Communications

Amino Effect on the Protonation of β-Aminoacrylonitrile

Hong Wu and Rainer Glaser*

Department of Chemistry, University of Missouri−Columbia, Columbia, Missouri 65211

Received August 5, 2004

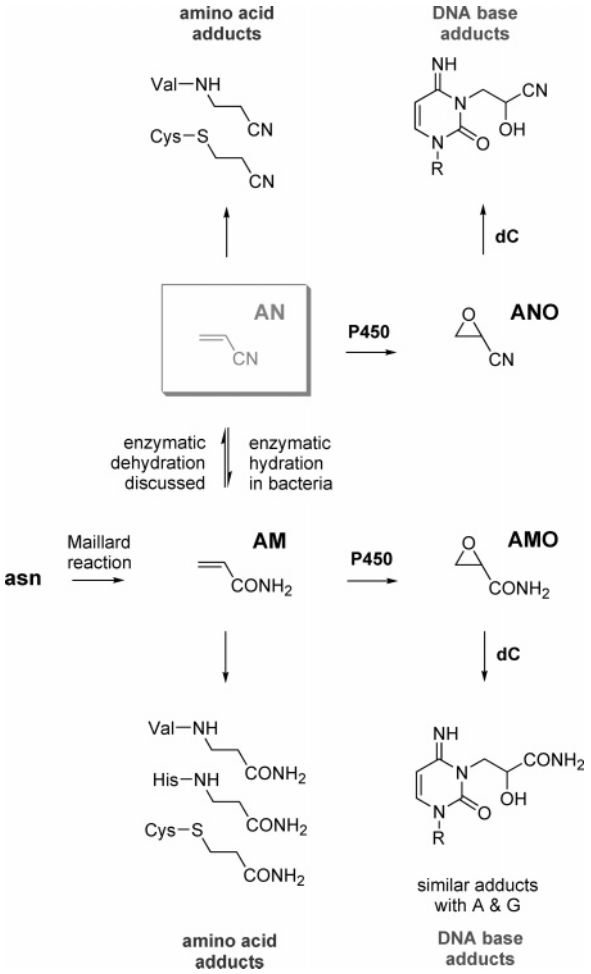
The protonation of acrylonitrile (**1**) and of *Z*- and *E*-3-aminoacrylonitrile (**5** and **9**) was studied with the Gaussian-2 method. Ammoniumacrylonitrile ion formation is not important. Nitrilium ion formation is preferred in all cases, and the proton affinities are about 80 kJ/mol higher for aminoacrylonitrile. Remarkably, it is found that C2-protonation of 3-aminoacrylonitriles to form iminium ions can compete with nitrilium ion formation. β-Aminoacrylonitriles thus show propinquity to both acrylonitriles and enamines, and mechanistic and toxicological implications are discussed.

Introduction

The toxicities of acrylonitrile (AN) and of acrylamide (AM) are documented in the NIOSH registry (*1*); both alkenes are carcinogens in animal models while there is inadequate evidence for their carcinogenicity in humans (*2*). AN and AM are group 2B and 2A carcinogens, respectively, and AN is possibly and AM is probably carcinogenic in humans. AN forms 2-cyanoethyl adducts with amino acids, such as valine (*3*), and AN adducts with hemoglobin are well-explored (*4*). Cysteine derivatives react with AN to form thioethers RS−CH_2−CH_2−CN (*5*). DNA damage occurs via cytochrome P450 (*6*) oxidation of AN to acrylonitrile epoxide (*7*), which forms adenine N1− and cytosine N3−CH_2−CH(OH)CN and guanine N7−CH_2−CHO adducts (*8*). The chemistry of AM is similar and was reviewed recently (*9*). AM has been in the public eye because of its occurrence in fried foods (*10*). AM has been discussed as a precursor to AN, but clear in vivo evidence is lacking (*11*).

