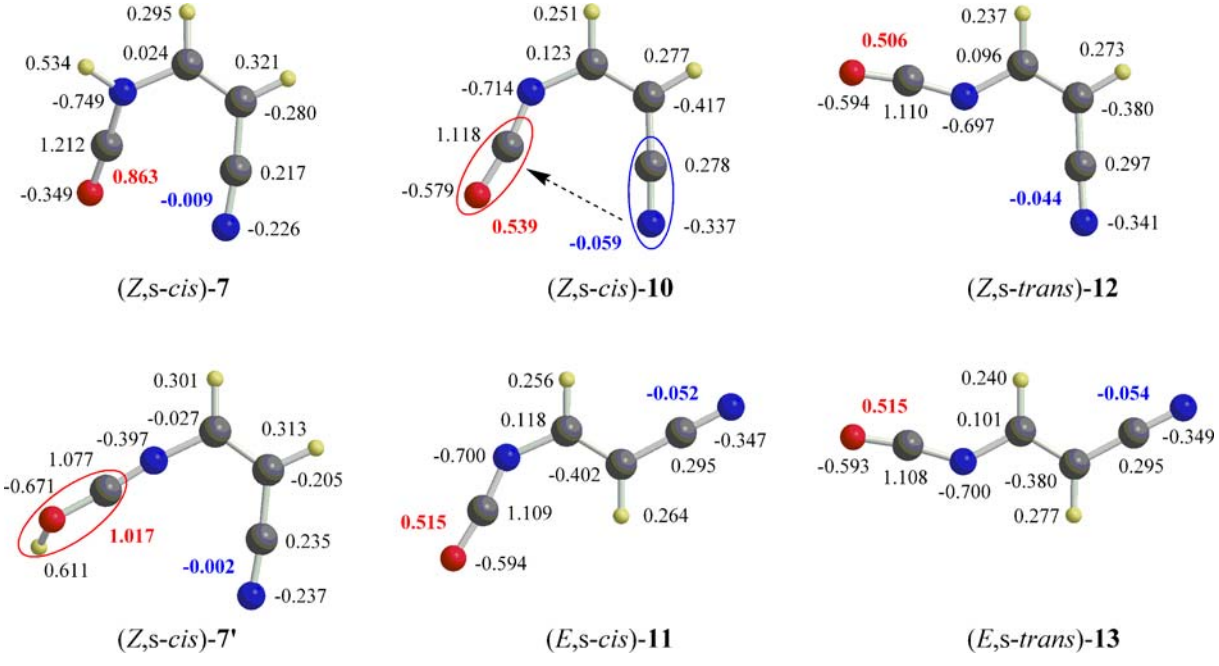

Fig. 4 NBO analysis of **7**–**13**. Numbers printed in bold are fragment charges

Fig. 5 Molecular models of the MP2(full)/6-31G** structures of TS1 and TS3

Total energies *E* (in Hartrees), vibrational zero-point energies *VZPE* (kcal mol^{-1}), thermal energies *TE* (kcal mol^{-1}, 298.15 K), and entropies *S* (cal mol^{-1} K^{-1}) are reported in Table 1. In Tables 2 and 3 are reported the values ΔE, $\Delta E_0 = \Delta E + \Delta VZPE$, $\Delta H_{298} = \Delta E + \Delta TE + \Delta RT$, and $\Delta G_{298} = \Delta H_{298} - 0.29815 \cdot \Delta S$. The discussion refers to Gibbs free energies unless otherwise noted.

Results and discussion

Stereochemical preferences Studies of α,β-unsaturated isocyanates are scarce [23, 24] and the stereochemistry of vinyl isocyanates with additional functionality remains unexplored. Structures **10**-**13** (Fig. 3) are almost isoenergetic (Table 2). (*Z*,*s-cis*)-**10** is the most stable isocyanatoacrylonitrile and the relative energies of isomers and conformers only are 0.1–0.9 kcal mol^{-1}. *Z*-preferences occur for the *s-cis*- and *s-trans*-conformers and they are higher for the *s-cis*-structures. If steric interactions dominated, one would expect preferences for the *E*-configuration and the *s-trans* conformation; (*E*,*s-trans*)-**13**. The calculated relative energies of 0.87 kcal mol^{-1} for (*E*,*s-cis*)-**11** *vs.* (*E*,*s-trans*)-**13** and of 0.28 kcal mol^{-1} for (*Z*,*s-trans*)-**11** *vs.* (*E*,s-*trans*)-**13** are in line with expectation based on steric demand. Assuming additivity, one would thus estimate an energy of about 1.15 kcal mol^{-1} for (*Z*,*s-cis*)-**10** relative to (*E*,*s-trans*)-**13** for steric reasons. Yet, (*Z*,*s-cis*)-**10** is 0.05 kcal mol^{-1}*more stable* than (*E*,*s-trans*)-**13** and, hence, **10** benefits from through-space neighboring group attraction of about 1.2 kcal mol^{-1}.

