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# Coordinated dissociative proton transfers of external proton and thiocarbamide hydrogen: MS experimental and theoretical studies on the fragmentation of protonated S-methyl benzenylmethylenehydrazine dithiocarboxylate in gas phase

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ABSTRACT

The dissociation chemistry of the protonated S-methyl benzenylmethylenehydrazine dithiocarboxylate, PhCH=N—NHC(=S)$SCH_3$, has been investigated by collision-induced dissociation (CID) mass spectrometry experiments in combination with density functional theory (DFT) calculations. Eliminations of $H_2S$, $CH_3SH$ and (NSC)$SCH_3$ were the three fragmentation reactions observed in the tandem mass spectra, witnessed by the MS/MS analysis of native $^{34}S$-isotopic ion and the D-labeling CID-MS experiment. Of the three fragmentations, both the added proton and the internal thiocarbamide hydrogen shift to the fragment ion ($m/z$ 106) in the dissociation of losing (NSC)$SCH_3$, while both of them shift to the neutral fragment $H_2S$ to generate the minor product ion at $m/z$ 177. In the case of the feasible fragmentation process of $CH_3SH$ elimination, one of the proton/the thiocarbamide hydrogen migrates to the fragment ion at $m/z$ 163, and the other migrates to the neutral specie. Calculated results show that thiocarbamide sulfur (S5) is the most thermodynamically favored position for protonation. The mechanisms of these reactions were postulated according to the theoretical results, and the reaction energy profiles were also constructed. These results indicated that fragmentation of the protonated molecule was viewed as a result of the coordinated migration of both the external proton and the thiocarbamide hydrogen.


## 1. Introductions

A full understanding of the dissociation chemistry of a gas phase ion is required for structural elucidation and has thus received considerable attention [1–5], with the advent of tandem mass spectrometry (MS/MS) in combination with soft ionization techniques, such as electrospray ionization (ESI) [6–8] and matrix-assisted laser desorption (MALDI) [9,10]. Upon low energy collisional activation, the protonated molecular ion isomerizes to produce many reactive precursors through migration of the added proton to the less favored protonation positions from the preferred one; it undergoes fragmentation triggered by the ionizing proton, leading to the "mobile proton" model [13–19]. In the past decades, this "mobile proton" model has been postulated to explain the dissociation of both flexible peptides and rigid molecules in tandem mass spectra.

Tautomerism, however, can also take place in the neutral compound in a reversible process through proton transfer (e.g., keto-enol, amide-iminol, thioamide-thioiminol, etc.) [20,21]. The neutral thiocarbamide hydrogen of primary amines can also precede gas phase elimination when heated and produce the corresponding isothiocyanate through transfer of the thiocarbamide hydrogen [22–25]. In addition, amide position protons are witnessed to behave mobility during CID of model peptides ions by IRMPD spectrum [26]. Even some methylene hydrogen can undergo migration to a reactive position prior to dissociation of the precursor anions [27,28]. Therefore, in the case of protonated thiocarbamide, an interesting question of dissociation mechanism arises: can the thiocarbamide hydrogen also shift efficiently in the protonated thiocarbamide? More importantly, between the added proton and the thiocarbamide hydrogen, migration of which proton results in the dissociation of the protonated compound?

S-methyl substituted-methylene hydrazine dithiocarboxylate, a derivative of thiocarbamide, is an important functional group of many drug molecules which shows particular biological activities, such as antibacterial and anticancer nature [29,30]. We recently communicated that the transfer of the thiocarbamide hydrogen leads to gas phase elimination in the neutral ketonic hydrazones

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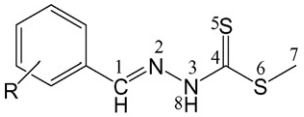
**Table 1**
Product ions observed in the ESI-MS/MS spectra of the protonated compounds **1**–**10**, at the normalization CID energy of 20%.



| Compounds | R | Precursor ions $[M+H]^+$ (relative abundance) | Relative abundances of product ions *m/z* (%) |
|---|---|---|---|
| **1** | H | 211 (39.1%) | 177 (6.0%), 163 (100%), 106 (52.7%) |
| **2** | *p*-$CH_3$ | 225 (23.6%) | 191 (4.2%), 177 (100%), 120 (42.8%) |
| **3** | *o*-$OCH_3$ | 241 (20.5%) | 207 (0.3%), 193 (100%), 136 (1.0%) |
| **4** | *m*-$OCH_3$ | 241 (35.8%) | 207 (8.2%), 193 (100%), 136 (87.7%) |
| **5** | *p*-$OCH_3$ | 241 (16.3%) | 207 (1.6%), 193 (100%), 136 (18.5%) |
| **6** | *p*-$N(CH_3)_2$ | 254 (76.1%) | 220 (0.2%), 206 (100%), 148 (1.6%) |
| **7** | *p*-F | 225 (41.7%) | 195 (8.8%), 181 (100%), 124 (39.8%) |
| **8** | *p*-Cl | 245 (63.2%) | 201 (4.6%), 197 (100%), 140 (29.2%) |
| **9** | *p*-Br | 289 (32.4%) | 255 (6.7%), 241 (100%), 184 (24.7%) |
| **10** | *p*-$NO_2$ | 256 (100%) | 222 (1.3%), 208 (12.3%), 151 (1.4%) |

