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Structure Fragmentation Studies for Ring Substituted N-Trifluoroacetyl-N-Benzylphenethylamines Related to the NBOMe Drugs

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/rcm.8593

Abstract

Rationale: The halogenated derivatives of N-(2-methoxy)benzyl-2,5-dimethoxyphenethylamine (25-NBOMe) such as the 4-bromo-analogue (25B-NBOMe) represent a new class of hallucinogenic or psychedelic drugs. The purpose of this study was to determine the role of the electron-donating groups (halogen and dimethoxy) on the pathway of decomposition for the distonic molecular radical cation in the EI-MS of the trifluoroacetamide (TFA) derivatives.

Methods: The systematic removal of substituents from the 4-halogenated-2,5-dimethoxy-phenethylamine portion of the N-dimethoxybenzyl NBOMe analogues allowed an evaluation of structural effects on the formation of major fragment ions in the EI-MS of the TFA derivatives.

All six regioisomeric dimethoxybenzyl substituted analogs (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethoxy) for the four series of phenethyl aromatic ring substitution patterns were prepared, derivatized and analyzed via GC/EI-MS.

Results: The analogues yield two unique radical cation fragments from the decomposition of the common distonic molecular radical cation. The substituted phenylethene radical cation (*m/z* 164) is the base peak or second most abundant ion in all six TFA-2,5-dimethoxyphenethylamine isomers. The dimethoxybenzyltrifloroacetamide radical cation (*m/z* 263) is the base peak or second most abundant ion in the 2- and 3MMPEADMB isomers. However, the 2- and 3-methoxyphenylethene radical cation (*m/z* 134) is among the five most abundant ions for each of these twelve isomers. Only one isomer in the PEADMB series yields the corresponding unsubstituted phenylethene radical cation at *m/z* 104.

Conclusions: The decomposition of the hydrogen rearranged distonic molecular radical cation favors formation of the dimethoxybenzyltrifloroacetamide (*m/z* 263) species for the less electron rich phenethyl aromatic rings. The addition of electron-donating groups to the aromatic ring of the phenethyl group as in the NBOMe-type molecules shifts the decomposition of the common distonic molecular radical cation to favor the formation of the electron-rich substituted phenylethene radical cation.

Introduction

The relative distributions of major fragment ions for trifloroacetyl derivatives of substituted phenethylamine-type compounds such as 4-iodo- and 4-bromo N-dimethoxybenzyl-2,5-dimethoxyphenethylamines are described in this report. The four series of compounds (Figure 1) compared in this study can be viewed as the sequential deletion of the electron-donating groups on the phenethylamine portion of the 4-halogenated 2,5-dimethoxyphenethyl-amines. Comparison of the major EI-MS fragment ions and their abundances for the TFA-derivatives for all six regioisomeric N-dimethoxybenzyl isomers in all four series of secondary amines will provide additional structure-fragment relationship data for this series of compounds.

The substituted N-benzyl derivatives of 4-halogenated 2,5-dimethoxyphenethylamines are examples of the expanding category of drugs of abuse sometimes referred to as new/novel psychoactive substances (NPS drugs). The 4-iodo- and 4-bromo-2,5-dimethoxyphenethylamines having a 2-methoxybenzyl substituent on nitrogen are among the most common hallucinogenic substances referred to as NBOMe drugs. These drugs have biological activity profiles and potency similar to those of lysergic acid diethylamide (LSD)[1-4]. Casele and Hayes[5] have reported the remarkable analytical similarity between 2, 3, and 4-methoxybenzyl regioisomers of the same 4-substituted 2,5-dimethoxyphenethylamine series. While the 4-substituent varies across a variety of NBOMe drugs[6, 7] all these compounds have the 2,5-dimethoxy substitution pattern in the aromatic ring of the phenethylamine portion of the NBOMe molecules.

Based on the commercial availability of precursor aldehydes the synthesis and analytical evaluation of the six regioisomeric N-dimethoxybenzyl NBOMe derivatives were

recently described for the 4-bromo-2,5-dimethoxyphenethylamine series[8]. The six isomers in each series displayed very little analytical uniquenesses and the major EI-MS fragment ions were essentially equivalent for each isomer.

The acylation of the secondary amine nitrogen in the 4-iodo- and 4-bromo N-dimethoxybenzyl-2,5-dimethoxyphenethylamines with trifluoroacetyl groups allowed differentiation of the six isomers based on some unique fragment ions and the relative abundance of other common ions[8]. The purpose of this study was to determine the role of the electron-donating groups (halogen and dimethoxy) on the pathway of decomposition (Figure 2) for the distonic molecular radical cation formed by hydrogen radical transfer from the phenethyl side-chain to the carbonyl of the TFA group.

Experimental

Instrumentation

The GC/MS system consisted of an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The gas chromatograph was operated in splitless injection mode with a helium (ultra-high purity, grade 5, 99.999%) flow rate of 0.48 mL/min and an injection volume of 1 uL. The mass spectrometer was operated in the electron ionization (EI) mode with an ionization energy of 70 eV, a scan rate of 2.86 scans/s and a source temperature of 230°C. The GC injector was maintained at 230°C and the transfer line at 230°C. The GC/MS chromatographic separations were carried out on a column (30 m ×0.25 mm i.d.) coated with 0.25 μm film of midpolarity Crossbond® silarylene phase similar to 50% phenyl, 50% dimethylpolysiloxane (Rxi®-17Sil MS) purchased from Restek Corporation (Bellefonte, PA, USA). The temperature program consisted of an initial hold at 70°C for 1.0 min, ramped up

to 250°C at 30°C/min followed by a hold at 250°C for 25 min, then increased to 340°C at 15°C/min.