In the course of our studies on DNA base deamination (*12*), we argued for the formation of 3-aminoacrylonitrile in nitrosative cytosine deamination (*12a*) by way of nitrosation, dediazoniation, pyrimidine ring opening, hydration, and decarboxylation as outlined in Scheme 2. The chemistry of β-aminoacrylonitriles is employed for the synthesis of a wide variety of heterocyclic compounds (*13*, *14*). Yet, despite this biomedical and industrial importance, the reaction mechanisms have not been studied in detail and the toxicology of β-aminoacrylonitriles is not known. Here, we report on the regiopreferences for the protonation of AN and 3-amino-AN. This information is essential to compare the reactivities and to clarify the mode of the addition to the alkene. While C2-protonation has been discussed for some β-alkylamino-β-phenylacrylonitriles (*15*), a study of β,β-bis(dialkylamino)-α-phenylacrylonitriles (*16*) provided evidence for nitrilium ion formation.

Scheme 1. Chemical Toxicology of AN and AM



Materials and Methods

The proton affinity is the negative enthalpy of the process B + H^+ → BH^+, and its determination requires the knowledge of the enthalpies *H* of B and BH^+. The Gaussian-2 theoretical

* To whom correspondence should be addressed. E-mail: glaserr@missouri.edu.

10.1021/tx049784a CCC: $30.25
Published on Web 01/06/2005

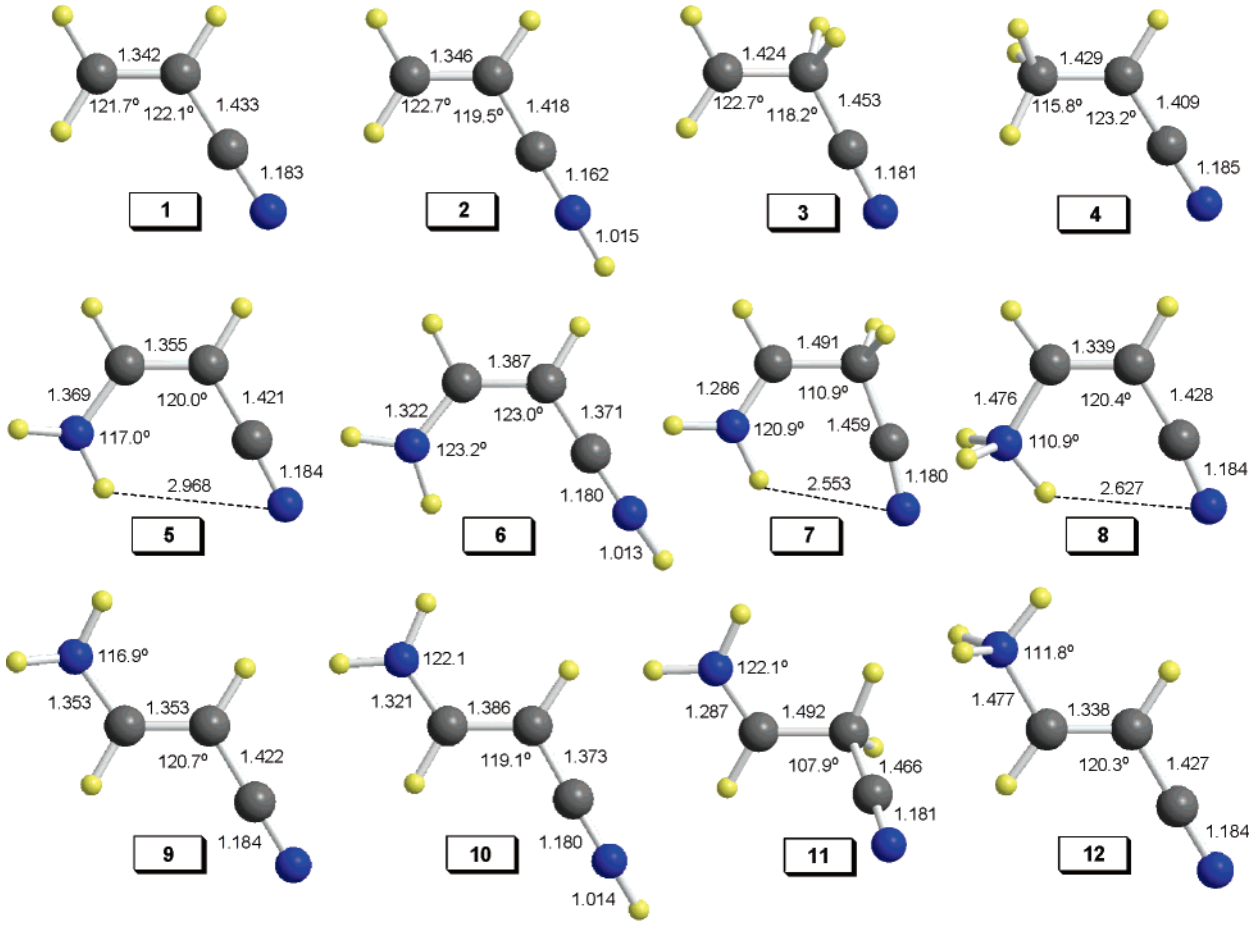
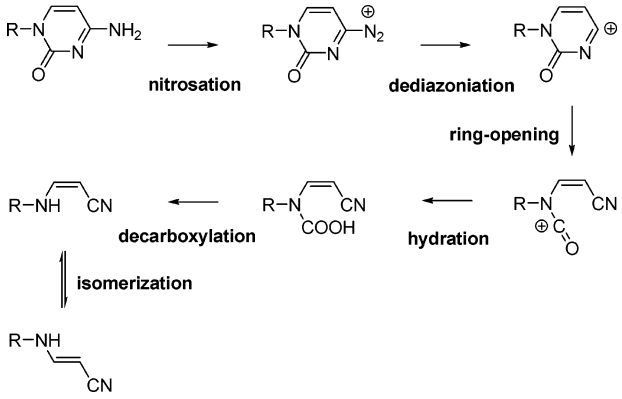


