
An Ion/Molecule Reaction for the Identification of Analytes with Two Basic Functional Groups

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A mass spectrometric method is presented for the identification of analytes with two basic functionalities and PA between 222 and 245 kcal/mol, including diamines. This method utilizes gas-phase ion–molecule reactions of protonated analytes with neutral 1,1-diethoxyethene (DEE) in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR). A variety of protonated mono-, bi-, and trifunctional analytes containing different functional groups, namely, amido, amino, N-oxide, hydroxy, carboxylic acid, keto, thio, thioether, alkene, phosphite, and phosphonate, were tested in the FT-ICR. The results demonstrate that basic protonated bifunctional compounds (PA between 222 and 245 kcal/mol) react selectively with DEE by forming a specific addition/elimination product ion (adduct – EtOH) (this product was also observed for lysine with three functionalities). The diagnostic reaction sequence involves proton transfer from the protonated analyte to the basic vinyl group in DEE, followed by addition of one of the functional groups of the analyte to the electrophilic α -carbon in protonated DEE. The next step involves proton transfer from this functionality to the other analyte functionality, followed by proton transfer to DEE and elimination of ethanol. Since the mechanism involves proton transfer between two functional groups of the analyte, the reaction does not occur for analytes where the two functionalities cannot be in close proximity (i.e., *meta*-phenylenediamine), and where no proton is available (i.e., dimethylaminoketone). (J Am Soc Mass Spectrom 2009, 20, 1251–1262) © 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

Development of analytical methods for rapid and accurate structural characterization of unknown organic compounds is important for many fields, including drug discovery [1]. Several techniques, including NMR, FT-IR, and X-ray crystallography, are commonly utilized for identification of previously unknown compounds [2a]. However, these techniques require relatively large quantities of analytes, and FT-IR and X-ray crystallography techniques are not applicable to complex mixtures [2b]. Tandem mass spectrometry (MS/MS) is a powerful tool for fast direct mixture analysis. Historically, MS/MS experiments rely on isolation of a protonated analyte and its exposure to activating collisions to generate structurally informative fragment ions (collision-activated dissociation or CAD) [3]. While extremely powerful in identification of known compounds, this method cannot usually provide detailed structural information for unknown analytes (not already in MS libraries), such as the type and number of different functionalities present in the analyte.

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MS/MS methods based on gas-phase ion–molecule reactions can provide very detailed structural information [4, 6a]. In some cases, this approach has been combined with collision-activated dissociation (CAD) of the ion–molecule reactions' products to obtain even more information [5]. Thus far, the majority of studies on structurally diagnostic ion–molecule reactions have focused on the identification of functionalities in neutral analytes by using selective ionic reagents [5]. For example, gas-phase reactions of dimethoxyborenyl ion followed by CAD and/or H/D exchange reactions have been demonstrated to allow the identification of functional groups present in neutral alcohols, aldehydes, ethers, ketones, and some biologically active molecules containing hydroxyl groups [5]. Much fewer studies have focused on the use of a neutral reagent to identify the functionalities in protonated analytes [6–8]. However, this approach is needed for mass spectrometric analyses involving the widely used electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or matrix-assisted laser desorption/ionization (MALDI) methods [7]. Neutral reagents have been developed for the identification of epoxy, primary N-oxide, amido, hydroxy, ether, ester, and keto functionalities in protonated mono- and polyfunctional analytes

[6]. For example, (*N,N*-diethylamino)dimethylborane can be used to identify the protonated amido functionality [6d]. Further, hydriodic acid has been reported to attach to neutral basic sites in polypeptide ions in the gas phase. These reactions can be used to determine the number of neutral basic sites, including lysine, arginine, histidine, and N-termini [8]. In the work presented here, 1,1-diethoxyethene (DEE) is demonstrated to react in a selective manner with protonated analytes with two basic functional groups, such as diamines, in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR).