Synthetic Methods

Precursor materials including the six dimethoxybenzaldehydes were purchased from Aldrich Chemical Company (Milwaukee, WI, USA) or VWR Chemical Company (Radnor, PA, USA). The synthesis of 2,5-dimethoxyphenylnitroethene was accomplished using 2,5-dimethoxybenzaldehyde, nitromethane and anhydrous ammonium acetate heated at reflux. The excess nitromethane was removed under reduced pressure and the resulting oil dissolved in isopropyl alcohol and the product isolated as needle shaped crystals by vacuum filtration. The structure of the product was confirmed by GC/MS and NMR spectroscopy.

The 2,5-dimethoxynitroethene was reduced in a suspension of $LiAlH_4$ to yield 2,5-dimethoxyphenethylamine and the product oil was converted to the hydrochloride salt via gaseous HCl. The N-(dimethoxy)benzyl-2,5-dimethoxyphenethylamine final products were prepared by treating 2,5-dimethoxyphenethylamine HCl with the appropriately substituted dimethoxybenzaldehyde followed by reduction with $NaBH_4$. The six N-(dimethoxy)benzyl-2-dimethoxyphenethylamines, N-(dimethoxy)benzyl-3-dimethoxyphenethylamines and N-(dimethoxy)benzyl-phenethylamines were prepared in an analogous manner.

Results and Discussion

The GC/MS studies in this report are designed to investigate structure-MS fragmentation relationships in dimethoxybenzyl NBOMe analogs. The compounds shown in Figure 1 were synthesized to represent the systematic removal of the halogen followed by one or both of the methoxy groups in the phenethyl aromatic ring of the classic 25XNBOMe structure. The 2,5-DMPEADMB series represents removal of halogen; 2-MMPEADMB

derivatives are analogs without the halogen and 5-methoxy group; and the 3-MMPEADMB derivatives represent analogs where the halogen and 2-methoxy group are removed. In addition, the ring unsubstituted phenethylamines (PEADMB) derivatives represent analogs where both the phenethyl ring methoxy groups and the halogen are removed. For each of these four series of phenethyl aromatic ring substitution patterns all six regioisomeric dimethoxybenzyl substituted analogs (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethoxy) were prepared, derivatized and analyzed via GC/MS. These compounds were synthesized by reductive alkylation reactions with 2,5-dimethoxy-, 2-methoxy, 3-methoxy- or unsubstituted phenethylamines and the corresponding six regioisomeric dimethoxy benzaldehydes as described in previous reports[8]. The EI mass spectra of the TFA derivatives of each of the 2,5-DMPEADMB, 2-MMPEADMB, 3-MMPEADMB and PEADMB series are shown in Figures 3-6 and the relative abundance of fragment ions are summarized in Table 1.

The major fragmentation in the EI-MS spectra for these TFA derivatives appears to be limited to three pathways yielding the dimethoxybenzyl cation along with the two radical cations shown in Figure 2. The dimethoxy benzyl cation at *m/z* 151 and its product ions (*m/z* 121 and *m/z* 91) can occur following initial radical cation formation at the dimethoxybenzyl aromatic ring. In addition, the *m/z* 151 dimethoxybenzyl cation can occur following initial hydrogen transfer to the carbonyl oxygen as shown for the distonic molecular radical cation in Figure 2. The fragmentation of this species along pathway "A" would yield the *m/z* 151 cation. Fragmentation via pathway "B" yields the phenylethene radical cation occurring at various masses (*m/z* 164, 134, 104) depending on the methoxy substituents on the phenethyl group. Decomposition of the common distonic radical cation along pathway "C" results in the formation of the *m/z* 263 radical cation (dimethoxybenzyltrifloroacetamide).

While the *m/z* 151 cation could occur via several fragmentation pathways, the substituted phenylethene radical cation and the *m/z* 263 ion appear to occur from the distonic molecular radical cation. Previous studies[8] have shown that the relative intensities of these three major fragments can be used to determine the dimethoxy group substitution pattern on the aromatic ring of the N-benzyl group for TFA-25X-NBOMe compounds. However, all these observations were made for molecules in which the phenethylamine portion of the NBOMe structure contained the electron-rich 4-halogen and 2,5-dimethoxyphenyl groups. These electron-donating substituent groups appear to play a role in the ease of formation of the distonic molecular radical cation and its subsequent decomposition along the perhaps competing pathways especially the formation of the *m/z* 263 fragment and the substituted phenylethene radical cation.

The electron rich substituted phenylethene radical cation fragments from pathway B are major ions for many of the dimethoxy benzyl substitution patterns in the 4-substituted (Br, I)-2,5-dimethoxyphenethylamine derivatives (25X-NBOMes). The *m/z* 263 and the substituted phenylethene radical cations each form by cleavage of the same chemical bond, the nitrogen to aliphatic carbon of the phenylethyl group. The heterolytic cleavage of the bond along Pathway B in Figure 2 yields the substituted phenylethene radical cation and the neutral substituted benzyl trifluoroacetamide. Breaking the same bond in a homolytic manner (Pathway C, Figure 2) yields the *m/z* 263 dimethoxybenzyl trifluoroacetamide radical cation and the neutral substituted phenylalkene. The substituted phenylethene radical cation occurs at various masses depending on the nature of the aromatic substituent on the phenethylamine portion of the structure while the substituted benzyl trifluoroacetamide radical cation always appears at *m/z* 263 since all the benzyl group aromatic ring substituents are dimethoxy. The TFA derivatives of the traditional 25XNBOMe compounds containing

only monomethoxybenzyl groups (at positions 2, 3 and 4) did not show any equivalent benzyl trifluoroacetamide radical cation at the expected mass of *m/z* 233.