from S-methyl dithiocarbazate [25]. In the present study, we select S-methyl benzenylmethylene hydrazine dithiocarboxylate as a model to carry out experimental and theoretical investigations on the migration of the external proton and the internal thiocarbamide hydrogen prior to fragmentation.

## 2. Experimental

Compounds **1**–**10** in Table 1, S-methyl arylmethylenehydrazine dithiocarboxylate, ArCH=N—NHC(=S)$SCH_3$, were synthesized by esterification of $H_2NNHCS_2K$, which was synthesized by reaction of $CS_2$ with $H_2NNH_2{\cdot}H_2O$ and KOH in i-PrOH solvent, with dimethyl sulfate, and then reacted with benzaldehydes to produce the corresponding hydrazone [29,30] (Scheme 1S). The products were then purified by wash with i-PrOH and recrystallization from $CH_2Cl_2$, and identified by MS and NMR.

Analysis of the samples was operated on an LCQ advantage mass spectrometer (ThermoFisher Company, USA), equipped with an ESI ion source in the positive ionization mode, with data acquisition using the Xcalibur software (Version 1.4). Typical parameters were used for operation of the ESI-MS as follows [31–35].

The compounds were dissolved in methanol in the normal collisional-induced dissociation (CID) experiment, while dissolved in methanol-d4 in the D-labeling experiment. The solutions were introduced into the source chamber via a length of 50 μm i.d. × 190 μm o.d. used-silica tubing at a flow rate of 5 $\mu L\,min^{-1}$. A potential of −4.5 kV, and a sheath gas of nitrogen at a pressure of 25 bar were employed. The heated capillary was maintained at 250 °C. The collision-induced dissociation (CID) mass spectra were obtained with helium as the collision gas after isolation of the precursor ions at the collision energy of 20%. More than 50 scans were summed to produce a mass spectrum.

The theoretical calculations were performed with the Gaussian 03 program [36]. The equilibrium geometries of reactants, transition states, intermediates and products were optimized, by using the density functional theory (DFT) method at the B3LYP/6-31+G(d,p) level, with calculated force constants. No symmetry constraint was imposed in the optimization. All reactants, intermediates and products were identified as true minima by the absence of imaginary frequencies. Transition state (TS), on the other hand, was identified by the presence of one single imaginary vibration frequency and the normal vibrational mode. In addition, transition states were confirmed by the intrinsic reaction coordinates (IRC) calculations. Vibrational frequencies and zero-point energies (ZPE) for all the key species were calculated at the same level of theory. The DFT optimized structures were shown by Gauss View (Version 3.09) software to give higher quality images of these structures.

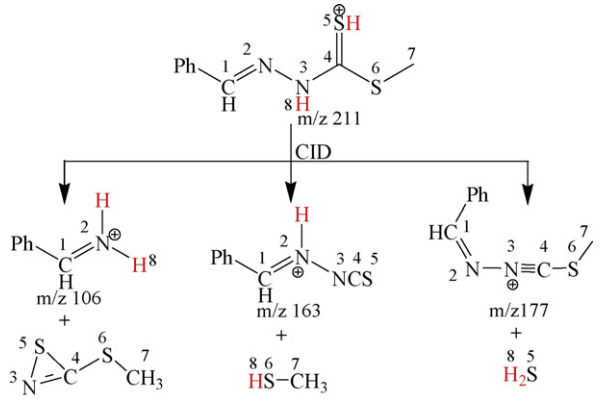
Hard data on geometries, electronic energies, as well as entropies and free energies (at 298 K) of all structures considered are available as Supporting Information. Potential energy hypersurfaces are presented using free energies at 298 K.

## 3. Results and discussion

### 3.1. Fragmentations in CID-MS

An abundant protonated molecular ion at *m/z* 211 was observed in a full scan mass spectrum of S-methyl benzenylmethylene hydrazine dithiocarboxylate (compound **1** in Table 1) under positive ion electrospray ionization conditions. As shown in Fig. 1a, the protonated molecular ion underwent decomposition upon collisional activation and generated two abundant product ions at *m/z* 163 and *m/z* 106, and a minor one at *m/z* 177. Protonated molecular ions of its derivatives (compounds **2**–**10**) had similar fragmentation behaviors, with the same neutral losses of 34 Da, 48 Da and 105 Da, respectively (Fig. 1S and Table 1), indicating that the fragmentation reactions occurred at the thiocarbonate moiety in the CID process.

