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Ion–molecule reactions of trimethylborate allow the mass spectrometric identification and counting of functional groups in protonated bifunctional oxygen-containing compounds and polyols

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Abstract

A mass spectrometric method has been developed for the identification of functional groups in unknown bifunctional oxygen-containing compounds and for the identification and counting of the hydroxyl groups in polyols. This method utilizes gas-phase ion–molecule reactions of protonated analytes with neutral trimethylborate (TMB) in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer. The diagnostic reaction sequence involves proton abstraction from the protonated analyte by TMB, followed by the addition of the analyte to TMB and elimination of methanol. The functional groups in bifunctional oxygen-containing compounds are identified based on the total number of TMB molecules that have added to the protonated analyte and/or the number of methanol molecules lost, or by sustained off-resonance irradiation collision-activated dissociation (SORI-CAD) of the reaction products. The number of hydroxyl groups in polyols is revealed by the number of methanol molecules lost during their reactions with TMB. Reactions of protonated carboxylic acids with TMB also lead to elimination of a methanol molecule. However, carboxylic acids can be differentiated from the isomeric hydroxyketones based on the loss of $HO\text{-}B(OCH_3)_2$ upon SORI-CAD of the reaction product. Reactions of protonated amides with TMB also lead to elimination of a methanol molecule. However, amides can be differentiated from bifunctional oxygen-containing compounds based on the loss of O=B–R from the reaction product upon SORI-CAD. Protonated amines do not react with TMB.

Keywords: Ion–molecule reaction; Mass spectrometry; Oxygen-containing compound; SORI-CAD

1. Introduction

A method for fast and accurate identification of unknown compounds directly in mixtures would facilitate various aspects of pharmaceutical drug development. Spectroscopic techniques that are currently utilized for these analyses, such as NMR, FT-IR and X-ray crystallography [1,2], are powerful but time consuming, and often require high-purity samples and relatively large quantities of analytes. Tandem mass spectrometry (MS/MS) is ideally suited for obtaining structural information on species present in mixtures because it is fast, highly sensitive, has a high specificity of detection, does not require pure compounds and consumes a minimal amount of sample [3].

MS/MS is currently utilized in various areas in pharmaceutical industry, including metabolite profiling, their structural elucidation [4,5], and drug discovery [6,7]. Typically, the experiments involve ionization of the analytes by protonation, followed by the mass-selection of the protonated analytes and their characterization by techniques such as exact mass measurement [8,9], collision-activated dissociation [10,11] (CAD) and/or H/D exchange reactions [12–14]. Though a wealth of information can be obtained from these experiments, unambiguous identification of the functional groups in unknown compounds is often difficult to achieve.

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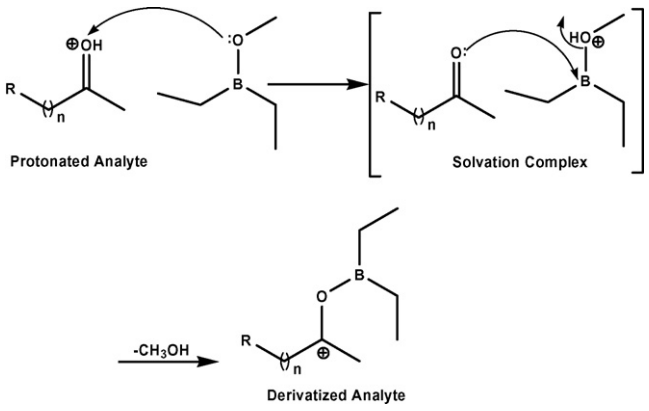
MS/MS based on gas-phase ion–molecule reactions presents a powerful approach for obtaining structural information for unknown compounds [15–20]. The advantages of this approach include low-energy conditions that do not lead to ion fragmentation or isomerization as readily as CAD, and the ability to use a variety of reagents to probe different structural features of the analyte. In some cases, ion–molecule reactions have been combined with collision-activated dissociation to obtain structurally informative product ions.

The majority of the past work on ion–molecule reactions has focused on obtaining functional group information for neutral analytes by using ionic reagents [15,21–25]. For example, gas-phase reactions of dimethoxyborenium ion, a major fragment ion of protonated trimethylborate (TMB), and of TMB molecular ion have been demonstrated to allow the identification of functional groups present in neutral alcohols, aldehydes, ethers, ketones, and some biologically active compounds containing hydroxyl groups [21–25]. Fewer studies have appeared wherein neutral reagents are used to identify functional groups in ionized analytes [15–17,26]. However, this approach is needed for MS/MS analysis of mixtures evaporated and ionized by ESI and MALDI.

Experiments involving ion–molecule reactions between neutral diethylmethoxyborane (DEMB) and protonated monofunctional oxygen-containing analytes, followed by CAD or H/D exchange reactions, have demonstrated that this approach can be used to distinguish between and identify these analytes [26]. The diagnostic reaction sequence involves proton abstraction from the protonated analyte by DEMB, followed by addition of the analyte to DEMB and elimination of a methanol molecule (Scheme 1). This reaction sequence results in the derivatization of the functional groups of the analytes by the boron reagent. Examination of the derivatized analytes by CAD or H/D exchange reactions allows the identification of their functionalities. However, DEMB failed to derivatize protonated bifunctional oxygen-containing compounds other than polyols due to the high proton affinity (PA) of these analytes compared to that of the reagent [27].

We demonstrate here the utility of trimethylborate (TMB) in the identification and counting of functional groups in protonated bifunctional oxygen-containing analytes and polyols in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR). TMB was chosen as the neutral reagent for this study because it has a higher PA than DEMB (PA of TMB is 195 kcal/mol; [28] PA of DEMB is 191 kcal/mol [26]) and more than one leaving group. These properties of the reagent aid in the derivatization of polyfunctional oxygen-containing compounds that have higher proton affinities than monofunctional oxygen-containing compounds, and more than one functionality that needs to be derivatized.



Scheme 1.

2. Experimental

The experiments were performed in an Extrel Model FTMS 2001 dual-cell FT-ICR mass spectrometer equipped with an Odyssey data station. This 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. The dual cell is aligned collinearly with the field of a 3 T superconducting magnet operated at ~2.7 T, and it is differentially pumped by two Balzers turbomolecular pumps (330 L/s), each backed by an Alcatel 2012 mechanical pump. A nominal baseline pressure of less than 1×10^{-9} Torr was measured by two MKS 290 ion gauges 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 Andonian leak valves. A manual solids probe was used to introduce solid samples into the instrument.

All chemicals were purchased from the Sigma-Aldrich Company and used without further purification. D-Mannitol was purchased from ICN Biomedicals and used as received. The analytes were protonated by self-chemical ionization (self-CI). This was achieved by allowing the ionic fragments and the molecular ion generated upon electron ionization (EI) of the analyte react with the neutral analyte molecules for a certain period of time (1–15 s). Typical ionization parameters were 0.1–1.0 s electron beam time, 25–70 eV electron energy and 7.0 μA filament current. Nominal base pressure of the neutral reagents in the cell varied between 6.0×10^{-8} and 1.2×10^{-7} Torr, as measured by the ion gauges. All the ions on the other side of the dual cell were removed prior to ion transfer by changing the remote trapping plate voltage from +2.0 to −2.0 V for 12 ms. The protonated analyte was transferred into the other cell by grounding the conductance limit plate (80–100 μs). The transferred ions were cooled by allowing IR emission [29] and by collisions with Ar present at about 10^{-5} Torr for a period of about 1 s. The protonated analyte was isolated by using a stored-waveform inverse Fourier transform [30,31] (SWIFT) excitation pulse to eject all unwanted ions, and allowed to react with TMB (reaction times typically varied from 0.1 to about 100 s; however, up to 500 s was used for very slow reactions). Some of the reaction products were further probed by sustained off-resonance irradiated collision-activated dissociation [32] (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 for about 1 s in the presence of an inert gas ($\sim 10^{-5}$ Torr of argon).

