Final Summary Overview, NIJ award 2016-DN-BX-0175

Project Title: Forensic Chemistry of Substituted N-Benzylmethoxy (N-BOMe) Phenethylamines: Isomeric Designer Drugs Related to 25I-N-BOMe

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Purpose of Project:

The goal of this work was to establish an analytical framework for the identification of individual N-benzylmethoxy-phenethylamine (NBOMe) isomers to the exclusion of all other possible isomeric and homologous forms of these compounds. The NBOMe compounds represent another recent addition to the new/novel psychoactive substances (NPS) category of drugs of abuse. Development of the desired analytical specificity was accomplished by: 1) chemical synthesis of complete sets of regioisomeric reference compounds for selected NBOMe isomers; 2) generation of an analytical profile for each compound; 3) chromatographic studies to separate/resolve all regioisomeric substances having overlapping analytical profiles; and 4) design and validation of confirmation level analytical methods to identify each compound to the exclusion of other regioisomeric forms.

A variety of NPS drugs of the NBOMe class have appeared in clandestine samples in recent years based on designer substituents of the aromatic ring of the phenethylamine portion of the molecule as well as the aromatic ring of the benzyl group. There are numerous compounds from this category already available on the clandestine drug market and each of these compounds can exist in homologous and isomeric forms often sharing the same mass spectrum, the most common method of confirmation of drug identity in forensic drug analysis.

The project has produced a significant amount of fundamental forensic chemical data resolving numerous issues related to regioisomerism in the NBOMe synthetic drugs. The results of this project have provided information and data sets to advance the forensic chemistry knowledge base and understanding of the relationship between NBOMe drug structure and specific analytical properties of these compounds. Three peer reviewed scientific publications from this project are in print at this time (see Appendix 1) and we expect to submit at least two others in the near future.

Design and Methods:

The availability of compounds to establish and validate the structure-retention, structure-fragmentation, and other structure-property analytical relationships was the first step in this forensic drug chemistry research. Over the course of this project about 100 NBOMe and related molecules were synthesized and evaluated in these structure-property analytical studies. The development of valid methods for differentiation can only be accomplished after an analytical evaluation of all the regioisomers in a series. The synthesis of aromatic ring substituted phenylnitroethenes such as 2,5-dimethoxy-phenylnitroethene was accomplished using 2,5-dimethoxybenzaldehyde, nitromethane, and anhydrous ammonium acetate and the structure of the product was confirmed by GC-MS and NMR spectroscopy. The 2,5-dimethoxyphenylnitroethene was reduced via a suspension of $LiAlH_4$ in dry tetrahydrofuran. The excess $LiAlH_4$ reagent was neutralized and the 2,5-dimethoxyphenethylamine base isolated by extraction. The 4-bromo- series was prepared via direct bromination of 2,5-dimethoxyphenethylamine. For the 4-iodo- series, the amine functionality was protected as the trifluoroacetamide followed by iodination with iodine monochloride and subsequent hydrolysis to yield the desired 4-iodo-2,5-dimethoxy-phenethylamine. Halogenation at the 4-position was

confirmed by proton NMR (see Appendix 2). The substituted N-benzyl group was added via reductive amination ($NaBH_4$) and the base form of the products were isolated by extraction and each compound was converted to the HCl salt.

The analytical studies included the collection of spectral data on each of the individual compounds, including GC-MS and GC-IR. Additional synthetic studies to label individual portions of the candidate molecules was necessary in order to fully understand and describe the mass spectral fragmentation chemistry. These labeled compounds (isotopic and homologous labels) were prepared as needed to complete this study. Derivatization methods via acylation of the secondary amine nitrogen were developed for additional structural characterization via GC-MS. The analytical methods focused on those techniques in routine use in forensic drug chemistry laboratories: GC, GC-MS, IR, GC-IR, and related techniques. Some exact mass GC-TOF-MS studies were used in specific applications for confirmation of the elemental composition of fragment ions. The use of exact mass GC-TOF-MS measurements is significant since the fragmentation process is the same for standard EI-MS and GC-TOF-MS.

Data Analysis:

The analytical data generated in this project were based primarily on gas chromatography with mass spectral and vapor phase infrared detection. The electron ionization (EI) mass spectral fragmentation pathways and products were characterized and confirmed using analogue, homolog, acylation, and stable isotope labeling techniques. Product ion MS/MS studies provided information on the source of a number of fragments while time of flight EI-MS gave accurate mass and elemental composition of fragment ions. The vapor phase infrared spectra obtained directly as the compounds elute from the GC column yielded data for the identification of any one regioisomer to the exclusion of other substances of mass spectral equivalence. The IR

spectra were derived from the compounds in the free base form at temperatures preventing intermolecular interactions such as polymorphism or other related issues of salt form and/or relative crystallinity. This lack of interferences from molecular variations has allowed the use of model compounds for direct spectral correlation and interpretation. The resolution and separation of the various regioisomeric forms of the compounds in this project were very successful on a number of gas chromatographic stationary phases.

Project Findings:

The project has produced a significant amount of fundamental forensic chemical data for NBOMe-type NPS molecules and their regioisomeric equivalents. The specific details of numerous individual project goals are in the publications listed in Appendix 1.

The goal of this work was to develop an analytical framework for the identification of individual substituted N-benzyl-phenethylamines to the exclusion of all other possible isomeric and homologous forms of these compounds. This analytical specificity was accomplished by 1) the chemical synthesis of a set of reference compounds for selected substituted N-benzyl-phenethylamines, 2) generation of an analytical profile for each compound, 3) chromatographic studies to separate/resolve regioisomeric N-benzyl-phenethylamines having overlapping analytical profiles, and 4) design and validation of confirmation level analytical methods to identify individual compounds to the exclusion of other regioisomeric forms. The information in Figure 1 shows that these compounds can be modified in five general structural regions: I) aromatic ring substitutents on the phenethyl group; II) alkyl substitution of the phenethyl chain; and III) alkyl substitution of the nitrogen; IV) aromatic ring substitution on the benzyl group; and V) halogenation of the phenethyl-aromatic ring. A wide variety of these compounds based on designer substituents already seen and developed in other series of drugs (such as the cathinones,

amphetamines and synthetic cannabinoids) are possible in these substituted N-benzyl-phenethylamines from readily available synthetic precursor substances.

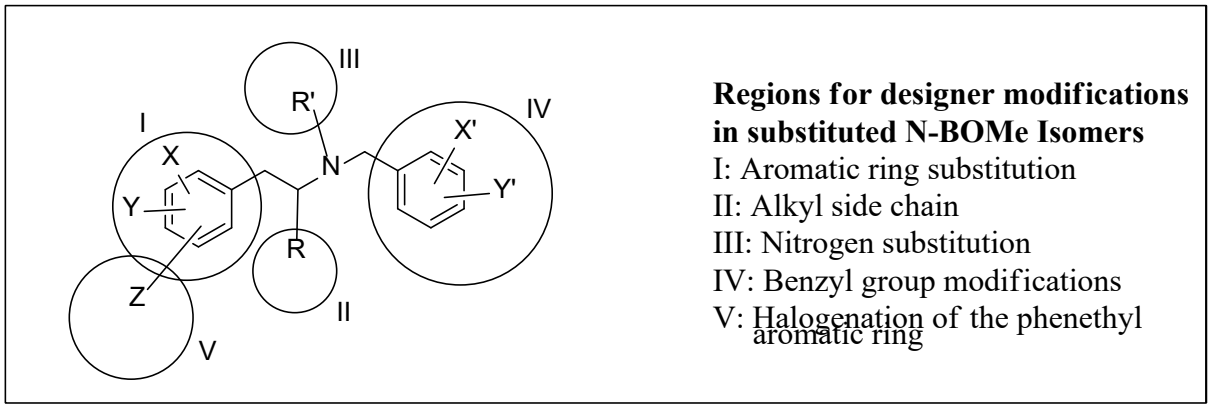


Figure 1. General regions of modification in substituted N-benzyl-phenethylamines.

The results of this project provide a scientific framework for understanding the spectroscopic properties (EI-MS and vapor phase IR) and chromatographic retention details for selected subsets of these N-BOMe compounds. These studies have provided synthetic methods and analytical reference spectra for rapid production of drug reference standards. Furthermore, the proper use and understanding of this comprehensive data set will produce a more scientifically prepared forensic expert to interact at the interface of the legal system and the science of forensic drug chemistry in the area of NBOMe drugs.

The compounds evaluated in this project represent variations in the substituents and pattern of the two aromatic rings of the basic N-benzyl-phenethylamine molecular skeleton. The project focuses on comparing the analytical properties resulting from the sequential increases in molecular complexity, as substituent groups are added, converting the basic N-benzylphen-ethylamine molecular skeleton to the various N-BOMe drugs, Figure 2.