Scheme 1 gave the plausible dissociation reactions of the protonated compound **1** ($[M+H]^+$) at *m/z* 211 observed in Fig. 1a. The fragment ion at *m/z* 106 is attributed to N-protonated phenylmethanimine $[PhCH{=}NH_2]^+$, formed by losing 3-(methylthio)-1,2-thiazirene (105 Da) from the molecular ion. The most abundant fragment ion at *m/z* 163 in the CID spectrum is assigned to be protonated N-isothiocyanato phenylmethanimine, which was generated by the methanethiol elimination (48 Da) from the precursor ion, meanwhile, the minor product ion at *m/z* 177 is 2-benzylidenehydrazono (methylthio)methenic cation, which cor-




**Scheme 1.** Three different fragmentation reactions in CID of protonated compound **1**.

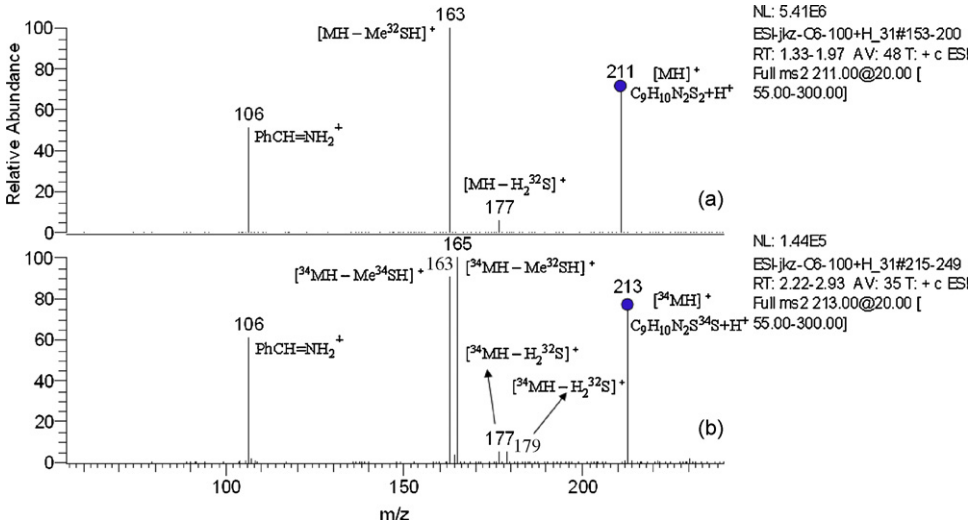



**Fig. 1.** The MS/MS spectra of $[M+H]^+$ ions of compound **1** ((a) $C_9H_{10}N_2S_2+H^+$, (b) $C_9H_{10}N_2S^{34}S+H^+$) in the positive ESI mode.

responds to the elimination of hydrogen sulfide from the precursor ion.

These postulated decomposition reactions were confirmed by the MS/MS analysis on the native $^{34}S$ isotopic ion. The sulfur element has two isotopes, $^{32}S$ and $^{34}S$ in nature, with the relative abundance at 100% and 4.4%, respectively. As shown in Fig. 2, decomposition of the mono isotope ion of $MH^+$ at $m/z$ 211 produces the fragment ion $[PhCH{=}N{-}NCS+H]^+$ at $m/z$ 163 by losing $CH_3SH$. The first $^{34}S$ isotope ion at $m/z$ 213, however, contains two isomeric structures due to the different position of $^{34}S$, $[PhCH{=}N{-}NHC(=^{34}S)^{32}SCH_3+H]^+$ (A) and $[PhCH{=}N{-}NHC(=^{32}S)^{34}SCH_3+H]^+$ (B). As expected, fragmentation of isomer A gives the product ion at $m/z$ 165, $[PhCH{=}N{-}NC^{34}S+H]^+$, through the neutral loss of $CH_3{}^{32}SH$; whereas dissociation of isomer B results in the product ion at $m/z$ 163, $[PhCH{=}N{-}NC^{32}S+H]^+$ via the neutral loss of $CH_3{}^{34}SH$. The almost identical abundance of the two product ions is attributed to the equal distribution of the $^{34}S$ atom in nature. Interestingly, elimination of $H_2S$ from the isotopic ion at $m/z$ 213 shows the similar fragmentation behaviors, with nearly equivalent abundance of the isotopic fragment ions at $m/z$ 177 and $m/z$ 179. The fragment ions at $m/z$ 177 is generated by the dissociation of isomer A through losing $H_2{}^{34}S$, while the product ions at $m/z$ 179 is formed *via* $H_2{}^{32}S$ elimination from isomer B. The product ion $[PhCH{=}NH_2]^+$ ($m/z$ 106), however, has no sulfur atom in the chemical formula, and thus no $^{34}S$ isotopic ion peak is observed in the CID spectrum.