Figure 1. Molecular models of the MP2(full)/6-31G* optimized structures.

Scheme 2. Postulated Production of *β*-Aminoacrylonitrile in Nitrosative Cytosine Deamination



model reproduces proton affinities consistently within 10 kJ/mol of experimental values (*17*), and we computed the enthalpies with this G2 method. The MP2(full)/6-31G* structures of **1**−**12** (Scheme 3) are shown in Figure 1, and *Z*-preference energies and proton affinities (H_{298}) are reported in Tables 1 and 2.

Results

In addition to the protonation of **1** to form the nitrilium ion **2** (*18*), we also considered the formations of the C2-ium ion (*19*) **3** and of the C3-ium ion **4**, respectively (*20*). The computed PA = 784 kJ/mol of **2** agrees well with the experimental value (794 kJ/mol) (*21*). C2-Protonation to **3** is greatly disadvantaged by 243 kJ/mol, and even the proton affinity for C3-protonation to **4** is still 177.38 kJ/mol lower than that of N-protonation (Table 2).

Scheme 3. AN (1), *Z*-3-Aminoacrylonitrile (5), and *E*-3-Aminoacrylonitrile (9) and Protonated Derivatives

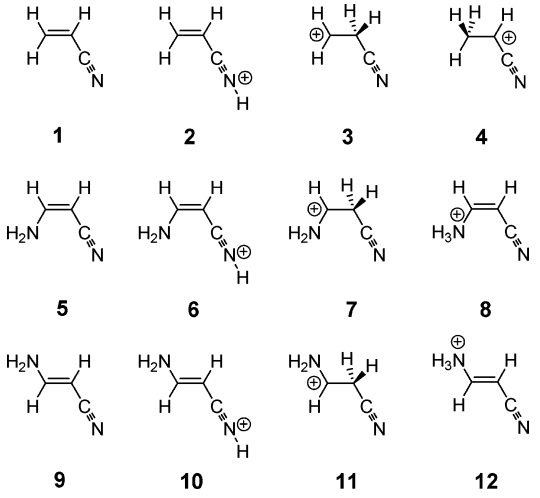


Table 1. *Z*-Preference Energies (kJ/mol)

	E_0	H_{298}	G_{298}
***E*-9 vs *Z*-5**	7.96	8.28	8.35
***E*-10 vs *Z*-6**	−12.56	−12.26	−12.33
***E*-11 vs *Z*-7**	20.76	21.26	19.09
***E*-12 vs *Z*-8**	17.74	18.23	17.92

The *Z*-isomer **5** of 3-amino-AN is more stable than the *E*-isomer **9**, with a computed *Z*-preference energy of ΔH_{298} = 8.3 kJ/mol (Table 1), and this preference has been attributed by intramolecular H-bonding in the *Z*-isomer

