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Identification of the Sulfone Functionality in Protonated Analytes via Ion/Molecule Reactions in a Linear Quadrupole Ion Trap Mass Spectrometer

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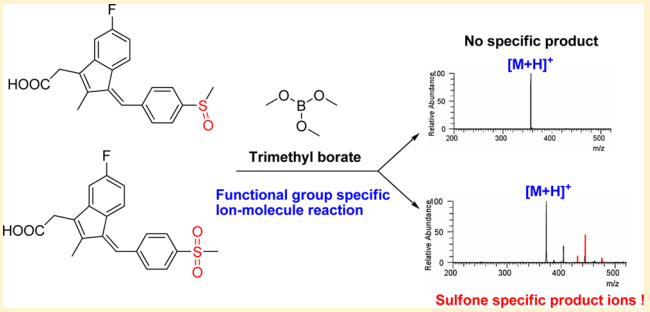
Supporting Information

ABSTRACT: A tandem mass spectrometric method is presented for the rapid identification of drug metabolites that contain the sulfone functional group. This method is based on a gas-phase ion/molecule reaction of protonated sulfone analytes with trimethyl borate (TMB) that yields a diagnostic product ion, adduct-Me_2O, at high reaction efficiency. A variety of compounds with different functional groups, such as sulfoxides, hydroxylamines, N-oxides, anilines, phenol, an aliphatic amine, and an aliphatic alcohol, were examined to probe the selectivity of this reaction. Except for protonated sulfones, most of the protonated compounds react very slowly or not at all with TMB. Most importantly, none of them give the adduct-Me_2O product. A mechanism that explains the observed selectivity is proposed for the diagnostic reaction and is supported by quantum chemical calculations. The reaction was tested with the anti-inflammatory drug sulindac and its metabolite, sulindac sulfone, which were readily distinguished. The presence of other functionalities in addition to sulfone was found not to influence the diagnostic reactivity.



INTRODUCTION

Oxidation of sulfur functionalities to sulfones is an important biotransformation pathway for many drugs.[1] Rapid identification of these drug metabolites is crucial because some of them have been reported to cause idiosyncratic drug reactions and they often go undiscovered until after the postmarketing stage.[2] However, the detection of these metabolites in the highly complex plasma is challenging for many analytical methods, such as NMR, FT-IR, and X-ray crystallography, because of their small quantity and because of stability issues of some metabolite molecules.[3] Tandem mass spectrometric methods involving collisionally activated dissociation (CAD) have been widely used for structure elucidation of unknown compounds directly in mixtures.[4] However, only a few CAD studies have been published on ionized sulfones. Some sulfones, such as deprotonated *N*-phenyl benzenesulfonamides, have been reported to lose SO_2 upon CAD.[5] Moreover, other oxidation products of N and S atom-containing drugs, such as sulfoxides, hydroxylamines, and N-oxides, may have the same molecular weight as the sulfone, which makes it difficult to identify the sulfone functionality unambiguously.[6]

Tandem mass spectrometric methods based on ion/molecule reactions hold great promise for being able to provide information useful in the identification of specific functional groups in analytes.[7] Our group has successfully developed methods based on ion/molecule reactions to identify several functionalities, such as epoxide,[8a] carboxylic acid,[8b] amido,[8c] polyol,[8d] primary, secondary and tertiary amino,[8e] hydroxyl,[8f] and N-oxide functionalities.[8g,h] In the work presented here, gas-phase ion/molecule reactions of trimethyl borate (TMB) are demonstrated to allow the differentiation of the protonated sulfone functionality from many other functional groups, including sulfoxide, hydroxylamino, N-oxide, aniline, amino, hydroxyl, and phenol functionalities. The reaction specificity is also demonstrated using a sulfoxide-containing anti-inflammatory drug, sulindac, and its metabolite, sulindac sulfone.