Selected examples representing the addition of alkyl groups to the methyl-ethylamine connecting linker/bridge between the two aromatic rings of the N-BOMe skeleton were also synthesized and evaluated in this project.

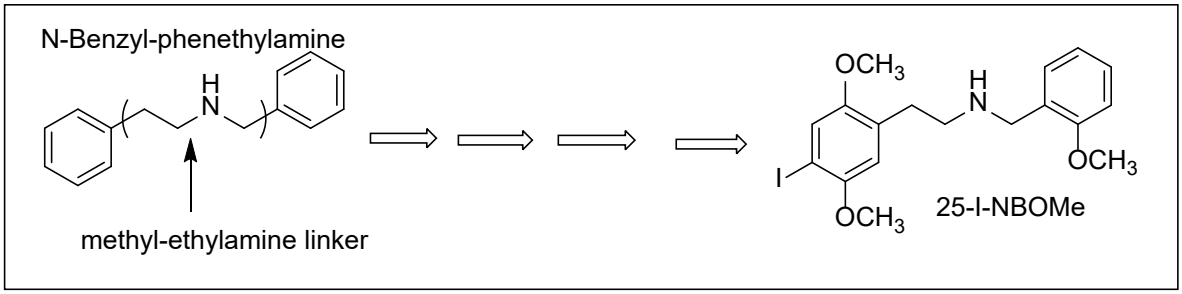


Figure 2. General modification scheme for NBOMe structure-property studies.

The initial phase of this work evaluated the common NBOMe drugs of abuse, N-(2-methoxybenzyl)-4-iodo-2,5-dimethoxyphenethylamine (25I-NBOMe) and N-(2-methoxy-benzyl)-4-bromo-2,5-dimethoxyphenethylamine (25B-NBOMe) and their regioisomeric equivalents, the 3- and 4-methoxybenzyl isomers. The precursor compounds for the synthesis of these regioisomers are commercially available from several sources. These isomers can be differentiated from their corresponding 3- and 4-methoxybenzyl isomers by gas chromatography with vapor phase infrared spectroscopy. The mass spectra for these regioisomeric compounds are essentially identical providing only the identity of the halogen, but no specific ions to identify the methoxy group position on the benzyl aromatic ring. However, relative intensity differences for some ions did provide some information for regioisomer differentiation.

The vapor phase IR absorption bands from single ring model compounds 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-iodophenethylamine (2C-I), and all three regioisomeric methoxy substituted benzylamines were used to assign each vapor phase absorption band to either the halogenated 2,5 dimethoxybenzene ring or the methoxybenzyl-amine ring. A pair of aromatic ring stretching bands reflect the position of methoxy group substitution on the benzyl aromatic ring. The 1608 cm^{-1} band is specific for the two regioisomeric 4-methoxy-benzylamine isomers and this band occurs at 1593 cm^{-1} for the 2-methoxy isomer in both the 25I and 25B series and shifts to1597/1598 cm^{-1} for 3-methoxybenzyl 25I and 25B. Asymmetric aryl-O stretching frequencies shift with a change in methoxy

substitution around the benzyl aromatic ring and occur at 1237/1238cm^{-1} for *ortho*, 1261 cm^{-1} for *meta*, and 1246 cm^{-1} for the *para* isomers. The overall shape and relative intensities of numerous bands in the vapor phase spectra provide clear identification for the individual methoxybenzyl isomers for the 25I-NBOMe and 25B-NBOMe series (see Appendices 3A and 3B).

The EI-MS and GC separation for the monomethoxybenzyl-25B-NBOMe series are shown in Appendices 4 and 5. The 25I NBOMe series show the equivalent fragmentation pattern and order of chromatographic elution. The order of elution on all GC phases was 2-methoxybenzyl eluting first followed by 3-methoxy and 4-methoxy eluting last. The EI-mass spectra clearly show the major fragmentation is controlled by the amine nitrogen with loss of the radical species containing the halogen substituent. Evidence for this fragmentation scheme was obtained by the synthesis and EI-MS evaluation of isomers containing the aromatic substituents on the opposite aromatic rings (see Appendix 6). A direct EI-MS comparison of the traditional 25B-NBOMe and its inverse isomer is shown in Appendix 7.

The six N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethylamine regioisomers (see Appendix 8) and the six N-(dimethoxybenzyl)-4-iodo-2,5-dimethoxyphenethylamine regioisomers are potential designer compounds related to the common NBOMe drugs representing the incorporation of one additional methoxy-group into the common NBOMe molecular framework. The general discussion here centers on the 25B-NBOMe series as an example; however, the equivalent data for the 25I-NBOMe series was generated and evaluated in this study. In addition to the dimethoxybenzyl regioisomers data for the 2,3- and 3,4-methylene-dioxybenzyl series for both 25B and 25I were collected and evaluated.

An example GC separation of the six N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethylamine regioisomers is provided in Appendix 9. The separation was achieved

using a midpolarity Crossbond® silarylene phase containing a 50% phenyl and 50% dimethyl polysiloxane polymer (Rxi®-17Sil MS). Under temperature programming conditions, the dimethoxybenzyl regioisomers eluted over a 2.0-minute window (approx.) in the 37-minute range and this chromatographic system allowed for baseline resolution for all six compounds. The isomers with a 2'-methoxy group (2',3'-, 2',4'-, 2',5'- and 2',6'-dimethoxy) eluted before the two regioisomers that did not contain a 2'-methoxy group. Furthermore, the two derivatives with the greatest degree of steric crowding relative to the benzyl side chain (2,3- and 2,6-dimethoxy) eluted prior to all other members of the series. The 3,5-isomer having the maximum distance between aromatic ring substituents eluted last.

The electron ionization mass spectra (EI-MS) for the compounds in this study are quite similar yielding nearly identical fragment ions as shown in Appendix 10. The most abundant ions in the EI-MS spectra for this series of dimethoxybenzyl isomers occurs at m/z 121, 151, and 180. The predominant ion at m/z 151 is the dimethoxybenzyl cation and the ion at m/z 180 is the iminium cation formed by the dissociation of bond between α- and β-carbon atoms of the ethylene linker group eliminating the 4-bromo-2,5-dimethoxybenzyl radical. Finally, the ion at m/z 121 likely forms from loss of CH_2O from the dimethoxybenzylcation. The molecular weight for each of the regioisomeric compounds in this study was confirmed by the $(M+H)^+$ ion at 410/412 using chemical ionization (CI) techniques since no molecular ion was observed in the EI-MS.

Only one isomer in this dimethoxybenzyl series (the 2',3'-dimethoxybenzyl regioisomer) gave a unique fragment ion of significant abundance in the EI-MS and this ion occurred at m/z 136. This characteristic ion is absent from the spectrum of the other five dimethoxybenzyl

regioisomers and corresponds to the loss of a methyl group (CH_3) from the m/z 151 dimethoxybenzyl cation.

The EI mass spectra for the TFA-derivatives of these six regioisomeric N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethylamines are shown in Appendix 11. Each of the TFA derivatives gave a molecular ion of significant abundance at m/z 505/507, unlike the parent compounds. Two major fragment ions common in the spectra of all six regioisomeric derivatives occur at m/z 242/244 and m/z 151. The m/z 242/244 ion contains bromine and is likely the phenylethyl radical cation formed by hydrogen migration followed by the dissociation of benzylic N-C bond from the phenylethyl side of the compound (see Appendix 12 and 13). The m/z 151 ion forms by the cleavage of the N-C bond yielding the methoxybenzyl cation as observed with the parent underivatized compounds. The competition between the formation of the m/z 263 and the m/z 242/244 radical cations formed the basis for the EI-MS differentiation of some of the TFA regioisomers in this series. This is the subject of the manuscript published in "Rapid Communications in Mass Spectrometry."

The vapor phase infrared spectra for the six *N*-(dimethoxybenzyl)- 4-bromo-2,5-dimethoxyphenethylamines are shown in Appendix 14. These spectra were obtained at the temperature of the GC transfer line directly as the compounds elute from the capillary column. These conditions prevent interference from water vapor and do not allow any salt forms of organic compounds. Thus, the GC-IR spectra in Appendix 14 are for the free base form of these dimethoxybenzyl 25I and 25B analogues. The IR spectra for each of the six isomers provide information for differentiation and specific identification.

