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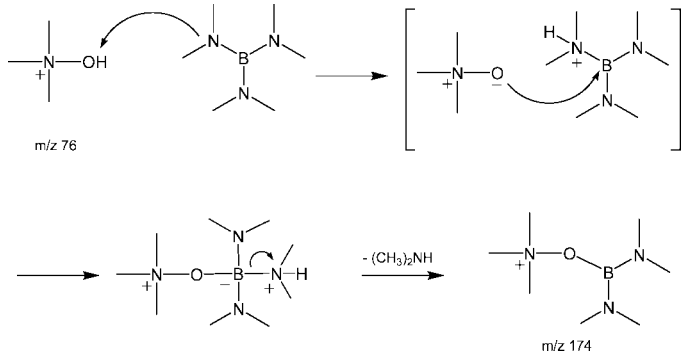
# Identification of Aliphatic and Aromatic Tertiary N-Oxide Functionalities in Protonated Analytes via Ion/Molecule and Dissociation Reactions in an FT-ICR Mass Spectrometer

Penggao Duan,[†] Mingkun Fu,[†] Todd A. Gillespie,[‡] Brian E. Winger,[‡] and Hilkka I. Kenttämaa*,[†]

*Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, and Eli Lilly and Company, Indianapolis, Indiana 46285*

*hilkka@purdue.edu*

*Received September 10, 2008*




A mass spectrometric method is presented for the identification of compounds that contain the aliphatic or aromatic N-oxide functional group. This method utilizes gas-phase ion/molecule reactions of tri(dimethylamino)borane (TDMAB), which rapidly derivatizes protonated aliphatic and aromatic tertiary N-oxides, amides, and some amines via loss of dimethylamine in a Fourier transform ion cyclotron resonance mass spectrometer. The mechanism involves proton transfer from the protonated analyte to the borane, followed by addition of the analyte to the boron center and elimination of dimethylamine. The derivatized analytes are readily identified on the basis of their *m*/*z* value which is 98 Th (thomson) greater than that of the protonated analyte, and the characteristic boron isotope patterns. SORI-CAD of the product ions (adduct-$(CH_3)_2NH$) yields different fragment ions for aliphatic tertiary N-oxides, aromatic tertiary N-oxides, amides, and pyridines. Therefore, these analytes can be identified based on their characteristic fragment ions. This method was tested by examining two drug samples, Olanzapine and Olanzapine-4′ *N*-oxide.

## Introduction

The oxidation of a N-atom to form a N-oxide is an important biotransformation pathway for many drugs and a commonly observed stress-induced oxidative degradation reaction in pharmaceuticals.[1] The ability to rapidly identify metabolites and their degradation products containing the N-oxide functionality is of interest since they are usually considered to be genotoxic.[2,3] Spectroscopic techniques, such as FT-IR, X-ray crystallography, and NMR, are commonly used to obtain information regarding the functional groups and elemental connectivity of analytes. However, due to their low sensitivity, these techniques usually require relatively large amounts of high-purity analytes.[4] Furthermore, the analysis of N-containing organic compounds by NMR is often hindered by the requirement of good solubility of the analytes[4] and by the low natural abundance of $^{15}N$ (0.37%) relative to $^{14}N$. In addition, the very small differences in

* Corresponding author. Phone: (765) 494-0882. Fax: (765)494-0239.
[†] Purdue University.
[‡] Eli Lilly and Company.

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10.1021/jo802001e CCC: $40.75 
Published on Web 01/09/2009

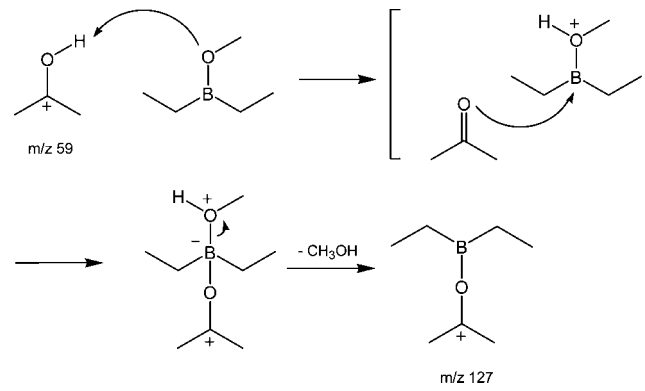
chemical shifts of many N-containing compounds complicate NMR spectral interpretation.[5]

Mass spectrometry/mass spectrometry (MS/MS) is a sensitive technique well-suited for obtaining structural information for organic compounds. The experiments typically involve ionization of the analyte by protonation followed by the mass-selection of the protonated analyte and its characterization by techniques such as collision-activated dissociation.[6,7] However, the CAD spectra of N-oxides are, in general, quite similar to those of many other nitrogen-containing species, which makes it difficult to unambiguously identify the N-oxide functionality.[4,8] For example, the CAD mass spectrum of protonated 4-amino-3,5-dichloropyridine *N*-oxide is identical with that of its isomer, protonated 4-amino-3,4-dichloro-2-hydroxypyridine.[8]

MS/MS experiments utilizing ion/molecule reactions have been used successfully for functional group identification.[9] For example, the epoxide and the acetal functionalities can be identified on the basis of gas-phase reactions with acylium ions,[10] and dimethoxyborenium ion can be used to identify the functional groups present in neutral alcohols,[11,12] aldehydes,[13] ketones,[13] ethers,[14] and esters.[15] However, the majority of the past work on ion/molecule reactions has focused on obtaining functionality information for neutral analytes by using ionic reagents. Only very few studies have appeared wherein neutral reagents are utilized to identify functionalities in protonated analytes, despite the wide use of ionization techniques such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) to produce protonated gaseous analytes. Such studies include, for example, the identification of protonated $\beta$-hydroxycarbonyl,[16] epoxide,[17] lactone,[18] carboxylic acid and ester[19] functionalities, as well as the head groups in different phospholipids.[20]

Recently, ion/molecule reactions involving neutral organoboranes (e.g., diethylmethoxyborane[21] (DEMB) and trimethoxylborane[22] (TMB)) were introduced for the identification and counting of O-containing functionalities in protonated mono-

**SCHEME 1**




functional and bifunctional analytes as well as polyols. The diagnostic reaction sequence involves proton transfer from the protonated analyte to the methoxy moiety of the organoborane, followed by nucleophilic addition of the analyte to the empty p-orbital of boron and elimination of methanol (Scheme 1). The derivatized analytes are readily identified on the basis of characteristic boron isotope pattern. Unfortunately, DEMB and TMB are not basic enough to deprotonate protonated N-oxides due to the high proton affinities (PAs) of these analytes (PA = 205−240 kcal/mol[23]) compared to those of the reagents (PA of DEMB is 191 kcal/mol[21] and PA of TMB is 195 kcal/mol[22]). We recently reported that dimethyl disulfide (DMDS) can be used to identify primary N-oxide functionality in protonated analytes via a functional group selective ion/molecule reaction.[24] However, the mechanism proposed for this reaction (Scheme 2) is not feasible for tertiary N-oxides. Indeed, protonated pyridine N-oxide was found to be unreactive toward DMDS. More recently, 2-methoxypropene was demonstrated to allow the identification of the aromatic tertiary N-oxide functional group in protonated analytes via the selective formation of a stable adduct.[25] However, 2-methoxypropene is unreactive toward protonated analytes containing the aliphatic tertiary N-oxide functional group and hence cannot be used to identify this functionality. Therefore, a different reagent is needed for the identification of the tertiary N-oxide functional group.