Table 2. Proton Affinities[a] (kJ/mol)

reaction	PA	ΔPA[b]	reaction	PA	ΔPA[b]
1 → **2** + H^+	783.99	0.00	**5** → **8** + H^+	796.44	−44.69
1 → **3** + H^+	540.98	−243.01	**9** → **10** + H^+	861.67	0.00
1 → **4** + H^+	606.61	−177.38	**9** → **11** + H^+	825.03	−36.63
5 → **6** + H^+	841.13	0.00	**9** → **12** + H^+	786.48	−75.18
5 → **7** + H^+	838.01	−3.12			

[a] PA = $-\Delta H = -[\Delta H_f(BH^+) - \Delta H_f(B) - \Delta H_f(H^+) + \Delta(pV)] = -[H(BH^+) - H(B)] - (5/2)RT$, where H is the enthalpy obtained from the calculation and $2.5 \cdot RT$ includes $\Delta(pV)$ for an ideal gas and the translational energy of the proton. [b] Relative data with respect to the preferred protonation of the same molecule or isomer.

(*22*). The synthesis of 3-amino-AN results in a 1:1 mixture of *Z*-**5** and *E*-**9** (for kinetic reasons), and the ratio increases during distillation (for thermodynamic reasons) until pure *Z*-**5** is obtained (*23*). Our own attempts to separate the isomers by alternative means also failed (*12a*), and this observation is consistent with the known facile isomerzation of push−pull alkenes (*24*).

For the *Z*- and *E*-isomers **5** and **9**, respectively, we considered N-protonation to **6** and **10**, C2-protonation to **7** and **11**, and the formations of the ammoniumacrylonitrile ions **8** and **12**, respectively (Scheme 3). The data in Tables 1 and 2 show that the ammoniumacrylonitrile ions **8** and **12** are not important and the discussion focuses on the N- and C2-protonation.

As with **1**, nitrilium ion formation is preferred. In fact, the proton affinity of 862 kJ/mol for the protonation of **9** to form **10** is 78 kJ/mol higher than for **1** and indicates a much higher reactivity of 3-amino-AN as compared to AN. N-protonation destroys the opportunity for H-bonding and reverses the isomer preference (Table 1) favoring the *E*-isomer **10** by 12.3 kJ/mol over *Z*-**6**.

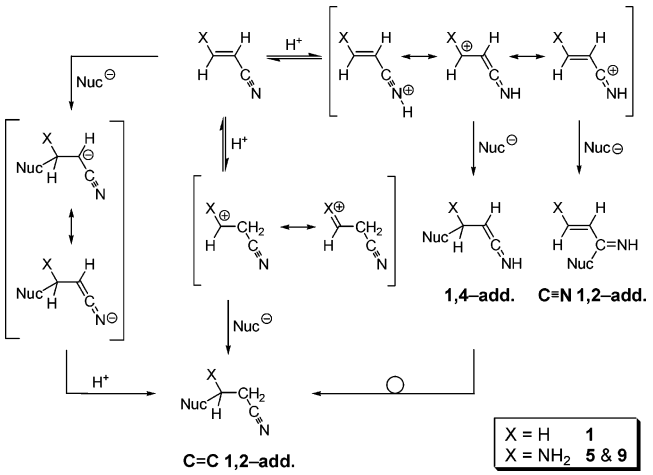
In contrast to **1**, C2-protonation leads to iminium ions **7** and **11** (see Scheme 3). The contribution of the iminium ion resonance structure is clearly manifested by the short C−N bond lengths of 1.29 Å in **7** and **11**. The proton affinity of vinylamine is about 920 kJ/mol (*25*), and it is much larger for the cyano-substituted derivatives. Nevertheless, the amino effect remains pronounced and allows C2-protonation to compete with nitrilium ion formation. The disadvantage of C2-protonation is diminished by 50% for the *E*-isomers; compare PA (**9**) to form **10** and **11**. For the *Z*-isomer, the disadvantage essentially vanishes and C2-protonation competes with N-protonation. Nevertheless, for *Z*-**5**, the proton affinity at C2 is only 3.1 kJ/mol lower than for the formation of the nitrilium ion **6**! Considering the preference of 8.3 kJ/mol for *Z*-**5** over *E*-**9**, the formation of the best protonated *E*-isomer **10** is only 12.2 kJ/mol preferred over the formation of the best protonated *Z*-isomer **6**.