The systematic removal of the electron-donating groups from the 4-halogenated-2,5-dimethoxyphenethylamine structure of 25X-NBOMes to yield the 2,5-dimethoxyphenethyl-amine, 2-methoxyphenethylamine, 3-methoxyphenethylamine, and phenethylamine allowed an evaluation of these structural effects on the formation of the phenylethene and *m/z* 263 radical cations. The EI-MS spectra for these isomers are shown in Figures 3-6. The 2,5-dimethoxyphenyl series of isomers showed a significant dimethoxybenzyl ion at *m/z* 151 as well as a significant peak for the dimethoxyphenylethene radical cation at *m/z* 164 (Figure 3). In fact, the phenylethene radical cation is the base peak for three of the six isomers in the 25H-NBOMe series and *m/z* 151 is the base peak for the others, while the *m/z* 263 fragment is not a prominent peak in any of the six spectra. However, in both the 2-MMPEADMB (Figure 4) and the 3-MMPEADMB (Figure 5) series of isomers containing only one of the electron-donating methoxy groups in the phenethyl aromatic ring, the *m/z* 263 radical cation becomes a more prominent fragment ion and the methoxyphenylethene radical cation at *m/z* 134 is relatively insignificant (see Table 1). These results suggest that homolytic cleavage of the N-C bond is the favored pathway leading to the formation of *m/z* 263 and the neutral 2- or 3-methoxyphenylethene, rather than the less favored heterolytic bond cleavage that would yield the methoxyphenylethene radical cation. This is further confirmed by the EI-MS spectra of the unsubstituted phenethyl PEADMB series (Figure 6) which shows no significant phenylalkene radical cation at *m/z* 104. The *m/z* 151 ion is the base peak in all six spectra of the PEADMBs and its product ions at *m/z* 121 and 91 are prominent ions in this series. The presence of the *m/z* 263 radical cation shows that the distonic molecular radical cation continues to form in this unsubstituted phenethylamine series. However, these results suggest

that the decomposition of this distonic molecular radical cation species strongly favors formation of the *m/z* 263 species with the elimination of the neutral phenylethene molecule. Furthermore, electron-donating groups on the aromatic ring of the phenethyl group appear to be necessary to allow formation of the substituted phenylethene radical cation. In fact, most of the spectra of the TFA-PEADMB compounds do not show the phenylethene (*m/z* 104) radical cation fragment and instead contain the phenethyl cation fragment at *m/z* 105 (Figure 6). Thus, the addition of electron-donating groups to the aromatic ring of the phenethyl group as in the NBOMe-type molecules shifts the decomposition of the corresponding distonic molecular radical cation to favor the formation of the electron-rich substituted phenylethene radical cation.

The mass spectra of the 2,3-dimethoxybenzyl isomer of 2,5-DMPEADMB, 2-MMPEADMB, 3-MMPEADMB and PEADMB (as well as the TFA derivatives for each series) each contain a *m/z* 136 fragment ion which is not present in the mass spectra of the other five N-(dimethoxy)benzyl regioisomers in each series. The *m/z* 136 ion is also a significant fragment in the 2,3-dimethoxybenzyl isomer for 25I- and 25B-NBOMe and their TFA derivatives. This unique ion appears to form in a fragmentation process resulting from loss of a methyl radical from one of the methoxy groups on the benzyl aromatic ring. The *m/z* 136 radical cation probably forms via methyl radical migration from a methoxy group of the 2,3-dimethoxybenzyl group to the initial molecular radical cation site on the oxygen of the carbonyl group to yield the distonic molecular radical cation. Direct heterolytic bond cleavage of this rearranged molecular radical cation would yield the unique *m/z* 136 fragment. For the underivatized NBOMe compounds, the *m/z* 136 ion can occur via migration of the methyl radical to the radical cation site on nitrogen followed by the analogous

fragmentation process. An equivalent fragment appears as the base peak in the EI mass spectra of the vicinal isomers 2,3- and 3,4-dimethoxybenzylamine.[9]

In the 2,3-dimethoxybenzyl 25X-NBOMe series (where X is 4-H, 4=Br or 4-I), the *m/z* 136 ion is the third most abundant after the substituted phenylethene radical cation (most abundant) and dimethoxybenzyl cation (second most abundant). For the 2,3-dimethoxy isomers of the 2- and 3-MMPEADMB series, the *m/z* 136 ion is less abundant than the TFA amide radical cation (*m/z* 263) and dimethoxybenzyl cation (*m/z* 151). However, in the mass spectra of the 2,3-dimethoxy isomer of the PEADMB series only the dimethoxybenzyl cation (*m/z* 151) is more abundant than the *m/z* 136 ion and the TFA amide radical cation (*m/z* 263) is formed in relatively low abundance.