In this study, we introduce tris(dimethylamino)borane (TDMAB) as a novel reagent that can be used to identify aliphatic and aromatic tertiary N-oxide functionalities in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer. TDMAB was chosen as the neutral reagent for this study because it has a higher calculated PA (PA = 230 kcal/mol, B3LYP/6-31G(d)) than DEMB and TMB, which should aid in the derivatization of tertiary N-oxides that have much higher PAs (220−240 kcal/mol) than monofunctional and bifunctional O-containing compounds.

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**SCHEME 2**

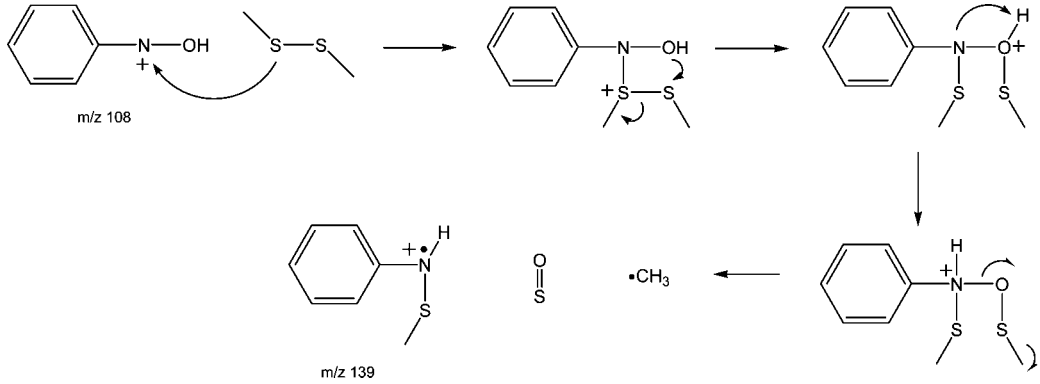
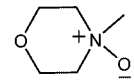
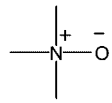
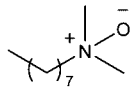
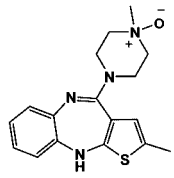
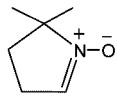



**TABLE 1. Observed Product Ions (Formation Reactions, *m/z* Values, Branching Ratios, and Reaction Efficiencies (Eff.)) Formed in Reactions of Protonated Aliphatic Tertiary N-Oxides with TDMAB (PA = 230 kcal/mol) and their SORI-CAD Fragments (Formation Reactions and *m/z* Values) in an FT-ICR Mass Spectrometer**

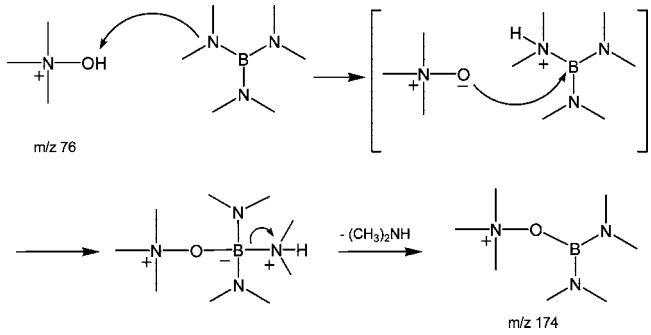
| Analyte (*m/z* of $[M+H]^+$) | Proton affinity (kcal/mol) | Observed product ions,[a] *m/z* (branching ratio %) | SORI-CAD fragments of derivatized analytes, *m/z* |
|---|---|---|---|
|  (118) | 224.6[b] | Adduct - $(CH_3)_2NH$, 216 (100)<br>Eff.[e] = 48% | 216 - $HOB(N(CH_3)_2)_2$, 100 |
|  (76) | 235.0[c] | Adduct - $(CH_3)_2NH$, 174 (100)<br>Eff. = 81% | 174 - $HOB(N(CH_3)_2)_2$, 58 |
|  (174) | Unknown | Adduct - $(CH_3)_2NH$, 272 (100)<br>Eff. not measured | 272 - $HOB(N(CH_3)_2)_2$, 156 |
|  (329) | Unknown | Adduct - $(CH_3)_2NH$, 427 (100)<br>Eff. not measured | 427 - $HOB(N(CH_3)_2)_2$, 311 |
|  (114) | 221.2[d] | Adduct - $(CH_3)_2NH$, 212 (66)<br>TDMAB + $H^+$, 144 (34)<br>Eff. = 93% | 212 - $CH_2{=}NCH_3$, 169 |

[a] Only derivatization products containing the most abundant $^{11}B$ isotope are listed (all products observed also contain a $^{10}B$ isotope present in an abundance of 25% relative to the most abundant isotope). [b] Calculated at the B3LYP/6-31G(d) level of theory by using an isodesmic reaction scheme involving trimethylamine *N*-oxide as a reference Brønsted acid. [c] Reference 23. [d] Calculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving pyridine *N*-oxide as a reference Brønsted acid. [e] Eff. = reaction efficiency = $(k_{reaction}/k_{collision}) \times 100\%$.

## Results and Discussion

**Reactions of TDMAB with Protonated Analytes Containing Different Types of Functionalities in an FT-ICR Mass Spectrometer.** A series of aliphatic and aromatic tertiary N-oxides, amides, amines, pyridines, and other O- and N-containing compounds (including primary and secondary N-oxides) were chosen as analytes for this study. The analytes were protonated by self-chemical ionization, transferred from one side of the dual cell into the "clean" side, isolated, and allowed to react with TDMAB for a variable period of time.