While *Z*-**6** is favored over *E*-**10**, the other tautomers of protonated **5** maintain intramolecular H-bonding and, in fact, the neighbor group interaction is enhanced because the positive charge strengthens the H-bond and because of stabilization gained because of charge-induced polarization. Thus, the *Z*-preferences are 21.3 kJ/mol for the 2-ium ion and 18.2 kJ/mol for the ammonium ion (Table 1).

Discussion

An overview of addition chemistry to ANs is provided in Scheme 4. Base catalysis can lead to Michael additions via carbanions (*5*, *9*), enzymatic catalysis can lead to additions via keteneimides stabilized in an oxyanion hole (*26*), and acid catalysis is most common in the addition chemistry. Catalysis by Lewis acids affords C=C 1,2-addition (*27*), and we are interested in the catalysis by protonation. The results show that AN and 3-amino-AN differ not merely quantitatively but there are significant qualitative differences. An addition to the alkene moiety of AN proceeds by 1,4-addition to form the keteneimine and its subsequent tautomerization. This analogous path remains possible for the 3-amino-substituted derivative. Yet, instead of reacting just like AN, 3-amino-AN also can behave like an enamine by way of C2-protonation and direct C=C 1,2-addition. No matter which path is taken, the magnitudes of the proton affinities demonstrate that 3-amino-AN will be more reactive than AN.

Scheme 4. Mechanisms of 1,2- and 1,4-Additions



Our results imply that 3-amino-AN could form protein adducts in analogy to AN and AM. In addition, the higher reactivity might allow adduct formation with the nucleobases C, A, and G without prior epoxidation. Because of the propinquity of 3-aminoacrylonitrile with ANs as well as enamines, the toxicology of 3-aminoacrylonitriles might parallel the known toxicology of other iminium ion progenitors (*28*). Hence, it is prudent to consider 3-amino-AN as a potential carcinogen and the appropriate care should be exercised when handling this compound.

Acknowledgment. This work was supported by the NIH (GM61027).

Supporting Information Available: One table of total energies and thermochemical data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) National Institute for Occupational Safety and Health (2004) *The Registry of Toxic Effects of Chemical Substances*, National Institute for Occupational Safety and Health (NIOSH). (a) Acrylonitrile, AT5250000. (b) Acrylamide, AS3325000.

(2) International Agency for Research on Cancer (IARC) (1999) Acrylonitriles. *Summaries and Evaluations*, Vol. 71, pp 43−45, IARC, Lyon, France.

(3) Farmer, P. B., and Sweetman, G. M. A. (1995) Mass spectrometric detection of carcinogen adducts. *J. Mass Spectrom. 30*, 1369−1379.

(4) (a) Perez, H. L., Segerback, D., and Osterman-Golkar, S. (1999) Adducts of acrylnitrile with hemoglobin in nonsmokers and in participants in a smoking cessation program. *Chem. Res. Toxicol. 12*, 869−873. (b) Bergmark, E. (1997) Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem. Res. Toxicol. 10*, 78−84. (c) Calleman, C. J.,