Each of the methyl groups of the 2,3-dimethoxybenzyl portion of the molecule was individually labeled with ^{13}C in an effort to determine which of the methyl groups is eliminated during the formation of the *m/z* 136 fragment. The *m/z* 130 to *m/z* 140 mass regions of the EI-MS spectra for both ^{13}C-labeled compounds are shown in Figures 7A and 7B. These results indicate that the ^{13}C methyl group was neither completely conserved nor completely eliminated for either of the labeled analogues in this set of experiments. However, both spectra support a slight preference for the migration and elimination of the methyl from the 3-methoxy group of the 2,3-dimethoxybenzyl isomer. Figure 7A shows the *m/z* 136 and 137 region for the labeled analogue having the ^{13}C at the 2-methoxy group, and the ion at *m/z* 137 is the more intense of the two ions. The analogue having the ^{13}C at the 3-methoxy group yields a fragment ion pattern (Figure 7B) indicating a more intense *m/z* 136. Thus, both ^{13}C-labeled compounds consistently support a slight preference for the migration and elimination of the methyl group from the 3-methoxy substituent. The two ^{13}C-labeled compounds used to

obtain the spectra in Figures 7A and 7B were prepared via reductive amination of the appropriately labeled 2,3-dimethoxybenzaldehydes. The ^{13}C-labeled 2,3-dimethoxybenzaldehydes were obtained by ^{13}C-methyl iodide treatment of 2-hydroxy-3-methoxybenzaldehyde or 2-methoxy-3-hydroxybenzaldehyde under basic conditions.

Conclusions

The EI-MS spectra for the trifloroacetamides of the N-dimethoxybenzyl NBOMe analogues yield two unique radical cation fragments from the decomposition of the common distonic molecular radical cation. The systematic removal of the electron-donating groups from the 4-halogenated-2,5-dimethoxyphenethylamine portion of the N-dimethoxybenzyl NBOMe analogues to yield the 2,5-dimethoxyphenethylamine, 2-methoxyphenethylamine, 3-methoxyphenethylamine, and unsubstituted phenethylamine allowed an evaluation of these structural effects on the formation of the substituted phenylethene (*m/z* 164, 134, 104) and *m/z* 263 dimethoxybenzyltrifloro-acetamide radical cations. The decomposition of this hydrogen-rearranged distonic molecular radical cation species favors formation of the dimethoxybenzyltrifloroacetamide *(m/z* 263) species with the elimination of the neutral phenylethene molecule, and the addition of electron-donating groups on the aromatic ring of the phenethyl group appear to be necessary to allow formation of the substituted phenylethene radical cation. The unique *m/z* 136 benzylic radical cation formed exclusively in the 2,3-dimethoxybenzyl regioisomer was unaffected by the nature of the substituent groups in the phenethylamine aromatic ring.

Acknowledgements

This project was supported by Award No. 2016-DN-BX-K0175, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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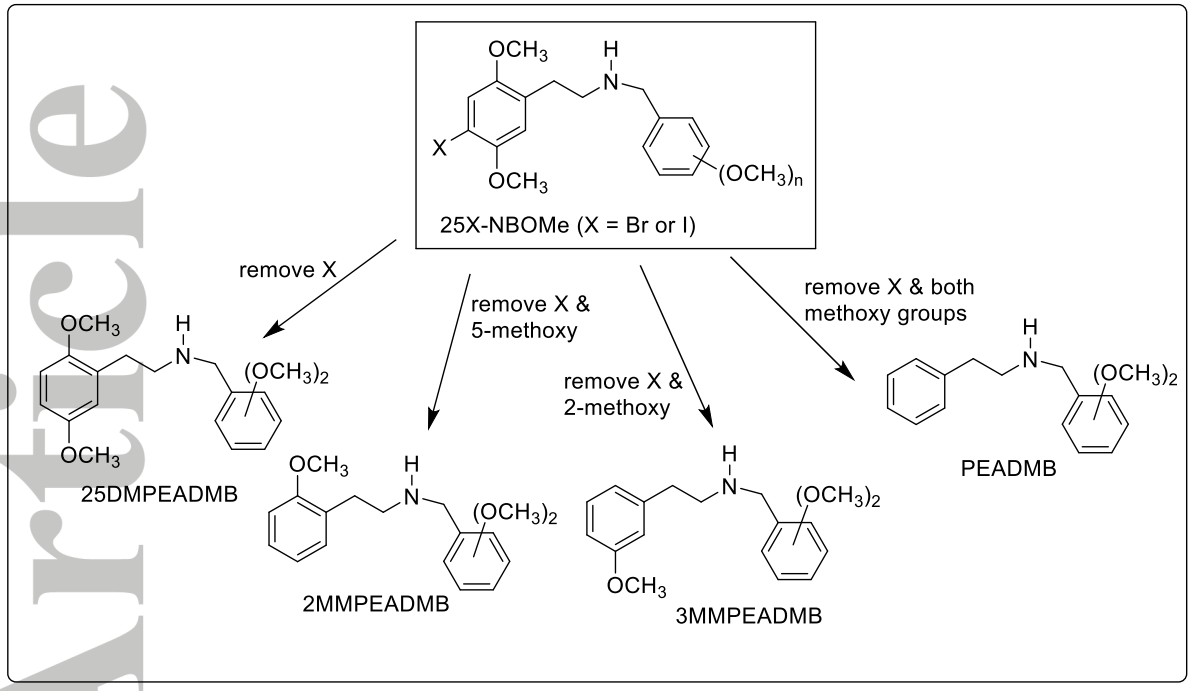

Figure 1. Substituted phenethylamines based on systematic removal of electron-donating groups.