**SCHEME 3**

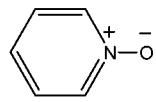
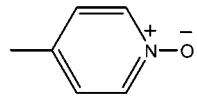
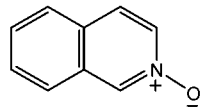
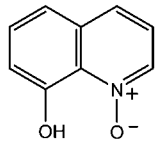



TDMAB was found to react at high efficiencies with the protonated aliphatic and aromatic tertiary N-oxides to yield product ions likely formed via a proton transfer/nucleophilic substitution mechanism (Scheme 3; Tables 1 and 2) similar to that shown in Scheme 1, forming boron-derivatized analytes (adduct-$(CH_3)_2NH$). These derivatized analytes can be easily differentiated from underivatized analytes based on the mass increase of 98 Th and the unique boron isotope ratio (25% of $^{10}B$ relative to $^{11}B$). Although several protonated amides and pyridines also form analogous products, these analytes can be distinguished from the aliphatic and aromatic N-oxides on the basis of the unique fragmentation patterns of their products upon SORI-CAD (Tables 3 and 4). No reaction products were observed for amines (triethylamine and ethylenediamine) that have PAs greater than TDMAB. All other protonated O- and N-containing analytes only transfer a proton to TDMAB due to their significantly lower PAs (186−211 kcal/mol)[23] than that of TDMAB (230 kcal/mol) with one exception (protonated propionaldehyde also yields another reaction product discussed later). The reactions of protonated analytes containing different types of functionalities (e.g., N-oxide, amido, amino, pyridine, and other O- and N-containing functionalities) with TDMAB is discussed in detail below.

**A. Aliphatic and Aromatic Tertiary N-Oxides.** Primary products, their branching ratios, and reaction efficiencies (Eff. $= k_{exp}/k_{coll}$) are given in Tables 1 and 2. These results reveal that protonated aliphatic and aromatic tertiary N-oxides react with TDMAB to form boron-derivatized analytes, presumably via a stepwise proton transfer/nucleophilic substitution mechanism (Scheme 3; Figure 1). In addition to nucleophilic substitution reactivity, some protonated aromatic tertiary N-oxides react with TDMAB via proton transfer (Table 2). The efficiencies of these reactions are very high (Eff. = 48−93%). 8-Hydroxyquinoline *N*-oxide, a substituted N-oxide, yields two boron-derivatized analytes, namely, adduct-$(CH_3)_2NH$ and adduct-$2(CH_3)_2NH$. This observation is readily rationalized by the fact that 8-hydroxyquinoline *N*-oxide is a bifunctional analyte (Scheme 4).

SORI-CAD of most derivatized aliphatic tertiary N-oxides studied yields a fragment ion via elimination of a $HOB(N(CH_3)_2)_2$ molecule (Table 1, first four analytes). For example, upon SORI-CAD, derivatized trimethylamine *N*-oxide (*m*/*z* 174) yields a fragment ion of *m*/*z* 58 by loss of

**TABLE 2. Observed Product Ions (Formation Reactions, *m*/*z* Values, Branching Ratios, and Reaction Efficiencies (Eff.)) Formed in Reactions of Protonated Aromatic Tertiary N-Oxides with TDMAB (PA = 230 kcal/mol) and Their SORI-CAD Fragments (Formation Reactions and *m*/*z* Values) in an FT-ICR Mass Spectrometer**

| Analyte (*m*/*z* of $[M+H]^+$) | Proton affinity (kcal/mol) | Observed product ions,[a] *m*/*z* (branching ratio %) | SORI-CAD fragments of derivatized analytes, *m*/*z* |
|---|---|---|---|
|  (96) | 220.7[b] | Adduct - $(CH_3)_2NH$, 194 (50)<br>TDMAB + $H^+$, 144 (50)<br>Eff.[d] = 93% | $OB(N(CH_3)_2)_2^+$, 115 |
|  (110) | 225.5[c] | Adduct - $(CH_3)_2NH$, 208 (63)<br>TDMAB + $H^+$, 144 (37)<br>Eff.= 86% | $OB(N(CH_3)_2)_2^+$, 115 |
|  (146) | 227.6[c] | Adduct - $(CH_3)_2NH$, 244 (100)<br>Eff. = 65% | $OB(N(CH_3)_2)_2^+$, 115 |
|  (162) | Unknown | Adduct - $(CH_3)_2NH$, 260 (3)<br>Adduct - $2(CH_3)_2NH$, 215 (11)<br>TDMAB + $H^+$, 144 (86)<br>Eff. = 67% | Not measured |

[a] Only derivatization products containing the most abundant $^{11}B$ isotope are listed (all products observed also contain a $^{10}B$ isotope present in an abundance of 25% relative to the most abundant isotope). [b] Reference 23. [c] Calculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving pyridine *N*-oxide as a reference Brønsted acid. [d] Eff. = reaction efficiency = $(k_{reaction}/k_{collision}) \times 100\%$.

**TABLE 3. Observed Product Ions (Formation Reactions, *m/z* Values, Branching Ratios, and Reaction Efficiencies (Eff.)) Formed in Reactions of Protonated Amides with TDMAB (PA = 230 kcal/mol) and Their SORI-CAD Fragments (Formation Reactions and *m/z* Values) in an FT-ICR Mass Spectrometer**

| Analyte (*m/z* of $[M + H]^+$) | Proton affinity (kcal/mol)[b] | Observed product ions,[a] *m/z* (branching ratio %) | SORI-CAD fragments of derivatized analytes, *m/z* |
|---|---|---|---|
| Acetamide (60) | 206.4 | TDMAB + $H^+$, 144 | N/A[d] |
| *N*,*N*-Dimethylacetamide (88) | 217.0 | Adduct-$(CH_3)_2NH$, 186 (45) | 186-$(CH_3)_2NH$, 141 |
| | | TDMAB + $H^+$, 144 (55) | $B(N(CH_3)_2)_2^+$, 99 |
| | | Eff. not measured | |
| *N*,*N*-Diethylacetamide (116) | 221.2 | Adduct-$(CH_3)_2NH$, 214 (13) | 214-$(CH_3)_2NH$, 169 |
| | | TDMAB + $H^+$, 144 (87) | $B(N(CH_3)_2)_2^+$, 99 |
| | | Eff.[c] = 93% | |
| *N*,*N*-Dimethylpropionamide (102) | Unknown | Adduct-$(CH_3)_2NH$, 200 (18) | 200-$(CH_3)_2NH$, 155 |
| | | TDMAB + $H^+$, 144 (82) | $B(N(CH_3)_2)_2^+$, 99 |
| | | Eff. not measured | |

[a] Only derivatization products containing the most abundant $^{11}B$ isotope are listed (all products observed also contain a $^{10}B$ isotope present in an abundance of 25% relative to the most abundant isotope). [b] Reference 23. [c] Eff. = reaction efficiency = $(k_{reaction}/k_{collision}) \times 100\%$. [d] N/A = not applicable.

