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Ion–molecule reactions facilitate the identification and differentiation of primary, secondary and tertiary amino functionalities in protonated monofunctional analytes in mass spectrometry

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ABSTRACT

A mass spectrometric method is presented that facilitates the identification and differentiation of primary, secondary and tertiary amino functionalities in protonated monofunctional analytes. This method utilizes gas-phase ion–molecule reactions of protonated analytes with neutral hexamethylphosphoramide (HMPA) and diethylmethylphosphonate (DEMP) in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR). A variety of protonated analytes containing different functional groups, namely, amino, amido, N-oxide and various oxygen-containing functional groups, were examined to demonstrate that protonated primary and secondary amines can be identified and differentiated by reactions with HMPA and DEMP. However, differentiation of tertiary amines from some N-oxides requires additional experiments. First, protonated secondary and tertiary amines can be differentiated from protonated primary amines, amides and oxygen-containing functionalities, as well as from each other (but not from protonated N-oxides), by using HMPA. Protonated primary amines, amides, some N-oxides and oxygen-containing analytes, most with a proton affinity (PA) < 229 kcal/mol, only transfer a proton to HMPA (PA = 229 kcal/mol). In contrast, protonated secondary amines also form two stable hydrogen-bound adducts (MH^+ + HMPA, MH^+ + 2HMPA; M: amine), and tertiary amines and some N-oxides (with PA ≥ 222 kcal/mol) react with HMPA by forming just one stable hydrogen-bound adduct (MH^+ + HMPA). Further, ion–molecule reactions with the other reagent, DEMP, allow the differentiation of protonated primary and secondary amines from tertiary amines and N-oxides and from protonated oxygen-containing analytes and amides. Protonated primary amines and secondary amines (most with PA ≥ 220 kcal/mol) react with DEMP (PA = 219 kcal/mol) by forming two stable hydrogen-bound adducts (MH^+ + DEMP and MH^+ + 2DEMP), while protonated oxygen-containing analytes and amides (with PA ≤ 221 kcal/mol) solely transfer a proton to DEMP. Protonated tertiary amines and N-oxides react yet differently, and yield only one stable hydrogen-bound adduct (MH^+ + DEMP) with DEMP. Protonated N-oxides can be differentiated from protonated tertiary amines by using previously reported methods based on diagnostic ion–molecule reactions of protonated N-oxides with 2-methoxypropene, dimethyl disulfide and/or tri(dimethylamine)borane.

1. Introduction

Development of methods for rapid and accurate identification of different types of amino groups in unknown analytes, preferably directly from mixtures, is of great interest to the chemical and pharmaceutical industry as well as to environmental scientists [1]. Amines are widely used as materials or intermediates in the production of explosives, rubber, epoxy polymers, plastics, coating additives, azo-dyes, aromatic polyurethane products, pharmaceuticals, cosmetics, ion-exchange resins, pesticides, additives in petroleum products, and corrosion inhibitors [2]. Due to their toxicity and carcinogenicity, amines are important environmental pollutants found in the air and water as a result of their widespread use [3]. In addition, analysis of biogenic amines is a topic of increasing interest in biological science and food chemistry [4]. For example, biogenic amines present in plants have been found to be associated with many cell processes, including cell division and differentiation, synthesis of nucleic acids and proteins, membrane stability, pH and thermic or osmotic stress responses, and defenses against herbivores and pathogens [4]. Further, biogenic polyamines present in animals and humans have been found to be essential for cell renewal [5]. For another example, biogenic amines