After reaction, all ions were excited for detection by using chirp excitation with a bandwidth of 2.7 MHz, amplitude of 124 V_{p-p} and a sweep rate of 3200 Hz μs^{-1}. The spectra were recorded as 128k data points by using one zero-fill prior to Fourier transform. Background spectra were recorded by removing the ion of interest by SWIFT ejection prior to reaction or SORI-CAD. All the spectra were background corrected by subtracting the background spectra from the reaction spectra.

During the ion–molecule reactions, the neutral reagent (TMB) was present at a constant pressure and its concentration 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 ion-molecule reaction's (IM) rate and the rate of the highly exothermic electron transfer reaction (ET) between argon radical cation and the neutral reagent (both measurements were carried out in the same day). Assuming that this exothermic electron transfer reaction proceeds at the collision rate ($k_{collision}$) that can be calculated, efficiencies of the ion–molecule reactions can be obtained by using Eq. (1) [33–36]. This equation is based on the ratio of the slopes ($k_{reaction}$ [TMB] = slope (IM) and $k_{collision}$ [TMB] = slope (ET); [TMB] = TMB concentration) of plots of the natural logarithm of the relative abundance of the reactant ion versus time determined for the ion–molecule (IM) and exothermic electron transfer (ET) reactions (thus eliminating the need to know [TMB]), masses of the ion (M_i), neutral reagent (M_n) and argon ($M_{(ET)}$), and the pressure of the neutral reagent during the ion–molecule reaction ($P_{n(IM)}$) and the electron transfer reaction ($P_{n(ET)}$).

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

All theoretical energies reported in this work were calculated with Gaussian 98 suite of programs [37]. Geometry optimizations and vibrational frequency calculations were performed using density functional theory at the B3LYP/6-31G(d) level. The proton affinities of the analytes were calculated by using protonated methanol as the Brønsted acid in an isodesmic reaction scheme. All stationary points were confirmed to have the correct number of imaginary frequencies. All theoretical energies are presented at 0 K and include zero-point vibrational energy corrections.

3. Results and discussion

3.1. Summary of the reactions of protonated bifunctional oxygen-containing analytes and polyols with TMB

Reactions of TMB with several protonated bi- and polyfunctional oxygen-containing compounds, including hydroxyketones, hydroxyethers, and polyols, were studied in an FT-ICR mass spectrometer. A few carboxylic acids, amides and amines were also examined. All analytes were protonated using self-chemical ionization, transferred into a clean cell, isolated and allowed to react with TMB for a variable period of time. These reactions lead to either partial or complete derivatization of the functional groups of the analytes, except for amines. The mechanisms of these reactions are likely to be similar to that proposed earlier for the reactions of protonated monofunctional oxygen-containing compounds with DEMB (Scheme 1) [26]. The first step involves proton transfer from the protonated analyte to the basic methoxy group of the boron reagent. This is followed by addition of the now-neutral analyte to the boron atom, and elimination of a methanol molecule. The derivatized analytes can be easily differentiated from underivatized analytes based on the unique boron isotope ratio (25% of ^{10}B relative to ^{11}B). The total number of functional groups present in a protonated analyte can be counted from the total number of methanol molecules lost during its reaction with TMB and during SORI-CAD of the singly derivatized analyte. Although protonated carboxylic acids and amides also form derivatization products, these analytes can be distinguished from the bifunctional analytes based on the unique product ions formed upon SORI-CAD of the singly derivatized carboxylic acids and amides. Amines do not react with TMB. This lack of reactivity can be attributed to their substantially higher PA ($\geq$ 20 kcal/mol) than that of TMB (195 kcal/mol; [28]).

3.2. Reaction kinetics

The efficiencies measured for the reactions of protonated oxygen-containing compounds with TMB are very high. For example, protonated *meso*-erythritol and xylitol react with TMB at an efficiency of 62%, *trans*-1,2-cyclohexanediol at near 56%, *cis*-1,2-cyclohexanediol at 50% and 1,3-butanediol at 25%. These reaction efficiencies suggest that the ion–molecule reactions are fast enough for practical applications.

3.3. Protonated bifunctional oxygen-containing analytes

3.3.1. Diols

The reactions of protonated acyclic and cyclic diols with TMB lead to the derivatization of both hydroxyl groups (Table 1; Fig. 1). The derivatization of the first hydroxyl group generates an acidic hydrogen that is abstracted by a second boron reagent, eventually resulting in the derivatization of the second functionality (Scheme 2). This reactivity is similar to that observed upon interaction of diethylmethoxyborane with protonated polyols, where a series of consecutive reactions derivatize the hydroxyl groups of the analyte [27].

Apart from forming the above derivatization products, the doubly derivatized cyclohexanediols (*m/z* 261) undergo a slow intramolecular methanol loss (forming an ion of *m/z* 229; Table 1). The reaction likely involves the migration of the acidic hydrogen from an oxygen atom of the doubly derivatized cyclic diol to one of the methoxy groups bonded to the boron center. This intramolecular proton transfer is then followed by nucleophilic addition at the boron atom by another methoxy oxygen attached to the adjacent boron atom, followed by methanol loss

Table 1
Derivatization products (formation reactions, *m/z* values and branching ratios) formed in reactions between protonated diols and TMB

Analyte (*m/z* of $(M+H)^+$)	PA[a] (kcal/mol)	Product ions (*m/z*)[b] (branching ratio)
1,2-Ethanediol (63)	195.0	Adduct − CH_3OH (135) (75%) Adduct + TMB–$2CH_3OH$ (207) (25%)
1,3-Propanediol (77)	209.4	Adduct − CH_3OH (149) (67%) Adduct + TMB–$2CH_3OH$ (221) (33%)
1,3-Butanediol (91)		Adduct − CH_3OH (163) (93%) Adduct + TMB–$2CH_3OH$ (235) (7%)
1,4-Butanediol (91)	218.8	Adduct − CH_3OH (163) (80%) Adduct + TMB–$2CH_3OH$ (235) (8%)
cis-1,2-Cyclohexanediol (103)		Adduct − CH_3OH (189) (63%) Adduct + TMB–$2CH_3OH$ (261) (33%) Adduct + TMB–$3CH_3OH$ (229) (4%)
trans-1,2-Cyclohexanediol (103)		Adduct − CH_3OH (189) (76%) Adduct + TMB–$2CH_3OH$ (261) (17%) Adduct + TMB–$3CH_3OH$ (229) (7%)

[a] Ref. [28].
[b] 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).