Implications for criminal justice policy and practice:

This project has added a significant amount of fundamental scientific information to the forensic analytical chemistry knowledge base. The information generated in this project primarily used common analytical tools available to the practicing forensic scientist. A proactive investigation of the forensic analytical chemistry of these potential designer substances was the objective of this project. The resulting analytical data and methods represent important advancements in forensic drug chemistry and provide the forensic chemistry community with significant fundamental chemical information for the substituted NBOMe class of compounds. This fundamental information includes gas chromatographic separations and elution order, mass spectra with confirmation of fragmentation mechanisms/structure, and infrared spectra for mass equivalent regioisomers. The synthesis and analytical evaluation of complete sets of regioisomeric NBOMe equivalents has allowed us to describe the chemical structure vs analytical properties for the current and likely future designer substances of this category. The relentless development of new designer substances of synthetic origin creates challenges in forensic drug identification due to the commercial availability of a variety of mass equivalent precursor substances. Regioisomeric forms of synthetic substances of equivalent elemental composition and yielding regioisomeric fragment ions of equal elemental composition present unique challenges in forensic drug identification using mass-based analytical methods (mass spectrometry). The results of this project have made available information and data sets to improve the forensic chemistry knowledge base and produce a scientifically skilled forensic expert available to interact at the interface of the legal system and the science of forensic drug chemistry of synthetic NPS drugs of the NBOMe class.

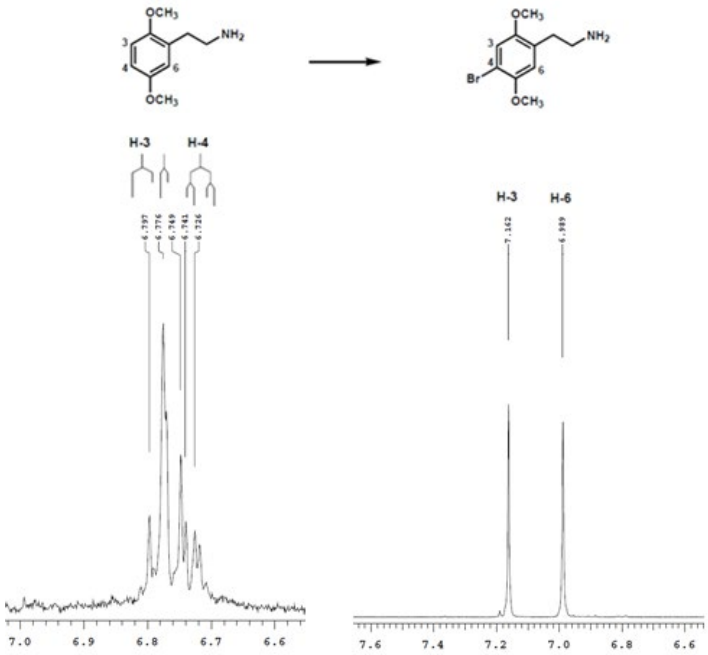
Appendix 1.
Bibliography of Project Publications:

1-Ahmad J. Almalki, C. Randall Clark and Jack DeRuiter, "GC-MS Analysis of Regioisomeric Substituted N-Benzyl-4-Bromo-2,5-Dimethoxyphenethylamines," Forensic Chemistry, 14, 100164 (2019).

2-Ahmad J. Almalki, Lewis Smith, C. Randall Clark and Jack DeRuiter, "Vapor phase GC-IR Identification of Regioisomeric N-Methoxybenzyl-4-Substituted-2,5-Dimethoxyphenethylamines (NBOMe)," Forensic Chemistry, 16, 100181 (2019).

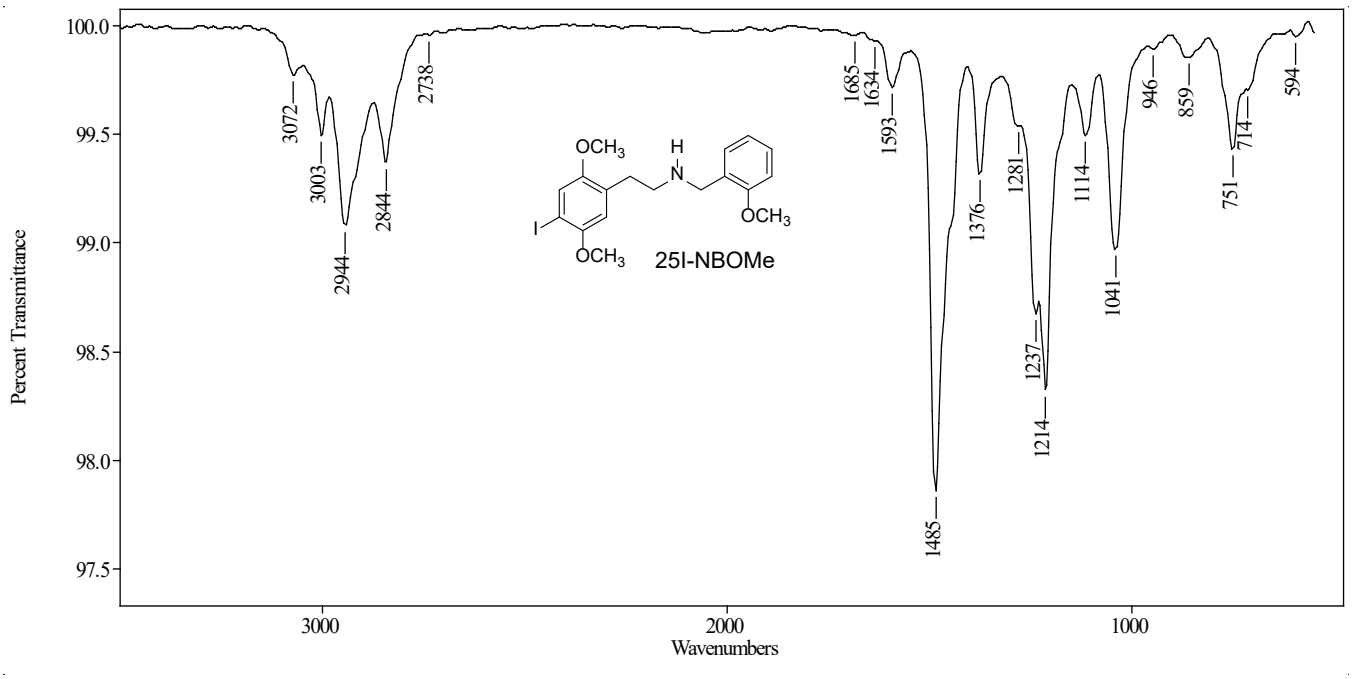
3-Ahmad J. Almalki, C. Randall Clark and Jack DeRuiter, "Structure Fragmentation Studies for Ring Substituted N-Trifluoroacetyl-N-Benzylphenethylamines Related to the NBOMe Drugs," Rapid Communications in Mass Spectrometry, accepted for publication, (2019).

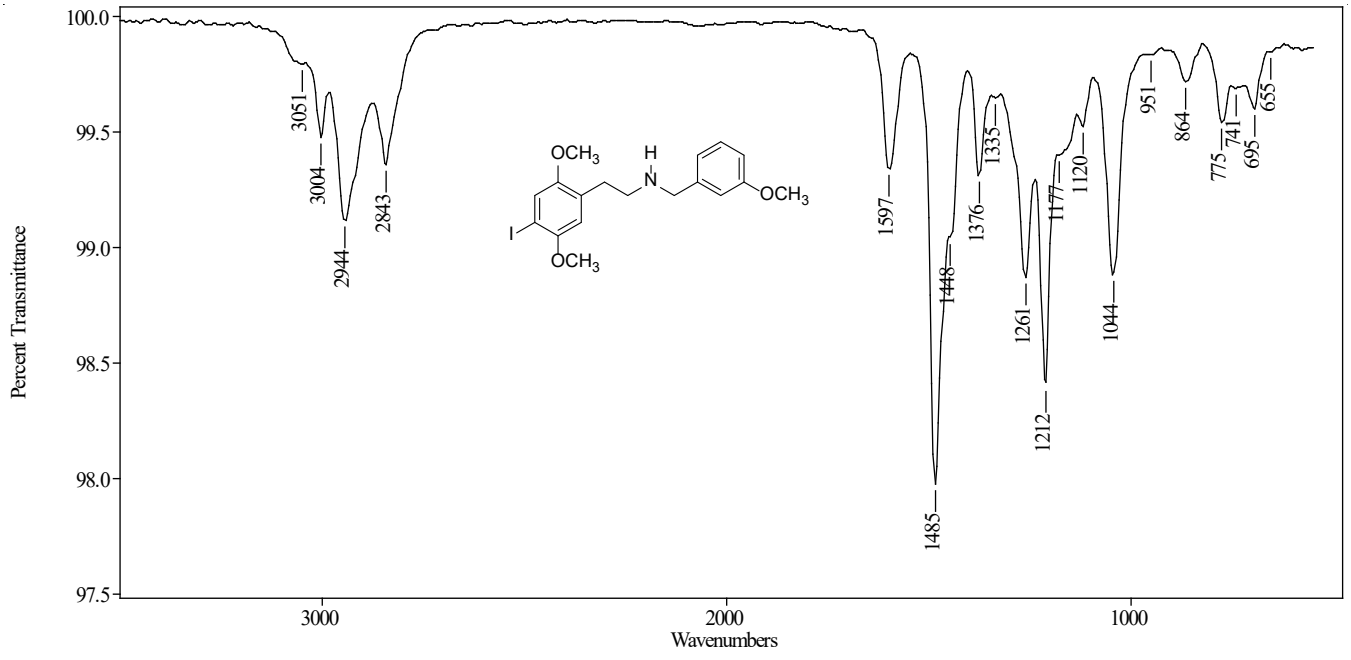
Appendix 2.
Proton NMR of the aromatic region for 2,5-dimethoxyphenethylamine and 4-bromo-2,5-dimethoxyphenethylamine.

