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Deprotonated *N*-(2,4-dinitrophenyl)amino acids undergo cyclization in solution and the gas phase

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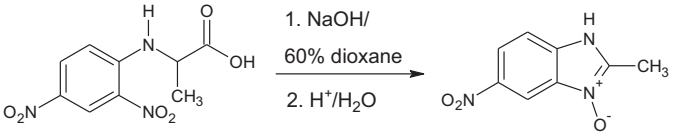
ABSTRACT

The collisionally activated mass spectral fragmentations of *N*-(2,4-dinitrophenyl)alanine and phenylalanine $[M - H]^-$ may be gas-phase analogs of the base-catalyzed cyclization of *N*-(2,4-dinitrophenyl)amino acids in aqueous dioxane. This latter reaction is one source of the 2-substituted 5-nitro-1*H*-benzimidazole-3-oxides, which are antibacterial agents. The fragmentation of both compounds, established by tandem mass spectrometric experiments and supported by molecular modeling using DFT methods, indicate that the $[M - H]^-$ ions dissociate via sequential eliminations of CO_2 and H_2O to produce deprotonated benzimidazole-*N*-oxide derivatives. The gas-phase cyclization reactions are analogous to the base-catalyzed cyclization in solution, except that in the latter case, the reactant must be a dianion for the reaction to occur on a reasonable time scale. The cyclization of *N*-(2-nitrophenyl)phenylalanine, which has one less nitro group, requires a stronger base for the cyclization than the compound with a second nitro group at the 4-position. Following losses of CO_2 and H_2O are expulsions of both neutral molecules and free radicals, the latter being examples of violations of the even-electron ion rule.

1. Introduction

Methods for synthesis of 2-substituted 5-nitro-benzimidazole-*N*-oxides have received significant attention because these compounds are potential anti-bacterial agents [1]. The synthesis of substituted benzimidazoles involves the construction of the imidazole ring via cyclization of appropriate *N*-substituted dinitroanilines by using sodium hydroxide in aqueous dioxane [2] or palladium catalysis [3]. In addition, the cyclization of *N*-substituted nitroanilines has been accomplished under thermal and photochemical conditions [4,5]. An efficient method for the synthesis of 2-methyl-5-nitrobenzimidazole-*N*-oxide involves the cyclization of *N*-(2,4-dinitriphenyl)-alanine by using sodium hydroxide in dioxane [6,7]. On the basis of kinetics and NMR studies of the reaction mixtures, a cyclization and elimination mechanism involving an intermediate dianion of the dinitrophenylalanine was proposed [7].



A mass spectrometer is a "chemical laboratory" for investigating unimolecular and ion–molecule reactions [8]. The anion of an amino acid can be generated in the gas-phase by ESI, and its unimolecular reactions can be effectively studied by a combination of accurate mass and tandem mass spectrometric experiments and by molecular modeling [9]. The mechanisms of unimolecular reactions taking place in the gas phase, however, are not necessarily the same as those in solution, and this has motivated comparisons of reactions that take place in solution with those in the gas phase by using ESI mass spectrometry and molecular orbital calculations. Examples of negative-ion ESI applications are investigations of the Smiles rearrangements [10,11] and the gas-phase denitration of nitro arenes with carbanions [12]. We reported earlier on the gas-phase Nazarov cyclization by using both empirical positive-ion ESI mass spectrometry and theoretical methods; in the gas phase, the mechanism is strictly unimolecular but not so in solution [13].

We report here a mass spectral study using negative-ion ESI of 2,4-dinitrophenylalanine and phenylalanine to explore whether a gas-phase cyclization reaction occurs for monoanions analogous

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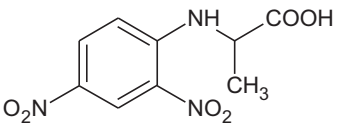
to that of the cyclization in solution. Because ESI deprotonation produces a monoanion whereas the solution-phase reaction may require a dianion, the gas-phase cyclization is an imperfect analog of the condensed-phase reaction. Nevertheless, the gas-phase reaction should provide insight on both the gas-phase and solution reactions. Our approach was to investigate the gas-phase mechanism by using tandem mass spectrometry, *d*-labeling, chemical substitution, and molecular modeling. The *N*-(2,4-dinitrophenyl)-alanine (**1**), *N*-(2,4-dinitrophenyl)-phenylanine (**2**), *N*-(2-nitrophenyl)-phenylalanine (**3**) and the *N*-ethylamide of **1** (**6**) were selected for study. The cyclizations of **1** and **2** were carried out in solution, and the benzimidazole-*N*-oxides **4** and **5** thus formed were purified and used as reference compounds to establish the structures of the gas-phase cyclization products. In the only previous reports on the mass spectrometry of these compounds, electron ionization and chemical ionization of 2,4-dinitrophenyl derivatives of amino acids were used for samples introduced to the ion source with a particle–beam interface [14].

We are pleased to contribute this article to an honor issue for Professor Tino Gaumann, who, during his productive career at Ecole Polytechnique Fédérale de Lausanne, made important contributions to the field of gas-phase ion chemistry.