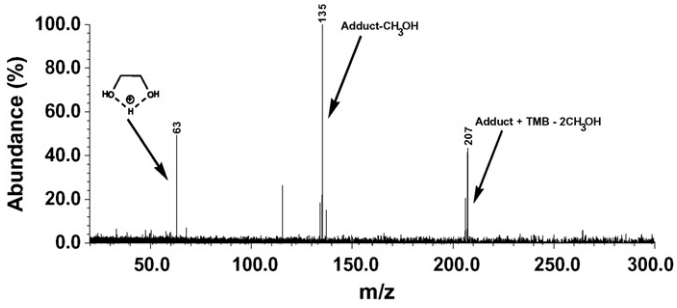
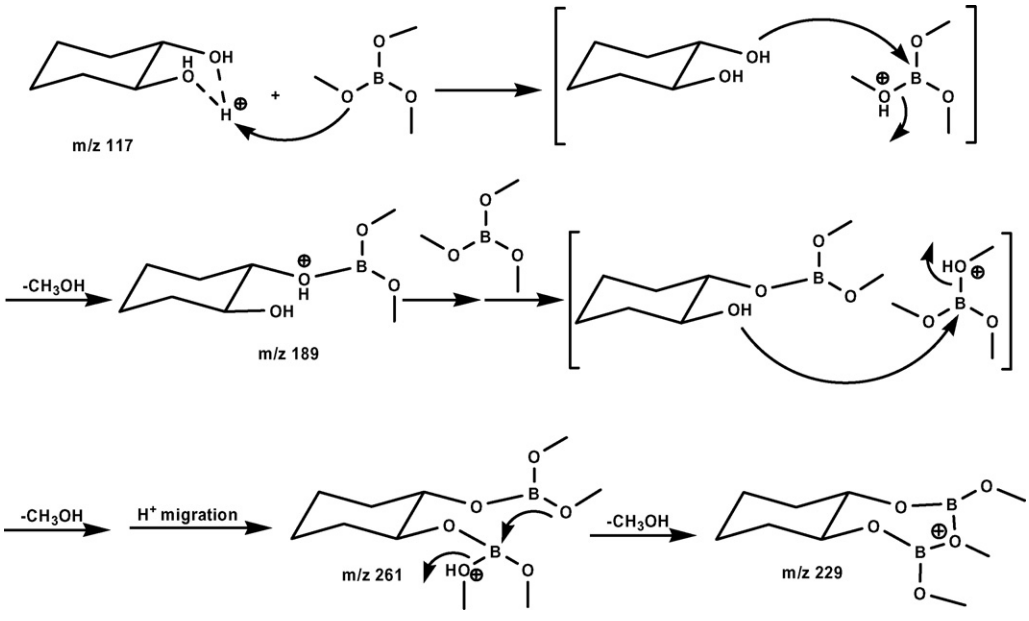


Fig. 1. A mass spectrum representing the reaction of TMB with protonated 1,2-ethanediol (*m/z* 63). This reaction leads to derivatization of both hydroxyl groups, resulting in the formation of product ions of *m/z* 135 and 207. The derivatized analytes are readily identified based on the ^{10}B isotope ions of *m/z* 134 and 206.

which likely leads to a cyclic product ion (Scheme 2). Steric hindrance in the doubly derivatized analyte may facilitate this reaction. The same product ion (*m/z* 229) was observed in an earlier study wherein neutral cyclic diols were allowed to react with protonated trimethylborate [25]. Doubly derivatized acyclic diols do not undergo methanol loss. Hence, the occurrence of intramolecular methanol loss reactions may be used to distinguish between acyclic diols and monocyclic diols.

In summary, all the protonated diols react with two molecules of TMB in the gas phase to derivatize the two hydroxyl groups via the elimination of two methanol molecules. However, in solution studies, one molecule of boronic acid was found to derivatize both the hydroxyl groups of the various neutral diols studied [38]. Molecular orbital calculations performed here at the B3LYP/6-31G(d) + ZPVE level of theory for the reaction of protonated ethylene glycol with TMB suggest that formation of a cyclic product in the gas phase is 12 kcal/mol endothermic (Fig. 2). On the other hand, derivatization of the second hydroxyl group by another TMB molecule is predicted to be exothermic by 13 kcal/mol (Fig. 3).



Scheme 2.

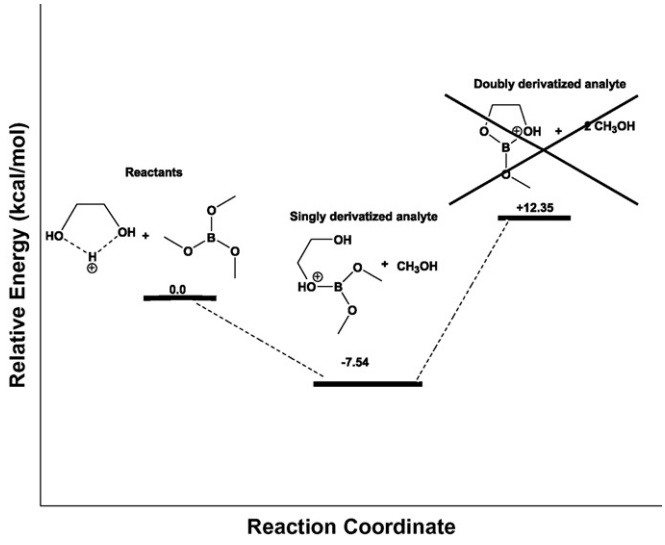


Fig. 2. Calculated potential energy surface for the reaction of protonated ethylene glycol with TMB (B3LYP/6-31G(d) + ZPVE).

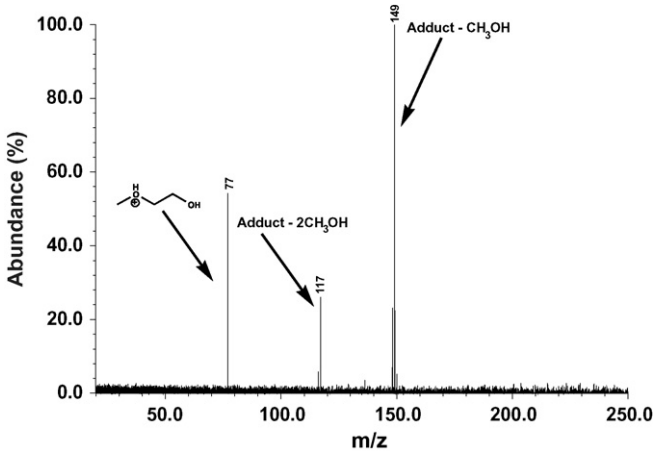


Fig. 4. A mass spectrum representing the reaction of TMB with protonated 2-methoxyethanol (*m/z* 77). Derivatization of the ether and the hydroxyl functional groups results in the formation of two products, ions of *m/z* 149 and 117, via loss of one and two methanol molecules, respectively. The derivatized analyte is readily identified based on the ^{10}B isotope ions of *m/z* 148 and 116.

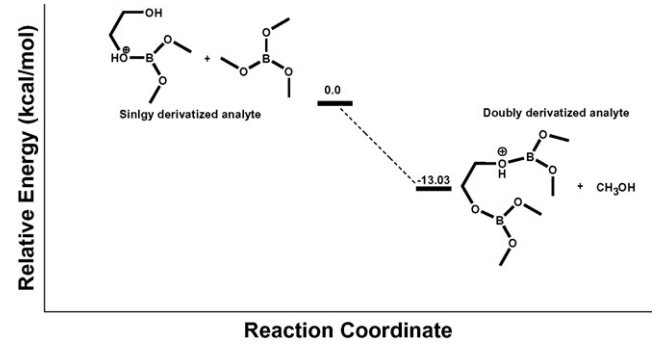


Fig. 3. Calculated potential energy surface for the formation of the doubly derivatized ethylene glycol upon reactions with TMB (B3LYP/6-31G(d) + ZPVE).