Experimental

Instrumentation

All experiments were performed using an Nicolet model FTMS-2000 dual-cell FT-ICR mass spectrometer equipped with a 3-Tesla superconducting magnet, and a Finnigan Odyssey data station, as described previously [9]. All chemicals used were purchased from the Sigma-Aldrich Company (St. Louis, Mo) and used as received. Analytes were introduced into one cell region by using a solids probe, a Varian (Palo Alto, CA) leak valve, or a Finnigan-built batch inlet system equipped with a variable leak valve. The analytes were protonated by self-chemical ionization, which was accomplished by allowing the molecular ion and the ionic fragments generated upon electron ionization (20 eV, 7 μ A, 50–500 ms) of the analyte, to react with the neutral analyte molecules for a certain period of time (\sim 3.0 s). Nominal pressure of the neutral analyte in the cell, as measured by a Bayard-Alpert ion gauge, varied between 0.5×10^{-8} and 9×10^{-8} Torr. After this, all the ions in the other side of the dual cell were removed by changing the remote trapping plate voltage from +2.0 V to -3.5 V for 12 ms. The protonated analyte was transferred into the other cell by grounding the conductance limit plate (75–140 μ s), followed by cooling for a period of about 1 s via IR emission [10] and collisions with Ar present at about 10^{-5} torr. The protonated analyte was isolated by using a stored-waveform inverse Fourier transform [11] (SWIFT) excitation pulse to eject all unwanted ions, and was allowed to react for a variable period of time with neutral DEE introduced into the same cell through a variable leak valve. Nominal pressure of DEE in the cell, as measured by a Bayard-Alpert ion gauge, was 1.1 – 6.9×10^{-8} Torr. Some of the derivatization products were further probed by subsequent isolation via SWIFT followed by sustained off-resonance irradiated collision-activated dissociation [12] (SORI-CAD). SORI-CAD experiments utilized off-resonance excitation of the isolated ion at a frequency \pm 1000 Hz off the cyclotron frequency of the ion. This experiment was carried out by subjecting the desired ion to an off-resonance excitation pulse with an amplitude between 0.19 and 1.1 V for 300 ms during which collisions with argon ($\sim 10^{-5}$ Torr) occur. After

reactions, all ions were excited for detection by using chirp excitation with a bandwidth of 2.7 MHz, and a sweep rate of $3200 \text{ Hz } \mu\text{s}^{-1}$. The spectra were recorded as 64 k data points and subjected to Hanning apodization, followed by augmentation of the data by using one zero-fill before Fourier transformation. All spectra were corrected by subtraction of a background spectrum recorded by removing the reactant ion by SWIFT ejection before reaction to make sure that the observed products were generated from the desired ion population.

Kinetics

In the ion–molecule reactions described above, the neutral reagent was present in vast excess to the ion of interest. As a result, these reactions inherently follow pseudo-first-order kinetics. Kinetic data were obtained by allowing the protonated analyte to react with the neutral reagent for variable periods of time (from 0.5 up to 50 s) at a constant pressure before excitation and detection. The second-order reaction rate constant (k_{reaction}) was derived from the concentration of the neutral reagent and the negative slope of the plot of the natural logarithm of the relative abundance of the protonated analyte ions versus time. The collision rates ($k_{\text{collision}}$) were estimated by using the parameterized trajectory theory of Su and Chesnavich [13]. The overall efficiency of each reaction is given as $k_{\text{reaction}}/k_{\text{collision}}$ (i.e., the percentage of collisions leading to product formation). The reagent pressure readings of the ion gauges were corrected for the sensitivity of the ion gauge towards the neutral reagent (DEE) and its distance from the center of the ICR cell [14]. These correction factors were obtained by measuring the rate of the highly exothermic electron-transfer reaction between $\text{CS}_2^{*\cdot+}$ and DEE. This highly exothermic electron-transfer reaction is assumed to proceed at the collision rate; therefore, its efficiency is assumed to be 100%.

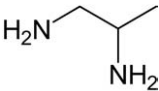
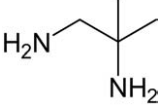
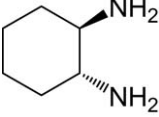
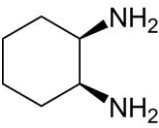



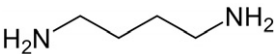
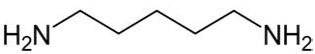

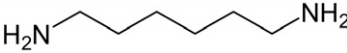

Computational Studies

Gaussian 2003 suite of programs was used in the calculations [15]. The proton affinity (PA = 236 kcal/mol) of DEE was calculated at the B3LYP/6-31G(d) level of theory by employing an isodesmic reaction scheme involving protonated methoxyethylene as the Brønsted acid [8d].

Results and Discussion

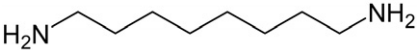
In a search for a suitable reagent, 2-methoxypropene (MOP) (PA^{8d} = 214 kcal/mol) appeared as a promising candidate since it is known to undergo a regioselective addition/MeOH elimination with protonated *ortho*-diamines (PA^{8d} = 214 kcal/mol) [6g]. However, MOP was found to be unreactive toward more basic compounds, e.g., *meta*-phenylenediamine [6g] (PA^{8d} = 222 kcal/mol⁸), diethylamine [6h] (PA^{8d} = 228 kcal/mol),

Table 1. Products (m/z values and branching ratios) formed in reactions between protonated aliphatic diamines and DEE (estimated PA = 238 kcal/mol)