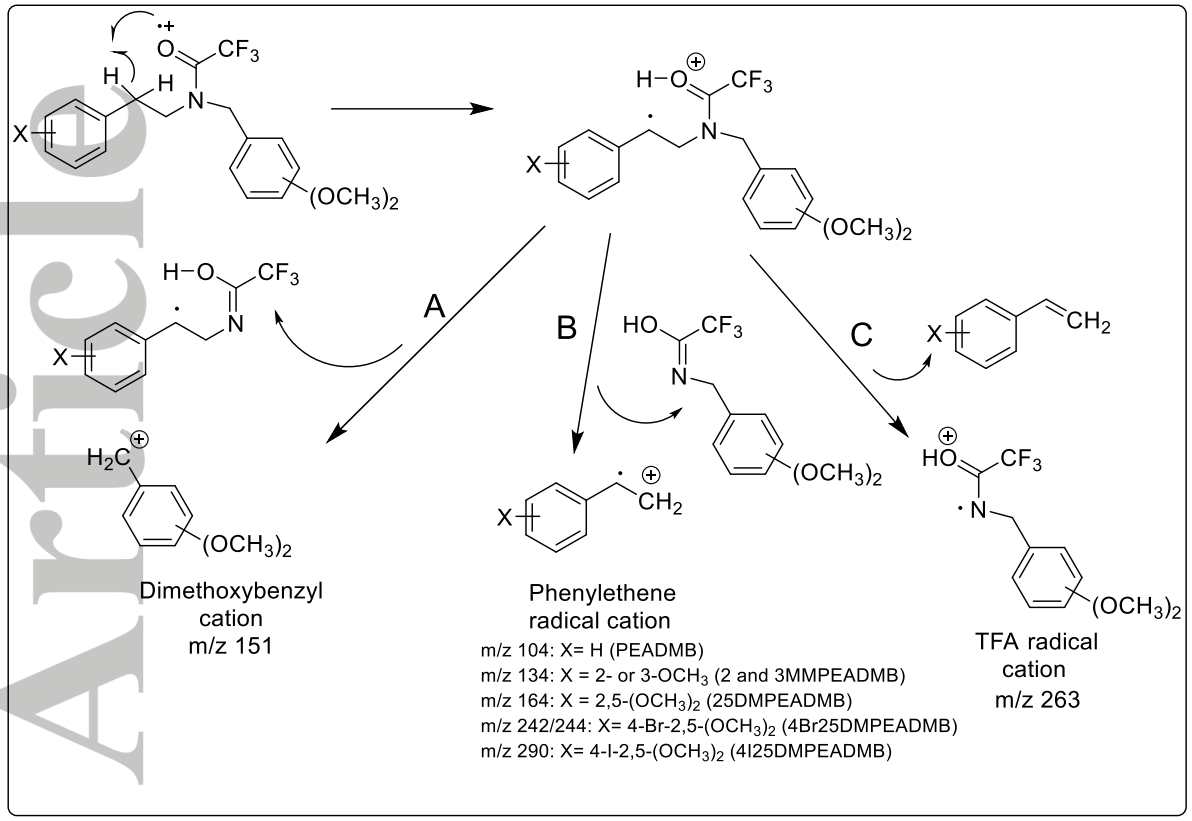

Figure 2. Major fragmentation pathways in the TFA-NBOMe analogues.