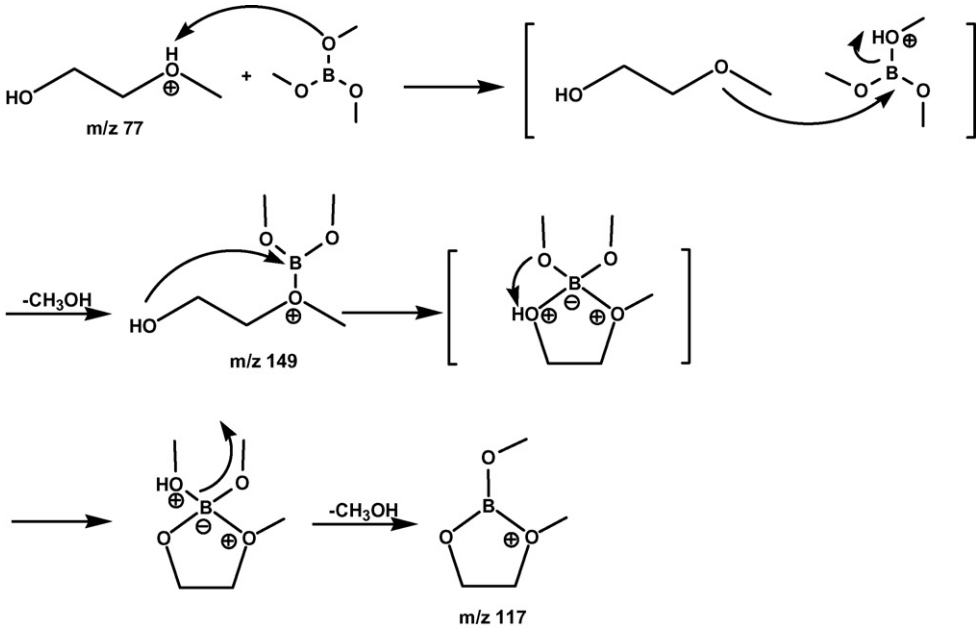
3.3.2. Hydroxyethers

The reactions of TMB with some protonated hydroxyethers lead to complete derivatization to yield two product ions (Table 2). For example, protonated 2-methoxyethanol and 2-hydroxymethyltetrahydropyran consume one molecule of TMB for derivatization of both functional groups (Fig. 4). Scheme 3 shows a plausible mechanism for the derivatization of the functional groups of protonated-2-methoxyethanol. However, protonated 3-hydroxytetrahydrofuran and protonated 4-hydroxytetrahydropyran do not undergo complete derivatization like the other hydroxyethers (Table 2). Instead, the latter two analytes undergo derivatization of only one functional group. This derivatization likely takes place at the ether functionality. If the hydroxyl group was derivatized first, an acidic hydrogen would be generated, which would allow the derivatization of the second functionality. In order to account for only partial derivatization, molecular orbital calculations were performed at the B3LYP/6-31G(d) level of theory for the singly derivatized 2-methoxyethanol and 3-hydroxytetrahydrofuran. The calculations indicate that the underivatized hydroxyl group and the boron atom in the singly derivatized 3-hydroxytetrahydrofuran are too far from each other to interact (Fig. 5). On the other hand, calculations performed on the singly derivatized 2-

Table 2
Derivatization products (formation reactions, *m/z* values and branching ratios) formed in reactions between protonated hydroxyethers and TMB, and their SORI-CAD fragments (formation reactions and *m/z* values)

Analyte (*m/z* of $(M+H)^+$)	PA[a] (kcal/mol)	Product ions (*m/z*)[b] (branching ratio)	SORI-CAD fragments of singly derivatized analytes (*m/z*)
2-Methoxyethanol (77)	183.7	Adduct – CH_3OH (149) (80%) Adduct – $2CH_3OH$ (117) (20%)	NN[c] NN[c]
2-Hydroxymethyl-tetrahydropyran (117)		Adduct – CH_3OH (189) (72%) Adduct – $2CH_3OH$ (157) (28%)	NN[c] NN[c]
3-Hydroxytetrahydrofuran (89)		Adduct – CH_3OH (161)	161–CH_3OH (129) 161–$HOB(OCH_3)_2$ (71)
4-Hydroxytetrahydropyran (103)		Adduct – CH_3OH (175)	175–CH_3OH (143)

[a] Ref. [28].
[b] 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).
[c] Not necessary.



Scheme 3.

methoxyethanol indicate that the boron atom and the hydroxyl oxygen are close enough to interact (Scheme 3; Fig. 6). This interaction leads to an acidic hydrogen, thus allowing the derivatization of the hydroxyl group.

SORI-CAD performed on singly derivatized 4-hydroxytetrahydropyran (Fig. 7) and 3-hydroxytetrahydrofuran leads to the derivatization of the second functionality via the loss of a second methanol molecule. This finding indicates that derivatization of the second functionality is endothermic for these two analytes. The second derivatization of 4-hydroxytetrahydropyran likely involves attack by the nucleophilic hydroxyl oxygen at the electrophilic boron center via a strained transition state, followed by proton transfer and methanol elimination (Scheme 4a). However, this mechanism is not possible for 3-hydroxytetrahydrofuran due to the distance between the boron atom and the hydroxyl group. It is concluded that the energy deposited into this singly derivatized analyte during SORI-CAD is sufficient to break the B–O bond, leading to the formation of a neutral 3-hydroxytetrahydrofuran and a dimethoxyborenium ion. The nucleophilic hydroxyl oxygen then likely attacks the

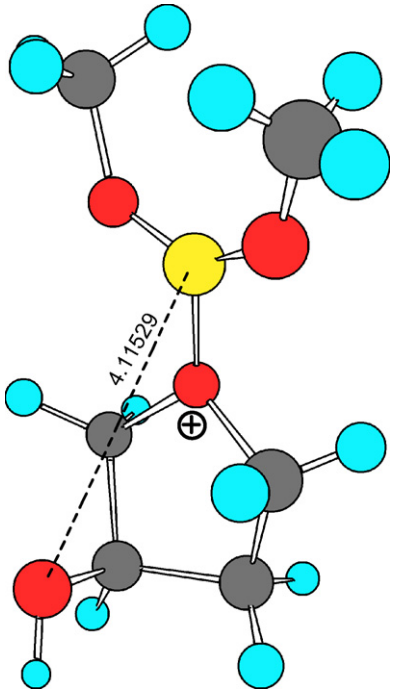


Fig. 5. Optimized (B3LYP/6-31G(d)) structure of the singly derivatized protonated 3-hydroxytetrahydrofuran. The boron center is too far away from the hydroxyl group to interact.

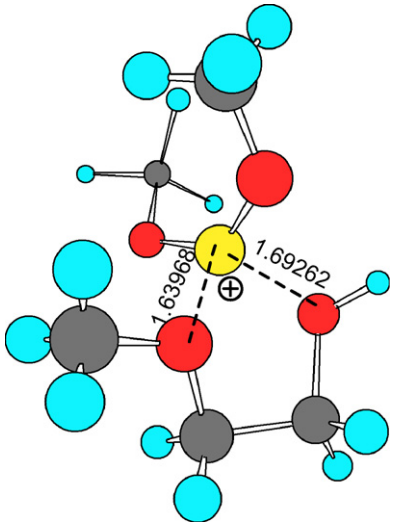


Fig. 6. Optimized (B3LYP/6-31G(d)) structure for the singly derivatized protonated 2-methoxyethanol. The boron center can interact with both oxygen functionalities, thus making the hydroxyl hydrogen acidic. This interaction allows derivatization of the second functionality, the hydroxyl, via the loss of a methanol molecule.