RESULTS AND DISCUSSION

Trimethyl borate (TMB) is known to deprotonate many protonated oxygen functionalities followed by addition of the analyte to the boron center and elimination of methanol (adduct-MeOH; Scheme 1).[8a,9,10] However, in order for the first proton transfer to occur within the gas-phase collision complex, the proton affinity (PA) of the analyte cannot be more than 10 kcal/mol greater than that of TMB (195 kcal/mol).[9] TMB was chosen to be the reagent to differentiate sulfones from sulfoxides and N-oxides because its PA is close to the PAs of sulfones (193−205

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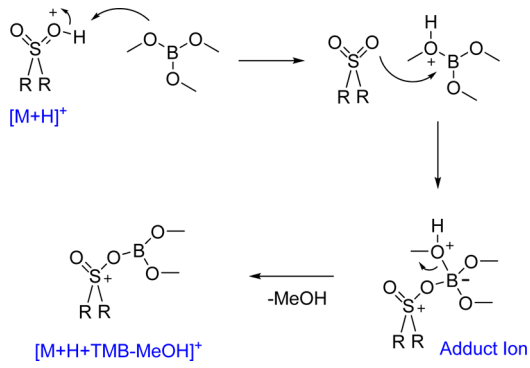
Scheme 1. Proposed Mechanism for Reaction of a Protonated Sulfone with TMB to Form Adduct and Adduct-MeOH



kcal/mol, Table 1), somewhat lower than the PAs of hydroxylamines (205−216 kcal/mol; Table 2), and substantially lower than the PAs of sulfoxides (215−220 kcal/mol; Table 1) and *N*-oxides (~220 kcal/mol; Table 2). Therefore, TMB is expected to react readily, as shown in Scheme 1, with protonated sulfones but not as readily with protonated hydroxylamines, sulfoxides, and N-oxides.

The reactions of TMB with many protonated model compounds with different functional groups, including sulfone, sulfoxide, hydroxylamino, N-oxide, aniline, amino, hydroxyl, and phenol, were examined in a linear quadruple ion trap mass spectrometer (LQIT). As shown in Table 1, most protonated sulfone model compounds react with TMB at high efficiencies (~100%) by predominant formation of the adduct-MeOH ion (Table 1), as expected. The lower reaction efficiency measured for protonated *N*-hydroxybenzenesulfonamide is explained by its relatively high PA (211.2 kcal/mol, Table 1).

For protonated sulfones, such as protonated methyl phenyl sulfone, additional product ions besides the adduct-MeOH ion were observed. These are a stable TMB adduct ion and, most importantly, adduct-Me_2O ion (Figure 1), which is diagnostic for protonated sulfones. No adduct-Me_2O product ion was found for any other protonated model compound studied here (Tables 1 and 2). On the basis of the literature, however, some protonated epoxides yield the adduct-Me_2O ion with a branching ratio ranging from 3 to 10% in the same instrument and under the same conditions as those used here (the other product is adduct-MeOH).[8a] Protonated epoxides can be distinguished from sulfones based on the lack of formation of TMB adduct ions for the epoxides.[8a] Therefore, the formation of adduct-Me_2O ions together with TMB adduct ions can be used to distinguish sulfones from all other compounds considered here.

A possible mechanism for the formation of adduct-Me_2O ion for protonated sulfones is shown in Figure 2. This mechanism is initiated by proton transfer from the analyte to the boron compound, just like the methanol elimination mechanism shown in Scheme 1. Nucleophilic addition of the sulfone to the boron center leads to the methanol elimination product as shown in Scheme 1. However, we propose that in some cases the sulfone instead undergoes nucleophilic substitution at the carbon atom of the protonated methoxy group and a methyl-transfer reaction takes place followed by nucleophilic substitution at the same carbon atom by a methoxy substituent of TMB, leading to another methyl cation transfer (Figure 2). The methyl cation affinities of TMB and sulfones are likely to be similar because of their similar PA values[8h] and hence these reactions should be nearly thermoneutral. Nucleophilic substitution has a more constrained TS than addition reactions, which explains why this pathway is minor compared to methanol elimination. Addition of the sulfone to the boron center in TMB with a methylated methoxy group has a very low calculated barrier (0.25 kcal/mol; Figure 2), and it is estimated to be exothermic by 1 kcal/mol. Elimination of dimethyl ether from this adduct also has a very low barrier (1.8 kcal/mol) and is exothermic by about 3 kcal/mol (Figure 2). Scheme S1 in the Supporting Information shows an analogous mechanism for the formation of adduct-Me_2O for a protonated epoxide, propylene oxide, with PA = 192.0 kcal/mol, which is close to that of TMB and sulfones.