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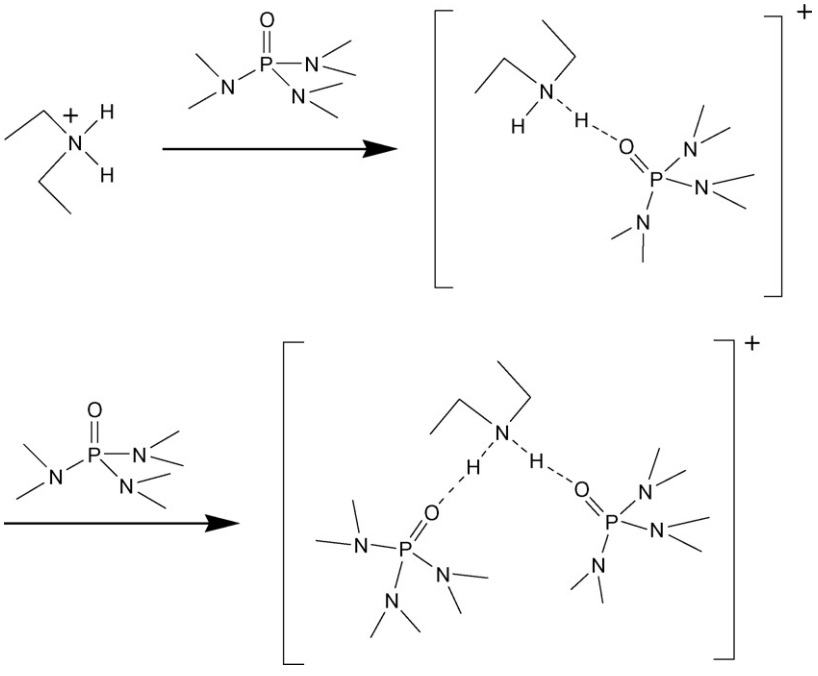
produced by lactic acid bacteria during the fermentation process of foods and beverages are present in cheese, salami, wine, beer, sauerkraut, fishery products and aged meat [6]. Therefore, accurate determination of the presence and the amounts of amines in various food products is important in the evaluation of their freshness, ripening level, and conditions of storage [7].

Several techniques have been employed to analyze known amines, including gas chromatography (GC), high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), capillary zone electrophoresis (CZE), paper electrophoresis (PE), thin layer electrophoresis (TLE), ultra-violet (UV) spectrophotometry, UV–vis combined with reflectance spectroscopy, and spectrofluorimetry [2d,5a,8]. However, to the best of our knowledge, none of these methods can be used to identify amino functionalities in previously unknown compounds or differentiate between the different types of amino functionalities in unknown analytes. Such powerful techniques as NMR and X-ray crystallography are usually needed to unambiguously identify an amino functionality in an unknown analyte [9a,9b]. However, these techniques require relatively large quantities of analytes and they are not applicable to complex mixtures [9a]. Moreover, NMR analysis of N-compounds is hindered by the requirement of good solubility [9a] and the low natural abundance of ^{15}N (0.37%) relative to ^{14}N. In addition, the small differences in chemical shifts of many N-containing compounds complicate NMR spectral interpretation [9c].

Mass spectrometric (MS) dissociation reactions can provide a wealth of information on previously unknown compounds. Furthermore, mass spectrometry (especially when combined with a separation technique or used in a tandem manner (MS/MS)) is particularly suitable for obtaining structural information on mixture components [10a–10d]. Indeed, MS has been recognized as the method of choice for the analysis of known amines in environmental, biological and food samples [2d,10d–10i]. However, the existing MS experimental approaches usually cannot provide information that allows the unambiguous identification of the presence of the amino functionality (as opposed to other types of N-functionalities, such as N-oxide or amido) or the type of the amino functionality present. Further, while electron ionization mass spectra often provide useful structural information for amines, in many cases the molecular ion is not stable and hence no molecular weight information is obtained [11a].

Recently, tandem mass spectrometry (MS/MS) experiments utilizing gas-phase ion–molecule reactions (instead of dissociation) have received attention as a possibly useful tool for obtaining detailed structural information for unknown compounds [12]. Thus far, several structurally diagnostic ion–molecule reactions (both class selective and functional group selective) have been developed [12]. However, the majority of these studies have focused on the identification of functionalities in neutral molecules by using selective ionic reagents (*e.g.*, ion–molecule reactions of dimethoxyborenium ion have been demonstrated to allow the identification of functional groups present in neutral alcohols, aldehydes, ethers, ketones, and biologically active molecules containing hydroxyl groups) [13]. One study has appeared in the literature on the use of ion–molecule reactions for the differentiation of primary, secondary and tertiary amines. This study is based on H/D exchange of amines by using deuterated ammonium ion as the chemical ionization reagent [11b].

Only a few studies have focused on the use of a neutral reagent to identify the functionalities in ionized analytes (*e.g.*, ion–molecule reactions of 2-methoxypropene (MOP) can be used to differentiate protonated *ortho*-, *meta*- and *para*-isomers of phenylene- and toluenediamines and to identify the protonated tertiary aromatic N-oxide functionality, and dimethyl disulfide can be used to identify the protonated primary N-oxide functionality) [14]. However, the approach of using a neutral reagent to analyze protonated (or deprotonated) analytes is needed for the mass spectrometric analysis of complex mixtures evaporated and ionized by the most commonly used methods, electrospray ionisation (ESI), atmospheric pressure chemical ionization (APCI) or matrix-assisted laser desorption/ionization (MALDI) [15]. Herein, we report ion–molecule reactions that facilitate the identification and differentiation of protonated primary, secondary and tertiary amino functionalities in unknown compounds (the differentiation of some N-oxides from tertiary amines has to be performed by using a literature method [14c,14e]).