Analyte (m/z of (M+H) ⁺)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
1,2-Propanediamine (75) 	228 ^b	Adduct – EtOH (145) (45%) DEE + H ⁺ (117) (55%)
2-Methyl-1,2-propanediamine (89) 	231 ^b	Adduct – EtOH (159) (61%) DEE + H ⁺ (117) (39%)
1,2- <i>trans</i> -Cyclohexanediamine (115) 	232 ^b	Adduct – EtOH (185) (64%) DEE + H ⁺ (117) (36%)
1,2- <i>cis</i> -Cyclohexanediamine (115) 	231 ^b	Adduct – EtOH (185) (62%) DEE + H ⁺ (117) (38%)
1,3-Propanediamine (75) 	236	Adduct – EtOH (145) (65%) DEE + H ⁺ (117) (35%)
2,2-Dimethyl-1,3-propanediamine (103) 	238 ^c	Adduct – EtOH (173) (94%) DEE + H ⁺ (117) (6%)
<i>N,N</i> -Dimethyl-1,3-propanediamine (103) 	245	Adduct – EtOH (173) (100%)
1,4-Butylenediamine (89) 	240	Adduct – EtOH (159) (100%)
1,5-Pentanediamine (103) 	239	Adduct – EtOH (173) (100%)
2-Methyl-1,5-pentanediamine (117) 	240 ^d	Adduct – EtOH (187) (100%)
1,6-Hexanediamine (117) 	239	Adduct – EtOH (187) (100%)
1,7-Heptanediamine (131) 	239	Adduct – EtOH (201) (100%)

(Continued)

Table 1. Continued

Analyte (m/z of $(M+H)^+$)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
1,8-Octanediamine (145) 	237 ^e	Adduct – EtOH (215) (86%) DEE + H ⁺ (117) (14%)

^aReference [8d].

^bCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 1,2-ethylenediamine as a reference Brønsted acid.

^cCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 1,3-propanediamine as a reference Brønsted acid.

^dCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 1,5-pentanediamine as a reference Brønsted acid.

^eCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 1,7-heptanediamine as a reference Brønsted acid.

and quinoline [6h] (PA^{8d} = 228 kcal/mol). A similar but more basic reagent is therefore needed to promote the identification of more basic compounds (i.e., bifunctional compounds). DEE was chosen as the neutral reagent for this study due to its high proton affinity (PA = 236 kcal/mol; calculated at the BLYP/6-31G(d) level of theory). Furthermore, DEE has two alkoxy groups that may act as leaving groups upon reactions with highly basic protonated analytes.

A brief summary of the results is provided here, followed by an in-depth discussion of the different analytes studied. Protonated aliphatic diamines were found to react with DEE to form a diagnostic addition/elimination product ion (adduct – EtOH) (Table 1). The efficiencies measured for the reactions of protonated aliphatic diamines with DEE are high. For example, protonated 1,3-propanediamine reacts with DEE at an efficiency of 21%, protonated 1,5-pentaneamine at 78%, protonated 2-methyl-1,5-pentanediamine at 75%, and protonated 2,2-dimethyl-1,3-propanediamine at 80%. These high reaction rates suggest that the ion–molecule reactions are fast enough for practical analytical applications. In addition, the observed proton transfer reactions suggest that the PA of DEE is actually somewhat greater than the calculated value (236 kcal/mol). The observation of proton transfer to DEE from protonated lysine (PA = 238 ± 2 kcal/mol) but not from protonated

1,5-pentaneamine (PA = 239 ± 2 kcal/mol) suggests that the PA of DEE is 238 ± 2 kcal/mol.

The examination of various additional analytes, including amides, N-oxides, mono- and triamines, aromatic diamines, amino alcohols, amino acids, amino ketone, amino thiol, amino thioether, amino alkene, phosphonate, phosphite, and oxygen-containing mono- and bifunctional compounds, suggest that protonated aliphatic bifunctional compounds (and one trifunctional compound, lysine) with PA between 222 and 245 kcal/mol react with DEE selectively by forming the addition/elimination product ion (adduct – EtOH). Protonated bifunctional analytes with PA lower than 222 kcal/mol, and monofunctional analytes with PA lower than 238 kcal/mol, predominantly transfer a proton to DEE, while protonated analytes with PA greater than 245 kcal/mol are unreactive. One protonated triamine (PA = 245 kcal/mol) was found to undergo addition and elimination of two ethanol molecules. The reactions of the different protonated analytes with DEE are discussed in more detail below.

Protonated Analytes

Aliphatic diamines. All protonated aliphatic diamines (PA ranges from 228 to 245 kcal/mol) react with DEE by forming the diagnostic addition/elimination product (adduct – EtOH) (Table 1). These product ions are easily recognized based on their m/z value that is 70 units greater than the m/z value of the protonated analytes (Figure 1). No secondary reaction products were observed. In addition to the addition/elimination reactivity, some of the aliphatic diamines (1,2-propanediamine, 2-methyl-1,2-propanediamine, 1,2-*trans*-cyclohexanediamine, 1,2-*cis*-cyclohexanediamine, 1,3-propanediamine, 2,2-dimethyl-1,3-propanediamine, and 1,8-octanediamine) react with DEE via proton transfer.