On the basis of previous research,[11] protonated compounds containing oxygen functionalities other than sulfone, such as ethers, ketones, carboxylic acids, and esters, only yield adduct-MeOH product ions with TMB, and they are formed as shown in Scheme 1. Here, the same was found to be true for protonated sulfoxides (Table 1) and hydoxylamines (Table 2), which also have substantially lower reaction efficiencies than protonated sulfones. Hence, sulfones are distinguished from sulfoxides and hydroxylamines based on the formation of the Me_2O elimination product for protonated sulfones only and based on their substantially greater reaction efficiencies. Protonated N-oxides are readily differentiated from sulfones, sulfoxides and hydroxylamines since they are unreactive toward TMB, as reported before.[8h] The observation of the Me_2O elimination product only for protonated sulfones (and some protonated epoxides) may be partially explained by their lower nucleophilicity compared to sulfoxides, N-oxides, and hydroxylamines (nucleophilicity commonly correlates[8h] with PA). Addition of the more nucleophilic analytes to the boron center of protonated TMB is likely to be exothermic enough to cause immediate elimination of methanol. However, for sulfones, this addition may be reversible. Furthermore, sulfones have very large dipole moments[16] (>4 D; comparable to those[16] of sulfoxides and N-oxides), which means that ion/molecule complexes containing a neutral sulfone lay low in energy, have a long lifetime, and contain excess internal energy. This likely enables a neutral sulfone to undergo competitive attack at both the boron atom and the most electrophilic methyl group in protonated TMB, ultimately resulting in elimination of both methanol as well as dimethyl ether (Scheme 1 and Figure 2).

The reactivity of TMB toward a protonated aliphatic amine and an alcohol as well as phenol and two anilines was also examined. As shown in Table 2, the protonated amine and the protonated anilines react only slowly with TMB (efficiencies <7%) via exclusive formation of a stable adduct (likely as shown in Scheme 1), whereas protonated butanol reacts rapidly by exclusive proton transfer (because of its low PA; 188.8 kcal/mol[16]). Protonated phenol (with PA close to that of TMB; Table 2) also transfers a proton to TMB, but, in addition, it forms a stable adduct and the MeOH elimination product (see Scheme 1 for both reactions). These findings suggests that formation of a stable adduct (just like the MeOH elimination product) is preceded by proton transfer from the protonated analyte to TMB because the adduct was observed only for analytes whose PAs are similar or greater than that of TMB (Tables 1 and 2). This finding is in agreement with the mechanisms shown in Scheme 1 and Figure 2. In summary, the protonated sulfone functional group can be easily differentiated from amino, aniline, hydroxyl, and phenol moieties via its formation of the adduct-Me_2O ion.

The application of this method for the identification of a sulfone metabolite of a sulfoxide drug was demonstrated using sulindac, a nonsteroidal anti-inflammatory drug, and its

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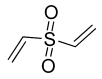
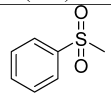
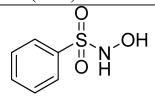
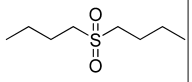
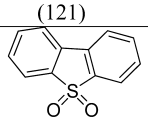
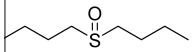
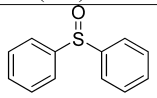
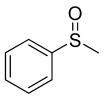
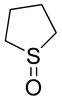
Table 1. Reaction Efficiencies and Products (m/z Values and Branching Ratios) for Reactions of Protonated Sulfones and Sulfoxides with TMB (PA = 195 kcal/mol)[a]