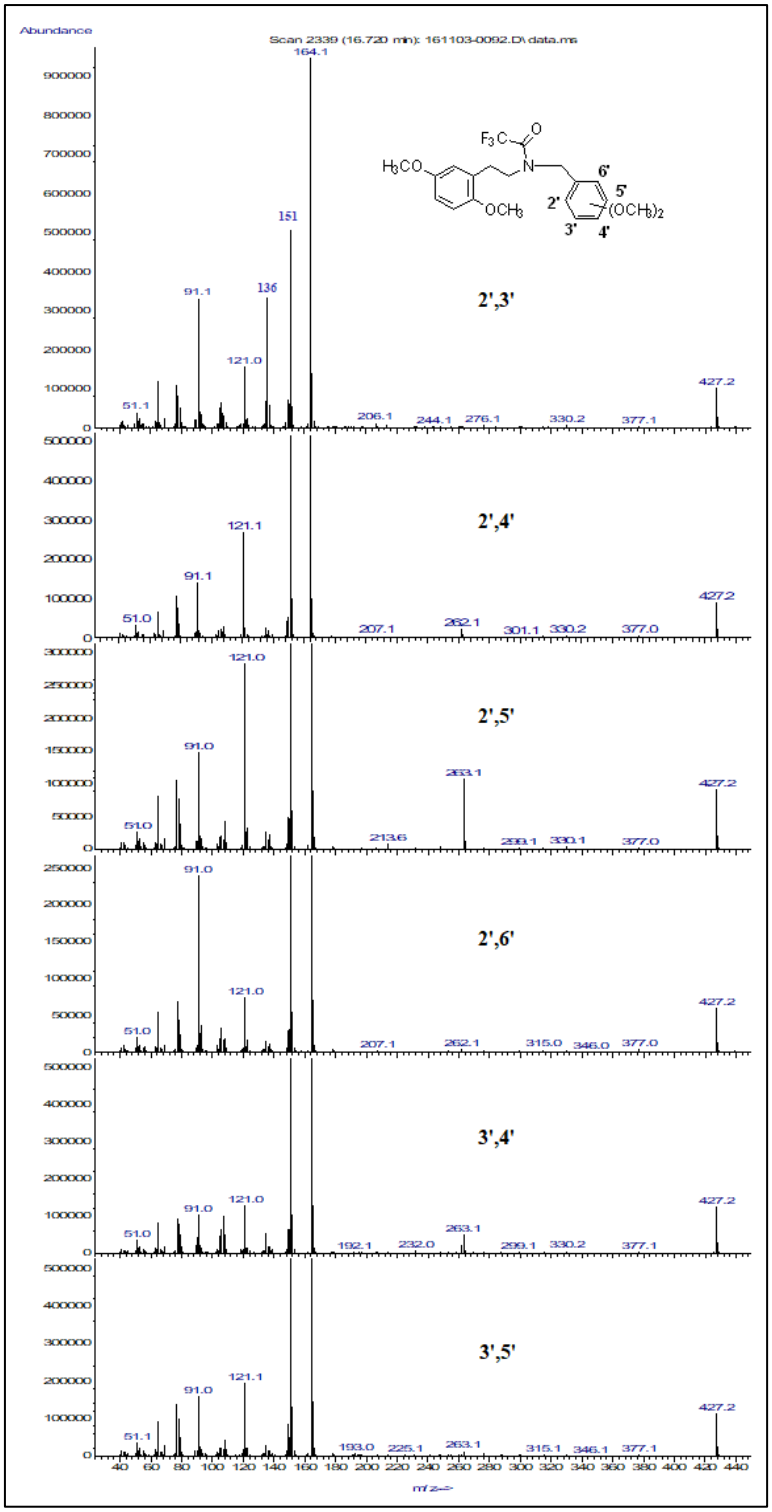

Figure 3. EI-MS of the 2,5-DMPEADMB regioisomers.

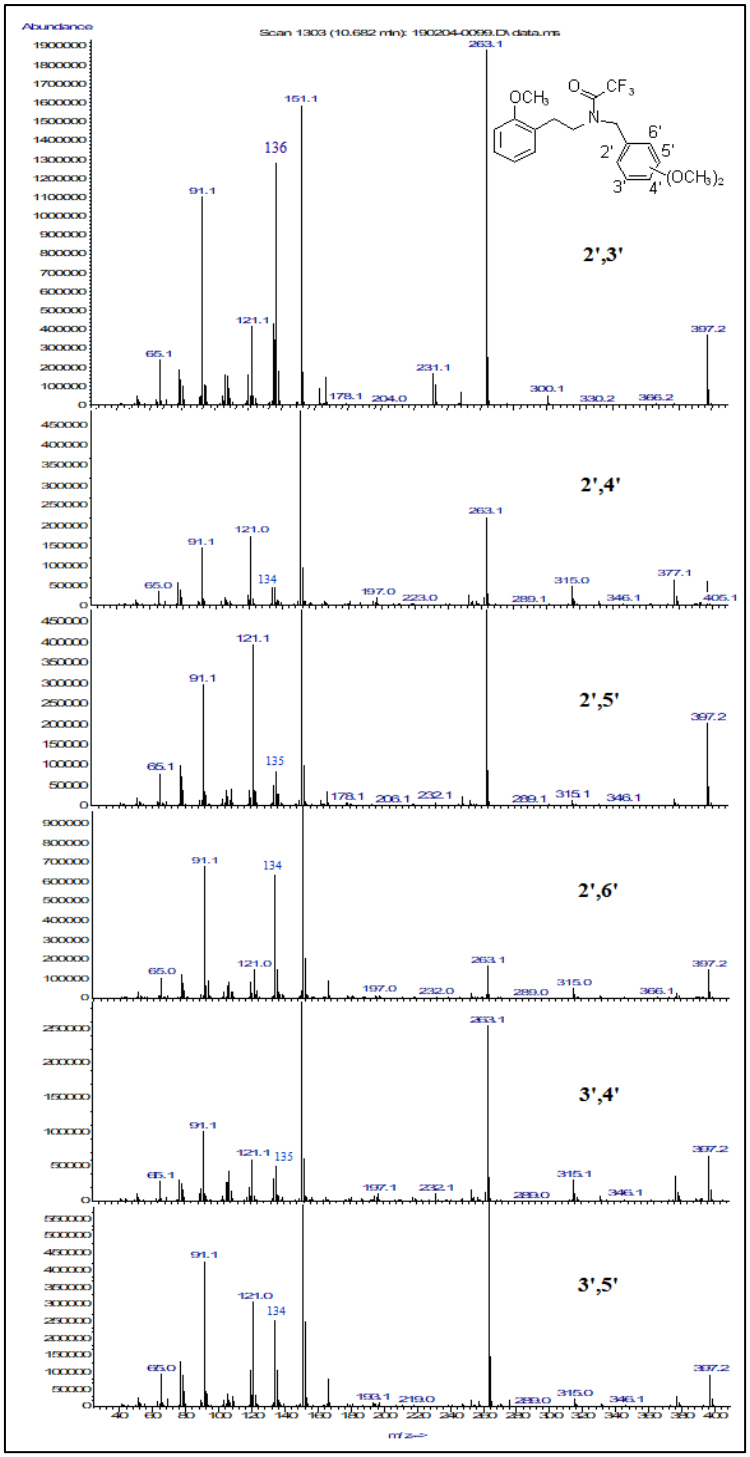

Figure 4 EI-MS of the 2MMPEADMB regioisomers.