The addition/EtOH elimination reaction sequence (Scheme 1) is likely initiated by proton transfer from the protonated analyte to the most basic site, the vinyl group, in DEE. This step is either slightly exothermic or endothermic by up to ~7 kcal/mol for these diamines (Table 1). Slightly endothermic reactions can occur within gas-phase ion–molecule complexes due to the solvation energy provided by solvation of the ion by the neutral molecule [6b, 6d]. However, when the PA of the analyte is greater than 249 kcal/mol, this reaction becomes too

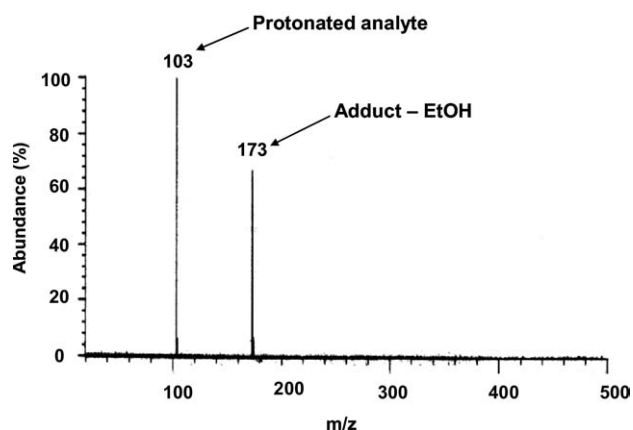
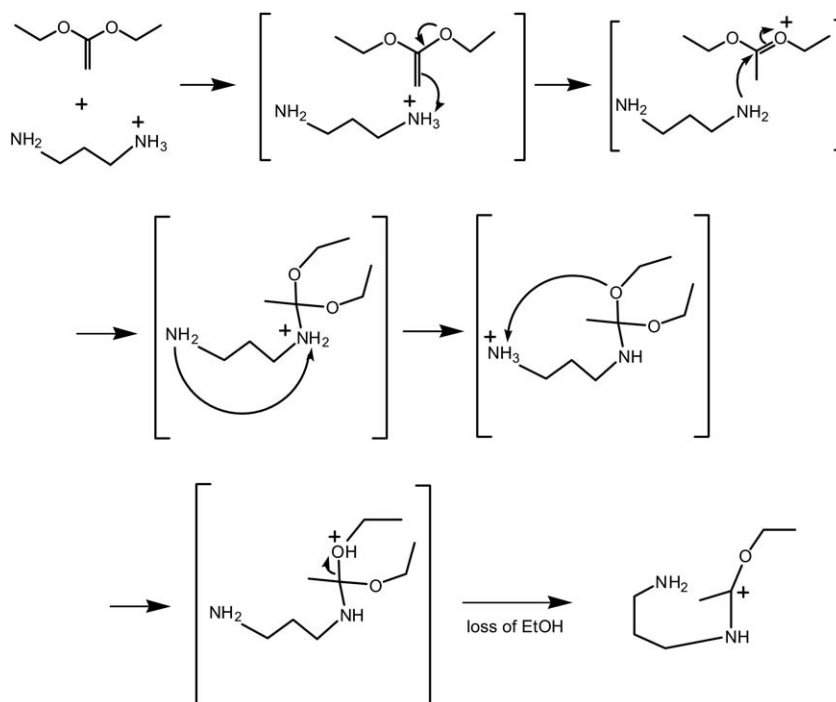


Figure 1. A mass spectrum measured after 3 s reaction of protonated 1,5-pentanediamine (m/z 103) with DEE (2.1×10^{-8} Torr) in FT-ICR. This reaction results in a derivatization product corresponding to addition accompanied by ethanol loss (adduct – EtOH) (m/z 173).



Scheme 1

endothermic to take place (see discussion below). Addition of an amino group of the analyte to the electrophilic α -carbon in protonated DEE follows. The reaction is completed by a proton transfer cascade that ultimately produces a protonated ethoxy group that cleaves off as neutral ethanol. Since this reaction was not observed for monoamines (see discussion below), both functional groups in diamines must play a critical role. Therefore, we propose that the proton transfer to the ethoxy group occurs via the second amino group of the analyte, instead of a direct transfer from the first amino group to the oxygen via a four-membered transition-state. The fact that both protonated *meta*-phenylenediamine and *N,N*-dimethylaminoacetone (Table 2; discussed in detail below) were not found to form this product ion provides further support for the occurrence of a proton transfer between the two functionalities in the analyte—for *meta*-phenylenediamine, the functional groups are oriented in such a manner that a proton transfer between them cannot take place; for *N,N*-dimethylaminoacetone, no proton is available to transfer after initial proton transfer and addition of the most nucleophilic group to the α -carbon of protonated DEE has taken place.