Analysis of the fragment ions from Table 1 showed that both the added proton and the internal thiocarbamide hydrogen precede migration prior to or in the dissociation reactions. As can be seen, both of them transfer to either the fragment ion at $m/z$ 106 via the neutral loss of $(NSC)SCH_3$, or to the neutral fragment $H_2S$ to generate the minor product ion at $m/z$ 177. In the most accessible reaction route of losing $CH_3SH$, one proton migrates to the fragment ion $[PhCH{=}N{-}NCS+H]^+$, while the other one shifts to the neutral specie.

### 3.2. D-labeling CID experiments

To further investigate the migration of both the added proton and the thiocarbamide hydrogen in the protonated ion, D-labeling CID experiments were carried out. An abundant deuterated molecular ion $[M+D]^+$ at $m/z$ 212 was generated by spraying the freshly prepared methanol-$d_4$ solution of compound **1** in positive mode. Di-deuterated ion $[MD_2–H]^+$ at $m/z$ 213 was also formed from the

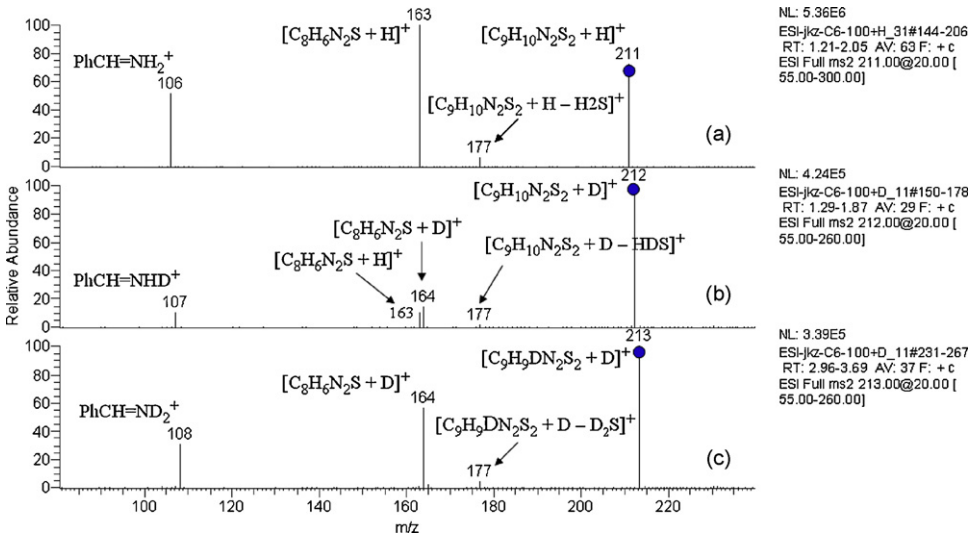



**Fig. 2.** The MS/MS spectra of $[M+H]^+$ ions (a), $[M+D]^+$ ions (b), and $[M+2D–H]^+$ ion (c) of compound **1**.

solution after some minutes. As displayed in Fig. 2, dissociation of the $MD^+$ and $[MD_2–H]^+$ show some different fragment ions to those of $MH^+$. Fragment ion $[PhCH{=}NH_2]^+$ contains both the external proton and the thiocarbamide hydrogen from the precursor ion. As expected, the decomposition of $[M+D]^+$ at $m/z$ 212 gave the corresponding fragment ion $[PhCH{=}NHD]^+$ at $m/z$ 107, 1 Da in molecular weight more than the analogous one from $[M+H]^+$. Similarly, dissociation of $[MD_2–H]^+$ ($m/z$ 213) generated the product ion $[PhCH{=}ND_2]^+$ at $m/z$ 108, which lies 2 Da in molecular weight above the analogous one from $[M+H]^+$. In comparison, the minor fragment ion at $m/z$ 177 has no added proton or the thiocarbamide hydrogen in the structure, because both of them transfer to the neutral fragment $H_2S$ in the fragmentation process. As a result, decomposition of the protonated molecular ion $[M+H]^+$, the deuterated ion $[M+D]^+$ and the di-deuterated ion $[MD_2–H]^+$ resulted in the same product ion at $m/z$ 177 via the neutral loss of $H_2S$, HDS and $D_2S$, respectively.