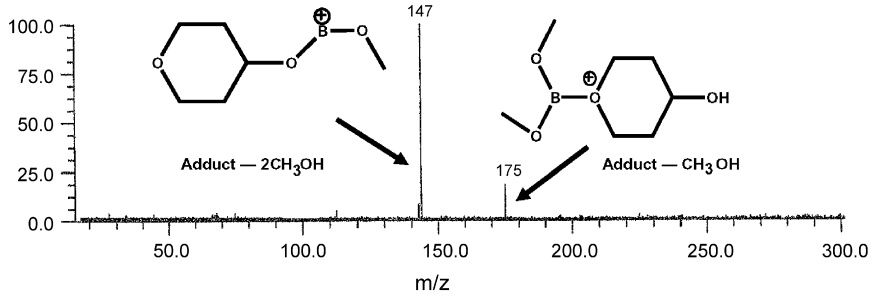


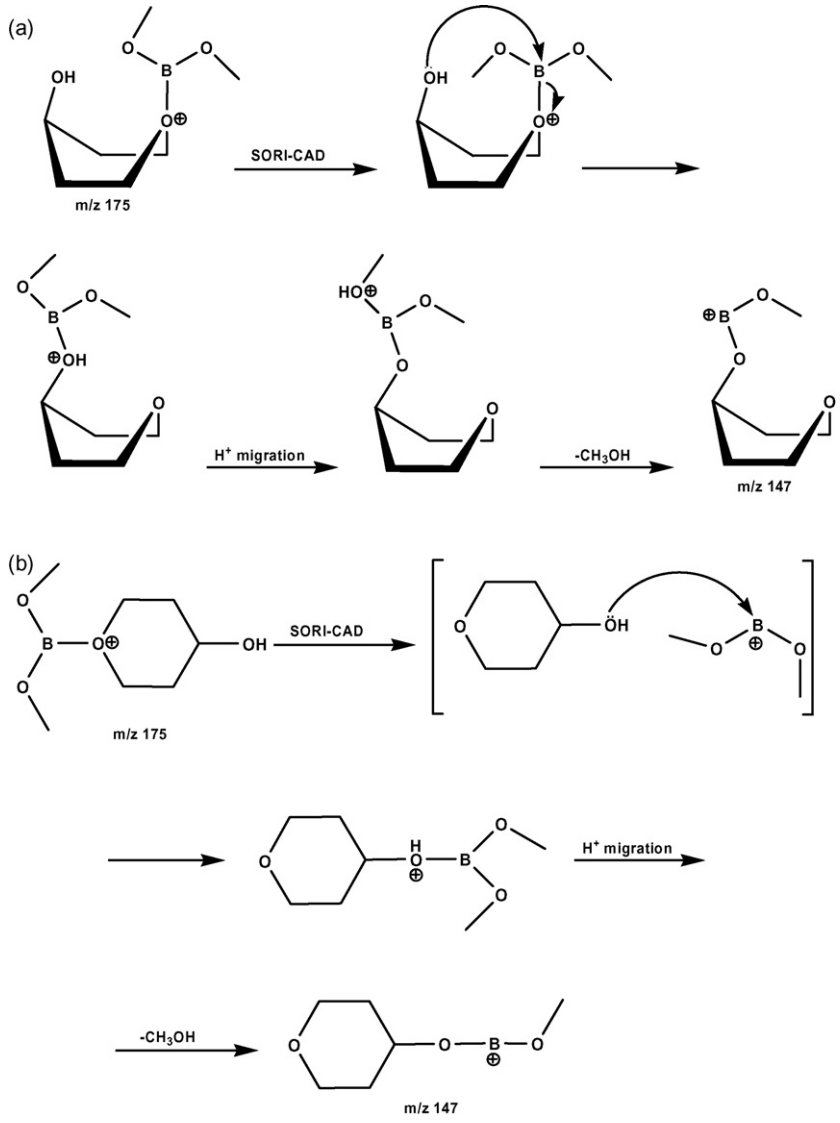
Fig. 7. SORI-CAD spectrum of the singly derivatized 4-hydroxy-tetrahydropyran (*m/z* 175).

electrophilic boron while these two species are still in the collision complex, generating an acidic hydrogen. This is followed by the migration of the proton to one of the methoxy groups attached to the boron center with a simultaneous elimination of the second methanol molecule (Scheme 4b).

In summary, both functional groups in protonated hydroxyethers can be derivatized by using the approach described above. If reactions with TMB do not derivatize both functionalities, derivatization of the second functionality can be achieved by performing SORI-CAD on the singly derivatized analyte.

3.3.3. Hydroxyketones

The reactions between protonated hydroxyketones and TMB lead to derivatization of only one functional group. Again, the



Scheme 4.

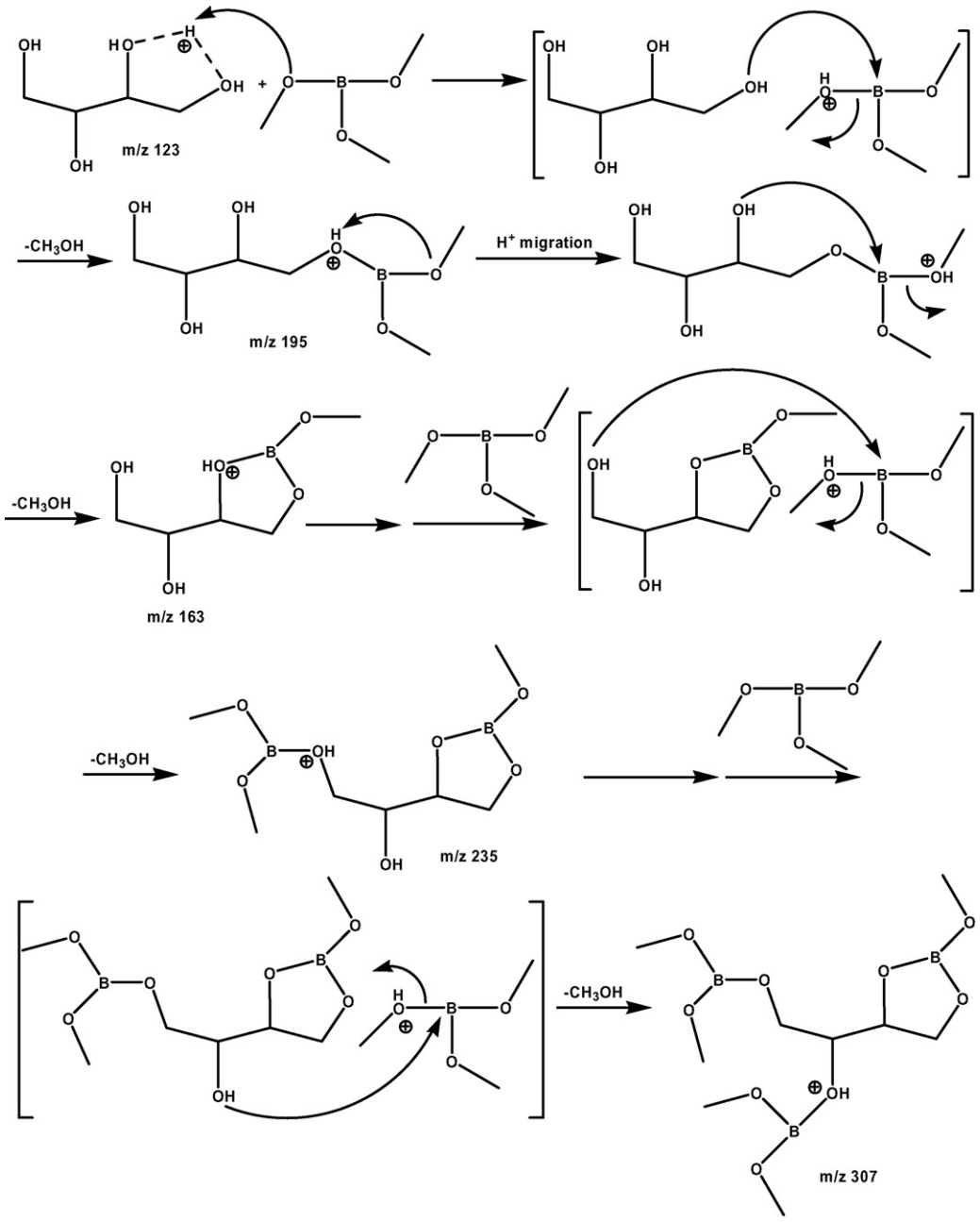
Table 3
Derivatization products (formation reactions, *m/z* values and branching ratios) formed in reactions between protonated hydroxyketones and TMB, and their SORI-CAD fragments (formation reactions and *m/z* values)