Bergmark, E., and Costa, L. G. (1990) Acrylamide is metabolized to glycidamide in the rat: Evidence from hemoglobin adduct formation. *Chem. Res. Toxicol. 3*, 406−412.
(5) Friedman, M., and Wall, J. S. (1966) Additive linear free-energy relationships in reaction kinetics of amino groups with α,β-unsaturated compounds. *J. Org. Chem*. *31*, 2888−2894.
(6) (a) Sumner, S. C. J., Fennell, T. R., Moore, T. A., Chanas, B., Gonzalez, F., and Ghanayem, B. I. (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol*. *12*, 1110−1116. (b) Guengerich, F. P., Kim, D., and Iwasaki, M. (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol*. *4*, 168−179.
(7) (a) Kedderis, G. L., Batra, R., and Koop, D. R. (1993) Epoxidation of acrylonitrile by rat and human cytochromes P450. *Chem. Res. Toxicol*. *6*, 866−871. (b) Hogy, L. L., and Guengerich, F. P. (1986) In vivo interaction of acrylonitrile and 2-cyanoethylene oxide with DNA in rats. *Cancer Res*. *46*, 3932−3938. (c) Guengerich, F. P., Geiger, L. E., Hogy, L. L., and Wright, P. L. (1981) In vitro metabolism of acrylonitrile to 2-cyanoethylene oxide, reaction with glutathione, and irreversible binding to proteins and nucleic acids. *Cancer Res*. *41*, 4925−4933.
(8) (a) Solomon, J. J., Cote, I. L., Wortman, M., Decker, K., and Segal, A. (1984) In vitro alkylation of calf thymus DNA by acrylonitrile. Isolation of cyanoethyl-adducts of guanine and thymine and carboxyethyl-adducts of adenine and cytosine. *Chem.-Biol. Interact*. *51*, 167−190. (b) Solomon, J. J., and Segal, A. (1989) DNA adducts of propylene oxide and acrylonitrile epoxide: Hydrolytic deamination of 3-alkyl-dCyd to 2-alkyl-dUrd. *Environ. Health Perspect*. *81*, 19−22.
(9) Friedman, M. (2003) Chemistry, biochemistry, and safety of acrylamide. A review. *J. Agric. Food Chem*. *51*, 4504−4526.
(10) (a) Mucci, L. A., Dickman, P. W., Steineck, G., Adami, H.-O., and Augustsson, K. (2003) Dietary acrylamide and cancer of the large bowel, kidney, and bladder: Absence of an association in a population-based study in Sweden. *Br. J. Cancer 88*, 84−89. (b) Mottram, D. S., Wedzicha, B. L., and Dodson, A. T. (2002) Acrylamide is formed in the Maillard reaction. *Nature 419*, 448. (c) Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., Robert, M.-C., and Riediker, S. (2002) Acrylamide from Maillard reaction products. *Nature 419*, 449. (d) Weiss, G. (2002) Acylamide in food: Uncharted territory. *Science 297*, 27.
(11) (a) Cowan, D., Cramp, R., Pereira. R., Graham, D., and Almatawah, Q. (1998) Biochemistry and biotechnology of mesophilic and thermophilic nitrile metabolizing enzymes. *Extremophiles 2*, 207−216. (b) Kobayashi, M., Nagasawa, T., and Yamada, H. (1992) Enzymatic synthesis of acrylamide: A success story not yet over. *Trends Biotechnol*. *10*, 402−408.
(12) (a) Rayat, S. (2003) Pyrimidine ring-opening in nitrosative DNA base deamination: Experiment and theory. Ph.D. Dissertation, University of Missouri—Columbia. (b) Qian, M., and Glaser, R. (2005) Demonstration of an alternative mechanism for G-to-G cross-link formation. *J. Am. Chem. Soc*., in press. (c) Rayat, S., Wu, Z., and Glaser. R. (2004) Nitrosative guanine deamination. ab initio study of deglycation of *N*-protonated 5-cyanoimino-4-oxomethylene-4,5-dihydroimidazoles. *Chem. Res. Tox*. *17*, 1157−1169. (d) Rayat, S., Majumdar, P., Tipton, P., and Glaser, R. (2004) 5-Cyanoimino-4-oxomethylene-4,5-dihydroimidazole and 5-cyanoamino-4-imidazolecarboxylic acid intermediates in nitrosative guanosine deamination. evidence from (18)O-labeling experiments. *J. Am. Chem. Soc*. *126*, 9960−9969. (e) Qian, M., and Glaser, R. (2004) 5-Cyanoamino-4-imidazolecarboxamide and nitrosative guanine deamination: Experimental evidence for pyrimidine ring-opening during deamination. *J. Am. Chem. Soc*. *126*, 2274−2275. (f) Hodgen, B., Rayat, S., and Glaser, R. (2003) Nitrosative adenine deamination: facile pyrimidine ring-opening in the dediazoniation of adeninediazonium ion. *Org. Lett*. *5*, 4077−4080. (g) Rayat, S., and Glaser, R. (2003) 5-Cyanoimino-4-oxomethylene-dihydroimidazole and nitrosative guanine deamination. A theoretical study of geometries, electronic structures and *N*-protonation. *J. Org. Chem*. *68*, 9882−9892. (h) Glaser, R., and Lewis, M. (1999) Single- and double-proton-transfer in the aggregate between cytosine and guaninediazonium ion. *Org. Lett*. *1*, 273−276. (i) Glaser, R., Rayat, S., Lewis, M., Son, M.