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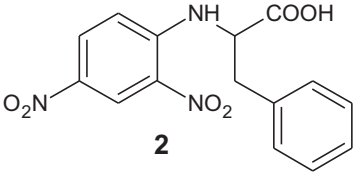

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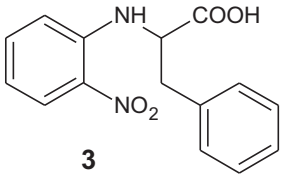

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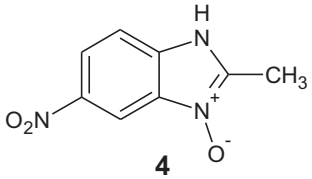

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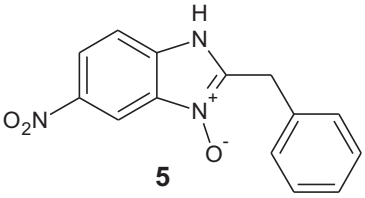

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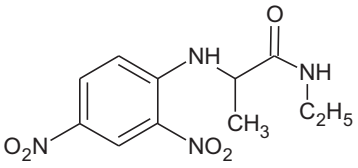

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2. Experimental

2.1. Synthesis

The *N*-(2,4-dinitrophenyl)amino acids **1** and **2** were synthesized by reacting the amino acids alanine and phenylalanine with 2,4-dinitrochlorobenzene, by means of a reported procedure [15,16]. Compound **3** was synthesized by the reaction between L-phenylalanine and 1-fluoro-2-nitrobenzene [16]. The 5-nitrobenzimidazole-*N*-oxides **4** and **5** were synthesized by refluxing the *N*-(2,4-dinitrophenyl)amino acids with 10% solution of NaOH in dioxane/water mixture (6:4) following a known procedure for the synthesis of **4** [7]. Compound **1** was treated with thionyl chloride followed by ethylamine to yield the *N*-ethylamide (**6**). The structures of synthetic compounds were confirmed by ^{1}H NMR, IR and HRMS data (Supplementary data).

2.2. Mass spectrometry

Formation of the negative-ion precursors, via deprotonation, was achieved by using ESI from 1:1 H_2O/acetonitrile mixture by direct infusion. ESI MS and tandem mass spectrometric experiments (CA MS/MS and MS^3) were performed by using the Thermo LCQ Deca Ion-Trap (San Jose, CA) at low mass resolving power or the Thermo LTQ-Orbitrap mass spectrometer (San Jose, CA) at high mass resolving power; both were operated in the negative-ion mode. The needle voltages were 3 kV, and the cone voltages were 90 V; the temperature of the capillaries for desolvation was 150 °C. All tuning parameters were optimized to achieve maximum sensitivity at the targeted mass resolving power. The high-resolution CAD mass spectra (both MS/MS and MS^3) were recorded at a mass resolving power of 30,000 for the product ions; collision voltages for inducing fragmentation of the precursor ions were in the range of 7–10 V. To track fragmentation pathways, $[M - D]^-$ ions were generated by ESI from 1:1 D_2O/acetonitrile mixture, introduced by direct infusion and analyzed by CA MS/MS and MS^3.

2.3. Theoretical calculations

Theoretical calculations were performed to characterize the potential-energy surface (PES) associated with fragmentation. The negative ion of 2,4-dinitrophenyl-alanine and its fragments were chosen instead of the phenylalanine analog based on size. Conformer spaces for precursors and intermediates were explored by Monte-Carlo/MMFF molecular mechanisms/dynamics methods. From these results, structures of precursors, intermediates, and scans for associated transition states were explored by using the PM3 semi-empirical [17,18] algorithm (Spartan for Linux: Wavefunction, Inc.). If necessary, scans were also performed by DFT: B3LYP/6-31+G(d,p). Minima and transition states were re-optimized by DFT (Density Function Theory, part of Gaussian 98/03 suites [19,20], Gaussian Inc.) to B3LYP/6-31+G(d,p) and confirmed by vibrational frequency analysis. In addition, connections of transition states to minima were examined by inspection, projections along normal reaction coordinates, and path calculations as necessary. Single-point energies were calculated at level B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) and scaled thermal-energy corrections were applied [21]. DFT was selected for high-level calculations because it requires less computational overhead than *ab initio* methods and performs adequately [22]. In addition for comparison purposes, single-point energies were calculated at levels B3LYP/and MP2(fc)/6-311+G(2d,p)//B3LYP/6-31+G(d,p), with the results being averaged [23] and scaled thermal-energy corrections applied. All results are reported in kJ/mol as enthalpies of formation relative to a selected, suitable precursor.

Calculations on radical products require unrestricted model chemistries, whereas the restricted format was used for all closed-shell systems. For fragmentation processes that involve the production of radical products from non-radical precursors, we employed multiconfiguration SCF calculations, specifically, CASSCF(6,8)/6-31G(d,p), which is included in the Gaussian 03 suite [20]. Transition states and minima were confirmed by vibrational frequency calculations, and thermal energy corrections were scaled and applied [21].

3. Results and discussion

3.1. N-(2,4-Dinitrophenyl)alanine

Collisional activation of the ESI-produced $[M - H]^-$ precursor ion of *N*-(2,4-dinitrophenyl)alanine (**1**) (*m/z* 254) produces a fragment ion of *m/z* 192 by elimination of the elements of carbonic acid, H_2CO_3 or more likely $CO_2 + H_2O$ (Fig. 1). The formation of the fragment ion of *m/z* 210 by expulsion of CO_2 from the $[M - H]^-$ ion suggests that the generation of the fragment ion of *m/z* 192 is likely stepwise, and this is supported by the observation that CA of the *m/z* 210 ion yields the *m/z* 192 product by means of water loss (data not shown). Accurate masses of the fragment ions confirm the elemental compositions (Table 1). Furthermore, the CAD mass spectrum of the $[M - H]^-$ also shows fragment ions of *m/z* 177, 175, 174 and 162, which were also obtained from the *m/z* 192 intermediate ion product as seen in an MS^3 experiment. The results show that these products originate, at least in part, from the ion $[H–CO_2–H_2O]^-$. The molecular formulae obtained via accurate mass measurement indicate that the fragment ions of *m/z* 177, 175, 174 and 162, which arise from both *m/z* 254 and 192 ions, are formed by the expulsions of $^{\bullet}CH_3$, $^{\bullet}OH$, H_2O and CH_2O (formaldehyde, *not* NO), respectively, from the ion of *m/z* 192 (Table 1).