Electronic structures and through-space interactions The NBO analysis shows highly polar electronic structures and the main effect of the isocyanato group on the acrylonitrile [14] is an increase of the electron-deficiency of the CH group to which it is attached (Fig. 4). The CN groups in **10**–**13** are highly dipolar but their overall charge is very small. On the other hand, the NCO groups in **10**–**13** are charged significantly, they carry charges of about −0.2, and they are extremely quadrupolar [25]. These findings suggest that the attractive neighboring-group interaction in **10** is due in part to polarization of the cyano group in the field of the overall charge of the isocyanato group and in part to dipole–dipole attraction. The dipole-dipole attraction between the CN bond dipoles in the NCO and CN groups appears to dominate the repulsion between CO bond dipole of the NCO group and the CN group dipole. The NBO charge analysis thus provides a consistent rationale based on simple and widely accepted concepts of interaction and reactivity. The proposed interactions can be quantified *via* fragment-interaction analysis [26, 27] and recent progress in the development of orbital deletion analysis [22, 28] also might provide for interesting tests of the proposed rationale and any possible hyperconjugation.

The major effect of the protonation of the NCO group is a withdrawal of electron density from the alkene fragment; in the cations the HNCO and alkene fragments carry charges of about 0.6 and 0.4, respectively. Note that the protonation of either one of the heteroatoms of the NCO group does very little to increase the electrophilicity of the heterocumulene's center. Instead, there is a rearrangement of electron density from one heteroatom to the protonated one.

Table 2 Relative energies, *trans*-preferences, *Z*-preferences and proton affinities (kcal mol^{-1})

Parameter	ΔE	ΔE_0	ΔH_{298}	ΔG_{298}
(*Z*,*s-trans*)-**12** *vs.* (*Z*,*s-cis*)-**10**	0.59	0.46	0.51	0.33
(*E*,*s-cis*)-**11** *vs.* (*Z*,*s-cis*)-**10**	1.11	1.00	1.10	0.92
(*E*,*s-trans*)-**13** *vs.* (*Z*,*s-cis*)-**10**	0.24	0.01	0.14	0.05
s-Trans-preference of *Z*-isomers	−0.59	−0.46	−0.51	−0.33
s-Trans-preference of *E*-isomers	0.87	0.99	0.96	0.87
Z-preference of *s-cis*-conformers	1.11	1.00	1.10	0.92
Z-preference of *s-trans*-conformers	0.35	0.45	0.37	0.28
PA(**10** → **7**)	179.84	172.59	173.27	166.41
PA(**10** → **7′**)	168.28	161.88	162.18	156.70

Table 1 Total energies and thermodynamical data

Molecule	Sym.	*E*	*VZPE*	TE	*S*	N
(*Z*,*s-cis*)-**7**	C_1	−337.692206	41.98	46.29	82.39	0
(*Z*,*s-cis*)-**7′**	C_1	−337.673798	41.14	45.82	85.89	0
(*Z*,*s-cis*)-**10**	C_s	−337.405620	34.73	38.83	81.51	0
(*Z*,*s-trans*)-**12**	C_s	−337.404675	34.60	38.74	82.10	0
(*E*,*s-cis*)-**11**	C_s	−337.403858	34.63	38.83	82.13	0
(*E*,*s-trans*)-**13**	C_s	−337.405230	34.49	38.73	81.82	0
(***Z***)-**TS1**	C_1	−337.401737	34.47	38.15	78.29	1
	C_s	−337.400516	34.31	37.61	75.62	2
(***E***)-**TS3**	C_1	−337.401528	34.35	38.15	78.86	1
	C_s	−337.400358	34.20	37.60	75.15	2

Table 3 Activation barriers for C–N rotation–inversion (kcal mol^{-1})

Process	ΔE	ΔE_0	ΔH_{298}	ΔG_{298}
(*Z*,*s-cis*)-**10** → (*Z*,*s-trans*)-**12**	2.44	2.17	1.72	2.72
	1.84	1.71	1.25	2.39
(*E*,*s-cis*)-**11** → (*E*,*s-trans*)-**13**	1.46	1.18	0.78	1.75
	2.32	2.18	1.74	2.62

Rotation-Inversion A priori the *s-cis*/*s-trans* interconversion might involve in-plane N-inversion or rotation about the OCN–CH bond. The N-inversion would involve an N-hybridization change from sp^2 to sp and shorten both bonds to N. Hybridization changes would be small along the rotation path and there would be some loss of π-conjugation.