-S., and Meyer, S. (1999) Theoretical studies of DNA base deamination. 2. Ab initio study of DNA base diazonium ions and of their linear, unimolecular dediazoniation paths. *J. Am. Chem. Soc*. *121*, 6108−6119.
(13) (a) Bigot, B., and Roux, D. (1981) Photochemical conversion of 1-amino-2-cyanoethylene to imidazole: An ab initio SCF-CI study. *J. Org. Chem*. *46*, 2872−2879. (b) Perris, J. P., Narang, R. S., Newton, T. A., and Rao, V. R. (1979) Mechanistic studies on the photochemical conversion of enaminonitriles to imidazoles. *J. Org. Chem. 44*, 1273−1278.
(14) Erian, A. W. (1993) The chemistry of β-enaminonitriles as versatile reagents in heterocyclic synthesis. *Chem. Rev. 93*, 1991−2005.
(15) Coward, J. K., and Bruice, T. C. (1969) Mechanism of hydrolysis of primary and secondary enamines. *J. Am. Chem. Soc*. *91*, 5329−5339.
(16) Dwyer, T. J., Jasien, P. G., Kirkowski, S. M., Buxton, C. M., Arruda, J. M., and Mitchell, G. W. (1998) Protanation of β-cyanoenamines: NMR spectroscopic and calculational studies of ketenimine amidium ion formation. *J. Am. Chem. Soc*. *120*, 12942−12949.
(17) Curtiss, L. A., Raghavachari, K., Trucks, G. W., and Pople, J. A. (1991) Gaussian-2 theory for molecular energies of first- and second-row compounds. *J. Chem. Phys*. *94*, 7221−7230.
(18) Smith, B. J., and Radom, L. (1993) Assigning absolute values to proton affinities: A differentiation between competing scales. *J. Am. Chem. Soc*. *115*, 4885−4888.
(19) Compound **3** is a transition state on the MP2(full)/6-31G* potential energy surface, and **3** serves as a model for comparison to **7** and **11**.
(20) http://www.chem.qmul.ac.uk/iupac/ions/RC821.html#p1.
(21) Lias, S. G., Bartmess, J. E., Liebman, J. F., Holmes, J. L., Levin, R. D., and Mallard, W. G. (1998) *J. Phys. Chem. Ref. Data Suppl*. *17*.
(22) Conn. J. J., and Taurins, A. (1953) The structure and Grignard reaction of the β-aminocrotononitrile. *Can. J. Chem*. *31*, 1211−1222.
(23) Sieveking, H. U., and Lüttke, W. (1969) Preparation of enamino dinitriles and pyridines by reduction of geminal dinitriles with $LiAlH_4$. *Angew. Chem. 81*, 432−433.
(24) Kleinpeter, E., Klod, S., and Rudorf, W.-D. (2004) Electronic state of push−pull alkenes: An experimental dynamic NMR and theoretical ab Initio MO study. *J. Org. Chem. 69*, 4317−4329.
(25) Ellenberger, M. R., Eades, R. A., Thomsen, M. W., Farneth, W. E., and Dixon, D. A. (1979) Proton affinities of ethylidenimine and vinylamine. *J. Am. Chem. Soc. 101*, 7151−7154.
(26) Torre, O., Alfonso, I., and Gotor, V. (2004) Lipase catalyzed Michael addition of secondary amines to acrylonitrile. *Chem. Commun*. 1724−1725.
(27) Xu, L., Li, L., and Xia, C. (2004) Transition-metal-based Lewis acid catalysis of aza-type Michael additions of amines of α,β-unsaturated electrophiles in water. *Helv. Chim. Acta 87*, 1522−1526.
(28) (a) Gorrod, J. W., and Aislaitner, G. (1994) The metabolism of alicyclic amines to reaction iminium ion intermediates. *Eur. J. Drug Metab. Pharmacokinet. 19*, 209−217. (b) Nguyen, T., Gruenke, L. D., and Castagnoli, N. (1976) Metabolic N-demethylation of nicotine. Trapping of a reactive iminium species with cyanide ion. *J. Med. Chem*. *19*, 1168−1169. (c) Hoag, M. K. P., Schmidt-Peetz, M., Lampen, P., Trevor, A., and Castagnoli, N. (1988) Metabolic studies on phencyclidine: Characterization of a phencyclidine iminium ion metabolite. *Chem. Res. Toxicol. 1*, 128−131.

TX049784A