The CAD mass spectrum of the $[M - H]^-$ ion (*m/z* 192) of 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**) (Fig. 2b), obtained in solution by cyclization of *N*-(2,4-dinitrophenyl)alanine by using NaOH in aqueous dioxane, showed the same fragment ions with a similar pattern of abundances as that obtained in an MS^3 experiment whereby the $[M - H–CO_2–H_2O]^-$ ion was interrogated (Fig. 2a). The close similarity indicates that the structure of the intermediate ion of *m/z* 192 is the deprotonated 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**). In addition, collisional activation of the $[M - D]^-$ precursor ion of d_2-*N*-(2,4-dinitrophenyl)alanine obtained by ESI from 1:1 D_2O/acetonitrile of 1 causes consecutive losses of CO_2 and HDO indicating the sole remaining D, attached to N, is lost as part of the HDO (Scheme 1). Hence, we propose that the formation of the *m/z* 192 fragment ion involves the gas-phase cyclization of the $[M - H]^-$ ion of *N*-(2,4-dinitrophenyl)alanine in a reaction analogous to the base-catalyzed cyclization of the same substrate in solution. The mechanism of the gas-phase cyclization likely involves stepwise elimination of CO_2 and H_2O (Scheme 1).

3.2. N-Ethylamide derivative of *1*

Collisional activation of the $[M - H]^-$ ion (*m/z* 281) of the *N*-ethylamide derivative of **1** (**6**) formed by negative-ion ESI also leads to an abundant fragment ion of *m/z* 192 in addition to fragment ions of *m/z* 263 (but not an ion of *m/z* 210), 177, 175 and 162 (Fig. A in Supplementary data and Table 1). These results suggest either consecutive losses of H_2O and then C_2H_5NCO or a single-step loss of 89 u ($C_2H_5NHCOOH$). An MS^3 experiment of the *m/z* 192 formed from the intermediate indicates it has the same structure as that obtained from **1** (data not shown).

3.3. N-(2,4-Dinitrophenyl)phenylalanine

The *m/z* 192 ions, either from collisional activation of the $[M - H]^-$ of *N*-(2,4-dinitrophenyl)alanine (**1**) or the ***N***-ethylamide derivative of **1** (**6**) or from deprotonation of 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**) all yield a product ion of *m/z* 162, which arises by elimination of formaldehyde. To investigate further this elimination, we studied *N*-(2,4-dinitrophenyl)phenylalanine (**2**), a molecule in which a benzyl group replaces the methyl group of **1**. Collisional activation of the ESI-generated $[M - H]^-$ of *m/z* 330 yields a fragment ion of *m/z* 268 from the elimination of the elements of carbonic acid, H_2CO_3 (62 u, likely $CO_2 + H_2O$), a fragmentation

Table 1
Negative-ion ESI mass spectral data of the $[M - H]^-$ ions and CA fragments of compounds **1**, **2**, **3** and **6**.

Compd	$[M - H]^-$	$[M - H - H_2CO_3/C_3H_7NO_2]^-$	$[M - H - H_2CO_3 - CH_3/C_7H_7]^-$	$[M - H - H_2CO_3 - OH]^-$	$[M - H - H_2CO_3 - CH_2O/C_7H_6O]^-$
1	254.0413	192.0409	177.0175	175.0383	162.0307
	$C_9H_8N_3O_6$	$C_8H_6N_3O_3$	$C_7H_3N_3O_3$	$C_8H_5N_3O_2$	$C_7H_4N_3O_2$
	(254.0413)	(192.040)	(177.0178)	(175.0381)	(162.0305)
2	330.0732	268.0727	177.0179	251.0699	162.0307
3	285	223 (100%)	132 (37%)	206 (68%)	117 (62%)
6	281	192 (100%)	177 (4%)	175 (1%)	162 (10%)

Figures in parentheses represent either calculated masses or, when followed by %, the relative abundances of the fragment ions.

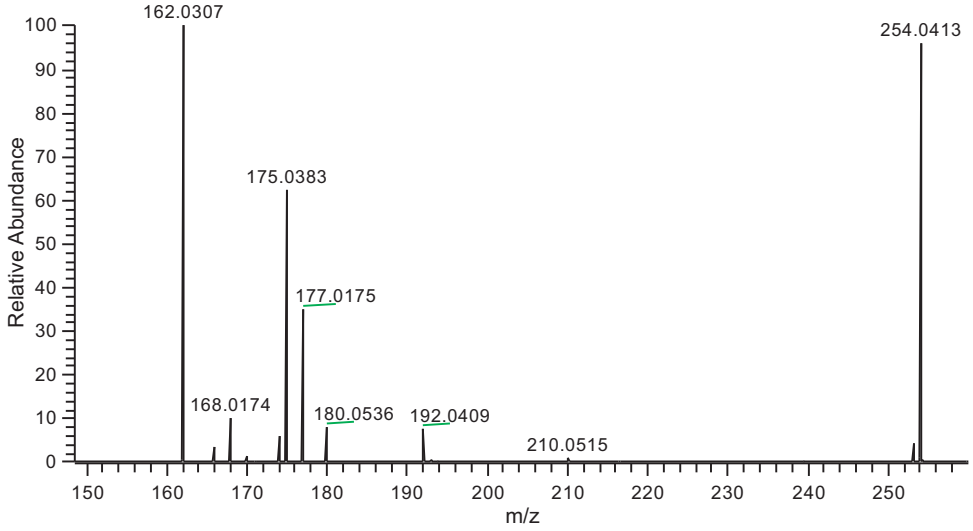


Fig. 1. CAD mass spectrum of ESI-produced [M - H]$^-$ ion (*m*/*z* 254) of *N*-(2,4-dinitrophenyl)alanine (**1**) obtained with an LTQ orbitrap mass spectrometer.