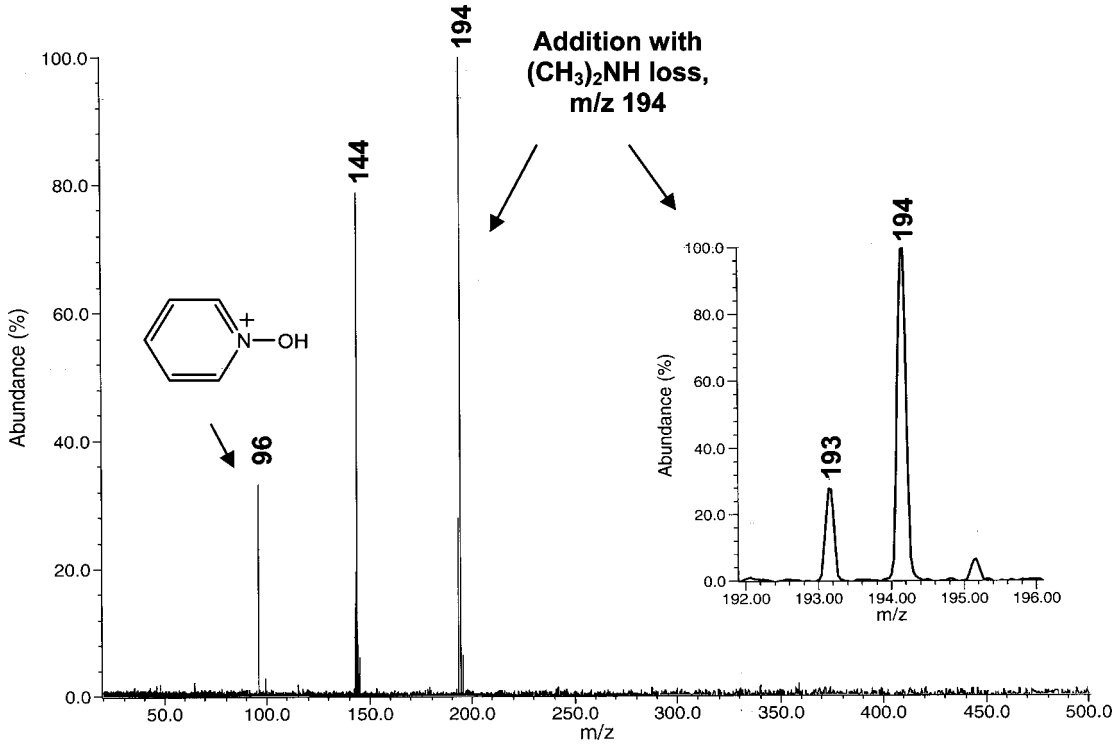



**FIGURE 1.** A mass spectrum measured after 5 s reaction of protonated pyridine *N*-oxide with TDMAB ($2.3 \times 10^{-8}$ Torr). The most abundant product ion (*m/z* 194) corresponds to the derivatized analyte. The derivatized analyte is readily identified based on the $^{10}B$ isotope ion of *m/z* 193 (see insert). The ion of *m/z* 144 is the result of proton transfer from protonated pyridine *N*-oxide to TDMAB.

$HOB(N(CH_3)_2)_2$ (Scheme 5; Figure 2). Interestingly, rather than eliminating $HOB(N(CH_3)_2)_2$, derivatized 5,5-dimethyl-1-pyrroline *N*-oxide yields a fragment ion of *m/z* 169 via elimination of a $CH_2{=}NCH_3$ molecule, possibly as shown in Scheme 6. Probing the structure of this fragment ion (*m/z* 169) by further SORI-CAD revealed loss of $O{=}BN(CH_3)_2$ (formation of an ion of *m/z* 98), which is in agreement with the structure proposed for the fragment ion of *m/z* 169 in Scheme 6.

In contrast, SORI-CAD of derivatized aromatic tertiary N-oxides leads to the cleavage of the N−O bond and loss of neutral amines, yielding exclusively a $OB(N(CH_3)_2)_2^+$ ion of *m/z* 115 (Figure 3). The formation of this ion is independent of the structure of the aromatic tertiary N-oxide and hence can be used as a diagnostic tool for the presence of the aromatic tertiary N-oxide functionality in analytes.

To test the feasibility of using TDMAB in practical applications, two real drug samples, Olanzapine and Olanzapine-4′ *N*-oxide (Figure 4), were examined. As expected, protonated Olanzapine-4′ *N*-oxide reacts with TDMAB to form a product ion of *m/z* 427 via elimination of a dimethylamine molecule from the adduct (Figure 5) while no products were observed for the protonated Olanzapine. As observed for all other aliphatic tertiary N-oxides tested, SORI-CAD of derivatized Olanzapine-4′ *N*-oxide (*m/z* 427) leads to the loss of a neutral $HOB(N(CH_3)_2)_2$ molecule, yielding a fragment ion of *m/z* 311 (Table 1).

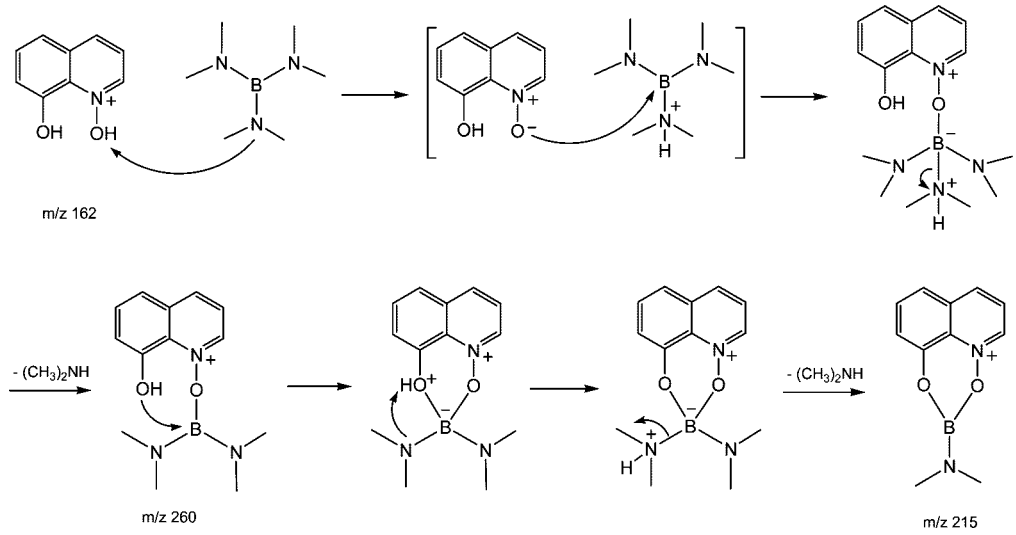
**B. Amides.** Analogous proton transfer/nucleophilic substitution products (adduct-$(CH_3)_2NH$) were observed for reactions of protonated amides with TDMAB, with one exception. Protonated acetamide only undergoes proton transfer due to its

**TABLE 4. Observed Product Ions (Formation Reactions, *m/z* Values, Branching Ratios, and Reaction Efficiencies (Eff.)) Formed in Reactions of Protonated Amines and Pyridines with TDMAB (PA = 230 kcal/mol) and Their SORI-CAD Fragments (Formation Reactions and *m/z* Values) in an FT-ICR Mass Spectrometer**