The most abundant fragment ion $[PhCH{=}N{-}NCS+H]^+$, formed by the $CH_3SH$ elimination of the precursor ion, contains the added proton (or the thiocarbamide hydrogen) in its structure. As expected, fragmentation of the di-deuterated ion $[MD_2–H]^+$ produced the $[PhCH{=}N{-}NCS+D]^+$ ion at $m/z$ 164, which is only 1 Da more than the analogous product ion from $[M+H]^+$. Dissociation of the deuterated ion $[M+D]^+$, however, generated two fragment ions, $[PhCH{=}N{-}NCS+D]^+$ at $m/z$ 164 and $[PhCH{=}N{-}NCS+H]^+$ at $m/z$ 163 (Fig. 2b), with the neutral loss of $CH_3SH$ and $CH_3SD$, respectively. The more abundant ion at $m/z$ 164 than that of $m/z$ 163 in the spectrum implied that it is more feasible for the thiocarbamide hydrogen to shift to the methyl mercapto group, than the added proton in the dissociative process.

### 3.3. Coordinated dissociative proton transfer

Theoretical calculations were invoked to further investigate the transfer of both the external and the internal thiocarbamide protons in the dissociation reactions of the protonated ion. The neutral compound 1 can undergo *thiocarbamide-thioiminol* tautomerism [21] and exist as the *thiocarbamide* form in methanol solvent according to the NMR results (Figs. 2S and 3S). Three main positions (N2, N3, and S5) in the *thiocarbamide* structure of compound 1 potentially accommodate the "ionizing" proton to generate MH-a, MH-b, and MH-c (Scheme 2S), respectively.

#### 3.3.1. Migration of the added proton and the thiocarbamide hydrogen

Structure MH-c1 is at the global minimum on our calculated potential energy surface (PES), indicating that the thiocarbonyl sulfur S5 is the most preferred site for protonation in the structure. The stability of MH-c1 is a consequence of the resonance in the planar thiocarbonate structure, which disperses the positive charge to thiocarbamide nitrogen N3 and S6 (Scheme 3S). Another structural contribution to the stability of MH-c1 is a long-range hydrogen bond (S5-H···N2) of 2.149 Å (Fig. 4S). In MH-c1, the added proton on S5 can easily be transferred by taking advantage of the S5-H···N2 hydrogen bond to the N2 atom to generate MH-a1. The energy barrier to this 1,4-proton shift via TS(c1-a1) is much small at 22.1 kJ/mol in terms of $\Delta G^\circ_{298}$, comparing to proton migration in protonated triglycine [13]. Structure MH-a1 is also stabilized by resonance (Scheme 4S), by dispersing the positive charge to the phenyl ring. It lies slightly higher in free energy than MH-c1 just by 3.3 kJ/mol. These results indicate that it is very accessible for the migration of the external proton between S5 and N2.

Protonation at N3, however, formally localizes the charge on the nitrogen atom in structure MH-b1, and even destroys the resonance of the thiocarbamic group and the hydrazonic moiety. As a result, N3 is the most unfavorable position for protonation of the above three protonation sites, and the protonated ion MH-b1 is much higher (85.6 kJ/mol) in free energy than MH-c1.

Amide position protons in peptides have been witnessed to undergo migration upon collisional activation by IRMPD spectrum [28]. In structure MH-a1, the added proton is located at N2, and the *thiocarbamide-thioiminol* tautomerism of the molecular ion can proceed via 1,3-proton shift of the thiocarbamide hydrogen (Scheme 2S) [21]. To undergo this 1,3-proton shift, it is required to rotate the thiocarbonic group (=S5) to be *trans* to the thiocarbamide hydrogen. The transition structure TS(a1–a2) for the rotation is 52.9 kJ/mol relative to the global minimum MH-c1, and the product minimum MH-a2 is 28.5 kJ/mol higher in free energy than MH-c1. The thiocarbamide hydrogen in MH-a2 can now be transferred to S5, and MH-a2 isomerizes to MH-d1 via TS(a2–d1). The energy barrier to this isomerization is 128.7 kJ/mol in free energy relative to MH-c1, and the product minimum MH-d1 is only 2.5 kJ/mol higher in free energy than the global minimum. The above results indicate that transfer of the thiocarbamide hydrogen via *thiocarbamide-thioiminol* tautomerism is also energetically accessible.

#### 3.3.2. Fragmentation to form $PhCH{=}NH_2^+$

The fragmentation route to generate $PhCH{=}NH_2^+$ is viewed as a classical "mobile proton" model [11–19], in which both the added proton and the thiocarbamide hydrogen are transferred to the fragment ion and located at the N2 atom. Upon collisional activation of the global minimum structure MH-c1, the added proton can be feasibly transferred to a less stable site N2 atom to give MH-a1. Fig. 3 shows the reaction profile for the fragmentation of MH-a1 to generate the product ion at $m/z$ 106 in the terms of $\Delta G^\circ_{298}$, and the corresponding $\Delta H^\circ_0$ values are shown in parentheses.