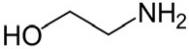
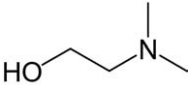

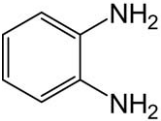
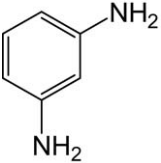
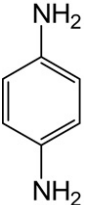
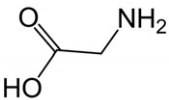
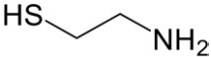
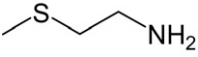
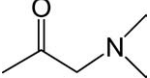
Other bifunctional analytes. To test whether the reaction discussed above (addition – EtOH) also occurs for other basic bifunctional analytes besides aliphatic diamines, various other bifunctional compounds, including aromatic diamines, amino alcohols, amino acids, amino ketone, amino thiol, amino thioether, hydroxyether, hydroxyketone, diol, and diketone, were examined (Table 2). The PAs of these analytes range from 202 to 230 kcal/mol, and hence are lower than

those of aliphatic diamines (228–245 kcal/mol) and DEE (~238 kcal/mol). Since proton transfer to DEE is exothermic for these analytes, it is not surprising that this was observed for all of them (Table 2). The results are detailed below.

All amino alcohols studied (PAs from 222 to 230 kcal/mol) were found to react with DEE by forming the addition/elimination product (adduct – EtOH), in addition to an abundant proton transfer product (Table 2). This finding was somewhat surprising because it reveals that even after a highly exothermic (up to 16 kcal/mol) proton transfer from the protonated analyte to DEE (first step in Scheme 1), the collision complex does not always dissociate to separated proton transfer products, but addition of the analyte to protonated DEE is able to compete, leading to the formation of the addition/elimination product.

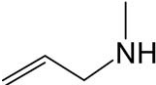

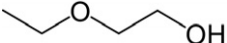
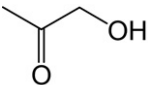
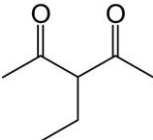
In sharp contrast to amino alcohols, the protonated amino acids, amino thiol, amino thioether, amino alkene, hydroxyether, hydroxyketone, diol, and diketone (PAs from 202 to 219 kcal/mol) exclusively transfer a proton to DEE to form the proton transfer product (m/z 117). This finding suggests that only analytes with PA greater or equal to 222 kcal/mol are able to undergo the diagnostic reaction. In addition to the proton transfer product, a fragment ion (m/z 89) of the protonated DEE (ethylene loss) was observed for the reaction of protonated 1-hydroxy-2-propanone with DEE due to the big difference in the PAs (Δ PA = 36 kcal/mol). Protonated *N,N*-dimethylaminoacetone has a high enough PA to undergo the diagnostic reaction (PA = 227 kcal/mol) but it exclusively transfers a proton to DEE due to the absence of a transferable proton after initial proton

Table 2. Products (m/z values and branching ratios) formed in reactions of various bifunctional analytes with DEE (estimated PA = 238 kcal/mol)

Analyte (m/z of (M+H) ⁺)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
2-Hydroxyethylamine (62) 	222	Adduct – EtOH (132) (13%) DEE + H ⁺ (117) (77%)
<i>N,N</i> -Dimethyl-2-hydroxyethylamine (90) 	227 ^b	Adduct – EtOH (160) (6%) DEE + H ⁺ (117) (94%)
3-Hydroxypropylamine (76) 	230	Adduct – EtOH (146) (54%) DEE + H ⁺ (117) (46%)
<i>ortho</i> -Phenylenediamine (109) 	214	DEE + H ⁺ (117) (100%)
<i>meta</i> -Phenylenediamine (109) 	222	Adduct – C ₆ H ₆ (147) (39%) Adduct (225) (3%) DEE + H ⁺ (117) (58%)
<i>para</i> -Phenylenediamine (109) 	217	DEE + H ⁺ (117) (100%)
Glycine (76) 	212	DEE + H ⁺ (117) (100%)
2-Thiolethylamine (78) 	217 ^b	DEE + H ⁺ (117) (100%)
2-Methylthioethylamine (92) 	219 ^b	DEE + H ⁺ (117) (100%)
<i>N,N</i> -Dimethylaminoacetone (102) 	227 ^b	DEE + H ⁺ (117) (100%)

(Continued)

Table 2. Continued

Analyte (m/z of $(M+H)^+$)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
N-Methylallylamine (72) 	219 ^b	DEE + H ⁺ (117) (100%)
1,3-Propylenediol (77) 	209	DEE + H ⁺ (117) (93%) Adduct – C ₂ H ₄ (165) (7%)
2-Ethoxyethanol (91) 	Unknown	DEE + H ⁺ (117) (100%)
1-Hydroxy-2-propanone (75) 	202	DEE + H ⁺ (117) (69%) DEE + H ⁺ – C ₂ H ₄ (89) (31%)
3-Ethyl-2,4-pentanedione (129) 	Unknown	DEE + H ⁺ (117) (100%)

^aReference [8d].^bCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 2-hydroxyethylamine as a reference Brønsted acid.

transfer and addition of the most nucleophilic group to the α -carbon of protonated DEE has taken place.