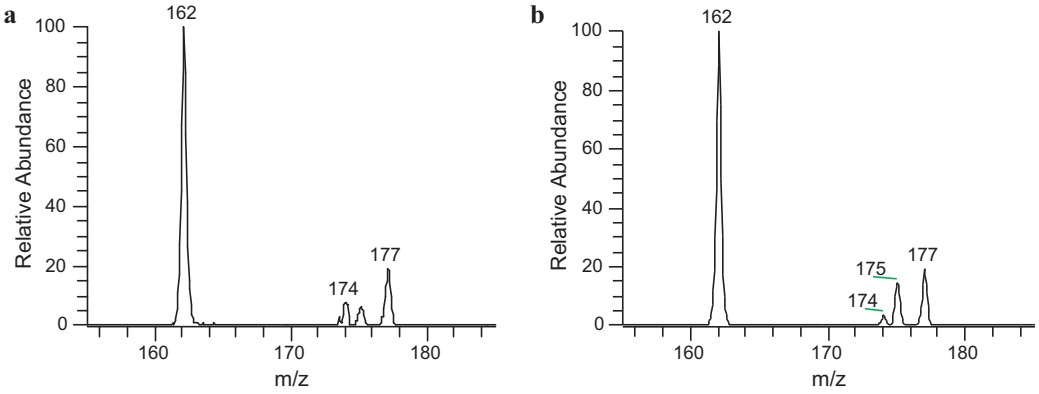
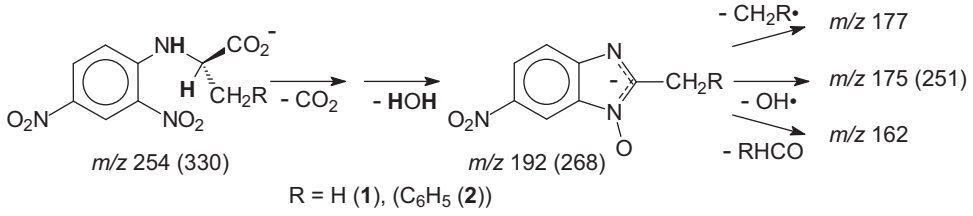


Fig. 2. The CAD mass spectra of the *m*/*z* 192 ion of the: (a) fragment ion from **1** obtained in an MS^3 experiment (b) [M - H]$^-$ of 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**) obtained with LCQ Deca mass spectrometer.

process similar to that occurring for **1**. No fragment ion from the elimination of CO_2, [M - H–CO_2]$^-$, however, is produced, raising the possibility that elimination of 62 u may be a one-step process. Other fragment ions are of *m*/*z* 251, 250, 177 and 162 formed by subsequent losses of •OH, H_2O, benzyl radical, and benzaldehyde from the ion of *m*/*z* 268, as verified by MS^3 experiments. The formulae of the fragment ions, as determined by accurate mass measurements, are consistent with the proposed pathways (Table 1). A minor fragment ion of *m*/*z* 175 from the *m*/*z* 268 ion arises from the elimination of phenoxyl radical. The elimination of formaldehyde observed in the case of compound **1** becomes the elimination of benzaldehyde for compound **2**, indicating that both compounds **1** and **2** undergo similar cyclization reactions.

Encouraged by the occurrence of a gas-phase cyclization, the cyclization of **2** in solution was attempted by using NaOH in aqueous dioxane under reflux conditions for 2 h. The reaction product was separated, purified and identified to be 2-benzyl-5-nitro-1*H*-benzimidazole-3-oxide (**5**) by NMR and accurate-mass measurement by ESI MS. To characterize this product, we collisionally activated the [M+H]$^+$ (*m*/*z* 270) and observed a fragment ion of *m*/*z* 91 (base peak), which is either benzyl or tropylium, confirming the presence of the benzyl group in the product. Collisional activation of the [M - H]$^-$ ion (*m*/*z* 268) of 2-the cyclized benzyl-5-nitro-1*H*-benzimidazole-3-oxide, however, gave a product-ion spectrum that is not a good match to that of the *m*/*z* 268 fragment from **2**, obtained by an MS^3 experiment (Fig. 3). CA of the cyclized product yields an abundant fragment ion of *m*/*z* 175, arising from loss of 93 u, probably a phenoxyl radical, whereas the *m*/*z* 268 ion from **2**, studied in an MS^3 experiment, yields instead an abundant ion of *m*/*z* 177 by the elimination of a benzyl radical,



Scheme 1. Proposed gas-phase cyclization and fragmentation of compounds **1** and **2**.