Scheme 1.

2. Experimental

2.1. Instrumentation

All experiments were performed in a Nicolet model FTMS 2000 dual-cell FT-ICR mass spectrometer equipped with a 3-T superconducting magnet. This instrument contains a dual cell [16] consisting of two identical cubic 2-in cells separated by a conductance limit plate. The conductance limit plate has a 2-mm hole in the center for the transfer of ions from one side into the other. The conductance limit plate and the two end trapping plates were maintained at +2.0 V unless otherwise stated. The two cells share a common trap plate that can be temporarily grounded to transfer desired ions from one cell into the other through the 2-mm hole in the center of this plate. The dual cell is aligned collinearly with the field of a 3-T superconducting magnet. A nominal baseline pressure of less than 1×10^{-9} Torr was measured by two Bayard-Alpert ionization gauges, one on each side of the dual cell. Liquid samples were introduced into the instruments either by using a Varian leak valve or a batch inlet system equipped with an Andonian leak valve. A manual solids probe was used to introduce solid samples into the instrument.

All chemicals used were purchased from the Sigma–Aldrich Company and used without further purification. The protonated analytes were generated by self-chemical ionization (self-CI). This was accomplished by allowing the molecular ion and the ionic fragments generated upon electron ionization (EI) (20 eV electron energy, 7 μA emission current, ~0.2 s) of the analyte to react with

Table 1
Product ions (m/z values and branching ratios) formed in reactions of protonated amines with HMPA (PA = 229 kcal/mol[23]).

Analyte (M) (m/z of $(M+H)^+$)	1°, 2°, or 3°	PA[a] (kcal/mol)	Product ions (m/z) (branching ratio)[b]	SORI-CAD fragments of the adduct ions (m/z) (branching ratio)[b]
Butylamine (74)	1°	220	HMPA + H^+ (180) (100%) (2°) 2HMPA + H^+ (359)[c]	NN[d]
tert-Butylamine (74)	1°	223	HMPA + H^+ (180) (100%) (2°) 2HMPA + H^+ (359)	NN
Cyclohexylamine (100)	1°	223	HMPA + H^+ (180) (100%) (2°) 2HMPA + H^+ (359)	NN
Hexylamine (102)	1°	222	HMPA + H^+ (180) (100%) (2°) 2HMPA + H^+ (359)	NN
Benzylamine (108)	1°	218	HMPA + H^+ (180) (100%) (2°) 2HMPA + H^+ (359)	NN
N-Ethylmethylamine (60)	2°	225	MH^+ + HMPA (239) (4%) (2°) MH^+ + 2HMPA (418) HMPA + H^+ (180) (96%) (2°) 2HMPA + H^+ (359)	NE[e]
Diethylamine (74)	2°	228	MH^+ + HMPA (253) (30%) (2°) MH^+ + 2HMPA (432) HMPA + H^+ (180) (70%) (2°) 2HMPA + H^+ (359)	253 – M (180) (100%) 432 – HMPA (253) (100%)
Diisopropylamine (102)	2°	232	MH^+ + HMPA (281) (100%) (2°) MH^+ + 2HMPA (460)	281 – HMPA (102) (77%) 281 – M (180) (23%) 460 – HMPA (281) (100%)
N-Benzyl-N-butyl-amine (164)	2°	231[f]	MH^+ + HMPA (343) (83%) (2°) MH^+ + 2HMPA (522) HMPA + H^+ (180) (17%) (2°) 2HMPA + H^+ (359)	343 – HMPA (164) (56%) 343 – M (180) (44%) 522 – HMPA (343) (100%)
Dihexylamine (186)	2°	Unknown	MH^+ + HMPA (365) (100%) (2°) MH^+ + 2HMPA (544)	365 – HMPA (186) (91%) 365 – M (180) (9%) 544 – HMPA (365) (100%)
Triethylamine (102)	3°	235	MH^+ + HMPA (281) (100%)	281 – HMPA (102) (100%)
Diisopropylethyl amine (130)	3°	238	MH^+ + HMPA (309) (100%)	309 – HMPA (130) (100%)
N,*N*-Dimethylben zylamine (136)	3°	232	MH^+ + HMPA (315) (13%) HMPA + H^+ (180) (87%) (2°) 2HMPA + H^+ (359)	NE
Tripropylamine (144)	3°	237	MH^+ + HMPA (232) (100%)	233 – HMPA (144) (100%)
Tributylamine (186)	3°	239	MH^+ + HMPA (365) (100%)	365 – HMPA (186) (100%)
Pyridine (80)	3°	222	MH^+ + HMPA (259) (2%) HMPA + H^+ (180) (98%) (2°) 2HMPA + H^+ (359)	NE
Isoquinoline (130)	3°	228	MH^+ + HMPA (309) (20%) HMPA + H^+ (180) (80%) (2°) 2HMPA + H^+ (359)	NE