To precede this dissociation, it is necessary for the thiocarbamide group to rotate around the N2—N3 bond to form a conformational isomer MH-a2, in which the N2—N3 bond is weakened by breaking the conjugation of the lone electron pair on N3 to the C1=N2 double-bond, witnessed by lengthening the N2—N3 bond to 1.397 Å from 1.341 Å in MH-a1. The thiocarbamide hydrogen in MH-a2 is parallel to the π-electron orbit of N2 with the dihedral angle *ΦC1-N2-N3-H8* at −90.3° (Fig. 4S), indicating that it is in an ideal position for the 1,2-proton migration of H8. The subsequent breakage of the N2—N3 bond *via* TS-a2, triggered by the formal charge on the external proton, occurs concomitantly with migration of the hydrogen to the N2 atom, which leads to P-a1, an ion-neutral complex of the phenylmethanimium ion ($m/z$ 106) and 3-(methylthio)-1,2-thiazirene ($CH_3S(CSN)$). Separation of P-a1 leads to the fragment ion at $m/z$ 106, and combination of the products lies 69.9 kJ/mol in free energy above MH-c1. The calculated energy barrier of the key step (TS-a2) is 169.4 kJ/mol in free energy relative to the global minimum MH-c1, which is proximate to the

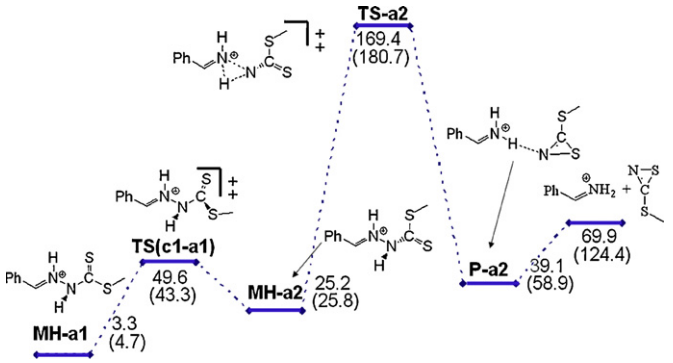



**Fig. 3.** Reaction profile for the fragmentation of the protonated compound 1 to the phenylmethaniminium ion at $m/z$ 106.

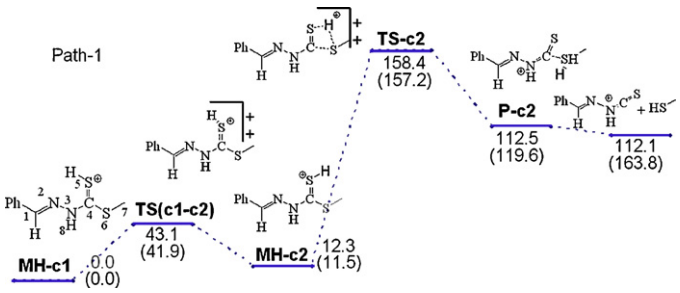



**Fig. 4.** Reaction profile for the most feasible fragmentation process of $CH_3SH$ elimination, triggered by migration of the external proton.

fragmentation of the b2 to the a1 ion for triglycine (163.9 kJ/mol, 39.3 kcal/mol) [14], indicating a feasible fragmentation channel.

### 3.3.3. Fragmentation with $CH_3SH$ elimination

Methanethiol elimination is the most favorable pathway in the decomposition of the protonated S-methyl substituted-benzenylmethylene hydrazine dithiocarboxylate, according to the CID-MS experimental results in Table 1. The D-labeling CID-MS experiment also indicated that the dissociative proton transfer of the added proton competes with that of the thiocarbamide proton in this process. We communicated that the neutral analogs can undergo $CH_3SH$ elimination when heated in the gas phase, triggered by the migration of the thiocarbamide hydrogen [25]. This paper provides two of the most feasible mechanistic decomposition routes, triggered by the transfer of the added proton and the thiocarbamide hydrogen, respectively.

Fig. 4 displays the mechanistic reaction profile (Path-1) for $CH_3SH$ elimination, in which the external proton is transferred to the methyl mercapto sulfur (S6) from the thiocarbonyl sulfur (S5). To proceed this dissociation, it is necessary to rotate the mercapto group to be *trans* to the methyl mercapto group *via* TS(c1–c2), which lies only 43.1 kJ/mol in free energy above MH-c1. In the resulting isomer MH-c2, which is 12.3 kJ/mol higher in free energy than MH-c1, the migrating external proton is in an ideal position for transfer, with the dihedral angle *ΦH-S5-C4-S6* at −175.2° (Fig. 5S). The subsequent 1,3-proton shift in the structure MH-c2 proceeds, and generates a loose ion-neutral complex, P-c2, with a long ion-neutral bond C4—S6 of 2.080 Å. The barrier to the proton transfer via TS-c2 is 158.4 kJ/mol relative to MH-c1, and P-c2 lies 112.5 kJ/mol above MH-c1. Decomposition of the loose complex to the product ion at *m*/*z* 163 is exoergic by 0.4 kJ/mol and the separated products lies 112.1 kJ/mol in free energy above MH-c1.