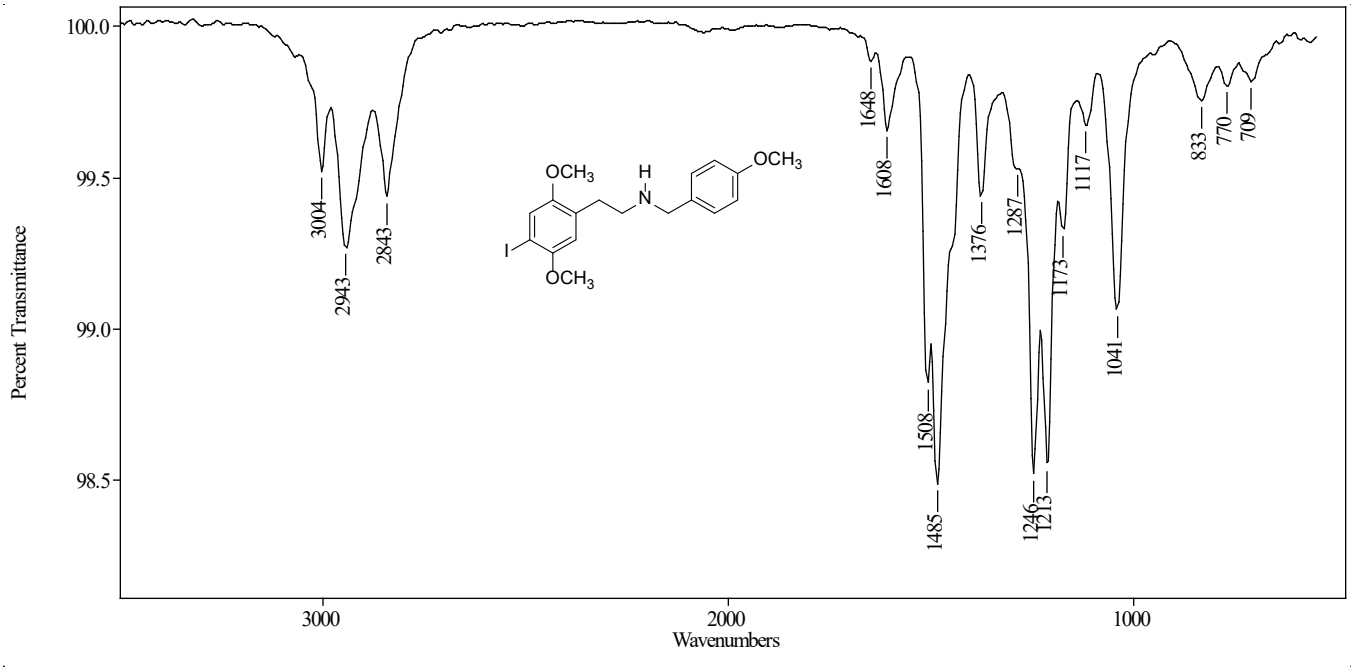


Appendix 2: Proton NMR of the aromatic region for 2,5-dimethoxyphenethylamine and 4-bromo-2,5-dimethoxyphenethylamine.

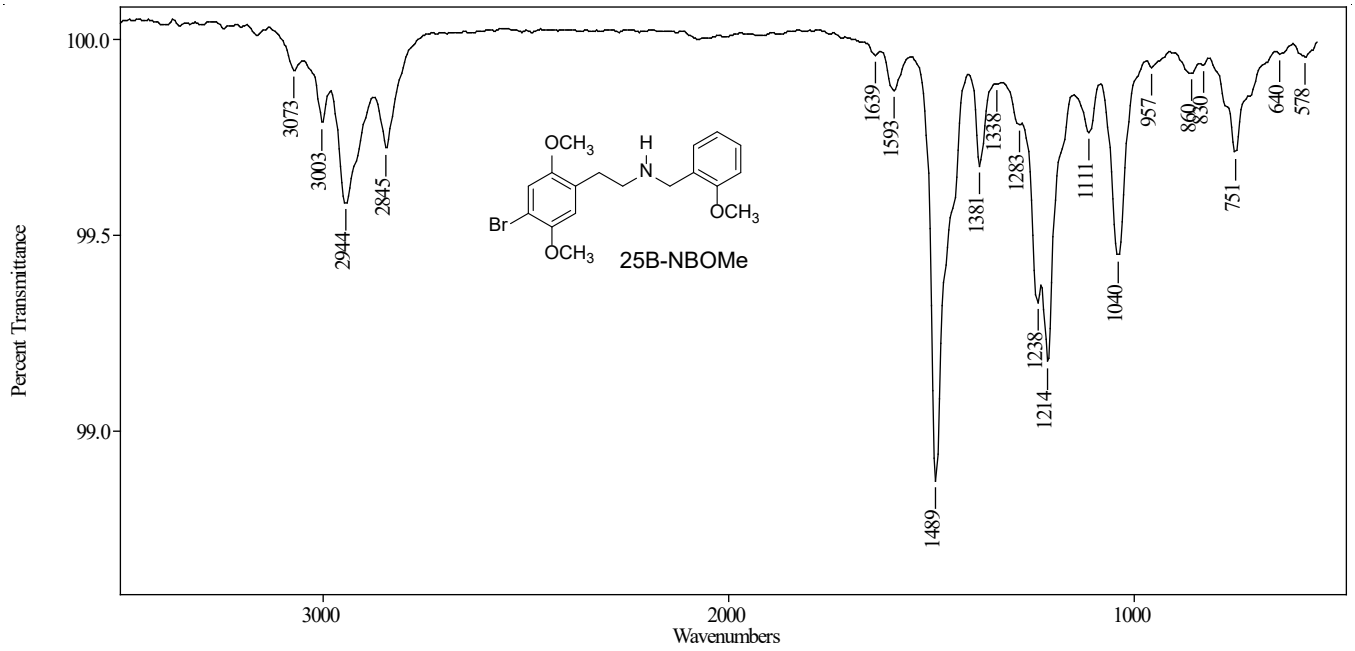
Appendix 3. Vapor phase IR of the NBOMe regioisomers.