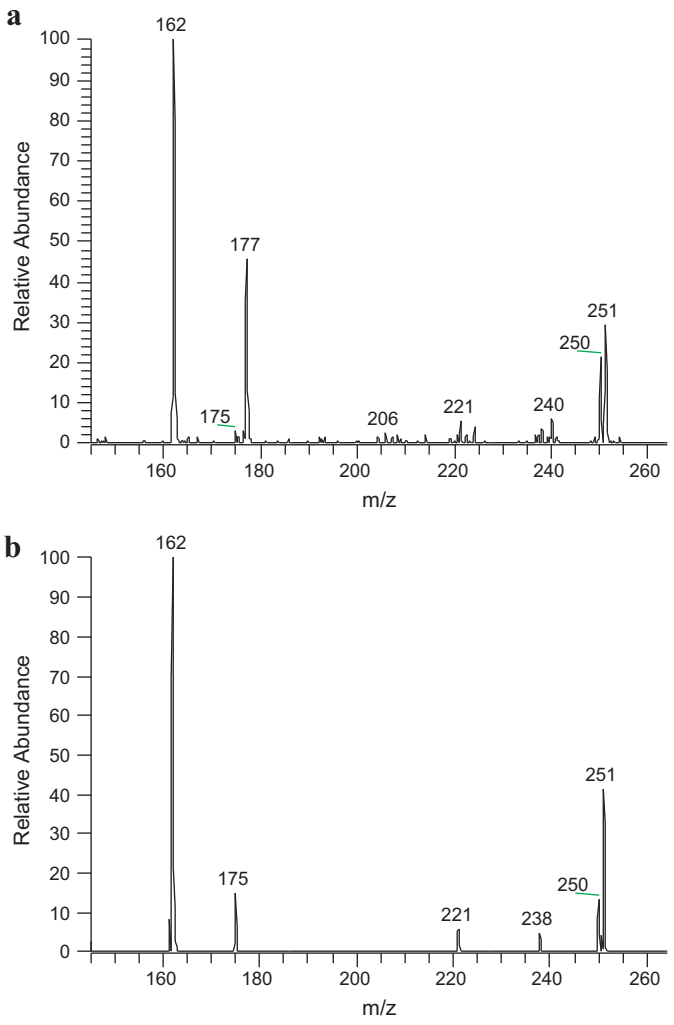


Fig. 3. CAD mass spectra of *m*/*z* 268 ion formed as (a) fragment ion from **2** in an MS^3 experiment and (b) as the $[M - H]^-$ ion of 2-benzyl-5-nitro-1*H*-benzimidazole-3-oxide (**5**) taken with an LCQ Deca Ion Trap mass spectrometer).

and only a minor product of *m*/*z* 175. Therefore, the *m*/*z* 268 fragment ion formed by the gas-phase cyclization likely arises from an isomeric structure in addition to deprotonated 2-benzyl-5-nitro-1*H*-benzimidazole-3-oxide structure.

3.4. *N-(2-Nitrophenyl)phenylalanine*

To understand the role of two electron-withdrawing nitro groups on the cyclization reaction, we investigated the cyclization of a mono-nitro compound, *N*-(2-nitrophenyl)phenylalanine (**3**), in the gas phase and in solution. Collisional activation of the $[M - H]^-$ ion (*m*/*z* 285) of **3** yields an *m*/*z* 223 product ion via elimination of CO_2 and H_2O. The *m*/*z* 223 ion goes on to generate second-generation fragment ions of *m*/*z* 206, 132, and 117 that arise from the expulsions of •OH, benzyl radical, and benzaldehyde, respectively. These reactions are analogous to the fragmentations of **1** and **2**, suggesting strongly the occurrence of a gas-phase cyclization reaction even when there is a single nitro group at the 4-position. This compound, however, does not cyclize in solution under the conditions of refluxing 10% NaOH in 60% aqueous dioxane, as did **1** and **2** that contain two nitro groups. The cyclization, however, does occur by refluxing with sodium ethoxide in ethanol for 5 h, yielding 2-benzyl-1*H*-benzimidazole-3-oxide. The nitro group at the 4-position is clearly important for cyclization in solution, in accord with a dianion mechanism [7] that is facilitated by the increased acidity of the N–H hydrogen owing to the presence of two nitro groups. Removal of one nitro group (for **3**) decreases the acidity of the N–H hydrogen and slows the reaction of **3** vs. **2**.

Table 2
Calculated relative enthalpies of formation and reaction for schemes (kJ/mol).

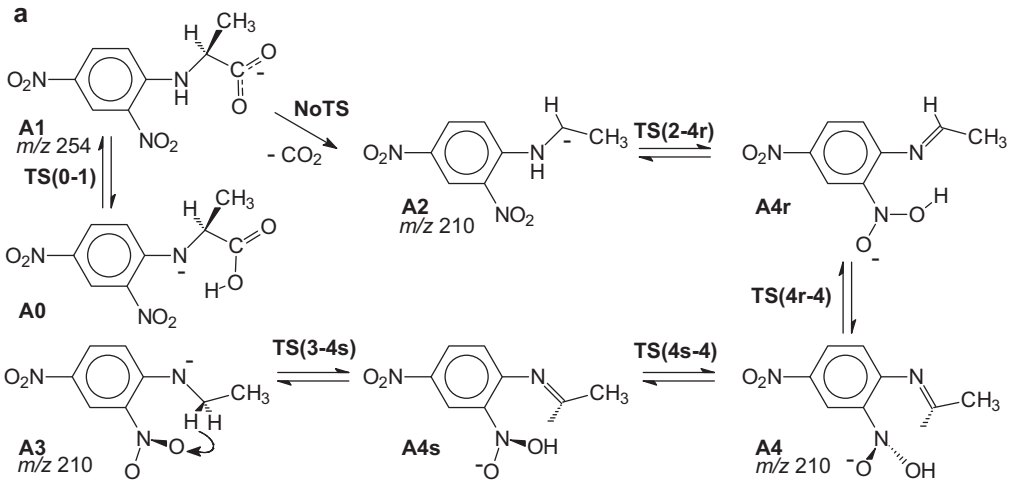
Minima		Transition States	
Label	$\Delta^2H_f/\Delta H_{rxn}$	Label	$\Delta\Delta H^{\ddagger}$
A0	−6.8	TS(0–1)	−0.9
A1	−16.3	TS(3–2r)	253.7
A2r	115.2	TS(2r–2x)	134.7
A2	73.2	TS(2x–P)	14.4
A2x	17.3	TS(2–P)	211.6
A3	0.0	TS(2–4r)	80.5
A4	84.7	TS(4r–4)	115.2
A4r	81.0	TS(3–4s)	109.9
A4s	112.6	TS(4s–4)	114.4
A4x	−54.0	TS(4–5)	103.6
A5	48.7	TS(5–9)	70.8
A6	−32.7	TS(4–4x)	100.2
A7	−2.0	TS(4x–6)	85.2
A8	−2.3	TS(6–7)	37.6
A9	−190.0	TS(7–8)	64.6
A10	−99.1	TS(8–12)	16.1
A11	−332.7	TS(9–10)	−55.1
A12	−248.3	TS(10–11)	−0.4
A13	−74.7	TS(11–12)	−2.5
A14	−79.2	TS(9–13)	−23.7
A15	−387.3	TS(13–14)	−66.8
A16	−1.9	TS(14–15)	−72.8
A17	−106.6	TS(15–18)	−176.8
A18	−204.1	TS(18–19)	−206.1
A19	−217.9	TS(19–20)	−140.8
A20	−230.6	TS(20–21)	−175.7
A21	−173.1	TS(21–22)	−76.3
A22	−137.6	TS(22–12)	−125.4