The ideal inversion process would involve a planar transition-state structure. We searched for these transition-state structures under the constraint to C_s-symmetry, and found the resulting structures C_s-**TS1** (***i***118, ***i***101 cm^{-1}) and C_s-**TS3** (***i***119, ***i***81 cm^{-1}) to be second-order saddle points on the potential-energy surface. The true transition-state structures **TS1** and **TS3** were located (Fig. 5) and the activation barriers are less than 3 kcal mol^{-1} (Table 3).

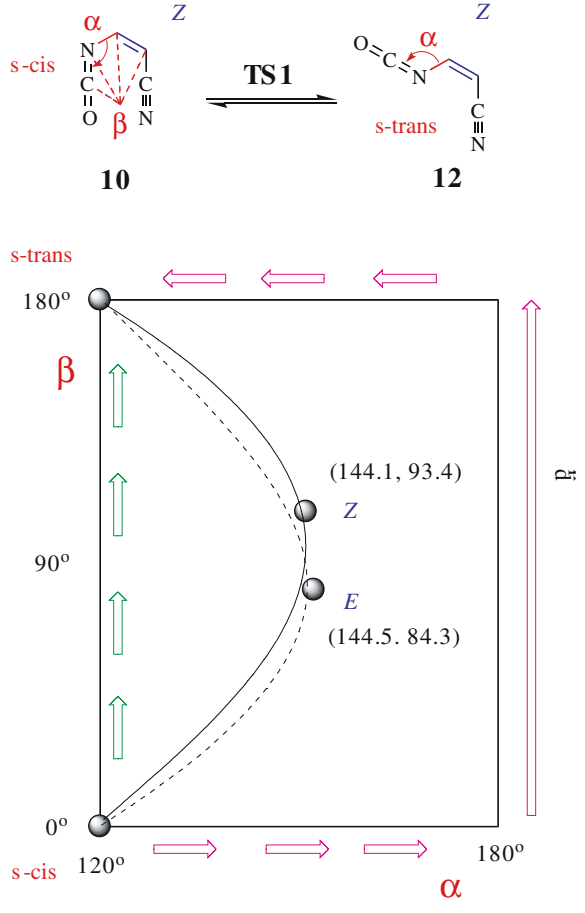

Fig. 6 Illustration of the rotation–inversion paths and the position of the transition state structures in the (α, β)-map. The "pure" rotation and inversion paths follow the *green* and *purple arrows*, respectively. (*Horizontal axis* is not drawn to linear scale)

The ∠(C–C–N–C) dihedral angles β in **TS1** and **TS3** are 93.4° and 84.3°, respectively, and close to 90°. However, the ∠(C–N–C) angles α in **TS1** and **TS3** are 144.1° and 144.5°, respectively, and it is clear that these are not the structures of "pure" rotational transition states.

The rotation–inversion paths are illustrated schematically in Fig. 6. The "pure" rotation would leave $\alpha \approx 120°$ relatively unchanged as the rotation proceeds from β=0° to β=180°. The "pure" inversion would change α from $\alpha \approx 120°$ to $\alpha \approx 180°$ while β=0° and then from $\alpha \approx 180°$ to $\alpha \approx 120°$ while β=180°. The change in β at α=180° causes the discontinuity on the right in Fig. 6. In the present case, neither of these ideal paths exists and rotation–inversion paths occur instead.

Proton affinities Isocyanate **10** shows a pronounced preference of about 10 kcal mol^{-1} for protonation at N rather than at O (Table 2). The proton affinity for N-protonation is 166.4 kcal mol^{-1} and very close to the proton affinity of water, which is about 167 kcal mol^{-1} [29]. Given that **7** is formed in aqueous solution, this result provides strong evidence that the reaction H_2O + **7** ⇄ **10** + H_3O^+ occurs under typical conditions of nitrosative deamination.

Conclusions

Pyrimidine ring-opening in nitrosative deamination of guanine, guanosine, and its nucleotide derivatives has been well established by theoretical study [20, 30, 31] and experimentation [32, 33]. The unimolecular dediazoniations of adeninediazonium and cytosinediazonium ions can proceed without ring-opening and it was realized only more recently that the cations which are produced by dediazoniation of adeninediazonium ion [34] and cytosinediazonium ion [9] can ring-open with very little kinetic hindrance.