An alternative efficient mechanistic route (Path-2, Fig. 5) involves the migration of the thiocarbamide hydrogen to the methyl mercapto sulfur (S6). Prior to fragmentation, the external proton can easily be transferred from S5 to N2 to give the structure MH-a1. The subsequent dissociation of MH-a1 occurs with $CH_3SH$ elimination by transferring H8 to the methyl mercapto S6 *via* TS-a1, which is higher in free energy than MH-c1 by 141.4 kJ/mol. The product minimum is P-a2, an ion-neutral complex of N2-pronated N-isothiocyanato(phenyl) methanimine ion and $CH_3SH$, and separation of P-a2 leads to the fragment ion at *m*/*z* 163. Combination of the separated products lies 37.2 kJ/mol in free energy above MH-c1.

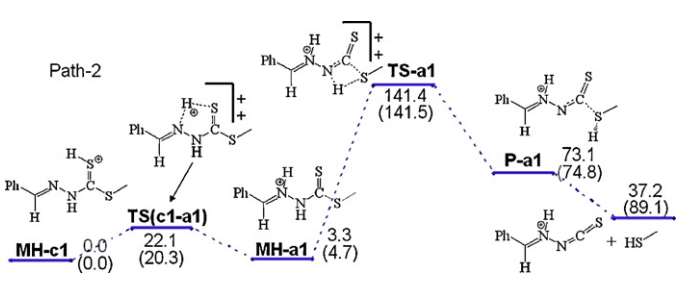



**Fig. 5.** Reaction profile for the most feasible fragmentation process of $CH_3SH$ elimination, triggered by migration of the thiocarbamide hydrogen.

Analysis of the reaction profiles of Path 1 and Path 2 indicates that the energy barriers of the $CH_3SH$ elimination channels is similar to dissociation of peptides [13–16], and is energetically more favorable than the dissociation channel to generate the product ion $PhCH{=}NH_2^+$. The fragmentation channel of Path 2 via TS-a1, however, shows kinetically and energetically more feasible than Path 1, with relative smaller energy barrier and lower free energy of the products, which is consistent with the D-labeling CID result. As a consequence, migration of the thiocarbamide hydrogen, rather than the added proton to the methyl mercapto group occurs in the $CH_3SH$ elimination, albeit the two channels co-exist in the fragmentation reaction.

### 3.3.4. Fragmentation with $H_2S$ elimination

The mechanistic route for $H_2S$ elimination has also been investigated as shown in Fig. 6. Due to resonance stabilization (Scheme 3S), some of positive charge in structure MH-c1 is delocalized to thiocarbamide N3, and then the thiocarbamide hydrogen H7 behaves partially as an ionizing proton. Amide position protons have also been witnessed by IRMPD spectrum to behave mobility during CID of peptides ions [26]. Transfer of H7 in MH-c1 to S6 atom produces an isomeric ion MH-e1, which is 122.3 kJ/mol higher in free energy than MH-c1. The energy barrier to this proton transfer *via* TS(c1–e1) is 162.6 kJ/mol in free energy, which is 21.1 kJ/mol higher than that in Path 2. Protonation at the S6 atom in MH-e1, however, does not directly lead to $CH_3SH$ elimination to give the fragment ion at *m*/*z* 163, with the length of 1.816 Å for the C4—S6 bond. The energy barrier to this fragmentation *via* TS-e1 is 169.3 kJ/mol higher in free energy relative to MH-c1, which is even kinetically more unfavorable than the Path 1 via TS-c2.

A conformational isomerization of MH-e1 to MH-e2 by rotation of the C4—S6 bond can be feasibly carried out, with an energy barrier of 15.3 kJ/mol relative to MH-e1. In MH-e2, the proton on the S6 atom is now in an ideal position to be transferred upon collisional activation to the S5 atom. The energy barrier to this 1,3-proton transfer *via* TS-e2 is 212.5 kJ/mol in free energy relative to MH-c1, and the product minimum P-e2 is inclined to form the final product ion at *m*/*z* 177 by losing $H_2S$, with exoergic by 57.6 kJ/mol. Combination of the separated products is 77.0 kJ/mol higher in free energy than MH-c1.

Analysis of the above calculation results show that there is an decreasing order in the calculated energy barrier to the three fragmentation reactions of losing $H_2S$, $CH_3S(CSN)$ and $CH_3SH$, indicating an increasing kinetically favorable order in reaction, which is in accordance with the increasing order in the relative abundance of the corresponding fragment ion the CID-MS spectra (Table 1).

