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APPLICATION NOTE

Gas-Phase Fragmentation of Protonated *N*,2-Diphenyl-*N*′-(*p*-Toluenesulfonyl)Ethanimidamides: Tosyl Cation Transfer Versus Proton Transfer

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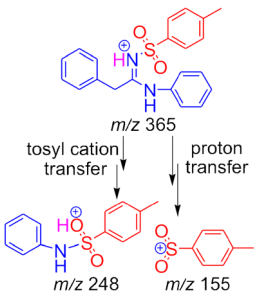

Abstract. The gas-phase dissociation chemistry of protonated *N*,2-diphenyl-*N*′-(*p*-toluenesulfonyl) ethanimidamides was investigated by electrospray ionization mass spectrometry in combination with density functional theory calculation. The protonated molecules underwent fragmentation via two main competing channels: (1) migration of the tosyl cation to the anilinic *N* atom and the subsequent loss of 2-phenylacetonitrile to afford protonated *N*-phenyl *p*-toluenesulfonamide (*m/z* 248); and (2) transfer of the ionizing proton to the anilinic *N* atom to give an ion/neutral complex of [tosyl cation / 2-phenylacetonitrile] (*m/z* 272) and the subsequent decomposition to yield tosyl cation (*m/z* 155). To the best of our knowledge, the gas-phase tosyl cation transfer has not been reported previously. For the *para*-substituted sulfonamides, the presence of electron-donating groups on the anilinic ring inhibits the reaction channel of the tosyl cation migration, whereas the presence of electron-withdrawing groups favors this pathway.

Keywords: Tosyl cation transfer, Proton transfer, *N*,2-diphenyl-*N*′-(*p*-toluenesulfonyl) ethanimidamides, The electronic effect of substituents, CID-MS, DFT

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Introduction

Since the invention of electrospray ionization (ESI) by Fenn in 1984 [1], MS has emerged as a powerful technique for analysis of polar and thermolabile compounds [2]. In particular, tandem mass spectrometry (MS) shows a unique superiority in structural characterization by providing substantial fragmentation data. However, such structural elucidation is often obscured because of widespread backbone rearrangement reactions; these reactions have also attracted the interest of many analysts [3–8].

Aromatic sulfonamides, with an important functional skeleton present in many drug candidates, have been extensively characterized by ESI-MS to facilitate structural elucidation. Several characteristic fragmentation patterns have been generalized, including typical losses of SO_2 [9–12] and SO [13, 14], as well as ion/neutral complex (INC)-mediated reaction to afford protonated *p*-toluenesulfonamide [15] or the complementary aniline radical cation [16]. In our previous work [8], transfer of the tosyl oxygen to the anilinic ring has been observed in fragmentation of protonated *N*-phenyl *p*-toluenesulfonamides. To the best of our knowledge, however, the gas-phase tosyl cation transfer has not been reported, although an intramolecular sulfonyl migration has been reported in the condensed phase [17, 18]. Fragmentation of protonated benzenesulfonamides can effectively afford an INC consisting of a sulfonyl cation and a neutral counterpart [15, 16]. With this in mind, a series of *N*,2-diphenyl-*N*′-(*p*-toluenesulfonyl)ethanimidamides, which contain a basic amino group in structure, were chosen as model molecules to probe whether the gas-phase tosyl transfer would occur and to elucidate the potential reaction mechanism if it does.

Experimental

N,2-diphenyl-*N*′-(*p*-toluenesulfonyl)ethanimidamides (compounds 1-7 in Scheme 1) were synthesized in our lab [19]. *N*-phenyl *p*-toluenesulfonamide (compounds 8 in Scheme 1) was synthesized by the classic method [20]. All compounds were

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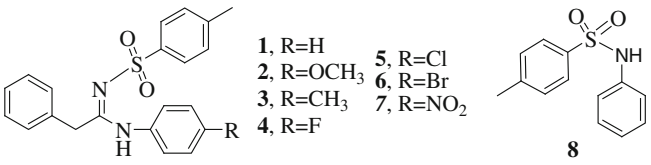


Scheme 1. Structures of compounds **1**-**8**

purified after synthesis, and their structures were further confirmed by MS and NMR (see Supplementary Figures S1–S7).