The proton affinity of **10** suggests that **10** and its isomers and conformers are formed in nitrosative cytosine deamination in aqueous solution. The possibility for recyclization of **10** to uracils has been explored experimentally and it is not likely [35]. Thus, toxicological studies of nitrosative cytosine deamination need to direct attention at 3-aminoacrylonitrile **9** and 3-isocyanatoacrylonitrile **10**. It remains to be seen whether **10** can be formed by hydrolytic deglycation from the respective cytidine derivatives.

Once **10** is formed, it can react with water to carbamic acid **8**, with alcohols to carbamates **14**, with amines to ureas **15**, and with thiols to carbamothioates **16**, etc. as shown in Fig. 7 for conformer **12** [36]. Note that this is a *second* path to **8** and on to **9**, and this path differs from the sequence **7** → **8** → **9** in one very important way: **10** has some lifetime and **8** and **9** would be formed at some distance from the site of the deamination event while **7** survives merely until a nucleophile diffuses to it (usually water). The 3-isocyanatoacrylonitrile thus has the potential to cause biological damage in a larger region around the site of nitrosation and this neutral carcinogen can be much

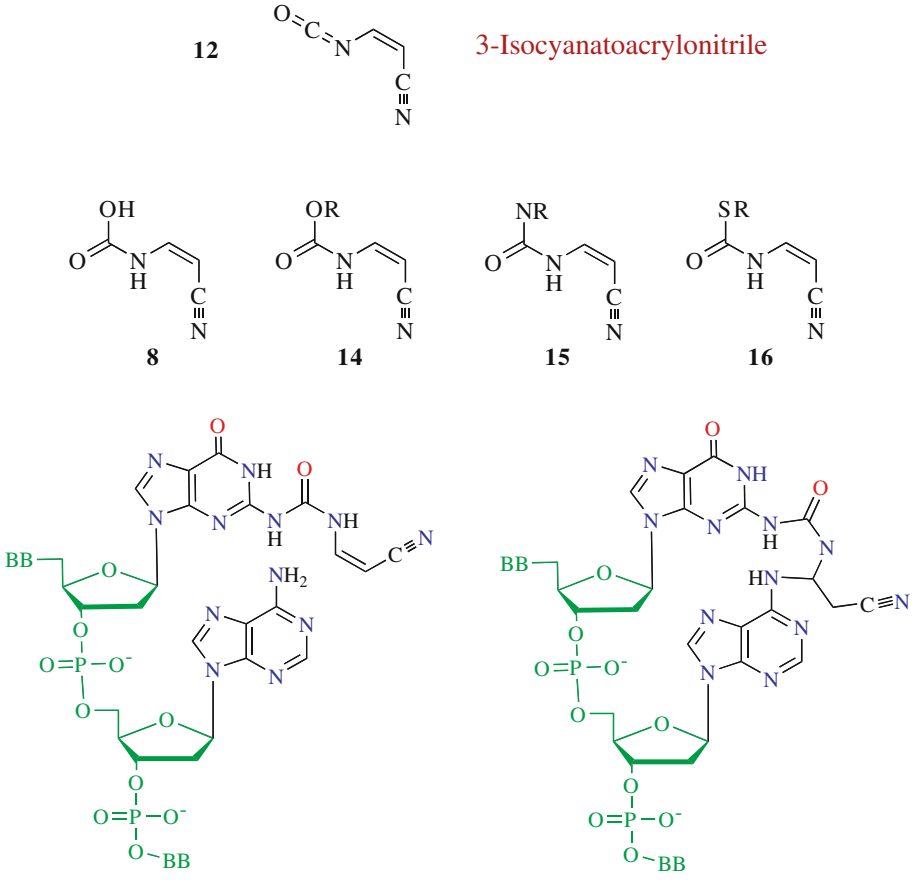

Fig. 7 Possible products of nucleophilic addition to **12** and examples for adduct and cross-link formation

more selective in its reactions. In addition to the types of isocyanate adducts shown in Fig. 7 (and their isomers and conformers), there also exist further possibilities for additions to the acrylonitrile moiety [14], either alternatively or subsequently. Fig. 7 provides an example for a urea adduct formation with dG and subsequent intrastrand dG-to-dA cross-link formation. The exploration of the chemistry of 3-isocyanatoacrylonitrile promises to be interesting and complicated.

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