Three aromatic diamines (*ortho*-, *meta*- and *para*-phenylenediamine) were also studied (Table 2). In sharp contrast to aliphatic diamines (Table 1), no addition/EtOH elimination products were observed for the protonated aromatic diamines. Protonated *ortho*- and *para*-phenylenediamines react with DEE only via proton transfer due to the low PA of these diamines (214 and 217 kcal/mol, respectively). In addition to the proton transfer reactivity, protonated *meta*-phenylenediamine reacts with DEE to form a new addition/elimination product (adduct – C₆H₆) and a stable adduct.

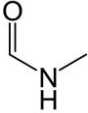
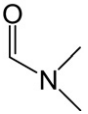
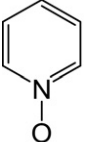
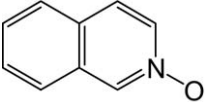
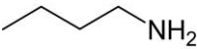
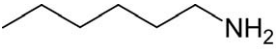
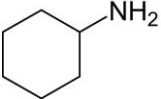

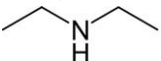
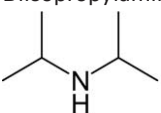
Nitrogen-, oxygen-, sulfur-, and phosphorus-containing monofunctional analytes. The selectivity of the neutral reagent (DEE) toward protonated basic bifunctional analytes (PA \geq 222 kcal/mol) was further probed by examining the reactivity of various protonated nitrogen-, oxygen-, sulfur-, and phosphorus-containing monofunctional compounds whose PAs range from 192 to 235 kcal/mol. All these ions transfer a proton to DEE to form the proton transfer product (m/z 117; Table 3), occasionally accompanied by a fragment ion of the proton transfer product (m/z 89). The diagnostic product ion (adduct – EtOH) was not observed for any of these analytes, including those with PA \geq 222 kcal/mol, which further

demonstrates that the formation of this product ion requires two functionalities in the analyte.

In addition to the proton transfer reactivity, some protonated amines (cyclohexylamine, hexylamine, and N-ethylmethylamine), pyridine and N-oxides react with DEE by forming a stable addition product (possibly a proton-bound dimer; Table 2). When the addition product (m/z 216) formed in the reaction of protonated cyclohexylamine with DEE was isolated and subjected to SORI-CAD, only protonated DEE (m/z 117) was formed. Hence, even energizing the reacting system does not result in formation of the diagnostic product ion for this monofunctional analyte.

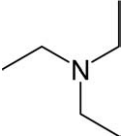
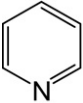
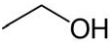

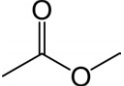

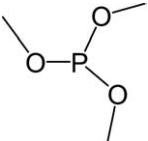
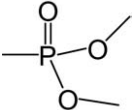
Trifunctional analytes. To further probe the selectivity of the addition/elimination reaction of DEE for bifunctional analytes with PA \geq 222 kcal/mol, various trifunctional compounds (PAs from 238 to 255 kcal/mol) were examined (Table 4). The analytes with PAs greater than 249 kcal/mol were found to be unreactive toward DEE, likely due to the excessively high endothermicity (>16 kcal/mol) of the initial proton transfer step in Scheme 1. One of the protonated triamines, 2,2'-diaminodiethylamine (PA = 245 kcal/mol), forms a product corresponding to addition and elimination of two ethanol molecules (adduct – 2 EtOH); Figure 2, possibly as shown in Scheme 2. This mechanism is analogous to that shown in Scheme 1, and it involves all three amino

Table 3. Products (m/z values and branching ratios) formed in reactions of protonated nitrogen-, oxygen-, sulfur- and phosphorus-containing monofunctional compounds with DEE (estimated PA = 238 kcal/mol)