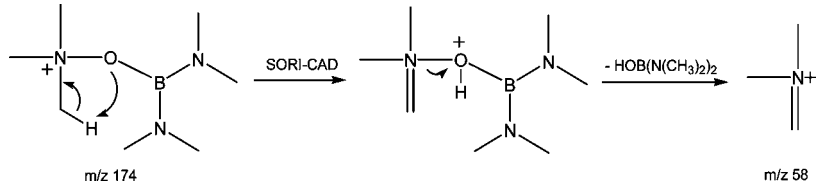
| Analyte (*m/z* of $[M + H]^+$) | Proton affinity (kcal/mol) | Observed product ions,[a] *m/z* (branching ratio %) | SORI-CAD fragments of derivatized analytes, *m/z* |
|---|---|---|---|
| Pyridine (80) | 222.0[b] | TDMAB + $H^+$, 144 | N/A[e] |
| Diethylamine (74) | 227.6[b] | TDMAB + $H^+$, 144 | N/A |
| 2-Methyl-1-pyrroline (84) | 228.1[c] | Adduct-$(CH_3)_2NH$, 182 (22)<br>TDMAB + $H^+$, 144 (78)<br>Eff.[d] = 85% | $B(N(CH_3)_2)_2^+$, 99 |
| 4-Methoxypyridine (110) | 229.9[b] | Adduct-$(CH_3)_2NH$, 208 (55)<br>TDMAB + $H^+$, 144 (45)<br>Eff. not measured | $B(N(CH_3)_2)_2^+$, 99 |
| Triethylamine (102) | 234.7[b] | No reaction | N/A |
| *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (117) | 242.1[b] | No reaction | N/A |

[a] Only derivatization products containing the most abundant $^{11}B$ isotope are listed (all products observed also contain a $^{10}B$ isotope present in an abundance of 25% relative to the most abundant isotope). [b] Reference 23. [c] Calculated at the B3LYP/6-31G(d) level of theory, using an isodesmic reaction scheme involving *N*-methyl methanimine as a reference Brønsted acid. [d] Eff. = reaction efficiency = $(k_{reaction}/k_{collision}) \times 100\%$. [e] N/A = not applicable.

**SCHEME 4**




**SCHEME 5**




significantly low PA (206.4 kcal/mol) relative to that of TDMAB (Table 3). All protonated amides studied also transfer a proton to TDMAB (Table 3). The observed reactions proceed very rapidly to completion (e.g., Eff. = 93% for protonated *N*,*N*-diethylacetamide). When subjected to SORI-CAD, the boron-derivatized amides primarily undergo another $(CH_3)_2NH$ loss to form adduct-2$(CH_3)_2NH$. For example, SORI-CAD of derivatized *N*,*N*-dimethylpropanamide yields a fragment ion of *m/z* 155 by loss of a second $(CH_3)_2NH$ molecule, possibly via the mechanism shown in Scheme 7 (path a). Further SORI-CAD of this fragment ion (*m/z* 155) occurs via the loss of $O{=}BN(CH_3)_2$, thus providing additional support to the structure proposed for the fragment ion (*m/z* 155). In addition to second $(CH_3)_2NH$ loss, a $B(N(CH_3)_2)_2^+$ ion of *m/z* 99 was also observed as a minor fragment ion (Scheme 7, path b). This dissociation behavior is different from those observed for derivatized aliphatic and aromatic tertiary N-oxides upon SORI-CAD. Therefore, amides can be differentiated from aliphatic and aromatic tertiary N-oxides by ion/molecule reactions with TDMAB followed by SORI-CAD.

**C. Amines and Pyridines.** Several protonated amines and pyridines were studied in order to test the selectivity of TDMAB toward tertiary N-oxide and amido functionalities. The analytes selected were pyridine, diethylamine, 2-methyl-1-pyrroline, 4-methoxypyridine, triethylamine, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (Table 4). The reactivity of these analytes toward TDMAB is varied and appears to be greatly affected by their proton affinity. For protonated pyridine and diethylamine, only proton transfer was observed due to the low PAs of these analytes (~222 and 228 kcal/mol,[23] respectively) relative to that of TDMAB (PA = 230 kcal/mol, B3LYP/6-31G(d)). Protonated triethylamine and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine are

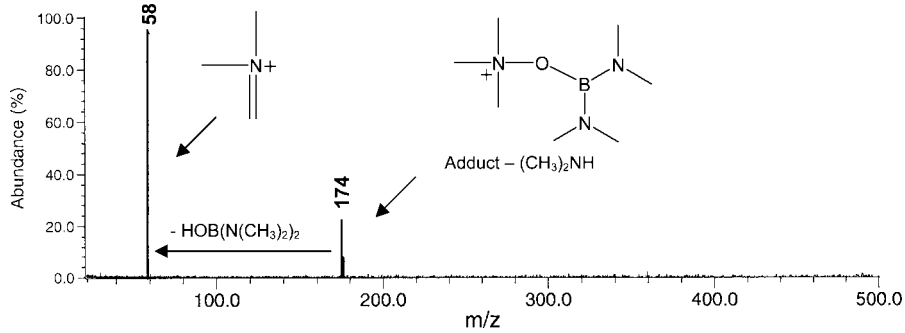
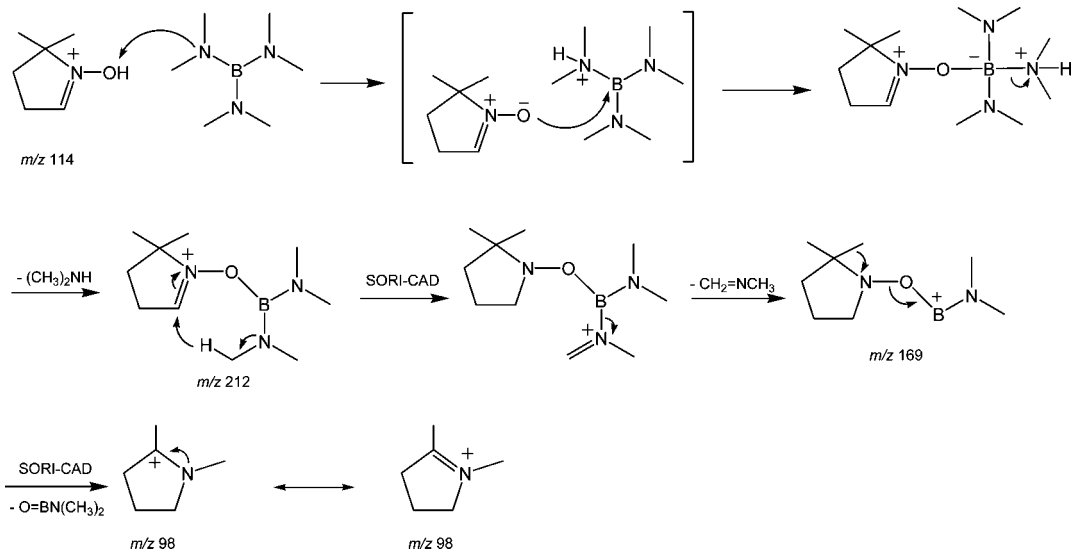



**FIGURE 2.** SORI-CAD spectrum of the derivatized trimethylamine *N*-oxide (adduct-$(CH_3)_2NH$, *m/z* 174).

**SCHEME 6**




unreactive. The lack of reactivity of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine may be attributed to its high PA (242 kcal/mol) relative to that of TDMAB, which likely prevents the proton transfer necessary for the derivatization reaction. The reasons for the lack of reactivity of protonated triethylamine are not clear at this time.