The ESI-MS experiments were carried out on an LCQ electrospray ionization quadrupole ion trap mass spectrometer (Thermo-Finnigan LCQ Advantage MAX, ThermoFisher Scientific Inc., San Jose, CA, USA), operated in the current method [5]. For deuterium labeling experiments, a methanol-d_4 solution of the target compound was used for ESI-MS analysis. High-resolution mass spectrometry experiments were performed on a micrOTOF (time-of-flight) mass spectrometer (Bruker, Billerica, MA, USA).

Theoretical calculations were carried out using the Gaussian 03 suite of programs [21]. The B3LYP DFT method was used with the 6-311+G(d,p) basis set. Optimized structures were visualized with Gauss View (Ver. 3.09) software. The energies discussed here are the sum of the associated electronic and thermal free energies under standard state.

Results and Discussion

The gas-phase tosyl cation transfer was explored by investigating the MS fragmentation behaviors of compound **1**. The fragment ion spectrum of $[\mathbf{1}+H]^+$ at *m/z* 365 (Figure 1a) shows three prominent peaks at *m/z* 155, 248, and 272. Formation of these three prominent peaks has also been supported by investigating the breakdown curves (see Supplementary Figure S8). The base peak at *m/z* 155 corresponds to the tosyl cation (**1-PI**). The fragment ion at *m/z* 248 is ascribed as protonated *N*-phenyl *p*-toluenesulfonamide (**2-PI**), resulting from the loss of 2-phenylacetonitrile from $[\mathbf{1}+H]^+$. The product ion at *m/z* 272, originating from the aniline elimination of the precursor ion, is attributed to an INC (**3-PI**) consisting of tosyl cation and 2-phenylacetonitrile. The elemental compositions of these fragment ions were determined by accurate mass measurements conducted on a high-resolution Q-TOF mass spectrometer (see Supplementary Table S1 and Figure S9). Noteworthy, the ion of **3-PI** cannot be detected in the accurate mass spectrum because of the much higher vacuum and activation in a collision cell in the Q-TOF mass spectrometer. Thus, the potential fragmentation pathways are proposed in Scheme 2. Interestingly, formation of **2-PI** at *m/z* 248 can only be rationalized as a result of transferring the tosyl cation to the aniline group.

The proposed dissociation pathways in Scheme 2 were supported by the MS/MS analysis of the protonated ^{34}S isotopologue. An increasing mass shift of 2 Da was observed for all of the product ion peaks (from 155 to 157 Da for **1-PI**, from 248 to 250 Da for **2-PI**, and from 272 to 274 Da for **3-PI**), in the fragment ion spectrum of the native ^{34}S-isotopologue of $[\mathbf{1}+H]^+$ at *m/z* 367 (Figure 1b). This shift indicates that all of these product ions contain the S atom, which originated from the tosyl moiety of the precursor ion.

The structure of **2-PI**, generated via the tosyl cation transfer, was confirmed by comparing its CID-MS with that of protonated *N*-phenyl *p*-toluenesulfonamide, $[\mathbf{8}+H]^+$ (see Supplementary Figure S10). Both of the CID-MS were identical, with four prominent product ions at *m/z* 93, 108, 109 and 155, which correspond to the aniline radical cation, the protonated imino cyclohexadienone, the radical hydroxyaniline, and tosyl cation (**1-PI**), respectively [8, 16].

The identity of **3-PI** at *m/z* 272, an INC consisting of tosyl cation and 2-phenylacetonitrile, was evidenced by the MS^3 experiments (see Supplementary Figure S11). The ion at *m/z* 272 cannot be effectively selected for further fragmentation at a narrow mass selection window of 2 Da. Nevertheless, it can be done at a broader window of 6 Da and afford **1-PI** even at a low normalized collisional energy of 13%. A reasonable explanation is the weak interaction between the two partners of the INC, which might obtain energy to undergo dissociation at the narrow mass selection window, when a supplementary AC voltage is applied to the end-cap electrodes of the trap to increase the kinetic energy of the non-target ions for exclusion in the mass selection step of the tandem MS experiment [22].