‡ indicate for transition states the equivalent of the relative enthalpy of formation for minima designated Δ^2H_f.

3.5. *Theoretical results*

We carried out theoretical calculations to provide more support for structures and fragmentation pathways. We surveyed the potential energy surface (PES) for minima and transition states involved in conversion of the precursor $[M - H]^-$ of *N*-(2,4-dinitrophenyl)alanine (**1**) and the related *N*-(2,4-dinitrophenyl)-ethylamine for the conversion to the intermediate ion corresponding to the $[M - H]^-$ of 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**) and its subsequent losses of •OH, $•CH_3$, H_2O and H_2CO (Section 2, Schemes 2 and 3, Table 2, and Supplementary data). We note the loss of radicals from closed-shell precursors violates the even-electron ion rule, but these processes are energetically tractable because the resultant radical anions are relatively stable.

The initial deprotonated *N*-(2,4-dinitrophenyl)alanine, $[M - H]^-$ (*m*/*z* 254) is comprised a pair of equilibrating forms via proton transfer (**A0**, **A1** in Scheme 2a) with **A1** not only being the more abundant but also primed for the direct loss of CO_2, a process which requires 110 kJ/mol enthalpy with no reverse-activation barrier (i.e., no transition state). The resulting intermediate **A2** undergoes a proton transfer from the amine N to an O of the 2-nitro group. The resulting product **A4r** after bond rotation becomes **A4**, which can readily undergo ring closure. Deprotonated *N*-(2,4-dinitrophenyl)-ethylamine (**A3**) reacts similarly by proton transfer from the ethylamine moiety to an O of the 2-nitro group yielding **A4s**, which becomes **A4** after –NO(OH) group rotation. Both **A4r** and **A4s** exist as minima according to the calculations, but upon adding thermal energy corrections, become shoulders to the adjoining transition states, Scheme 3.



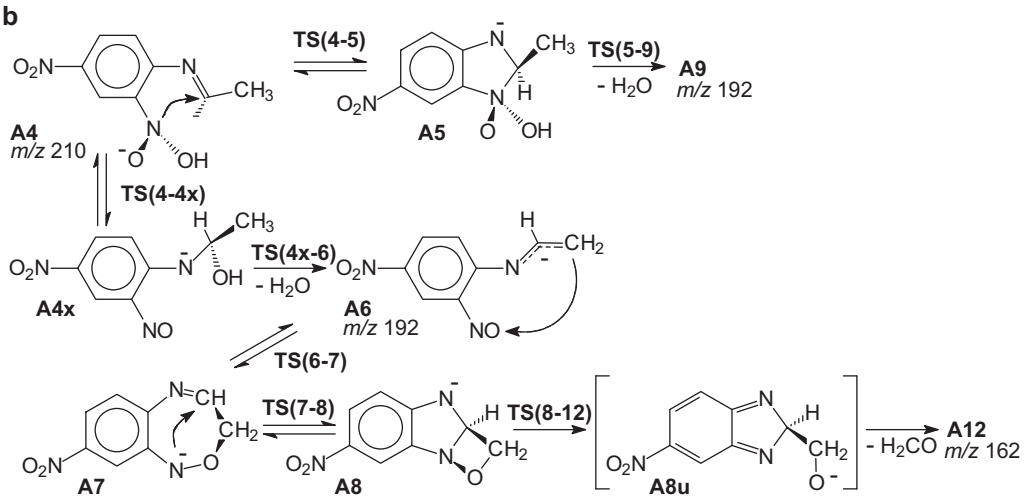
Scheme 2a. Proposed structures and fragmentation mechanisms: formation of intermediate **A4** (*m*/*z* 210). *Note*: **A0**, **A1**, [M - H]$^-$ of *N*-(2,4-dinitrophenyl)alanine (1, *m*/*z* 254); **A3**, [M - H]$^-$ of *N*-(2,4-dinitrophenyl)ethylamine (*m*/*z* 210).

The intermediate **A4** has an activated nitro group, owing to protonation of one of the oxygens, and that protonated group initiates a nucleophilic attack effecting ring closure to cyclic **A5**, which in turn loses H_2O via 1,2-elimination yielding **A9** (Scheme 2b). The composite reaction trajectory (**A2**, **A3**) → **A4** → **A5** → **A9** presents only modest energetic barriers; the maximum transition state is of ~120 kJ/mol relative enthalpy (Scheme 3 and Table 2). We note that **A3** does not directly interconvert to **A2**, but instead both eliminate acetaldehyde via transition states of large energetic barriers (Supplementary data), processes that occur to a modest extent at best (Fig. 1). This mechanism is in accord with *d*-labeling results in that the single deuterium in the [M - H]$^-$ of **A1** (**1**) is eliminated solely as HDO. In addition, collisional activation of the [M - H]$^-$ of *N*-(2,4-dinitrophenyl)ethylamine yields results consistent with this scheme (data not shown).