[a] Ref. [23].
[b] Only primary products' branching ratios are listed.
[c] Secondary products (2°) are shown after the primary products that formed them.
[d] Not necessary.
[e] Not examined.
[f] Calculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving methylamine as a reference Brønsted acid.

the neutral analyte molecules for a certain period of time (~3.0 s). Nominal pressure of the neutral analyte in the cell varied between 0.5×10^{-8} and 1.5×10^{-7} Torr, as measured by the Bayard-Alpert ionization gauges. All the ions in the other side of the dual cell were removed prior to ion transfer 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 μs). Following transfer into the other cell, the transferred ions were cooled for a period of 1 s by allowing IR emission and by collisions with Ar present at about 10^{-5} Torr [17]. The protonated analyte was isolated by using a stored-waveform inverse Fourier transform [18] (SWIFT) excitation pulse to eject all unwanted ions, and allowed to react with neutral reagents introduced into the same cell through a variable leak valve. The nominal pressure of the neutral reagents in the cell was $1.1–6.9 \times 10^{-8}$ Torr. Some of the reaction products were further studied by isolating them and subjecting them to sustained off-resonance irradiated collision-activated dissociation [19] (SORI-CAD). SORI-CAD experiments utilized off-resonance excitation of the isolated ion at a frequency ±1000 Hz off the cyclotron frequency of the ion. This experiment was carried out by subjecting the desired ion to off-resonance excitation pulse with an amplitude between 0.19 and 1.1 V for 300 ms while allowing the ion to undergo collisions with argon (~10^{-5} Torr) for 0.6 s.

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 Hz μs^{-1}. The spectra were recorded as 64k data points by using one zero-fill prior to Fourier transformation. Background subtraction was applied to all spectra to make sure that the observed products were generated from the desired ion population. Background spectra were recorded by removing the reactant ion by SWIFT ejection prior to the reaction time.

2.2. Kinetics

Kinetic data were obtained by allowing the protonated analytes to react with the neutral reagents for variable periods of time at a constant pressure prior to excitation and detection. Under the conditions described above, the neutral reagent was present in excess of the ion of interest. Hence, these reactions inherently follow pseudo-first order kinetics. The second-order reaction rate constants ($k_{reaction}$) were derived from the negative slopes of the plots of the natural logarithm of the relative abundances of the protonated analytes versus time. The collision rates ($k_{collision}$) were estimated using the parameterized trajectory theory of Su and Chesnavich [20]. The overall efficiency of each reaction was given as $k_{reaction}/k_{collision}$ (*i.e.*, the percentage of collisions leading to product formation). Pressure readings of the ion gauges were corrected for the sensitivity of the ion gauge towards the neutral reagents (DEMP and HMPA) and their distance from the center of the ICR cell [21]. These correction factors were obtained by measuring the rates of highly exothermic electron-transfer reactions between $CS_2^{\bullet+}$ and the neutral reagents, DEMP and HMPA. These exothermic electron transfer reactions are assumed to proceed at the collision rate. Therefore, the efficiency of these reactions is assumed to 100%.

2.3. Computational studies

Gaussian 03 suite of programs was used in calculations [22]. Geometry optimizations and vibrational frequency calculations

Table 2
Product ions (*m*/*z* values and branching ratios) formed in reactions of various protonated oxygen-containing analytes, amides and N-oxides with HMPA and DEMP.