The pathways in Scheme 2 are supported by the deuterium labeling experiments. The deuterated molecule ($[\mathbf{1}+D]^+$, *m/z* 366) was obtained by spraying a diluted methanol-d_4 solution of **1,** which was freshly prepared, while ESI of the same solution, which had been allowed to stand for several minutes, afforded

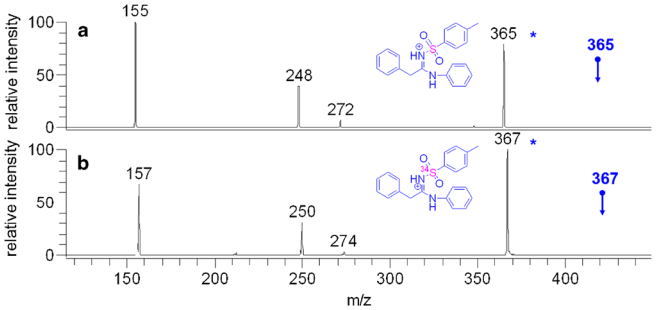
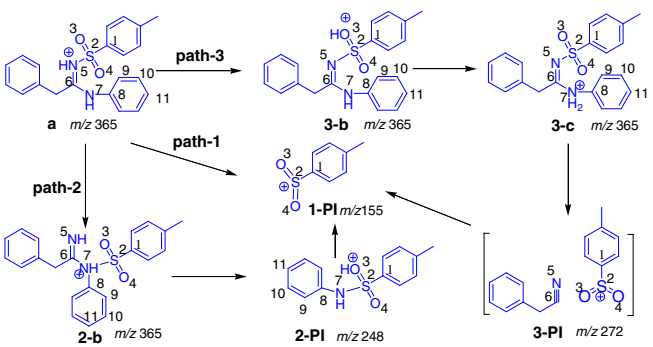


Figure 1. CID-MS of (**a**) $[\mathbf{1}+H]^+$ at *m/z* 365, and (**b**) the corresponding ion of the native ^{34}S-isotopologue at *m/z* 367, $[C_{21}H_{20}N_2O_2{}^{34}S+H]^+$



Scheme 2. Proposed mechanism for fragmentation of $[\mathbf{1}+H]^+$

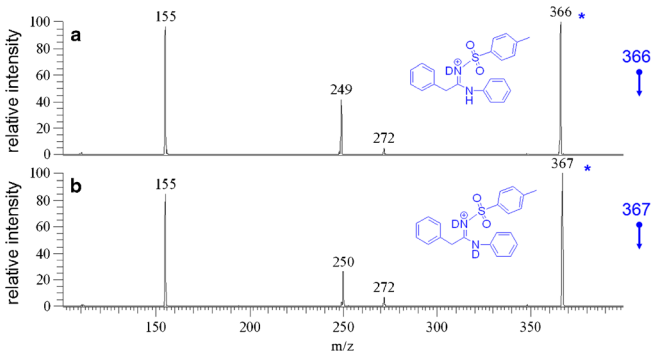


Figure 2. CID-MS of (**a**) $[\mathbf{1}+D]^+$ (*m/z* 366) and (**b**) $[\mathbf{1}\text{-}H+2D]^+$ (*m/z* 367)

the di-deuterated molecule ($[\mathbf{1}\text{-}H+2D]^+$, *m/z* 367) (see Supplementary Figure S12). As shown in Figure 2, decomposition of the $[\mathbf{1}+H]^+$, $[\mathbf{1}+D]^+$, and $[\mathbf{1}\text{-}H+2D]^+$ species resulted in the tosyl cation with the same mass (i.e., 155 Da), indicating that no external proton or sulfamidic hydrogen exists in the structure of **1-PI**. Similarly, **3-PI** keeps the same mass (272 Da) in the CID-MS of $[\mathbf{1}+H]^+$, $[\mathbf{1}+D]^+$, and $[\mathbf{1}\text{-}H+2D]^+$. The fragment ion **2-PI** contains both external proton and sulfamidic hydrogen from the precursor ion. As expected, the corresponding mass (248 Da) of **2-PI** shifts to 249 Da, originating from $[\mathbf{1}+D]^+$, and to 250 Da from $[\mathbf{1}\text{-}H+2D]^+$. Interestingly, no H/D exchange occurs for these fragment ions in the CID process.