Reagent (m/z of $[M+H]^+$)	PA[b] (kcal/mol)	Product ions (m/z) and branching ratios		Reaction efficiency
 (119)	206.3	Adduct–MeOH (191) Adduct–Me_2O (177) Adduct (223)	85% 10% 5%	107%
 (157)	201.4	Adduct–MeOH(225) Adduct–Me_2O(215) Adduct (261)	87% 10% 3%	107%
 (174)	211.6	Adduct–MeOH (246) Adduct–Me_2O (232) Adduct (278)	74% 12% 14%	57%
 (179)	203.7	Adduct–MeOH (251) Adduct–Me_2O (237) Adduct (281)	89% 9% 1%	118%
 (95)	193.5	Adduct–MeOH (167) Adduct–Me_2O (153) Adduct (199) Proton Transfer (105)	83% 8% 3% 6%	101%
 (121)	198.3	Adduct–MeOH (193) Adduct–Me_2O (179) Adduct (225)	79% 14% 7%	111%
 (217)	205.0	Adduct–MeOH (289) Adduct–Me_2O (276) Adduct (321)	89% 10% 1%	104%
 (163)	220.1	Adduct–MeOH (235)	100%	0.7%
 (203)	222.5	Adduct–MeOH (275)	100%	0.4%
 (141)	219.8	Adduct–MeOH (213)	100%	0.9%
 (104)	219.6	Adduct–MeOH (177) Adduct (209)	64% 36%	2%

[a]Ref 9. [b]PA calculated at the B3LYP/6-31G(d) level of theory.

metabolite, sulindac sulfone (Figure 3). As expected, protonated sulindac sulfone reacts quickly (with 44% efficiency) with TMB to give TMB adduct, adduct-MeOH, and adduct-Me_2O, whereas protonated sulindac reacts slowly (10% efficiency) with TMB and only shows a small amount of adduct-MeOH product ion. Their reactivities and product ions are similar to the analogous compounds in Table 1. However, when sulindac and sulindac sulfone were protonated and subjected to CAD, both of these molecules fragment by losses of water, carbon dioxide, and methyl radical, with no fragmentations indicative of either a sulfoxide or a sulfone functionality.

Lastly, it is important to note that a reactivity diagnostic of a sulfone functionality was observed for reactions of TMB with two protonated compounds with more than one functionality, *N*-hydroxybenzenesulfonamide and sulindac sulfone. These findings indicate that the diagnostic reactivity is not quenched by the presence of additional functionalities, although the reaction efficiency may depend on the functionalities present in the analyte.

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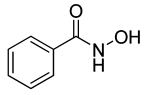
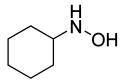
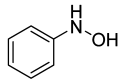
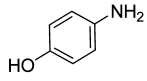
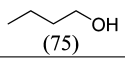
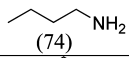
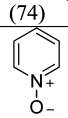
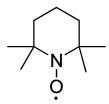
Table 2. Reaction Efficiencies and Products (m/z Values and Branching Ratios) Formed in Reactions between Protonated Hydroxylamines, N-Oxides, Anilines, an Aliphatic Amine, an Aliphatic Alcohol, and Phenol with TMB (PA = 195 kcal/mol)[a]

Reagent (m/z of $[M+H]^+$)	PA[b] (kcal/mol)	Product ions (m/z) and branching ratios	Reaction efficiency
 (138)	211.7	Adduct–2MeOH (178) 45% Adduct (242) 29% Adduct–MeOH(228) 26%	6%
 (116)	215.9	Adduct (220) 100%	4%
 (110)	204.6	Adduct (214) 100%	1%
 (110)	212.9	Adduct (214) 100%	1%
 (94)	209.4	Adduct (198) 100%	2%
 (95)	194.6	Adduct–MeOH (167) 85% Adduct (199) 3% Proton Transfer (105) 12%	5%
 (75)	187.6	Proton Transfer (105) 100%	60%
 (74)	220.9	Adduct (178) 100%	1%
 (96)	219.2	No reaction	No reaction
 (203)	--	No reaction	No reaction

[a]Ref 9. [b]PA calculated at the B3LYP/6-31G(d) level of theory.