Analyte (M) (*m*/*z* of (M + H)$^+$)	PA[a] (kcal/mol)	Product ions for HMPA (*m*/*z*) (branching ratio)[b]	Product ions for DEMP (*m*/*z*) (branching ratio)[b]
Ethanol (47)	186	HMPA + H$^+$ (180) (87%)[c] (2°) 2HMPA + H$^+$ (359)[d]	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
tert-Butyl methyl ether (89)	201	HMPA + H$^+$ (180) (72%)[c] (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Acetone (59)	194	HMPA + H$^+$ (180) (64%)[c] (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Butyraldehyde (87)	190	HMPA + H$^+$ (180) (38%)[c] (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Methyl acetate (75)	196	HMPA + H$^+$ (180) (34%)[c] (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Butyric acid (89)	188[e]	HMPA + H$^+$ (180) (100%)[c] (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Acetamide (60)	206	HMPA + H$^+$ (180) (100%) (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Methylacetamide (74)	212	HMPA + H$^+$ (180) (100%) (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Dimethylformamide (74)	212	HMPA + H$^+$ (180) (100%) (2°) 2HMPA + H$^+$ (359)	DEMP + H$^+$ (153) (100%) (2°) 2DEMP + H$^+$ (305)
Pyridine N-oxide (96)	221	HMPA + H$^+$ (180) (100%) (2°) 2HMPA + H$^+$ (359)	MH$^+$ + DEMP (248) (100%)
Isoquinoline N-oxide (146)	228[f]	MH$^+$ + HMPA (325) (17%) HMPA + H$^+$ (180) (83%) (2°) 2HMPA + H$^+$ (359)	MH$^+$ + DEMP (298) (100%)

[a] Ref. [23].
[b] Only primary products' branching ratios are listed.
[c] Due to the large difference in the basicities (ΔPA > 28 kcal/mol) of these oxygen-containing analytes compared to HMPA, the product of the exothermic proton transfer (HMPA + H$^+$) fragments to yield ions of *m*/*z* 46, *m*/*z* 58 and *m*/*z* 103.
[d] Secondary products (2°) are shown after the primary products that formed them.
[e] Calculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving formic acid as a reference Brønsted acid.
[f] Ref. [14f].

were performed using density functional theory at the B3LYP/6-31G(d) level. Stationary points were characterized by frequency calculations to confirm a correct number of imaginary frequencies. Minimum energy structures have no imaginary frequencies. All theoretical energies are presented at 0 K and include zero-point vibrational energy corrections. The proton affinity (PA) of DEMP was calculated at the B3LYP/6-31G(d) level of theory by employing an isodesmic reaction scheme involving protonated trimethylphosphate as the Brønsted acid [23,14e].

2.4. Hazard warning

HMPA is a known carcinogen and should be handled with care [24].

3. Results and discussion

3.1. Reactions of protonated amines with HMPA

Protonated primary amines exclusively transfer a proton to HMPA (by forming the proton transfer product HMPA + H^+) due to the low PAs of primary amines relative to that of HMPA (Table 1). This proton transfer product (HMPA + H^+) reacts further with another HMPA molecule to form a secondary product (2HMPA + H^+).

In sharp contrast to protonated primary amines, protonated secondary amines react with HMPA to form two stable adducts, a primary (MH^+ + HMPA) and a secondary product (MH^+ + 2HMPA), likely by the mechanism shown in Scheme 1. These adducts are likely to have hydrogen-bound structures (as indicated in the Scheme) instead of covalently N–P bound structures since the hydrogen-bound complex of NH_4^+ and $O{=}PH_3$ was found to be 17.3 kcal/mol more stable than the covalently bound adduct of NH_3 and $HO{=}PH_3^+$ (B3LYP/6-31G(d)). No tertiary adduct (MH^+ + 3HMPA) formation was observed, possibly due to the absence of an acidic proton in the secondary adduct. In addition to this reactivity, some protonated secondary amines, the protonated N-ethylmethylamine, diethylamine, and N-benzyl-N-butylamine, were found to react with HMPA by forming proton transfer products (HMPA + H^+ and 2HMPA + H^+) due to their low PAs relative to HMPA.

In contrast to protonated primary and secondary amines, protonated tertiary amines react with HMPA only by forming one hydrogen-bound adduct (MH^+ + HMPA). In summary, protonated primary, secondary, and tertiary amines can be differentiated from each other by reactions with HMPA (Fig. 1).

Since both secondary and tertiary amines form stable hydrogen-bound adducts upon reaction with HMPA, some of these hydrogen-bound adducts were isolated and subjected to sustained off-resonance irradiation collision-activated dissociation (SORI-CAD) in the FT-ICR (Table 1). While the secondary hydrogen-bound adducts (MH^+ + 2HMPA) of all studied protonated secondary amines only undergo loss of HMPA upon SORI-CAD, most of the primary hydrogen-bound adducts (MH^+ + HMPA) fragment by two different ways, via elimination of HMPA or the neutral amine (M)

Table 3
Product ions (*m*/*z* values and branching ratios) formed in reactions of protonated amines with DEMP (PA = 219 kcal/mol).