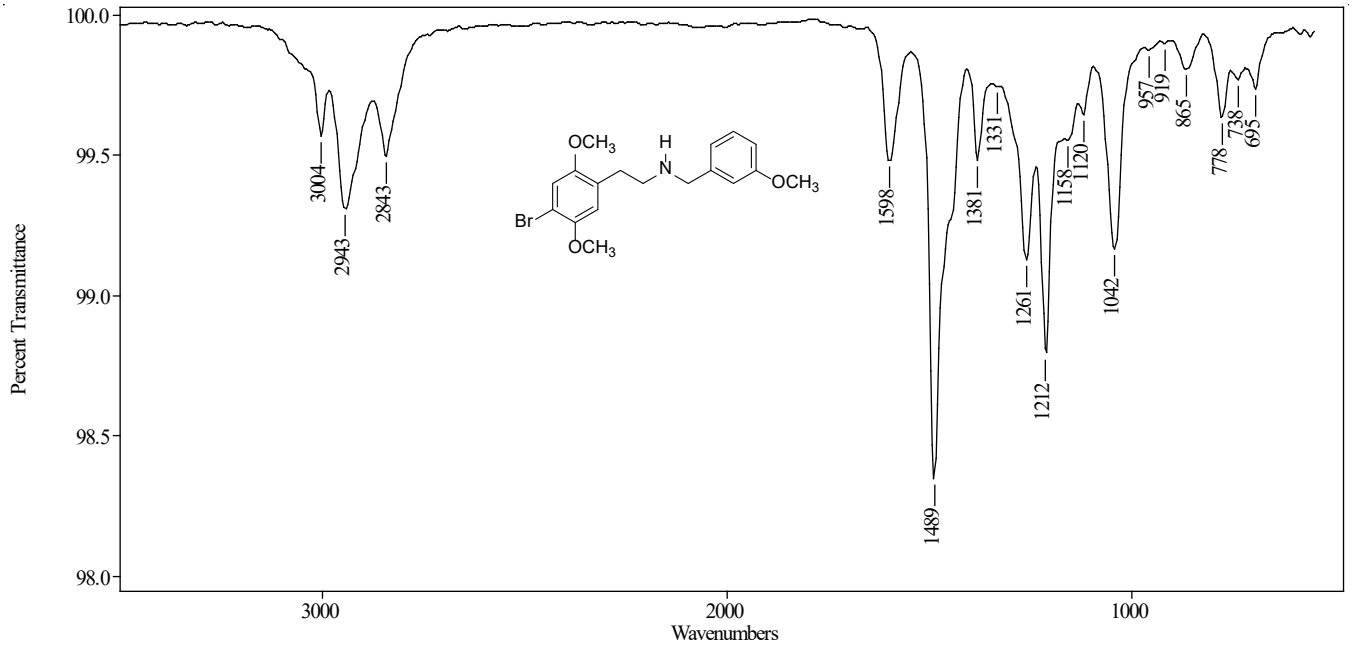


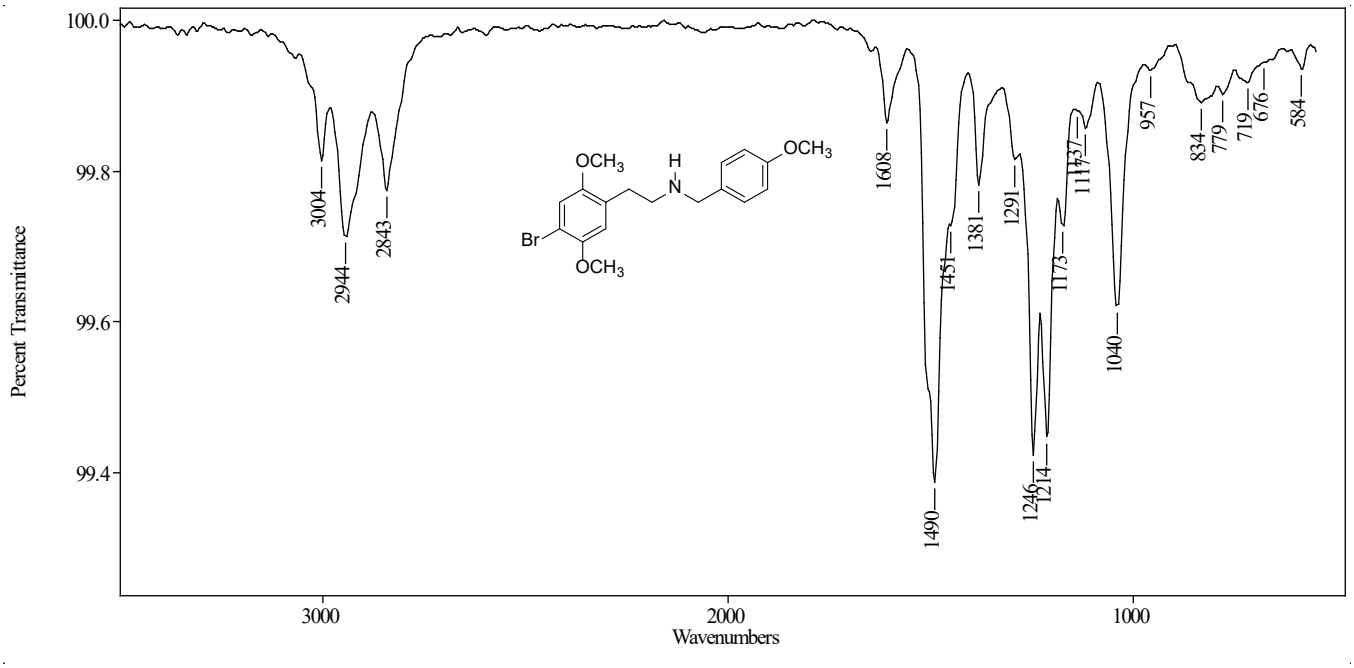




Appendix 3A. Vapor phase IR of the *N*-(monomethoxy)benzyl-4-iodo-2,5-dimethoxyphenethylamines.

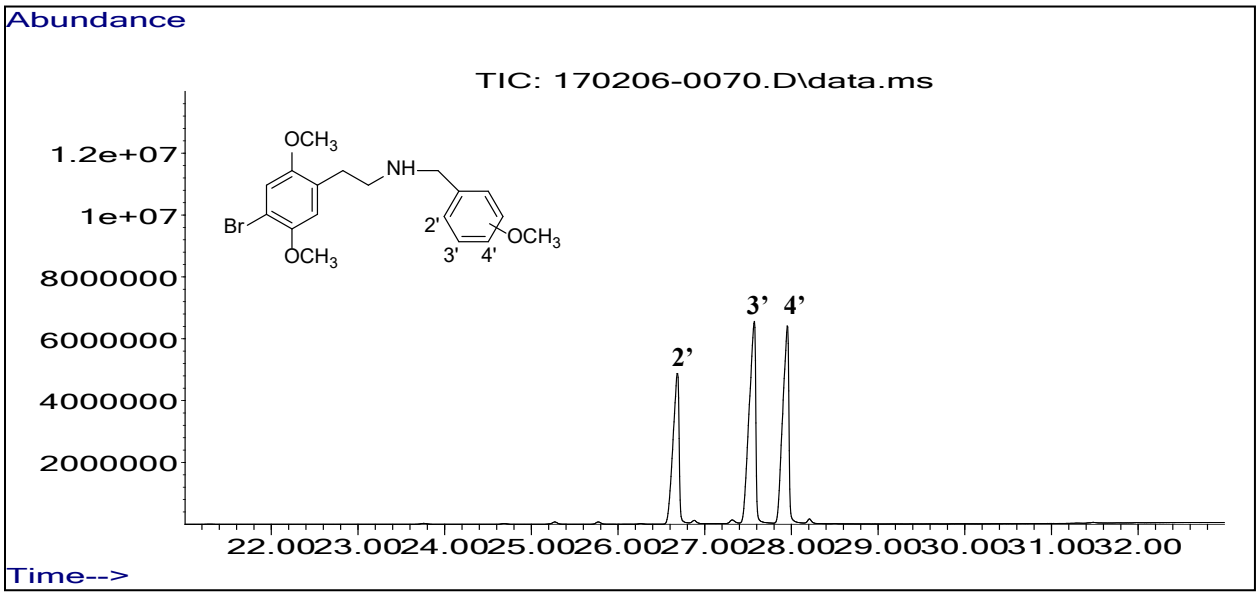






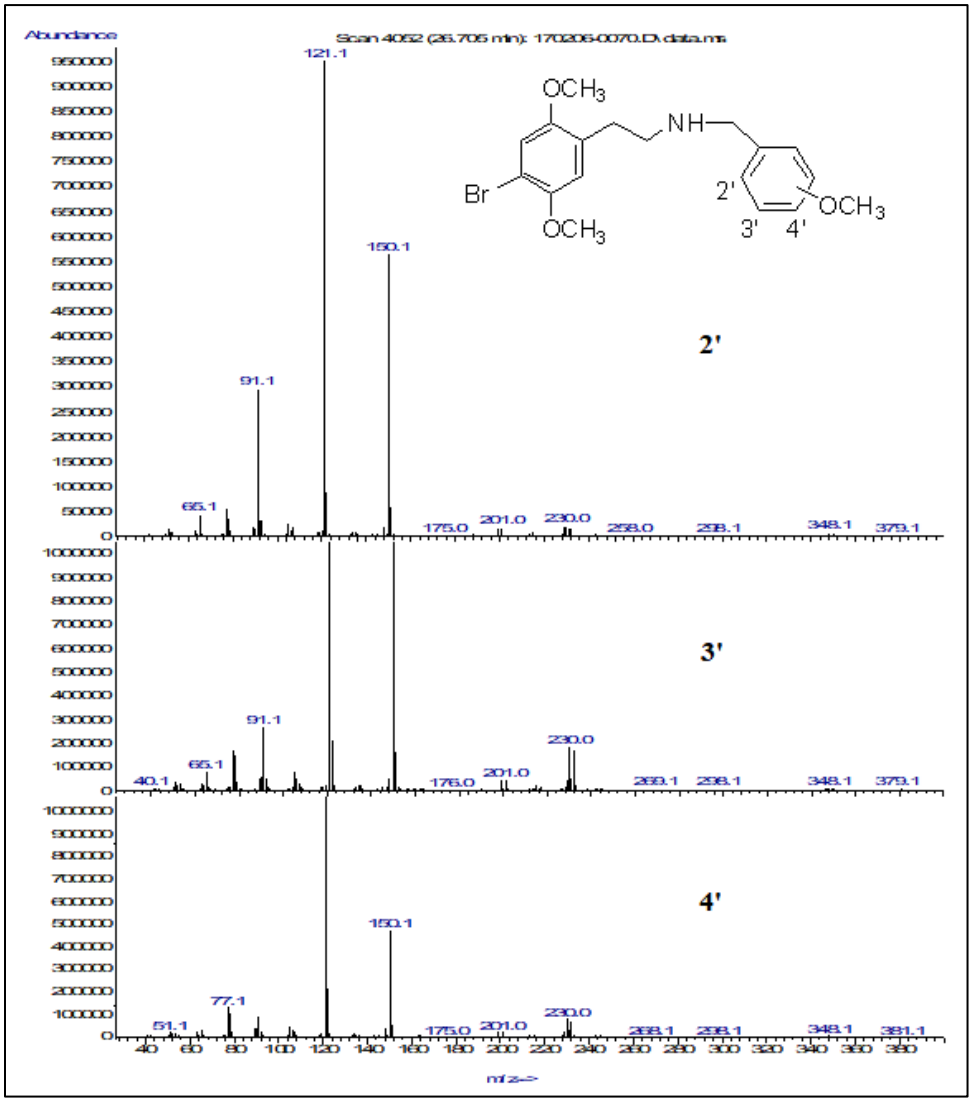
Appendix 3B. Vapor phase IR of the *N*-(monomethoxy)benzyl-4-bromo-2,5-dimethoxyphenethylamines.