CONCLUSIONS

The ability to use functional-group-selective ion/molecule reactions in a linear quadrupole ion trap mass spectrometer to identify compounds with the sulfone functionality has been demonstrated. All protonated sulfone model compounds were found to react with TMB to form the diagnostic product ion, adduct-Me_2O, at high efficiency. Other protonated compounds, including sulfoxides, hydroxylamines, and N-oxides, react with TMB only very slowly or not at all via adduct formation or MeOH elimination from the adduct, and none of them give the adduct-Me_2O product ion. Similar results were obtained for anilines and an aliphatic amine. Phenol yields a proton-transfer product in addition to the adduct-MeOH product and the stable adduct. Protonated butanol differs from the other ions studied in that it rapidly and exclusively transfers a proton to TMB because of its high acidity. On the basis of the literature,[8a] some protonated epoxides form the adduct-Me_2O product ion but can be differentiated from sulfones because of the lack of formation of a stable adduct. The sulfone-selective reactivity was observed even in the presence of additional functionalities. A mechanism is proposed that rationalizes the selectivity of the addition/Me_2O elimination reaction for protonated sulfones and that is supported by quantum chemical calculations. Lastly, the results obtained for sulindac and sulindac sulfone suggest that this method is applicable to sulfone-containing drugs and drug metabolites.

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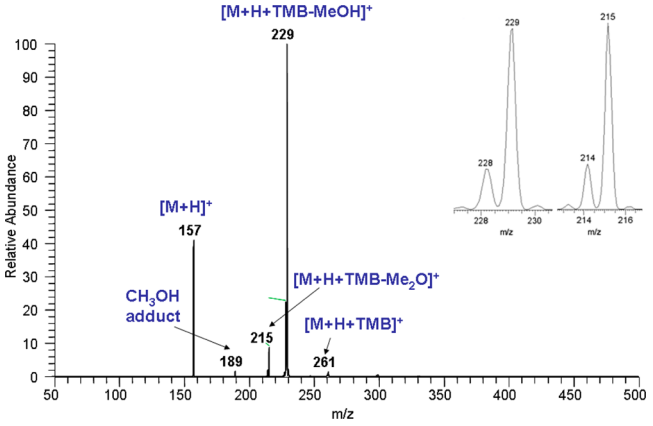

Figure 1. Mass spectrum measured after 30 ms reaction of protonated methyl phenyl sulfone (m/z 157) with TMB. The most abundant product ion (m/z 229) corresponds to adduct-MeOH. The other two product ions of TMB correspond to the adduct-Me_2O (m/z 215) and TMB adduct (m/z 261). The presence of boron in ions of m/z 229 and 215 was verified by the presence of ^{10}B isotope peaks. Ion of m/z 189 corresponds to a methanol adduct (verified by using ethanol as a solvent instead of methanol and observing a 14 unit shift in the m/z value).

EXPERIMENTAL SECTION

Chemicals. All chemicals were purchased and used without further purification.