Analyte (m/z of $(M+H)^+$)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
Methylformamide (60) 	204	DEE + H ⁺ (117) (100%)
Dimethylformamide (74) 	212	DEE + H ⁺ (117) (100%)
Pyridine N-oxide (96) 	221	DEE + H ⁺ (117) (79%) Adduct (212) (21%)
Isoquinoline N-oxide (146) 	Unknown	DEE + H ⁺ (117) (52%) Adduct (262) (27%) Adduct - CH ₃ COOC ₂ H ₅ (174) (21%)
Butylamine (74) 	220	DEE + H ⁺ (117) (100%)
Hexylamine (102) 	221	DEE + H ⁺ (117) (99%) Adduct (218) (1%)
Cyclohexylamine (100) 	223	DEE + H ⁺ (117) (91%) Adduct (216) (9%)
N-Ethylmethylamine (60) 	225	DEE + H ⁺ (117) (94%) Adduct (176) (6%)
Diethylamine (74) 	228	DEE + H ⁺ (117) (100%)
Diisopropylamine (102) 	232	DEE + H ⁺ (117) (100%)

(Continued)

Table 3. Continued

Analyte (m/z of $(M+H)^+$)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
Triethylamine (102) 	235	DEE + H ⁺ (117) (100%)
Pyridine (80) 	222	DEE + H ⁺ (117) (95%) Adduct (196) (5%)
Ethanol (47) 	196	DEE + H ⁺ (117) (80%) DEE + H ⁺ - C ₂ H ₄ (89) (20%)
Acetone (59) 	194	DEE + H ⁺ (117) (87%) DEE + H ⁺ - C ₂ H ₄ (89) (13%)
Methyl acetate (75) 	196	DEE + H ⁺ (117) (90%) DEE + H ⁺ - C ₂ H ₄ (89) (10%)
1-Butanethiol (91) 	192	DEE + H ⁺ (117) (81%) DEE + H ⁺ - C ₂ H ₄ (89) (19%)
Trimethyl phosphite (125) 	222	DEE + H ⁺ (117) (100%)
Dimethyl methylphosphonate (125) 	Unknown	DEE + H ⁺ (117) (100%)

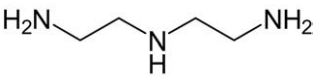
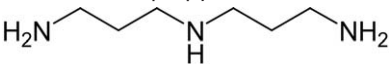
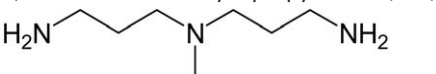
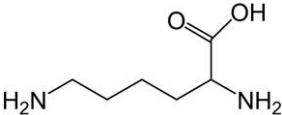
^aReference [8d].

groups since no such products were observed for mono- or diamines.

Only protonated lysine, with the lowest PA of the trifunctional analytes studied (238 kcal/mol), was found to undergo the diagnostic reaction with DEE (addition - EtOH). Although lysine contains three basic functionalities (two amino groups and one carboxylic acid group), it does not undergo elimination of two ethanol molecules like the triamine discussed above. However, SORI-CAD of the singly derivatized lysine (adduct - EtOH) results in elimination of EtOH

(and also elimination of NH = C(CH₃)OEt, which demonstrates that the first derivatized site in lysine was one of the amino groups). Clearly, activation of the first derivatization product is needed to overcome the endothermicity of proton transfer from a basic amino group to the much less basic carboxylic acid group in the singly derivatized analyte (that has lost some of the initial solvation energy during ethanol loss), which is required for the elimination of the second ethanol molecule (Scheme 2). This finding suggests that in addition to basic bifunctional ana-

Table 4. Products (m/z values and branching ratios) formed in reactions of protonated triamines with DEE (estimated PA = 238 kcal/mol)

Analyte (m/z of (M+H) ⁺)	PA ^a kcal/mol	Product ions (m/z) (branching ratio)
2,2'-Diaminodiethylamine (104) 	245 ^b	Adduct – 2 EtOH (128) (100%)
3,3'-Diaminodipropylamine (132) 	255 ^c	No products
3,3'-Diamino-N-methyldipropylamine (146) 	250 ^c	No products
Lysine (147) 	238	Adduct – EtOH (217) (61%) DEE + H ⁺ (117) (39%)

^aReference [8d].^bCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 1,2-ethylenediamine as a reference Brønsted acid.^cCalculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving 1,3-propanediamine as a reference Brønsted acid.

lytes, also trifunctional analytes with two highly basic and one less basic functionality may undergo the diagnostic reaction.

Conclusions

Protonated aliphatic bifunctional analytes with PA between 222 and 245 kcal/mol (as well as lysine with three functionalities) have been shown to react with neutral DEE in a FT-ICR mass spectrometer in a selective manner by forming an addition/elimination