Appendix 4. Example GC separation



Appendix 4. GC separation of the *N*-(monomethoxy)benzyl-4-bromo-2,5 dimethoxyphenethylamines.

Appendix 5. EI-MS of monomethoxybenzyl 2,5-B-NBOMe isomers.



Appendix 5. Mass Spectra of the *N*-(monomethoxy)benzyl-4-bromo-2,5-dimethoxyphenethylamines.

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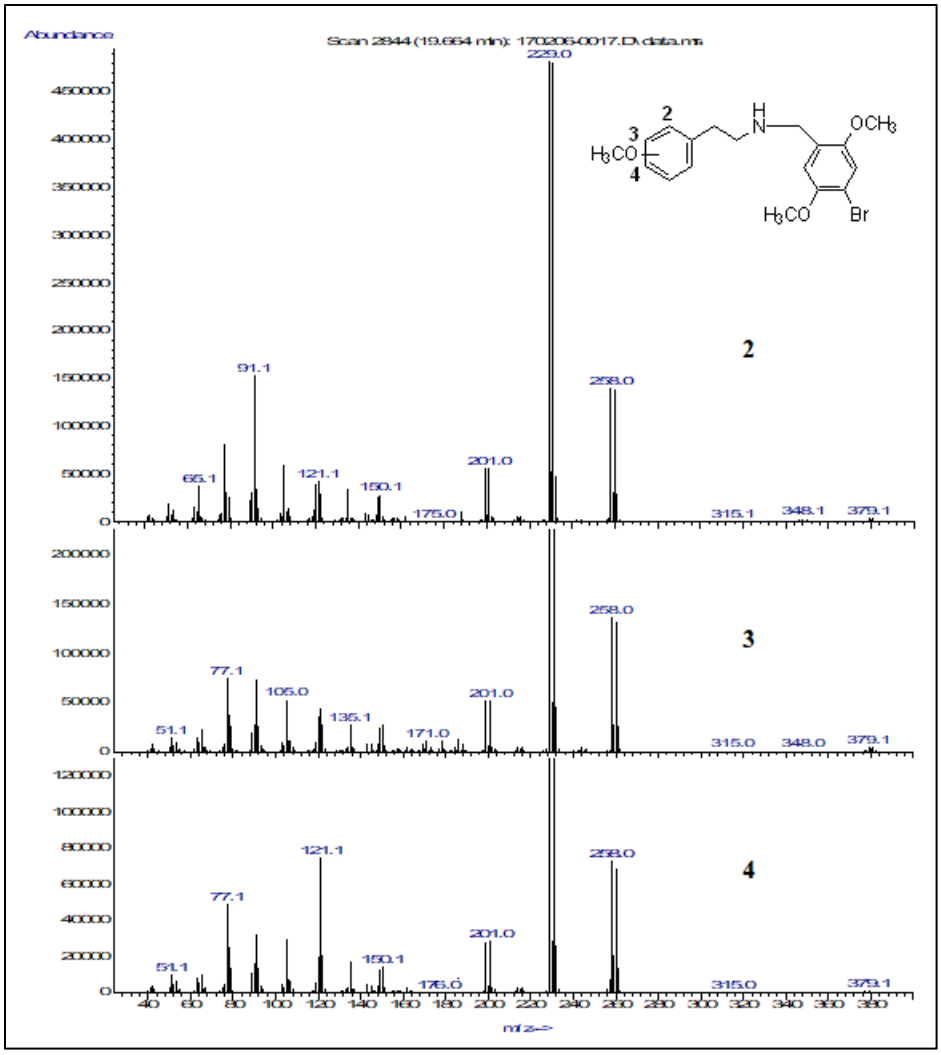
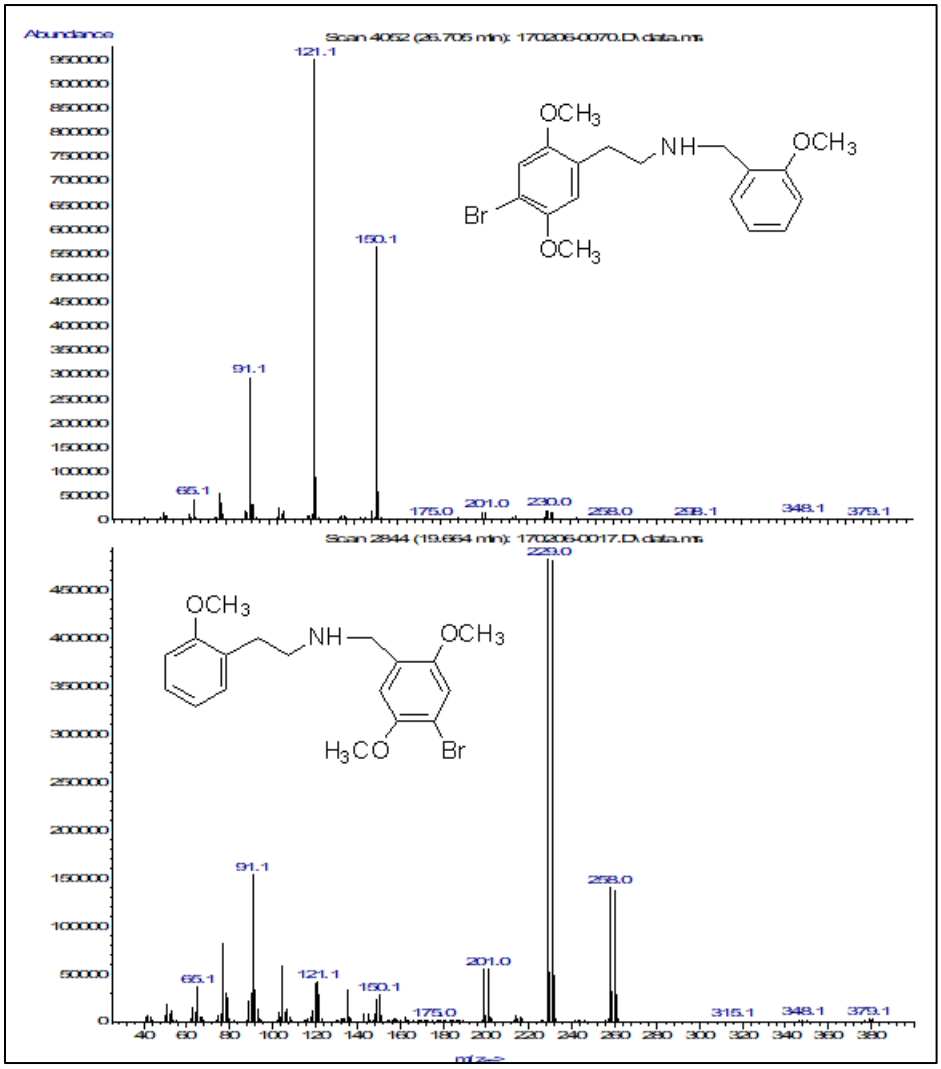
Appendix 6. EI-MS of the inverse NBOMe isomers.