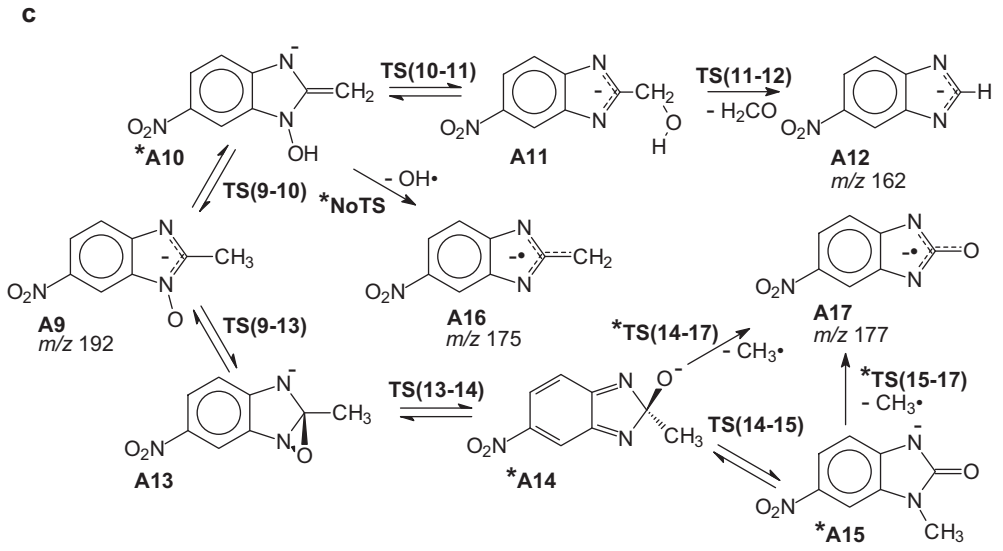
We conclude that loss of CO_2 must precede the loss of H_2O en route to the formation of the deprotonated 2-methyl-5-nitrobenzimidazole-3-*N*-oxide of *m*/*z* 192 (**4**, **A9**), in the case of deprotonated *N*-(2,4-dinitrophenyl)alanine. We were unable to find on the PES any competitive route for the loss of carbonic acid, $OC(OH)_2$, as a single species. There is precedent for carbonic acid loss in which a cupric ion mediates the formation of the incipient carbonic acid species, but in the gas phase, there is no such stabilization [24]. In addition, we searched for stabilization of the incipient carbonic acid via by incorporation into a ring structure but were unsuccessful. Consequently, by analogy CO_2 likely precedes the loss of H_2O for *N*-(2,4-dinitrophenyl)phenylalanine, giving an intermediate, [M - H–CO_2]$^-$, that is of insufficient abundance to be detected.

One possible reaction trajectory from **A4** to **A12** is **A4** → **A4x** → **A6** → **A7** → **A8** → **A12** (Scheme 2b), wherein H_2O is eliminated from the acyclic form **A4x** to yield **A6** where the negative charge is resonant between N and the terminal vinyl C. Attack of the methide C upon the 2-NO oxygen forms a seven-membered ring species, **A7**, which then undergoes ring contraction to **A8** followed by stepwise extrusion of H_2CO to yield **A12**. This route would be competitive for fragmentation from **A1** or **A3** (Scheme 3).

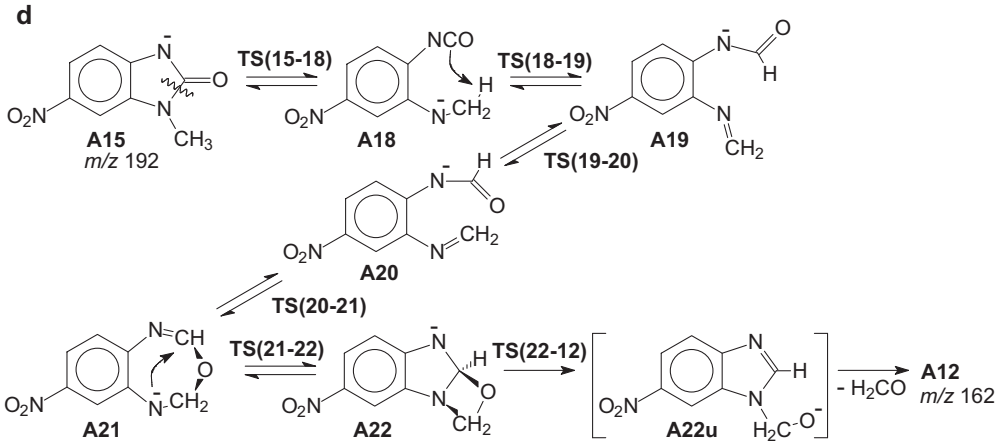
Another route from **A4** to **A12** involves deprotonated 2-methyl-5-nitrobenzimidazole-3-*N*-oxide of *m*/*z* 192 (4, **A9** – Scheme 2c) and would be a candidate for fragmentation of **A9** to **A12**. The intermediate **A9** is the most stabilized intermediate on the PES up to this point in the proposed mechanism (Scheme 3 and Table 2). Loss of H_2CO from **A9** must necessarily involve migrations and/or other rearrangements. In this case, •H transfers from the methyl group to ON forming **A10**; this is followed by •OH back migration to the methylene C forming **A11** that eliminates



Scheme 2b. Proposed structures and fragmentation mechanisms: from intermediates **A4** to **A9** and alternate route to **A12** of *m*/*z* 162.