Protonated 2-methyl-1-pyrroline (PA = 228.1 kcal/mol, B3LYP/6-31G(d)) and 4-methoxypyridine (PA = 229.9 kcal/mol[23]) were found to react with TDMAB to yield derivatized analytes. On the basis of this finding, 4-methoxypyridine cannot be differentiated from the isomeric 4-methylpyridine *N*-oxide (Table 2). However, SORI-CAD of the derivatized pyridine results in the cleavage of the N−B bond and the loss of the original pyridine, yielding a $B(N(CH_3)_2)_2^+$ ion of *m/z* 99 (Scheme 8). This dissociation pattern is different from those of tertary N-oxides and amides. Therefore, pyridines can probably be differentiated from tertiary N-oxides and

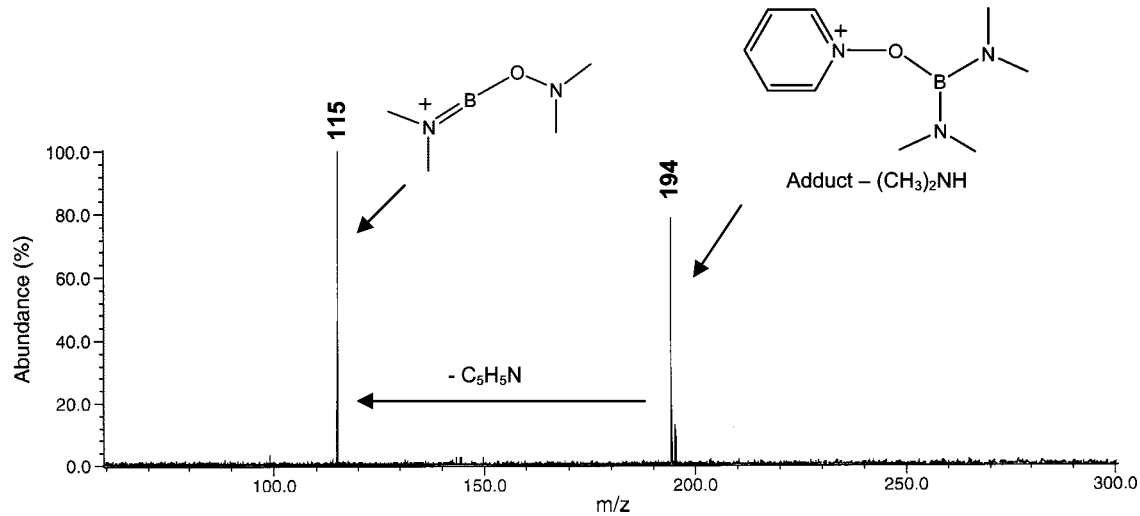



**FIGURE 3.** SORI-CAD spectrum of the derivatized pyridine *N*-oxide (adduct-$(CH_3)_2NH$, *m/z* 194).

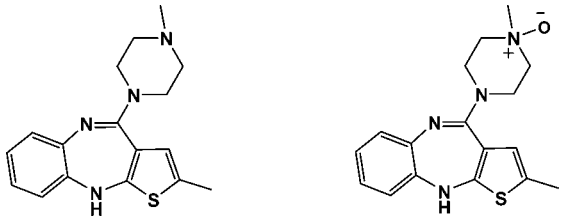


**FIGURE 4.** Structures of Olanzapine (left) and Olanzapine-4′ *N*-oxide (right).

amides by ion/molecule reactions with TDMAB followed by SORI-CAD.

**D. Other O- and N-Containing Analytes.** The reactivity of TDMAB toward various additional protonated molecules containing O- or N-functional groups was also examined (Table 5). The PAs of these compounds range from ~186 to 211 kcal/mol[23] and hence are substantially lower than the PA of TDMAB (230 kcal/mol). Such differences in PA lead to fast proton transfer. Protonated propionaldehyde is an exception. Protonated propionaldehyde also undergoes another reaction, addition accompanied by the loss of $HOB(N(CH_3)_2)_2$, that likely involves a rearrangement, possibly as shown in Scheme 9.

**TABLE 5. Observed Product Ions (Formation Reactions, *m/z* Values, and Branching Ratios) Formed in Reactions of Various Protonated Analytes with TDMAB (PA = 230 kcal/mol) in an FT-ICR Mass Spectrometer**

| Analyte (*m/z* of $[M + H]^+$) | Proton affinity (kcal/mol)[a] | Observed product ions, *m/z* (branching ratio %) |
|---|---|---|
| Ethanol (47) | 185.6 | TDMAB + $H^+$, 144 (100) |
| Acetonitrile (42) | 186.2 | TDMAB + $H^+$, 144 (100) |
| Propionaldehyde (59) | 187.9 | TDMAB + $H^+$, 144 (88)<br>Adduct-$HOB(N(CH_3)_2)_2$, 86 (12) |
| Propanoic acid (75) | 190.5 | TDMAB + $H^+$, 144 (100) |
| Acetone (59) | 194.0 | TDMAB + $H^+$, 144 (100) |
| Diethyl ether (75) | 198.0 | TDMAB + $H^+$, 144 (100) |
| Ethyl acetate (89) | 199.7 | TDMAB + $H^+$, 144 (100) |
| Nitrosobenzene (108) (1° *N*-oxide) | 204.2 | TDMAB + $H^+$, 144 (100) |
| 2,2,6,6-Tetramethyl-piperidine *N*-oxide (157) (2° *N*-oxide) | 210.9 | TDMAB + $H^+$, 144 (100) |

[a] Reference 23.