Analyte (M) (*m*/*z* of $(M+H)^+$)	1°, 2°, or 3°	PA[a] (kcal/mol)	Product ions (*m*/*z*) (branching ratio)[b]
Butylamine (74)	1°	220	MH^+ + DEMP (226) (100%) (2°) MH^+ + 2DEMP (378)[c]
tert-Butylamine (74)	1°	223	MH^+ + DEMP (226) (100%) (2°) MH^+ + 2DEMP (378)
Cyclohexylamine (100)	1°	223	MH^+ + DEMP (253) (100%) (2°) MH^+ + 2DEMP (405)
Hexylamine (102)	1°	222	MH^+ + DEMP (254) (100%) (2°) MH^+ + 2DEMP (406)
Benzylamine (108)	1°	218	MH^+ + DEMP (260) (86%) (2°) MH^+ + 2DEMP (412) DEMP + H^+ (153) (14%) (2°) 2DEMP + H^+ (305)
N-Ethylmethylamine (60)	2°	225	MH^+ + DEMP (212) (100%) (2°) MH^+ + 2DEMP (364)
Diethylamine (74)	2°	228	MH^+ + DEMP (226) (100%) (2°) MH^+ + 2DEMP (378)
Diisopropylamine (102)	2°	232	MH^+ + DEMP (254) (100%) (2°) MH^+ + 2DEMP (406)
N-Benzyl-*N*-butylamine (164)	2°	231[d]	MH^+ + DEMP (316) (100%) (2°) MH^+ + 2DEMP (468)
Dihexylamine (186)	2°	Unknown	MH^+ + DEMP (338) 100%) (2°) MH^+ + 2DEMP (490)
Triethylamine (102)	3°	235	MH^+ + DEMP (254) (100%)
Diisopropylethylamine (130)	3°	238	MH^+ + DEMP (282) (100%)
N,*N*-Dimethylbenzylamine (136)	3°	232	MH^+ + DEMP (288) (100%)
Tripropylamine (144)	3°	237	MH^+ + DEMP (296) (100%)
Tributylamine (186)	3°	239	MH^+ + DEMP (338) (100%)
Pyridine (80)	3°	222	MH^+ + DEMP (232) (100%)
Isoquinoline (130)	3°	228	MH^+ + DEMP (282) (100%)

[a] Ref. [23].
[b] Only primary products' branching ratios are listed.
[c] Secondary products (2°) are shown after the primary products that formed them.
[d] Calculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving methylamine as a reference Brønsted acid.