Appendix 6. Mass Spectra of the *N*-(4-bromo-2,5-dimethoxybenzyl)methoxyphenethylamines.

Appendix 7. Direct comparison of 25B-NBOMe and the inverse isomer



Appendix 7. EI-MS of 25BNBOMe and the inverse isomer.

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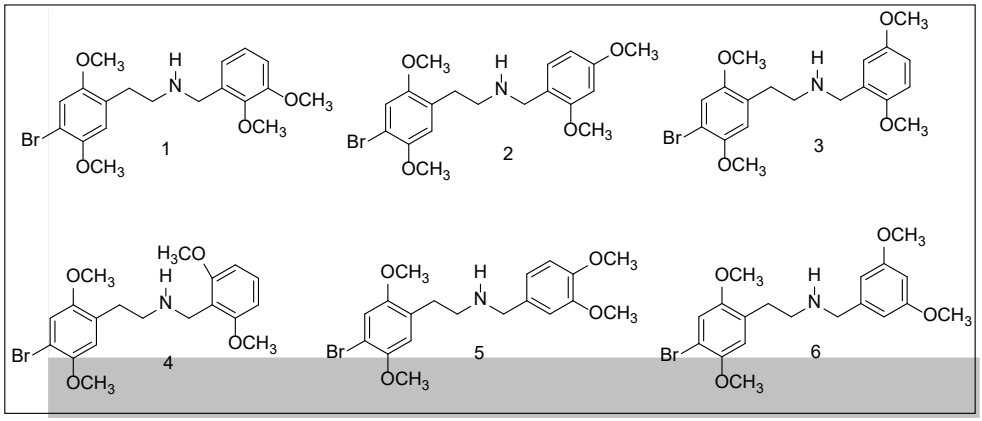
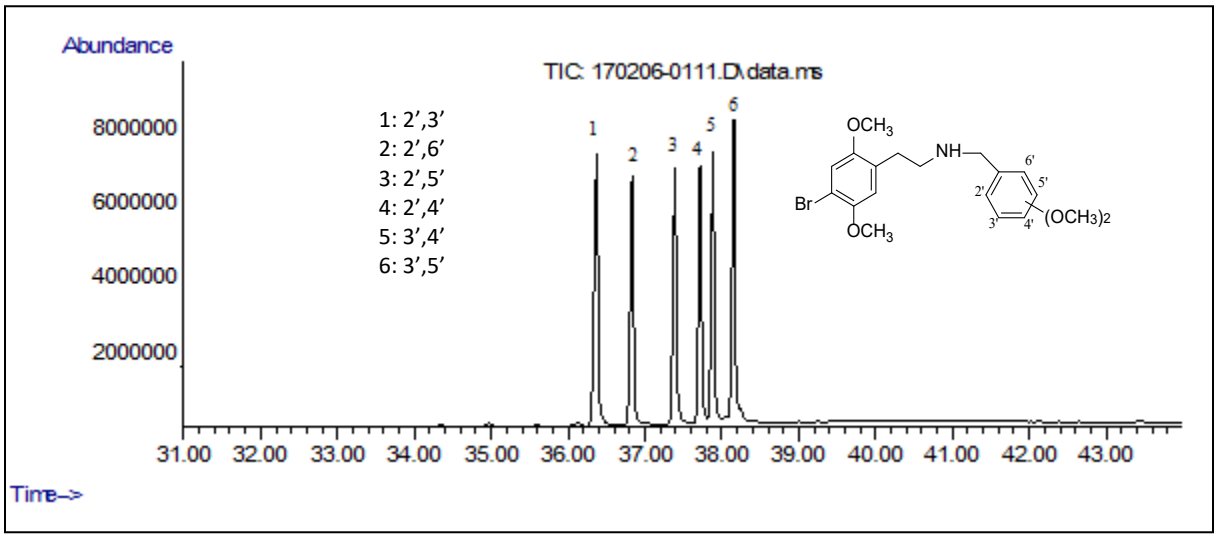
Appendix 8. The six N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethylamines.



Appendix 8. Structures of the N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethylamines.

Appendix 9. GC separation of the dimethoxy-25BNBOMe derivatives



Appendix 9. Gas chromatographic separation of the N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethylamines.