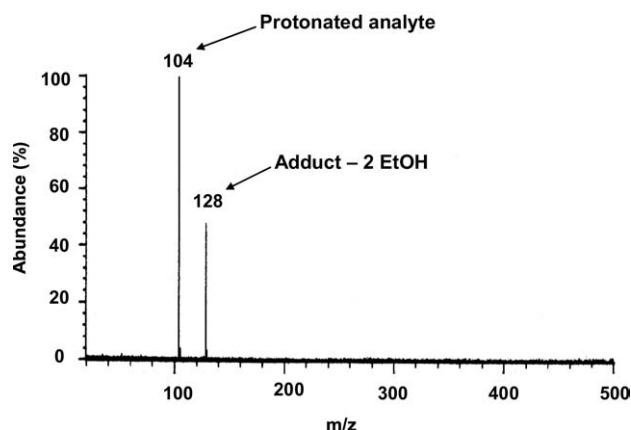
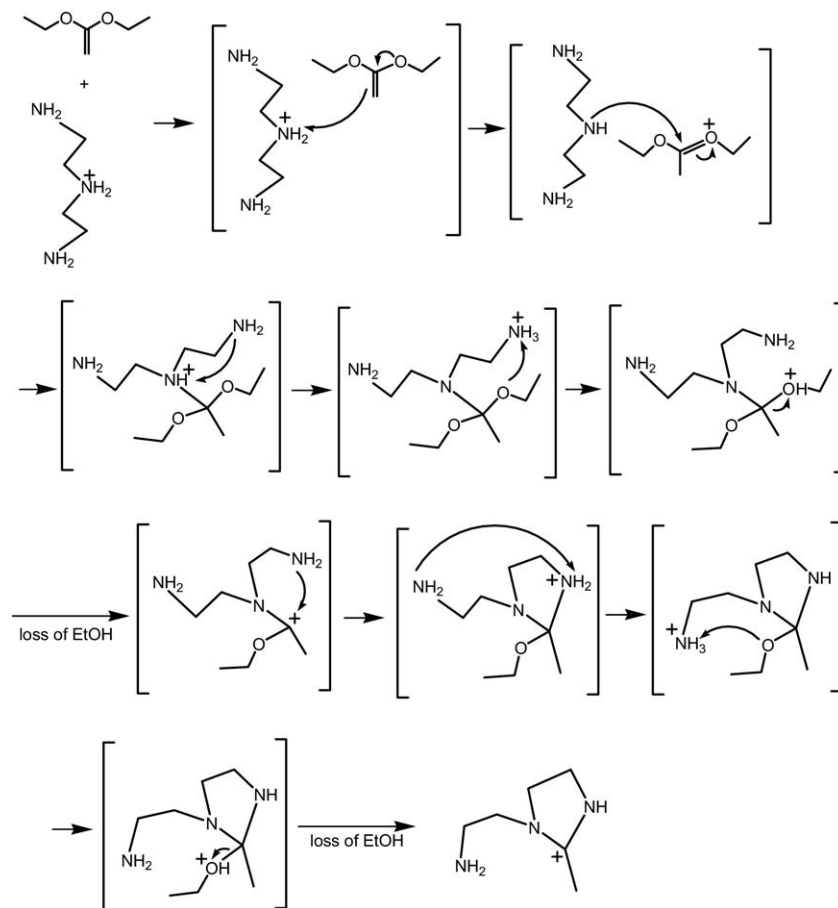


Figure 2. A mass spectrum measured after 5 s reaction of protonated 2,2'-diaminodiethylamine (m/z 104) with DEE (3.1×10^{-8} Torr) in FT-ICR. This reaction results in a product that corresponds to addition with the loss of two ethanol molecules (adduct – 2 EtOH) (m/z 128).

product ion (adduct – EtOH). The diagnostic reaction sequence involves proton transfer from the protonated analyte to the basic vinyl group in DEE, followed by the addition of the analyte to protonated DEE. The second step involves proton transfer from the addition site to the second functional group of the analyte, followed by transfer of a proton from this group to the ethoxy group of DEE (converting this into a good leaving group), which leads to elimination of an ethanol molecule (adduct – EtOH). Examination of the reactions of DEE with various mono-, di-, and trifunctional protonated analytes containing amido, amino, N-oxide, hydroxy, carboxylic acid, keto, thio, thioether, alkene, phosphate, and phosphonate functionalities, demonstrates that this reaction is selective for analytes with two basic functional groups and PA between 222 and 245 kcal/mol. However, the reaction does not occur for analytes wherein the functional groups cannot get into close proximity, such as some aromatic compounds, or for analytes wherein no transferable proton is available after initial proton transfer and addition of the most nucleophilic group to the α -carbon of protonated DEE has taken place, such as dimethylaminoketone, since the mechanism involves proton transfer between the functional groups in the analyte. Bifunctional analytes with PA lower than 222 kcal/mol, and mono-functional analytes with PA lower than 238 kcal/mol, predominantly transfer a proton to DEE, while analytes with PA greater than 249 kcal/mol are unreactive.



Scheme 2

Acknowledgments

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Appendix A Supplementary Material

Supplementary material associated with this article may be found in the online version at doi: [10.1016/j.jasms.2009.02.020](https://doi.org/10.1016/j.jasms.2009.02.020).

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