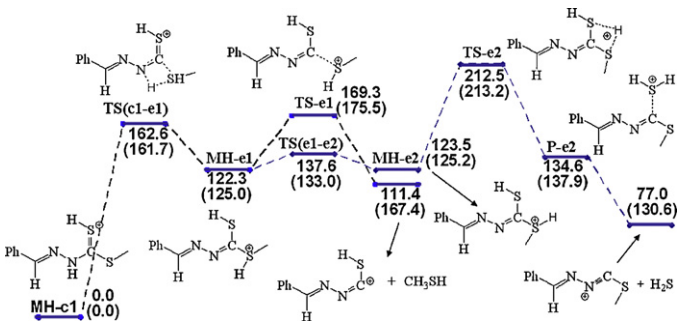



**Fig. 6.** Reaction profile for the fragmentation of MH-c1 by losing $H_2S$.

Dissociation of $H_2S$ has a much considerable energy barrier and therefore the minor fragment ion ($m/z$ 177, 6.0%) in the CID-MS. These results validate the rationality of the postulated mechanistic fragmentation channels and also indicate that the dissociation reactions of the protonated title compound are kinetically controlled in the process of CID.

The CID-MS experimental results have substantiated that both the added proton and the internal thiocarbamide hydrogen undergo migration prior to or in the dissociation reactions. Theoretical investigation of the mechanistic routes also indicates both the external proton and the thiocarbamide hydrogen play different roles in the three fragmentation reactions. In the process of losing $CH_3S$(CSN), the external (mobile) proton migrates to the imine N2 from the most favorable protonation site S5, and triggers the breakage of the N2—N3 bond, concomitantly with migration of the thiocarbamide hydrogen to imine N2 to form the fragment ion at $m/z$ 106. The fragmentation of losing $CH_3SH$ is induced by migration of either the added proton or the thiocarbamide hydrogen to the methyl mercapto S6; whereas, the most favorable channel is migration of the external proton to imine N2 is prior to charge-remote fragmentation, triggered by transfer of the thiocarbamide hydrogen to S6. In the $H_2S$ elimination reaction, the thiocarbamide hydrogen behaves as an ionizing proton due to resonance when the thiocarbamide S5 accepts the external proton, and then it migrates to the S5 via the methyl mercapto S6 to induce fragmentation. As a conclusion, fragmentation of the protonated title molecule is viewed as a result of the coordinated migration of both the external proton and the thiocarbamide hydrogen.

As shown in Table 1, a number of the protonated analogs of the title compound behave the similar fragmentations, with the same neutral losses of 34 Da, 48 Da and 105 Da, respectively, indicating that the coordinated migration of both the external proton and the thiocarbamide hydrogen occur in the CID process. Amide position protons have also been witnessed to behave mobility during CID of peptides ions by IRMPD spectrum [26]. Coordinated dissociative proton transfers therefore might be a reasonable interpretation of the fragmentation of the protonated compound containing thioamide or amide hydrogen, in which some fragmentations is beyond the classical mobile proton model, e.g., charge-remote peptide fragmentation pathways [19].

## 4. Conclusion

The dissociation chemistry of protonated S-methyl benzenylmethylenehydrazine dithiocarboxylate was investigated by tandem mass spectrometry and theoretical calculation. Two main fragmentation reactions, elimination of $CH_3S$(CSN) and $CH_3SH$, and a minor decomposition one with losing $H_2S$ were observed in the CID-MS spectra, and evidenced by MS/MS analysis of the native $^{34}S$ isotopic ion and the D-labeling CID-MS.

The calculated results at the B3LYP/6-31+G(d,p) level indicated that the thiocarboxyl sulfur S5 is the most preferred position for protonation in the gas phase to generate a protonated ion MH-c1, which can undergo isomerization by migration of the added proton or the thiocarbamide hydrogen. In the mechanistic fragmentation pathway of losing $CH_3S$(CSN), the added proton shifts first to the imine nitrogen N2, followed by migration of the thiocarbamide hydrogen to N2 and then the breakage of the N2—N3 bond, which produces the fragment ion at $m/z$ 106. In the case of the dissociation of $CH_3SH$ elimination, either the added proton or the thiocarbamide hydrogen is transferred to the methyl mercapto sulfur S6, leading to the fragment ion at $m/z$ 163. For the fragmentation to generate the product ion at $m/z$ 177, the thiocarbamide hydrogen in MH-c1 behaves as an ionizing proton due to resonance, and migrates to the protonated thiocarboxyl sulfur atom S5 *via* the methyl mercapto sulfur S6, followed by the neutral loss of $H_2S$ via breaking the C4—S5 bond. The above results indicated that fragmentation of the protonated molecule is a result of the coordinated migration of both the external proton and the thiocarbamide hydrogen.

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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.2009.12.017.

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