For further mechanistic investigation on the tosyl cation migration, density functional theory calculations were performed for fragmentation of $[\mathbf{1}+H]^+$. A schematic potential energy diagram for this reaction is shown in Figure 3, and details of the corresponding structures are available in the Supplementary Figure S13. The imine *N5* atom is the most preferred site for protonation in the structure of Compound **1** (Figure 3). Decomposition of the *N5*-protonated **1 (a)** via cleavage of the N5–S2 bond affords the tosyl cation at *m/z* 155 (**1-PI**) in the reaction channel of path 1. This process is unlikely under low energy CID as it is endergonic by 215.3 kJ mol^{-1}. The *Mulliken* charge distribution analysis for **1-PI** shows that the positive charge (1.273) of the tosyl cation is

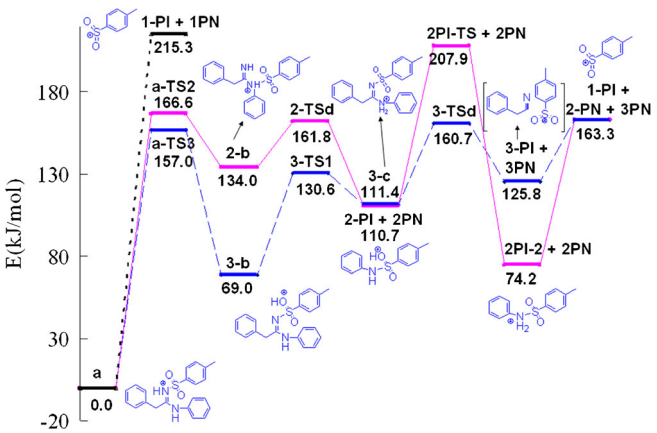


Figure 3. The schematic potential energy diagram involved in fragmentation of **a**

Table 1. The Collision-Induced Dissociation Mass Spectra Data of the Protonated **1-7** at the normalized collisional energy of 27%

Comp.	R	$[M+H]^+$ *m/z* (%)	1-PI *m/z* (%)	2-PI *m/z* (%)	3-PI *m/z* (%)
1	-H	365 (89.9)	155 (100)	248 (42.9)	272 (6.4)
2	$-OCH_3$	395 (100)	155 (16.4)	278 (0.2)	272 (0.9)
3	$-CH_3$	379 (100)	155 (36.2)	262 (7.4)	272 (0.2)
4	-F	383 (78.3)	155 (100)	266 (23.5)	272 (7.7)
5	$-^{35}Cl$	399 (99.2)	155 (100)	282 (27.5)	272 (5.4)
6	$-^{79}Br$	443 (29.7)	155 (100)	326 (39.1)	272 (3.7)
7	$-NO_2$	410 (41.5)	155 (100)	293 (63.6)	272 (1.2)

mainly localized on the S atom, indicating a preferred site to undergo electrophilic attack during the tosyl cation transfer.

In path 2, the formed tosyl cation undergoes 1,3-migration to the anilinic N7 atom to afford an isomer **2-b**, which surmounts an energy barrier of 166.6 kJ mol^{-1} (**a-TS2**). The anilinic N7 in **2-b** is a charged tetravalent nitrogen atom, which triggers breakage of the C6–N7 bond and the loss of 2-phenylacetonitrile (**2-PN**). This process is accompanied with migration of the imine H5 to the tosyl O3, which results in **2-PI** (*m/z* 248). Noteworthy, *N7*-protonated *N*-phenyl *p*-toluenesulfonamide (**2PI-2**) is located at 36.5 kJ mol^{-1} below **2-PI** in free energy. Isomerization of **2-PI** can lead to **2PI-2**, which subsequently undergoes dissociation to afford **1-PI**. Nevertheless, the subsequent dissociation of **2-PI** is unfeasible in the MS^2 process because of a considerable energy barrier (207.9 kJ mol^{-1}) via **2PI-TS**. In path 3, the ionizing proton in **a** migrates to the tosyl O3 to afford an isomer **3-b**, with a barrier of 157.0 kJ mol^{-1} (**a-TS3**). The subsequent 1,5-H migration in **3-b** occurs effectively with an energy barrier of 130.6 kJ mol^{-1} (**3-TS1**) to afford **3-c**. The elimination of aniline from **3-c** results in the product ion (**3-PI**) at *m/z* 272, which overcomes an energy barrier of 160.7 kJ mol^{-1}. **3-PI** is an INC consisting of tosyl cation and 2-phenylacetonitrile, which can easily undergo further decomposition to give **1-PI** at *m/z* 155, with an endoergicity of 37.5 kJ mol^{-1}, which is in good agreement with the tandem MS experiments (see Supplementary Figure S11).