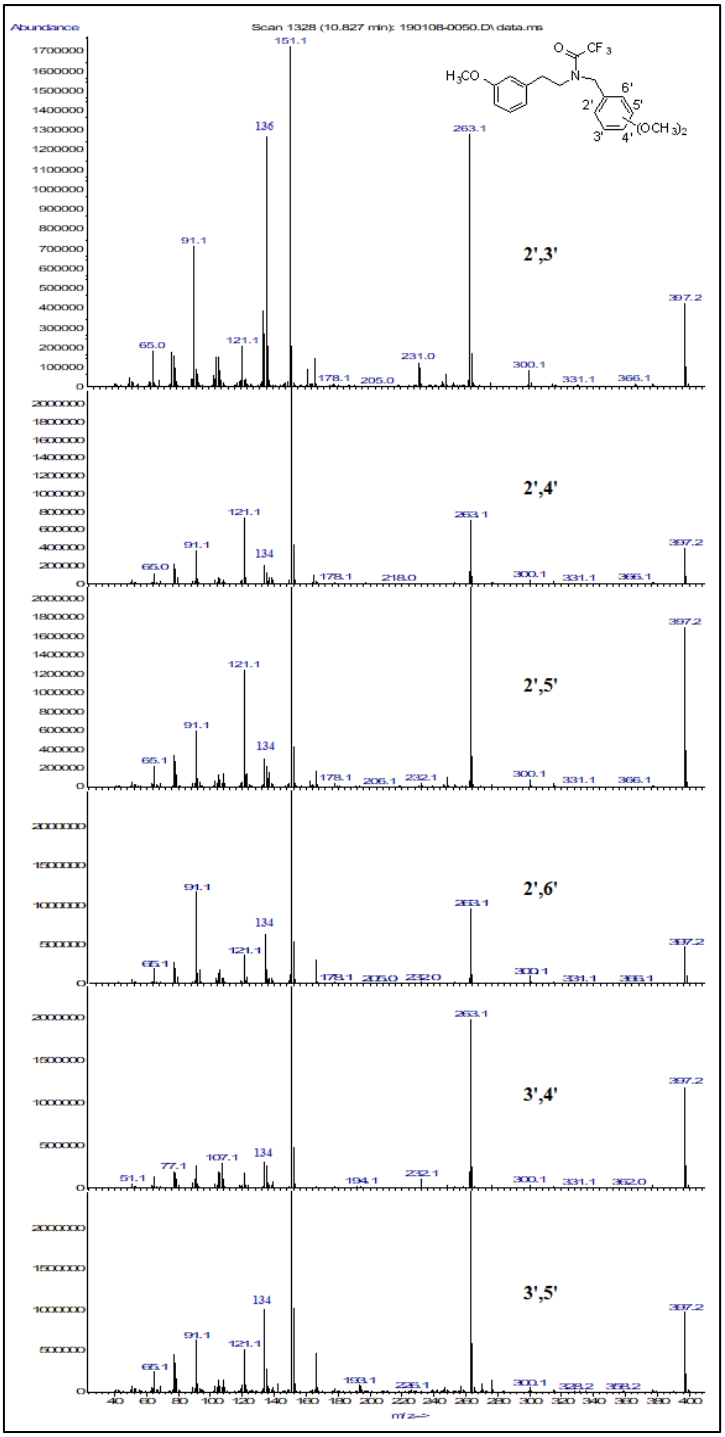

Figure 5 EI-MS of the 3MMPEADMB regioisomers.