Instrumentation. All mass spectrometry experiments were performed using a linear quadruple ion trap (LQIT) equipped with an APCI source. Sample solutions were prepared at analyte concentrations ranging from 0.01 to 1 mg/mL in methanol. An integrated syringe drive directly infused the solutions into the APCI source at a rate of 20 μL/min. In the APCI source (operated in positive ion mode), the vaporizer and capillary temperatures were set at 400 and 265 °C, respectively. The sheath gas (N_2) flow was maintained at about 30 arbitrary units. The voltages for the ion optics were optimized for each analyte by using the tune feature of the LTQ Tune Plus interface. The detection mass range was from m/z 50 to 500. The manifold used to introduce reagents into the helium buffer gas line was first described by Gronert.[12,13] A diagram of the exact manifold used in this research was published by Habicht et al.[8b] TMB was introduced into the manifold via a syringe pump maintained at a flow rate of 5 μL/h. A known amount of He (0.8 L/h) was used to dilute TMB. The syringe port and surrounding area were heated at ~70 °C to ensure evaporation of TMB. Before entering the trap, the He/reagent mixture was split using two Granville–Phillips leak valves instead of the standard flow splitter. This allowed for better control over the amount of the mixture introduced into the instrument. One leak valve was set to establish a helium pressure of ~3 mTorr in the ion trap by allowing ~2 mL/min of the mixture into the trap,[14] whereas the other leak valve controlled the amount of flow diverted to waste. A typical nominal pressure of TMB in the trap during the experiments was 0.38×10^{-5} Torr. After the experiments were completed each day, the manifold was isolated from the instrument and placed under vacuum to remove any remaining reagent.

Kinetics. During the ion/molecule reactions, the 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 (efficiency = $(k_{reaction}/k_{collision})100$ = the fraction of ion/molecule collisions that results in the formation of products) were determined by measuring each reaction's rate (IM) (by monitoring the abundance of the protonated analyte as a function of time for up to 1 s) and the rate of the highly exothermic proton-transfer reaction (PT) between protonated methanol and the reagent (TMB) under identical conditions on the same day. 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 using eq 1. This equation is based on the ratio of the slopes ($k_{reaction}$[TMB] = slope (IM) and $k_{collision}$[TMB] = slope (PT); [TMB] = TMB concentration) of the 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 [TMB]), masses of the ion (M_i), neutral reagent (M_n), and methanol ($M_{(PT)}$), and the nominal pressure of the neutral reagent