Analyte (*m/z* of $(M+H)^+$)	PA[a] (kcal/mol)	Singly derivatized analyte (*m/z*)[b]	SORI-CAD fragments of singly derivatized analytes (*m/z*)
3-Hydroxy-2-propanone (75)	198	Adduct − CH_3OH (147)	147–CH_3OH (115)
4-Hydroxy-2-butanone (89)	202	Adduct − CH_3OH (161)	161–CH_3OH (129)
5-Hydroxy-2-pentanone (103)	220	$MH^+ - H_2O$ (85)	NN[c]
6-Hydroxy-2-hexanone (117)	204	Adduct − CH_3OH (189)	189–CH_3OH (157)

[a] PA calculated at the B3LYP/6-31G(d) level of theory. The PA of the analytes was calculated by using protonated methanol as the Brønsted acid in an isodesmic reaction scheme.

[b] 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).

[c] Not necessary.



Scheme 5.

Table 4
Derivatization products (formation reactions and *m/z* values) formed in reactions between protonated polyols and TMB

Analyte (*m/z* of $(M+H)^+$)	PA[a] (kcal/mol)	Products (*m/z*)[b]
Glycerol (93)	209.1	Adduct − CH_3OH (165) Adduct + TMB–$2CH_3OH$ (237) Adduct + TMB–$3CH_3OH$ (205)
Erythritol (123)		Adduct − CH_3OH (195) Adduct − $2CH_3OH$ (163) Adduct + TMB–$3CH_3OH$ (235) Adduct + 2TMB–$4CH_3OH$ (307)
Xylitol (153)		Adduct − CH_3OH (225) Adduct − $2CH_3OH$ (193) Adduct + TMB–$3CH_3OH$ (265) Adduct + TMB–$4CH_3OH$ (233) Adduct + 2TMB–$5CH_3OH$ (305)
Mannitol (183)		Adduct − CH_3OH (255) Adduct − $2CH_3OH$ (223) Adduct + TMB–$3CH_3OH$ (295) Adduct + TMB–$4CH_3OH$ (263) Adduct + 2TMB–$5CH_3OH$ (335) Adduct + 2TMB–$6CH_3OH$ (303)

[a] Ref. [28].
[b] 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).

lack of generation of an acidic hydrogen upon derivatization of the first functional group hinders the successive derivatization of the second functional group, as observed for some of the hydroxyethers. However, when the singly derivatized hydroxyketones were subjected to SORI-CAD, derivatization of the second functional group took place, via the elimination of a second methanol molecule (Table 3). The dissociation of the singly derivatized hydroxyketones may follow the mechanism shown in Scheme 4b.

In summary, the reactivities of protonated hydroxyketones and some of the protonated hydroxyethers are similar. Hence, some hydroxyethers cannot be distinguished from hydroxyketones by reactions with TMB followed by SORI-CAD.

Protonated 5-hydroxy-2-pentanone displays reactivity different from that of the other hydroxyketones discussed here. This analyte does not undergo a derivatization reaction with TMB. Instead, it slowly loses a water molecule. The lack of derivatization may be attributed to the substantially higher PA of this analyte (PA 220–222 kcal/mol; calculated at the B3LYP/6-31G(d) level of theory) than TMB (195 kcal/mol [28]). The difference in PA between the two is much greater than the

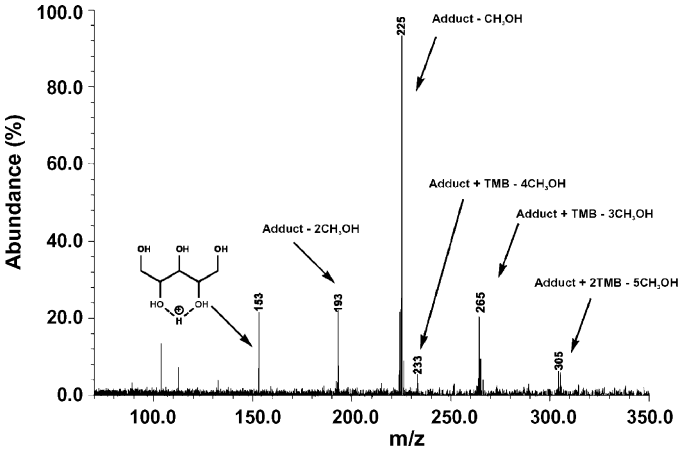


Fig. 8. A mass spectrum representing the reaction of TMB with protonated xylitol (*m/z* 153). This reaction leads to derivatization of all five hydroxyl groups present in xylitol. The number of hydroxyl groups can be determined by simply counting the total number of methanol molecules lost.

solvation energy that can be gained during the formation of the ion–molecule collision complex. Hence, the initial proton transfer step of the derivatization reaction is hindered. However, the boron reagent somehow facilitates proton transfer from the carbonyl to the hydroxyl group since water loss is observed.

3.4. Polyols

Previously [27], diethylmethoxyborane was used to derivatize and count the hydroxyl groups in polyols containing four or fewer hydroxyl groups. However, derivatization of all the hydroxyl groups in higher polyols was not achieved, presumably due to a low gas-phase acidity of the fourth and fifth derivatization products. This prevents the counting of hydroxyl groups in these polyols by simply determining the total number of methanol molecules lost. However, the total number of hydroxyl groups could be indirectly determined by counting the number

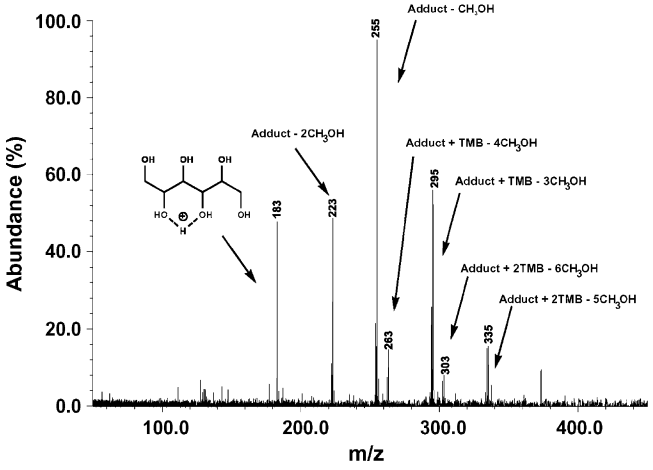


Fig. 9. A mass spectrum representing the reaction of TMB with protonated mannitol (*m/z* 183). This reaction leads to derivatization of all six hydroxyl groups present in mannitol. The number of hydroxyl groups can be determined by simply counting the total number of methanol molecules lost.