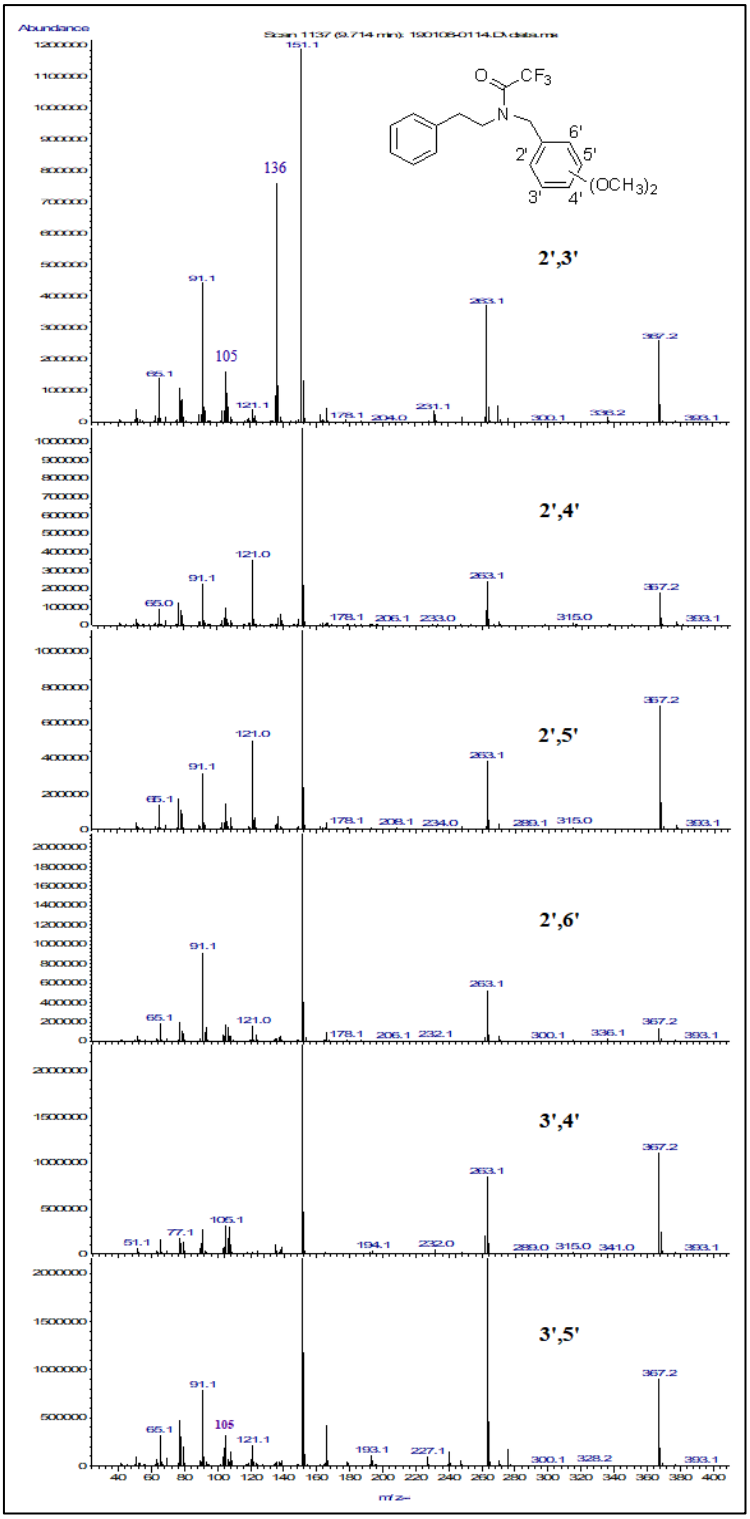

Figure 6 EI-MS of the PEADMB regioisomers.

A B

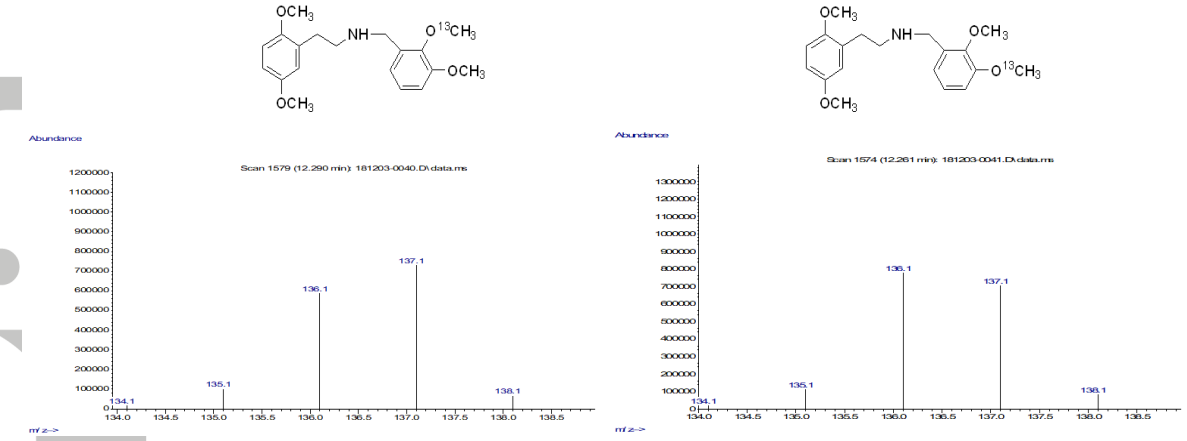

Figure 7. The m/z 136 and m/z 137 region of the EI-MS for N-(2-^{13}C-methoxy-3-methoxybenzyl)-2,5-dimethoxyphenylamine (7A) and N-(2-methoxy-3-^{13}C-methoxybenzyl)-2,5-dimethoxyphenylamines (7B).

Table 1 Relative abundance of fragment ions in the EI-MS of TFA-2,5-DMPEADMBs, TFA-2-MMPEADMBs, TFA-3-MMPEADMBs and TFA-PEADMBs.

2,5DMPEADMB Regioisomers	**Base Peak**	**2nd Most Abundant Ion**	**3rd Most Abundant Ion**	**4th Most Abundant Ion**	**5th Most Abundant Ion**
2,3-Isomer	164	151	136	91	121
2,4-Isomer	151	164	121	91	77
2,5-Isomer	164	151	121	91	263 = 77
2,6-Isomer	151	164	91	121	77
3,4-Isomer	151	164	121	91	77
3,5-Isomer	164	151	121	91	77
2MMPEADMB Regioisomers	**Base Peak**	**2nd Most Abundant Ion**	**3rd Most Abundant Ion**	**4th Most Abundant Ion**	**5th Most Abundant Ion**
2,3-Isomer	263	151	91	136	134
2,4-Isomer	151	263	121	91	134
2,5-Isomer	151	263	121	91	134
2,6-Isomer	151	91	134	263	121
3,4-Isomer	151	263	91	121	134
3,5-Isomer	263	151	91	121	134
3MMPEADMB Regioisomers	**Base Peak**	**2nd Most Abundant Ion**	**3rd Most Abundant Ion**	**4th Most Abundant Ion**	**5th Most Abundant Ion**
2,3-Isomer	263	151	136	91	134
2,4-Isomer	151	263	121	91	134
2,5-Isomer	151	263	121	91	134
2,6-Isomer	151	263	91	134	121
3,4-Isomer	151	263	134	91	121
3,5-Isomer	263	151	134	91	121
PEADMB Regioisomers	**Base Peak**	**2nd Most Abundant Ion**	**3rd Most Abundant Ion**	**4th Most Abundant Ion**	**5th Most Abundant Ion**
2,3-Isomer	151	136	91	263	105
2,4-Isomer	151	121	263	91	105
2,5-Isomer	151	121	263	91	105
2,6-Isomer	151	91	263	105	121
3,4-Isomer	151	263	104	91	121
3,5-Isomer	151	263	91	105	121