## Conclusions

The ability to use functional group-selective ion/molecule reactions in an FT-ICR mass spectrometer to identify compounds with an aliphatic or aromatic tertiary N-oxide functionality has been demonstrated. All protonated aliphatic and aromatic tertiary N-oxides, most amides, and some amines and pyridines were found to react with TDMAB to form an adduct that has lost $(CH_3)_2NH$. SORI-CAD of this product ion yields fragments unique for aliphatic tertiary N-oxides, aromatic tertiary N-oxides, amides, and pyridines. Hence, these analytes can be differentiated from each other based on the characteristic fragment ions. Almost all other protonated N- and O-containing analytes examined in this work were found to react by proton transfer with TDMAB. A summary of the diagnostic reactions observed for analytes with tertiary N-oxide, amido, amino, or pyridine functionality is given in Table 6. The results obtained for Olanzapine-4′ *N*-oxide and Olanzapine suggest that this method is applicable to drug analysis. Since this method involves ionization of the analyte by proton transfer, it is applicable to tandem mass spectrometers that are equipped with an ESI, APCI, or CI ion source.

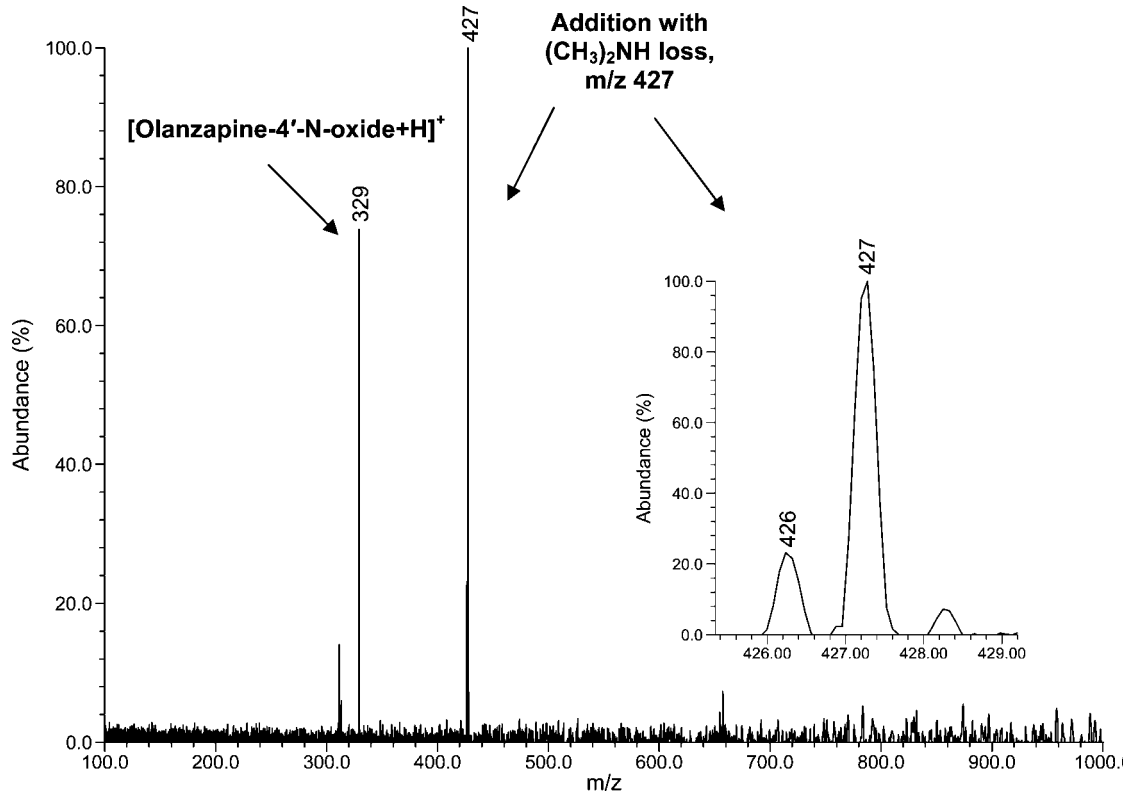
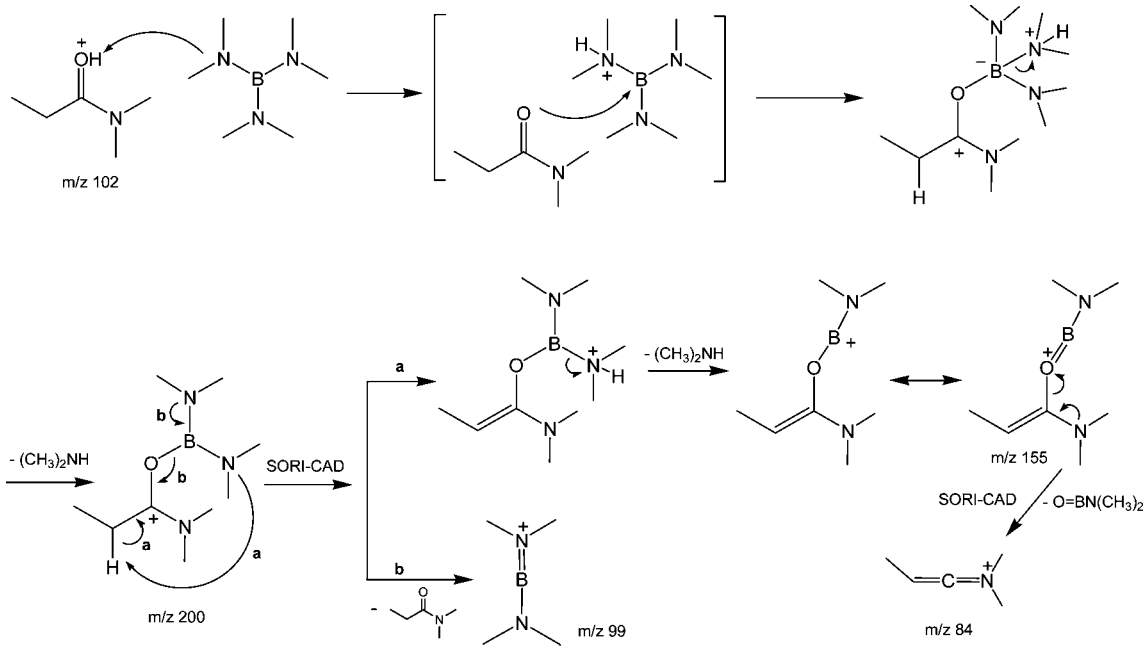
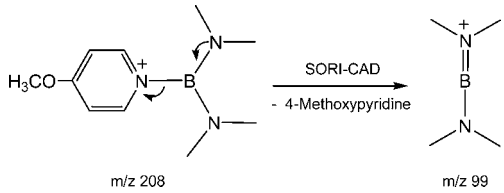



**FIGURE 5.** A mass spectrum measured after 200 s reaction of protonated Olanzapine-4′ *N*-oxide with TDMAB ($1.2 \times 10^{-8}$ Torr). The most abundant product ion (*m/z* 427) corresponds to the derivatized analyte. This N-oxide is likely to be substantially more basic than the other N-oxides studied here, which may explain its low reactivity.

**SCHEME 7**




**SCHEME 8**




## Experimental Section

**Chemicals.** All compounds studied were purchased and used without further purification. The purity of all reagents was verified by mass spectrometry.