Analysis of the potential energy diagram in Figure 3 shows that the reaction channels of paths 2 and 3, rather than path 1, occur effectively in dissociation of **a**, in which the fragment ion at

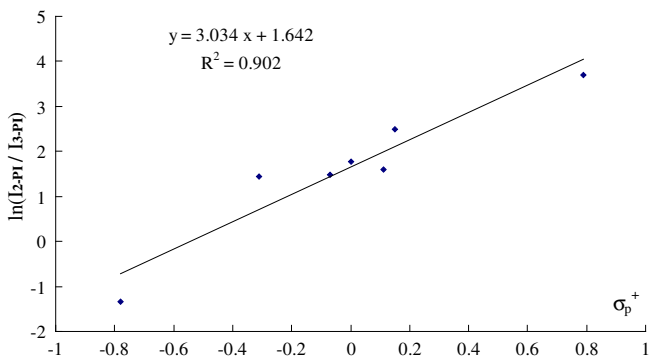


Figure 4. Plot of $\ln(I_{\mathbf{2\text{-}PI}}/I_{\mathbf{3\text{-}PI}})$ *versus* the Hammett substituent constants σ_p^+

m/z 248 is formed in path 2, and formation of the ions at *m/z* 272 and 155 occurs via path 3. Thereby, two competing reaction channels, namely, tosyl cation transfer (path 2) and proton transfer (path 3), occur in the gas-phase fragmentation of $[\mathbf{1}+H]^+$.

To test the universality of the gas-phase tosyl cation transfer, a series of *N*,2-diphenyl-*N*′-(*p*-toluenesulfonyl)-ethanimidamides (compounds **2-7** in Scheme 1) were investigated by ESI-MS. The ions of **1-PI** and **2-PI** were also obtained in the ESI-Q-TOF MS analysis (see Supplementary Figure S9 and Table S1). As summarized in Table 1, all of these compounds exhibited similar fragmentation patterns, whereas the relative intensities of the product ions varied with the changes in the substituents. The presence of an electron-donating group (such as methoxy) enhances the stability of the protonated molecule, whereas the presence of an electron-withdrawing group promotes its dissociation. As for paths 2 and 3, the presence of electron-donating groups on the anilinic ring favors formation the fragment ion **3-PI**, whereas the presence of electron-withdrawing groups promotes generation of **2-PI**. As shown in Figure 4, the logarithmic abundance ratios of these two product ions $\ln(I_{\mathbf{2\text{-}PI}} / I_{\mathbf{3\text{-}PI}})$ are in line with the Hammett substituent constants σ_p^+ [23]. The significant substituent effect on the competing fragmentation pathways can be explained as a result of the stability of the intermediate **3-c** being influenced by the electronic effect of the substituent.

Conclusion

A novel 1,3-tosyl cation migration was first observed in the gas-phase fragmentation of the protonated *N*,2-diphenyl-*N*′-(*p*-toluenesulfonyl)ethanimidamides. The mechanistic pathways were investigated by theoretical calculations. Moreover, the presence of electron-donating groups on the *para* site of the anilinic ring was found to inhibit the reaction channel of the tosyl cation migration, whereas the presence of electron-withdrawing groups favors this pathway.

Acknowledgments

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