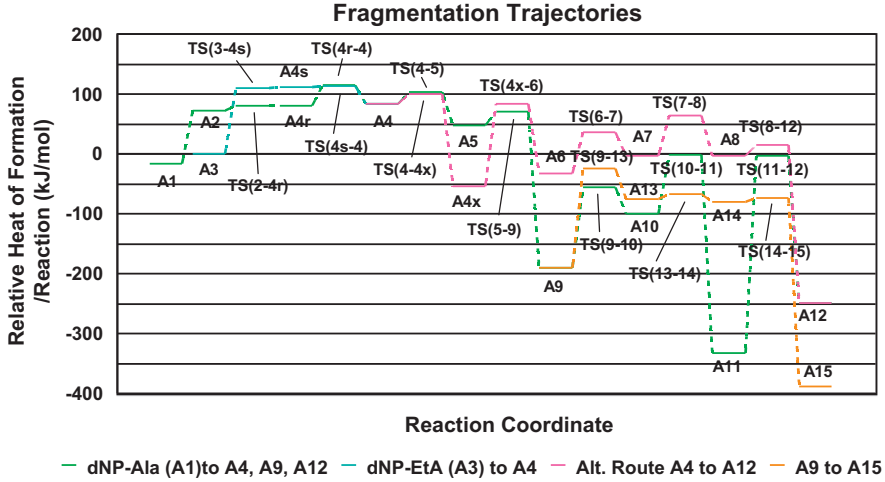


Scheme 2c. Proposed structures and fragmentations of *m*/*z* 192 ion (**4**, **A9**). *Notes*: *Indicates species that were calculated by CASSCF(6,8)/6-31+G(d,p) to assess transition states with radical character: barrier height for transition state TS(14–17) of 126 kJ/mol relative to **A14** and for TS(15–17), 292 kJ/mol relative to **A15**.



Scheme 2d. Proposed structures and fragmentations of *m*/*z* 192 (**A15**).

H_2CO (**A9** → **A10** → **A11** → **A12**). Competitive fragmentation from **A9**, however, leads to the formation of **A15**, the most stable species that we discovered on the PES; it is formed by an O migration from N to the adjacent C forming **A14** followed by CH_3 moiety back migration to the N forming **A15**, on the reaction trajectory: **A9** → **A13** → **A14** → **A15** (Scheme 3). Formation of **A15** would be favored over **A11** → **A12** starting from **A9** (Fig. 2), owing to lower transition state barriers.



Scheme 3. Calculated reaction trajectories: $[M - H]^-$ (**A1**) to production of the *m*/*z* 162 ion (**A12**).

A candidate fragmentation route consistent with the product-abundance patterns obtained via low-energy CAD of **A9** involves fragmentation of **A15** (Scheme 2d). This route starts with ring opening of **A15** (**A18**) followed by proton transfer (**A19**), group rotations (**A20**), and ring formation (**A21**) and contraction to **A22**. The intermediate **A22** is similar to **A8** but with the CH_2O moiety reversed in attachments to the benzimidazole core, a difference that is >130 kJ/mol more stable according to calculations. In addition, the largest transition state barriers on the route from **A9** to **A12** through **A15** are less by 20 kJ/mol the route through **A11**; thus this route would predominate (Table 2).

We propose that the eliminations of $^{\bullet}OH$ and $^{\bullet}CH_3$ originate from **A10** and **A14**, **A15**, respectively; the loss of $^{\bullet}CH_3$ involves transition states barriers that were calculated via CASSCF(6,8)/6-31+G(d,p) to require 126 kJ/mol, relative to **A14** and 292 kJ/mol relative to **A15** (Scheme 2c), the latter being more favorable and greater than any for the formation of **A12** from **A15**. Given that the calculations were performed by very different methods, however, this relative ranking is only suggestive; nevertheless, it is consistent with empirical data in which the *m*/*z* 162 ion predominates over the ions of *m*/*z* 175 and *m*/*z* 177 (Figs. 1 and 2).

The theoretically determined paths for fragmentation are feasible as based on relative enthalpies of formation of the minima and energetic barriers for the transition states, and, in addition, are consistent with the results of *d*-labeling.

4. Conclusions

Collisional activation of the $[M - H]^-$ of *N*-(2,4-dinitrophenyl)alanine (**1**) promotes a two-step fragmentation to lose the elements of carbonic acid as CO_2 and H_2O. The resulting fragment ion has the structure of deprotonated 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**) as verified by comparison with the authentic N-oxide that we prepared by base-catalyzed cyclization. Furthermore, the *N*-ethylamide of **1** (**6**) undergoes a similar cyclization reaction in the gas-phase, suggesting that elimination of CO_2 need not be a requirement for the cyclization. Experiments conducted by using *N*-(2,4-dinitrophenyl)-phenylalanine (**2**) and *N*-(2-nitrophenyl)-phenylalanine (**3**) demonstrate that both compounds undergo cyclization in both the gas phase and solution, resulting in the formation of 2-benzyl-5-nitro-1*H*-benzimidazole-3-oxide (**5**) and 2-benzyl-1*H*-benzimidazole-3-oxide, respectively. The latter (**3**), however, requires more basic conditions in solution to effect cyclization.

The facile cyclization reaction of these various monoanions in the gas phase is likely due to a lack of solvation of the critical anion, imparting to it more nucleophilicity. In solution, the anions are well solvated, attenuating their reactivity and requiring more stringent conditions for the reaction (i.e., use of strong base).

Theoretical calculations of the potential energy surface (PES) for deprotonated *N*-(2,4-dinitrophenyl)alanine (**1**) indicate that cyclization occurs after loss of CO_2 and before loss of H_2O in the reactions that form the deprotonated 2-methyl-5-nitro-1*H*-benzimidazole-3-oxide (**4**).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2011.01.007.

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