**Instrumentation.** The experiments were performed in an FT-ICR mass spectrometer equipped with a 3-T superconducting magnet and an Odyssey data station. The instrument contains a dual cell 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. Liquid samples were introduced into the instrument by using either a Varian leak valve or an adjustable leak valve. An automatic solids probe was used to introduce solid samples into the instrument.

The protonated analytes were generated in one of the cells by self-protonation chemical ionization (self-CI). This was achieved by allowing the molecular ions and the ionic fragments of each analyte, generated upon electron ionization (EI) (25−70 eV, 7 $\mu$A, 0.1−1.0 s) of the analyte, to react (0.2−6.0 s) with the neutral analyte present in the same cell (pressures varied from $1.2 \times 10^{-8}$ to $6.0 \times 10^{-8}$ Torr, as measured by an ion gauge). The protonated analytes were subsequently transferred into the other cell (by grounding the conductance limit plate for 85−170 $\mu$s) where the neutral reagent was present typically at a pressure of $1.0 \times 10^{-8}$ to $2.6 \times 10^{-8}$ Torr (nominal) and were cooled for 1 s by allowing them to undergo IR emission[26] and collisions with argon gas pulsed into that cell (nominal peak pressure ~$1.0 \times 10^{-5}$ Torr). The desired protonated analyte was isolated by using a stored-waveform inverse Fourier transform[27] (SWIFT) excitation pulse to eject all unwanted ions, and allowed to react with the neutral reagent (reaction times varied from 0.2 to 200 s; however, up to 400 s was used in cases where no reactions were observed). Some of the reaction products were further probed by sustained off-

**SCHEME 9**

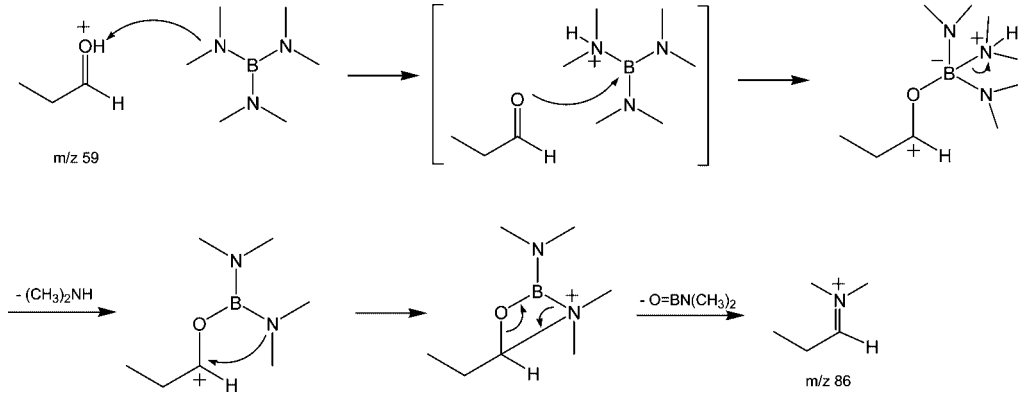


**TABLE 6. A Summary of the Diagnostic Reactions Observed for Analytes with N-Oxide, Amido, Amino, and Pyridine Functionalities**

| Observed product ions in ion/molecule reactions with TDMAB, *m/z* | Characteristic SORI-CAD fragments of derivatized analytes | Possible analyte (M) |
|---|---|---|
| Adduct-$(CH_3)_2NH$, *m/z* ($MH^+ + 98$) | Adduct-$(CH_3)_2NH$-$HOB(N(CH_3)_2)_2$ | Aliphatic tertiary N-oxide |
| | $OB(N(CH_3)_2)_2^+$, *m/z* 115 | Aromatic tertiary N-oxide |
| | Adduct-2$(CH_3)_2NH$ (major), $B(N(CH_3)_2)_2^+$, *m/z* 99 (minor) | Amide |
| | $B(N(CH_3)_2)_2^+$, *m/z* 99 | Pyridine |
| TDMAB + $H^+$, 144 | N/A[a] | Other O- and N-containing compound |
| No reaction | N/A[a] | Amine |

[a] N/A = not applicable.

resonance irradiation collision-activated dissociation[28] (SORI-CAD). SORI-CAD involved off-resonance excitation of the isolated ion at a frequency ±1000 Hz off the cyclotron frequency of the ion for about 1 s in the presence of an argon gas (nominal peak pressure $\sim 10^{-5}$ Torr). All the spectra were background-corrected by subtracting the background spectra from the reaction spectra. Background spectra were recorded by removing the ion of interest by SWIFT ejection prior to reaction or SORI-CAD.

**Kinetics.** During the ion/molecule reactions, the neutral reagent (TDMAB) was present at a constant pressure and itsconcentration was in excess of that of the ion of interest. Hence, these reactions follow pseudo-first-order kinetics. The reaction efficiencies (Eff. = $k_{reaction}/k_{collision}$ = the fraction of ion/molecule collisions that results in the formation of products) were determined by measuring each reaction's rate (IM) and the rate of the highly exothermic proton-transfer reaction (PT) between protonated acetone and the neutral reagent (TDMAB). Assuming that this exothermic proton-transfer reaction proceeds at a collision rate ($k_{collision}$) that can be calculated, the efficiencies of the ion/molecule reactions can be obtained by using eq 1.[29−31] This equation is based on the ratio of the slopes ($k_{reaction}$[TDMAB] = slope (IM) and $k_{collision}$[TDMAB] = slope (PT); [TDMAB] = TDMAB concentration) of plots of the natural logarithm of the relative abundance of the reactant ion versus reaction time for the ion/molecule (IM) and exothermic proton-transfer (PT) reactions (thus eliminating the need to know [TDMAB]), masses of the ion ($M_i$), neutral reagent ($M_n$), and acetone ($M_{(PT)}$), and the pressure of the neutral reagent during the ion/molecule reaction ($P_{n(IM)}$) and the proton-transfer reaction ($P_{n(PT)}$).

$$\text{Efficiency} = \frac{\text{slope(IM)}}{\text{slope(PT)}}\left(\frac{M_i(M_{(PT)} + M_n)}{M_{(PT)}(M_i + M_n)}\right)^{1/2}\left(\frac{P_{n(PT)}}{P_{n(IM)}}\right)100\% \quad (1)$$

**Computational Studies.** All theoretical calculations in this work were performed with the Gaussian 03 suite of programs.[32] Geometry optimizations and vibrational frequency calculations were performed by 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 affinities of the analytes and TDMAB were calculated by using protonated trimethylamine as the Brønsted acid in an isodesmic reaction scheme.

**Acknowledgment.** The authors gratefully acknowledge Eli Lilly and Company for providing financial support for this work.

**Supporting Information Available:** Tables of Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

JO802001E

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