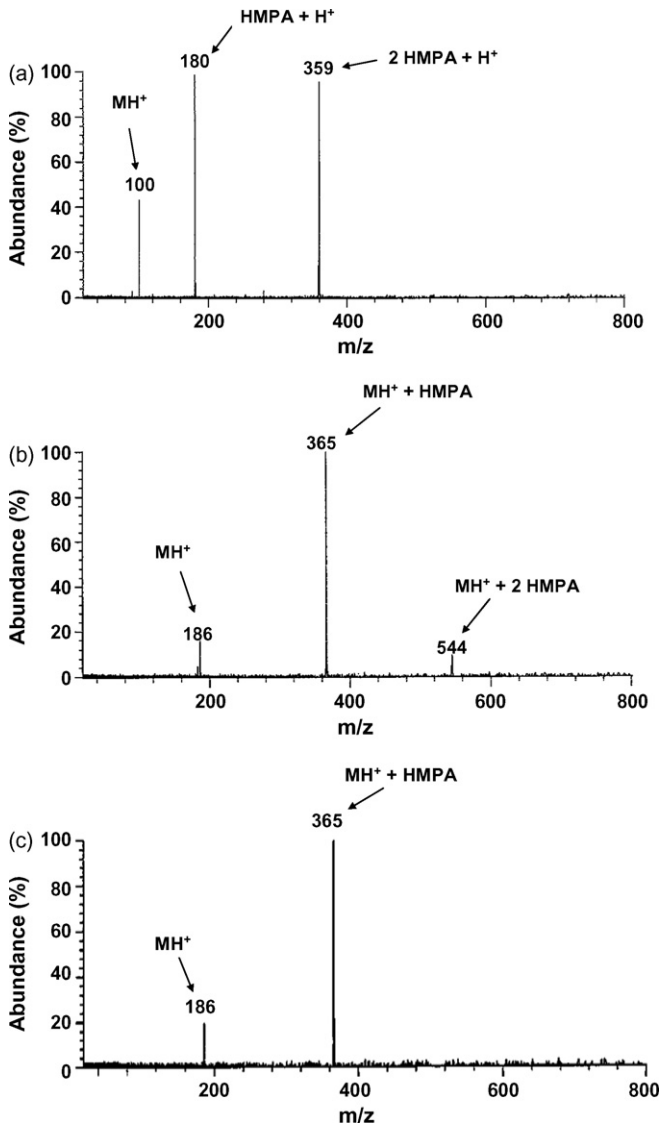


Fig. 1. Mass spectra showing the ionic products formed upon reactions of protonated primary, secondary, and tertiary amines (MH^+) with HMPA. (a) A mass spectrum measured after 5 s reaction of protonated cyclohexylamine (MH^+, m/z 100) with HMPA, leading to two proton transfer products, HMPA + H^+ (m/z 180) and 2HMPA + H^+ (m/z 359). (b) A mass spectrum measured after 20 s reaction of protonated dihexylamine (MH^+, m/z 186) with HMPA, which results in two stable hydrogen-bound adducts (MH^+ + HMPA (m/z 365) and MH^+ + 2HMPA (m/z 544)). (c) A mass spectrum measured after 20 s reactions of protonated tributylamine (MH^+, m/z 186) with HMPA, forming one hydrogen-bound adduct (MH^+ + HMPA (m/z 365)).

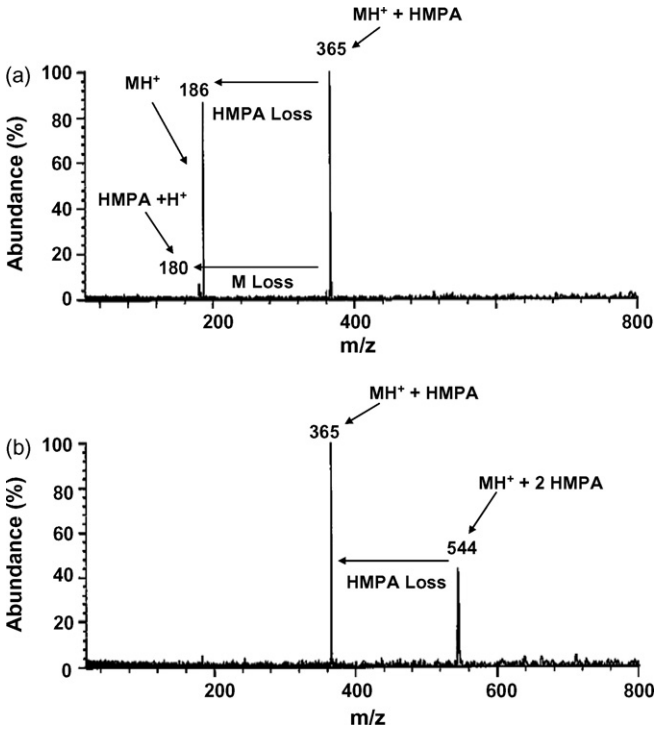


Fig. 2. SORI-CAD spectra of the hydrogen-bound adducts formed in a dual cell FT-ICR in the reaction between protonated dihexylamine (MH^+) and HMPA. (a) SORI-CAD spectrum of the primary hydrogen-bound adduct (MH^+ + HMPA) (m/z 365). (b) SORI-CAD spectrum of the secondary hydrogen-bound adduct (MH^+ + 2HMPA) (m/z 544).

(for example, see Fig. 2). Only one primary hydrogen-bound adduct, that of the protonated secondary amine with PA lower than that of HMPA, does not fragment by loss of HMPA. In sharp contrast to the fragmentation behavior of the primary hydrogen-bound adducts of secondary amines, the primary hydrogen-bound adducts (MH^+ + HMPA) of protonated tertiary amines only undergo loss of HMPA upon SORI-CAD (Table 1) due to their high PAs relative to that of HMPA. This dissociation behavior further facilitates the differentiation of primary, secondary and tertiary amines.