Table 5
Derivatization products (formation reactions and *m/z* values) formed in reactions between protonated carboxylic acids and TMB, and their SORI-CAD fragments (formation reactions and *m/z* values)

Analyte (*m/z* of $(M+H)^+$)	PA[a] (kcal/mol)	Singly derivatized analyte (*m/z*)[b]	SORI-CAD fragments of singly derivatized analyte (*m/z*)
Propionic acid (75)	190.5	Adduct − CH_3OH (147)	147–$HOB(OCH_3)_2$ (57)
Butanoic acid (89)		Adduct − CH_3OH (161)	161–$HOB(OCH_3)_2$ (71)
Hexanoic acid (117)		Adduct − CH_3OH (189)	189–$HOB(OCH_3)_2$ (99) 189–$HOB(OCH_3)_2$–CO (71)
Heptanoic acid (131)		Adduct − CH_3OH (203)	203–$HOB(OCH_3)_2$ (113) 203–$HOB(OCH_3)_2$–CO (85)

[a] Ref. [28].
[b] 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).

of consecutive ethane losses from the observed derivatization products.

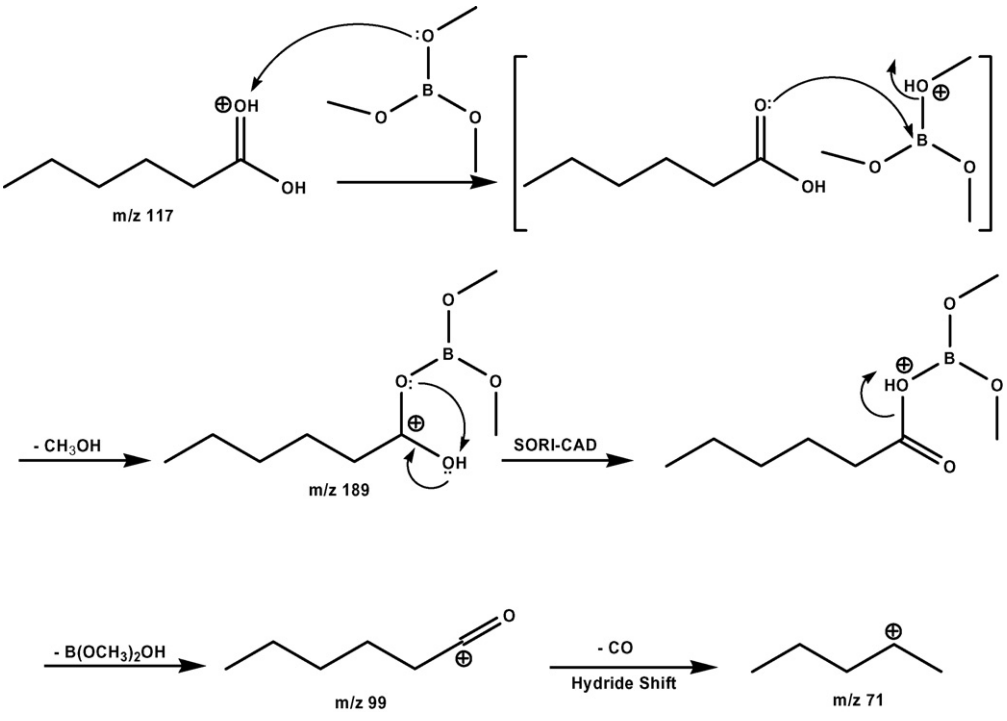
In contrast, reactions of TMB with protonated glycerol, erythritol, xylitol and mannitol lead to derivatization of all the hydroxyl groups (Table 4; Figs. 8 and 9). After derivatization of the first functional group, the product ion undergoes either an intramolecular methanol elimination to derivatize another functional group, or proton transfer to another borate molecule followed by addition/elimination that derivatizes the next hydroxyl group (Scheme 5). The number of hydroxyl groups in a polyol can be determined from the total number of methanol molecules lost upon reactions with TMB molecules.

3.5. Specificity of the method

To examine whether the method discussed here can be applied for the unambiguous identification of functional groups in bifunctional oxygen-containing analytes and polyols, carboxylic acids and nitrogen containing analytes were also examined. Carboxylic acids were chosen for the specificity studies since these analytes are isomeric to hydroxyketones.

3.5.1. Carboxylic acids

The reactions of protonated carboxylic acids with TMB lead to a singly derivatized analyte (Table 5), just like for protonated hydroxyketones and some hydroxyethers. Hence, hydroxyketones and some hydroxyethers cannot be differentiated from carboxylic acids via ion–molecule reactions with TMB. SORI-CAD of all singly derivatized carboxylic acids forms a product via elimination of $HOB(OCH_3)_2$. The singly derivatized longer chain carboxylic acids also undergo a consecutive CO loss (Table 5; Scheme 6). These results are different from those obtained for the singly derivatized hydroxyketones and hydroxyethers which lose a second molecule of methanol

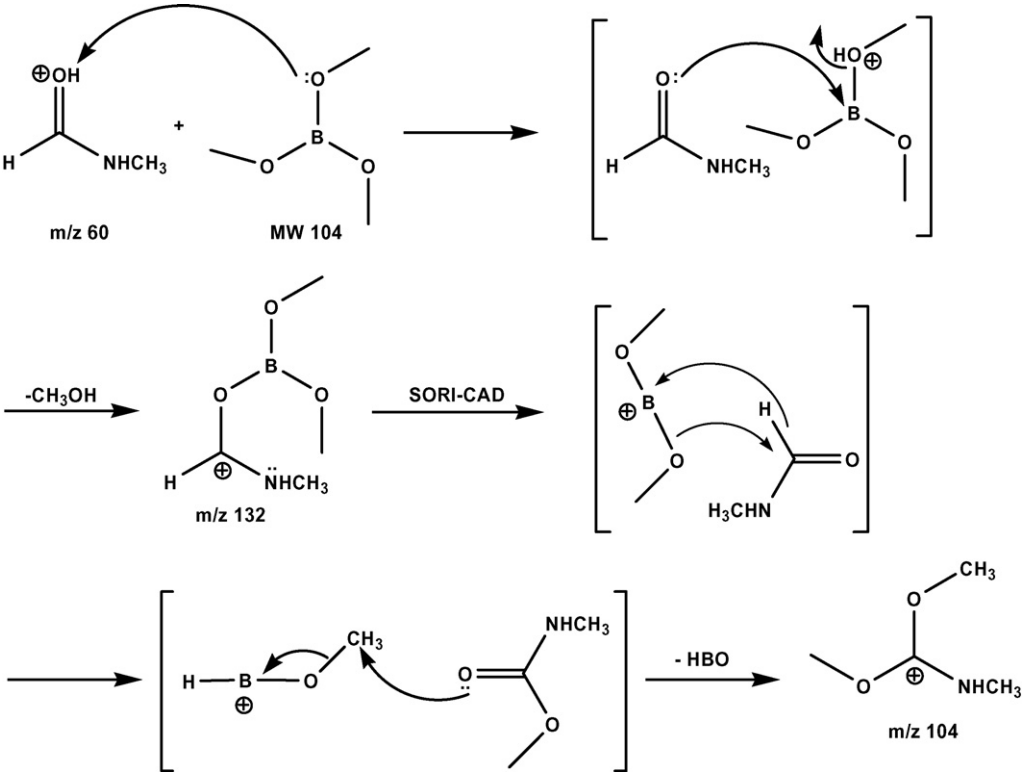


Scheme 6.