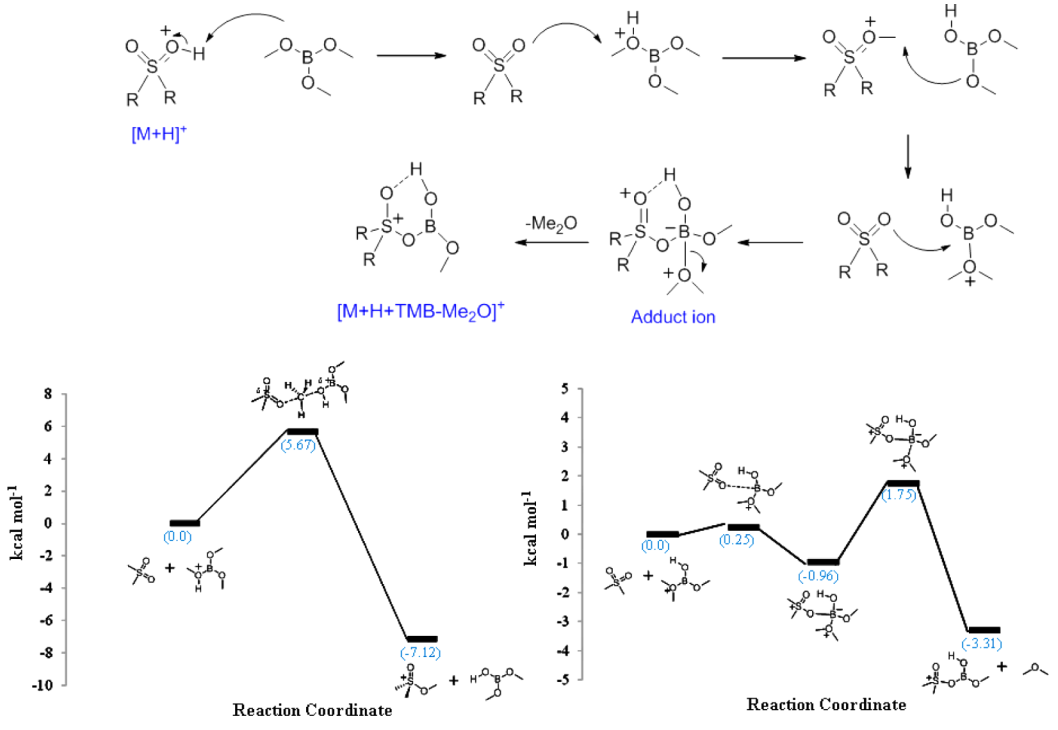

Figure 2. Proposed mechanism for reaction of a protonated sulfone with TMB to form adduct-Me_2O and the calculated (B3LYP/6-31G(d,p)) free-energy surface for the second step (left) and the last two steps (right) of the mechanism.

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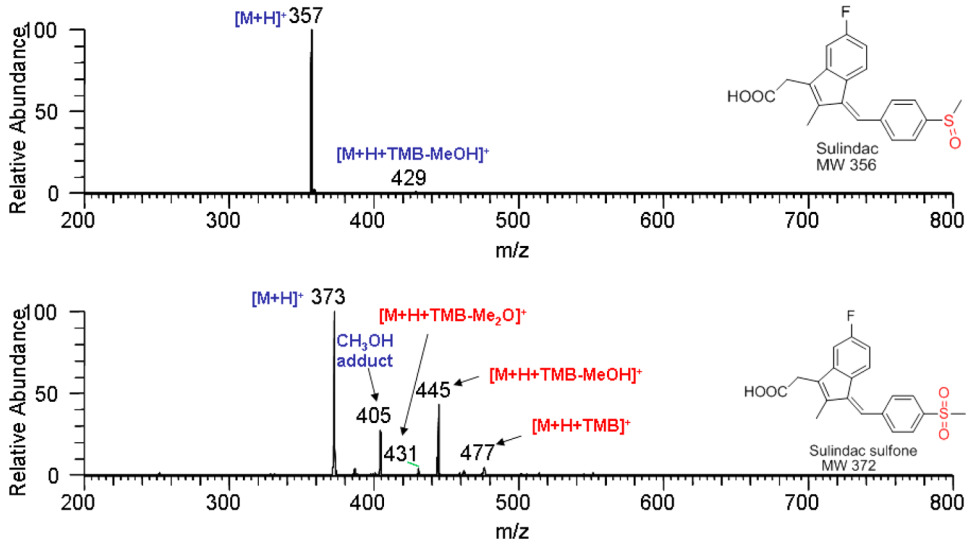

Figure 3. Upper mass spectrum was measured after 1 s reaction of protonated sulindac with TMB. Lower mass spectrum was measured after 100 ms reaction of protonated sulindac sulfone with TMB. The ion of m/z 405 in the bottom spectrum corresponds to a methanol adduct, which was confirmed by using ethanol as the solvent instead of methanol and observing a 14 unit shift in the m/z value.

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. The Gaussian 03 suite of programs was used for all calculations.[15] Proton affinities were calculated at the B3LYP/6-31G(d) level of theory. For protonation of an oxygen site, protonated methanol was used as the Brønsted acid in isodesmic reaction schemes.[16] For protonation of the phenyl ring, protonated benzene[16] was used as the reference acid. For protonation of a nitrogen site, ammonium[16] was used as the reference acid. An excellent agreement of the calculated values with experimental data, where available, was found. For example, methyl phenyl sulfone, dimethyl sulfone, diphenyl sulfoxide, and methyl phenyl sulfoxide have calculated PAs of 201.4, 193.5, 222.5, and 219.8 kcal/mol, whereas the experimentally[17] determined values are 200.0, 193.5, 218.8, and 214.9 kcal/mol. The free-energy surfaces shown in Figure 2 were calculated at the B3LYP/6-31G(d,p) level of theory.

■ ASSOCIATED CONTENT

Supporting Information

Proposed mechanism for reaction of a protonated epoxide with TMB to form adduct-Me_2O and dtailed computational data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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