3.2. Reactions of protonated oxygen-containing analytes, amides and N-oxides with HMPA

The selectivity of HMPA toward protonated amino functionalities was probed by examining the reactions of protonated oxygen- and nitrogen-containing analytes, including an alcohol, an ether, a ketone, an aldehyde, an ester, a carboxylic acid, amides and N-oxides (Table 2). The PAs of these compounds range from 186 to 212 kcal/mol, and hence are substantially lower than that of HMPA (PA = 229 kcal/mol). Such big differences in basicity allow only fast proton transfer to take place. Indeed, only proton transfer products (HMPA + H^+, 2HMPA + H^+) were observed for these protonated analytes, with one exception (isoquinoline N-oxide). These proton transfer products in some cases included dissociation products (m/z 46 ($(H_2N(CH_3)_2^+$), m/z 58 (likely $H_2C{=}N(CH_3)_2^+$), and m/z 103 (unknown)) of protonated HMPA). The observed dissociation products are due to the large differences in the basicities (ΔPA > 18) of some of the analytes compared to HMPA, which makes the proton transfer highly exothermic. The only protonated analyte that was found to form a stable hydrogen-bound adduct with HMPA, *i.e.*, react like protonated tertiary amines, is protonated isoquinoline N-oxide.

In summary, ion–molecule reactions of HMPA allow the identification of protonated secondary and tertiary amines and their differentiation from primary amines, oxygen-containing analytes and amides. However, the reactivity of protonated isoquinoline N-oxide toward HMPA was found to be similar to the reactivity of protonated tertiary amines. This does not cause a problem, however, since the protonated N-oxide functionality can be differentiated from the protonated amino functionality by using previously developed ion–molecule reaction based methods [14c,14e].

3.3. Reactions of protonated analytes with DEMP

Primary amines can be differentiated from oxygen-containing analytes, amides and N-oxides by using another phosphorus reagent, DEMP (Table 3). This reagent has a lower PA (calculated to be 219 kcal/mol at the B3LYP/6-31G(d) level of theory) than HMPA (229 kcal/mol), primary amines (218–220 kcal/mol), and N-oxides (>220 kcal/mol) but higher than oxygen-containing analytes and amides (186–212 kcal/mol). Hence, this reagent was expected

to react with oxygen-containing analytes and amides by proton abstraction but with primary amines (as well as secondary and tertiary amines and N-oxides) by hydrogen-bound adduct formation. Indeed, all protonated primary and secondary amines were found to react with DMEP by forming two stable hydrogen-bound adducts (MH^+ + DEMP and MH^+ + 2DEMP; a minor proton transfer product was also formed for the least basic primary amine), while protonated tertiary amines and N-oxides react with DEMP by forming only one hydrogen-bound adduct (MH^+ + DEMP). In sharp contrast, all other protonated oxygen-containing analytes, including amides, transfer a proton to DEMP by forming two proton transfer products (DEMP + H^+ and 2DEMP + H^+) due to their low PAs relative to that of DEMP (Table 2).

3.4. Reaction kinetics

The efficiencies measured for the reactions of protonated amines with HMPA and DMEP are high. For example, protonated N-benzyl-N-butylamine reacts with HMPA at an efficiency of 92%, protonated N,N-dimethylbenzylamine at 72%, protonated diisopropylethylamine at 59%, and protonated diethylamine at 79%. Protonated *tert*-butylamine reacts with DEMP at an efficiency of 77%, protonated dimethylbenzylamine at 49%, protonated N-butylbenzylamine at near 62%, and protonated hexylamine at 74%. The above reaction efficiencies suggest that these ion–molecule reactions are fast enough for practical applications.

4. Conclusions

Ion–molecule reactions of protonated analytes with two phosphorus reagents, HMPA and DEMP, have been demonstrated to facilitate the identification of amino functionalities, and the differentiation of primary, secondary and tertiary amino functionalities. Protonated secondary and tertiary amines can be differentiated from protonated primary amines, amides and oxygen-containing functionalities, as well as from each other (but not from protonated N-oxides), by using HMPA. Further, ion–molecule reactions with the other reagent, DEMP, allow the differentiation of protonated primary and secondary amines from tertiary amines and N-oxides and from protonated oxygen-containing analytes and amides. The observed reactivity can be partially rationalized by the proton affinities of the phosphorus reagents. While the reactivity of one protonated N-oxide toward HMPA and DEMP was found to be similar to the reactivity of protonated tertiary amines, this does not present a problem since the N-oxide functionality can be differentiated from amino functionalities by using previously developed ion–molecule reaction-based methods [14c,14e,14f].

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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.2009.01.018.

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