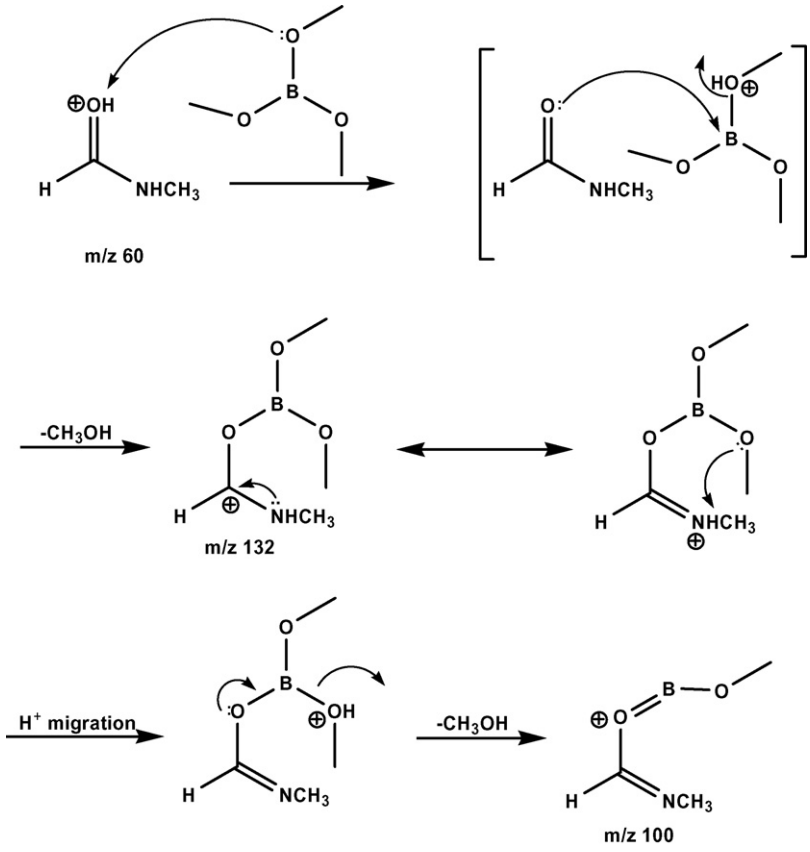
Table 6
Derivatization products (formation reactions, *m/z* values and branching ratios) formed in reactions between protonated amides and amines with TMB, and their SORI-CAD fragments (formation reactions and *m/z* values)

Analyte (*m/z* of $(M+H)^+$)	PA[a] (kcal/mol)	Singly derivatized analyte (*m/z*)[b] (branching ratio)	SORI-CAD fragments of singly derivatized analytes (*m/z*)
Acetamide (60)	206.4	Adduct – CH_3OH (132)	132–O=B–CH_3 (90) 132–CH_3OH (100) $^+B(OCH_3)_2$ (73)
N-Methylformamide (60)	203.5	Adduct – CH_3OH (132)	132–O=B–H (104) 132–CH_3OH (100) $^+B(OCH_3)_2$ (73) MH^+ (60) $(HCNCH_3)^+$ (42)
N-Methylacetamide (74)	212.4	Adduct – CH_3OH (146) (76%) 146–O=B-CH_3 (104) (24%)	NN[c]
N,*N*-Dimethylformamide (74)	212.1	Adduct – CH_3OH (146)	146–O=B–H (118) $^+B(OCH_3)_2$ (73)
N,*N*-Dimethylacetamide (88)	217.0	Adduct – CH_3OH (160)	160–O=B–CH_3 (118) $^+B(OCH_3)_2$ (73)
N-Methylpropionamide (88)	214.6	Adduct – CH_3OH (160)	160–O=B–C_2H_5 (104) 160–CH_3OH (128)
Isopropylamine (60)	220.8	No reaction observed	
N,*N*-Diethylamine (74)	222.2	No reaction observed	
Triethylamine (102)	234.7	No reaction observed	

[a] Ref. [28].
[b] 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).
[c] Not necessary.



Scheme 7.



Scheme 8.

upon SORI-CAD. Therefore, carboxylic acids can be differentiated from hydroxyketones and hydroxyethers by ion–molecule reactions followed by SORI-CAD.

3.5.2. Amides and amines

Reactions of protonated amides with TMB lead to the derivatization of the amido functionality (Table 6). Protonated *N*-methylacetamide also forms a product via the loss of O=B–CH_3 from the singly derivatized analyte. SORI-CAD of all the singly derivatized amides results in the elimination of O=B–R, where R=H, CH_3 and C_2H_5 for formamide, acetamide and propionamide, respectively (Scheme 7). These results suggest that protonated amides may be differentiated from protonated bifunctional oxygen-containing compounds based on the loss of O=B–R either during the ion–molecule reactions or during SORI-CAD.

SORI-CAD of singly derivatized 1° and 2° amides also forms another product via elimination of a CH_3OH molecule. This finding suggests that resonance delocalization of the charge to the nitrogen atom facilitates abstraction of the nitrogen-bound hydrogen by one of the methoxy groups attached to the boron center, which is followed by elimination of a methanol molecule (Scheme 8).

Protonated amines do not react with TMB (Table 6). This lack of reactivity may be attributed to their higher PAs ($\geq$ 20 kcal/mol) than that of TMB. These results are similar to those obtained when examining the reactions between protonated amines and diethylmethoxyborane [26]. In this earlier study, molecular orbital calculations were used to rationalize the lack of reactivity between the two.

4. Conclusions

Reactions of protonated bi- and some polyfunctional oxygen-containing compounds with TMB in an FT-ICR mass spectrometer lead to either complete or partial derivatization of the functional groups, which aids in the identification and counting of the functional groups present in these analytes. Protonated diols react with two molecules of TMB to achieve complete derivatization, whereas most protonated hydroxyethers require only one molecule of TMB for complete derivatization of both the functional groups. For some of the hydroxyethers where the two functional groups are far away from each other, ion–molecule reactions with TMB followed by SORI-CAD of the singly derivatized analyte is required to achieve complete derivatization. Reactions between protonated hydroxyketones and TMB lead to derivatization of only one functional group. However, SORI-CAD of the singly derivatized hydroxyketones leads to derivatization of the second functionality. Finally, the identity and number of hydroxyl groups present in polyols can be directly determined from the total number of methanol molecules lost during the reaction with TMB.

In summary, based on the number of molecules of TMB consumed, the number of methanol molecules lost during the ion–molecule reactions, and SORI-CAD results, it is possible to identify and count the functional groups in the above protonated

bifunctional oxygen-containing compounds, with a few exceptions. Protonated hydroxyethers with functional groups far apart cannot be differentiated from protonated hydroxyketones.

To verify that the method presented here can be used for the unambiguous identification of the above oxygen-containing compounds, carboxylic acids, amides and amines were also examined. Reactions of protonated carboxylic acids with TMB lead to the formation of a singly derivatized analyte just like for protonated hydroxyketones and some of the protonated hydroxyethers. However, when subjected to SORI-CAD, the singly derivatized carboxylic acids lose a $HOB(OCH_3)_2$ molecule, unlike singly derivatized hydroxyketones and hydroxyethers, which lose a second methanol molecule. Reactions of protonated amides with TMB lead to the formation of a singly derivatized analyte which upon SORI-CAD loses O=B–R. The high PA of the amines hinders their reactions with TMB. Hence, the unique products obtained upon SORI-CAD of the singly derivatized carboxylic acids and amides, and the lack of reactivity of protonated amines toward TMB, suggest that this method can be used for the identification and counting of the number of functional groups present in bifunctional oxygen-containing compounds and polyols. In the future, the applicability of this methodology to sodiated analytes will be tested since some analytes are readily ionized by sodium addition during MALDI and ESI.

Acknowledgments

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