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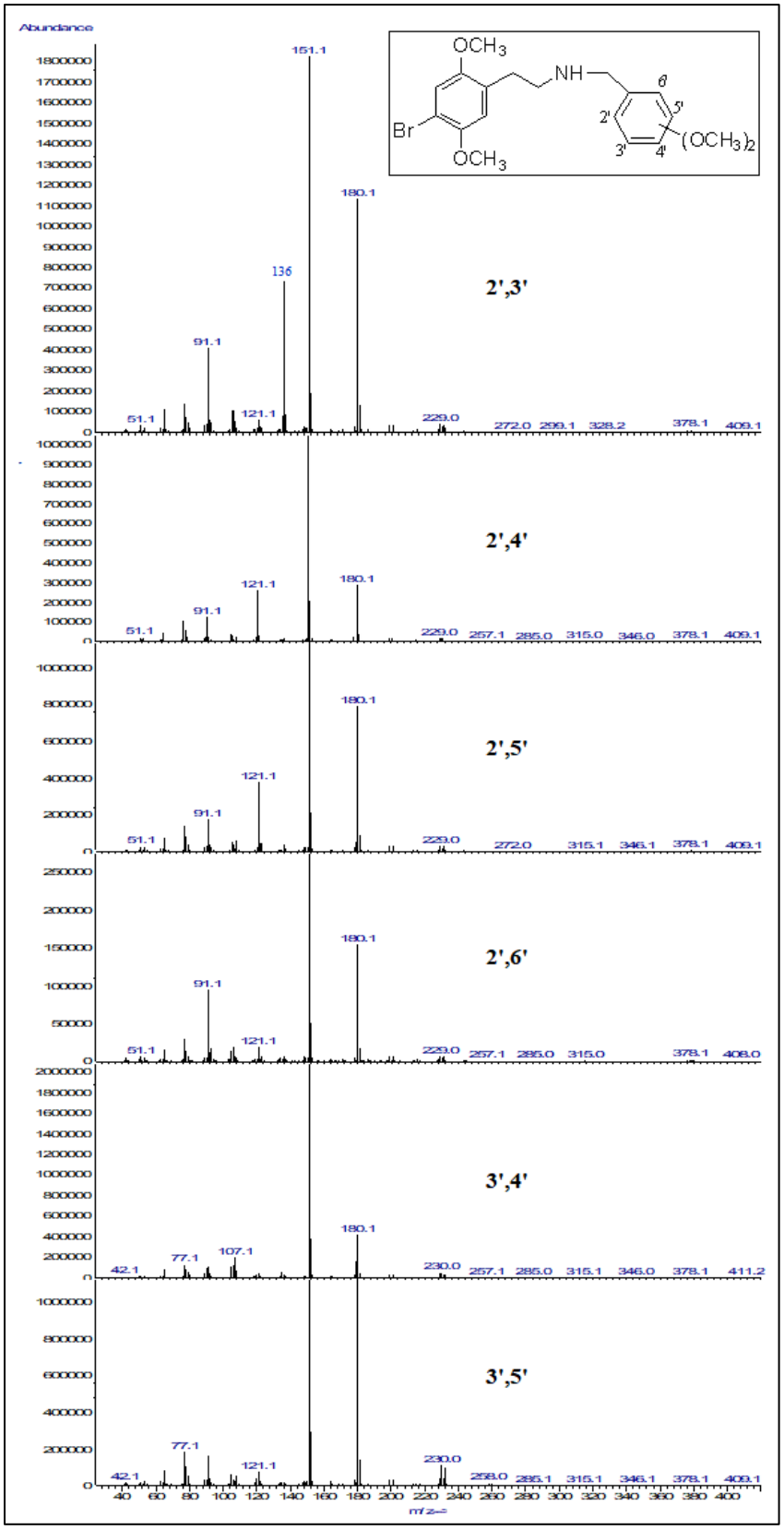
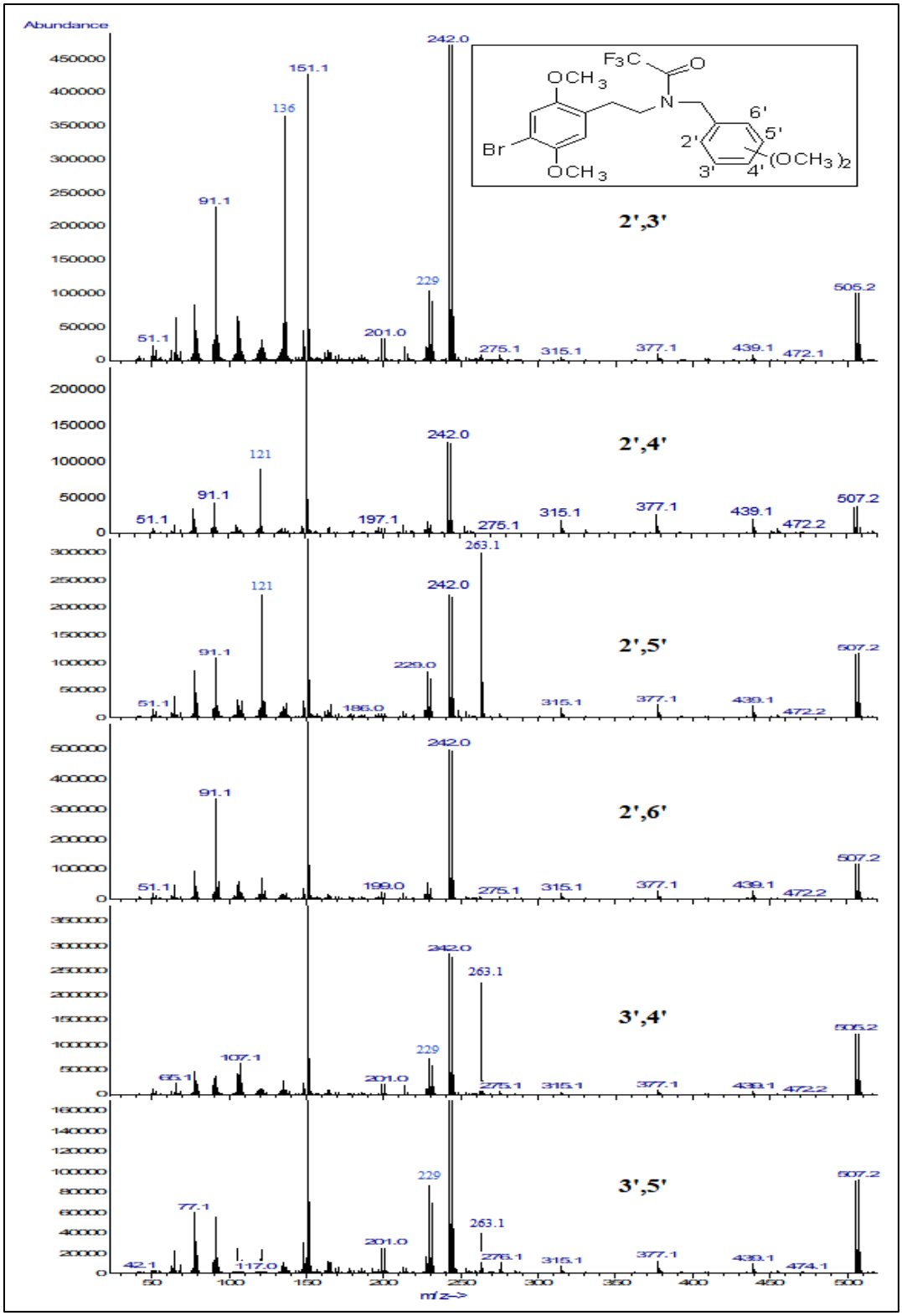
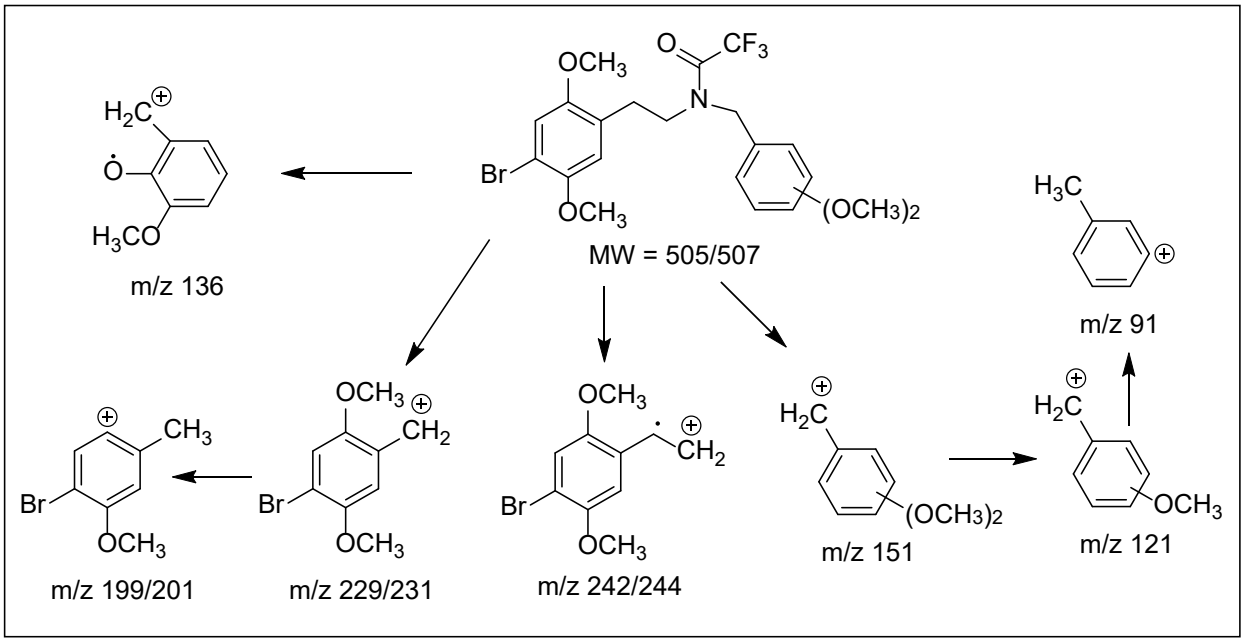
Appendix 10. Mass Spectra of the dimethoxy 25BNBOMe isomers.



Appendix 11. Mass Spectra of the TFA-dimethoxy 25BNBOMe isomers.

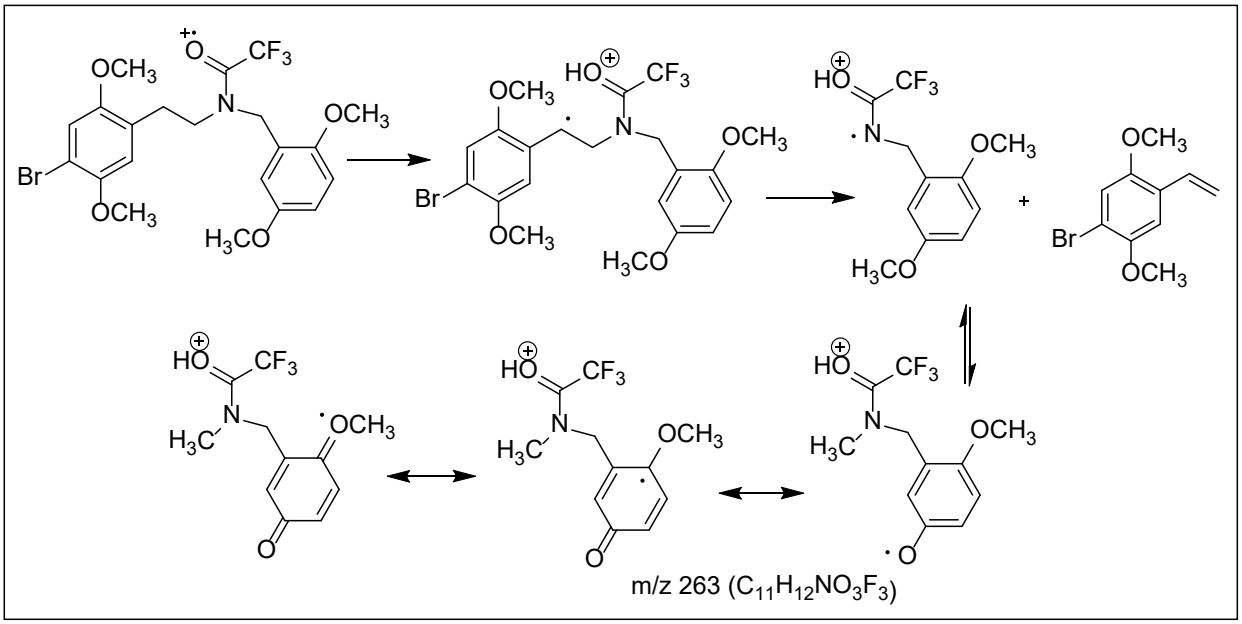


Appendix 12. Fragmentation pathway for TFA-dimethoxy-25BNBOMe isomers.



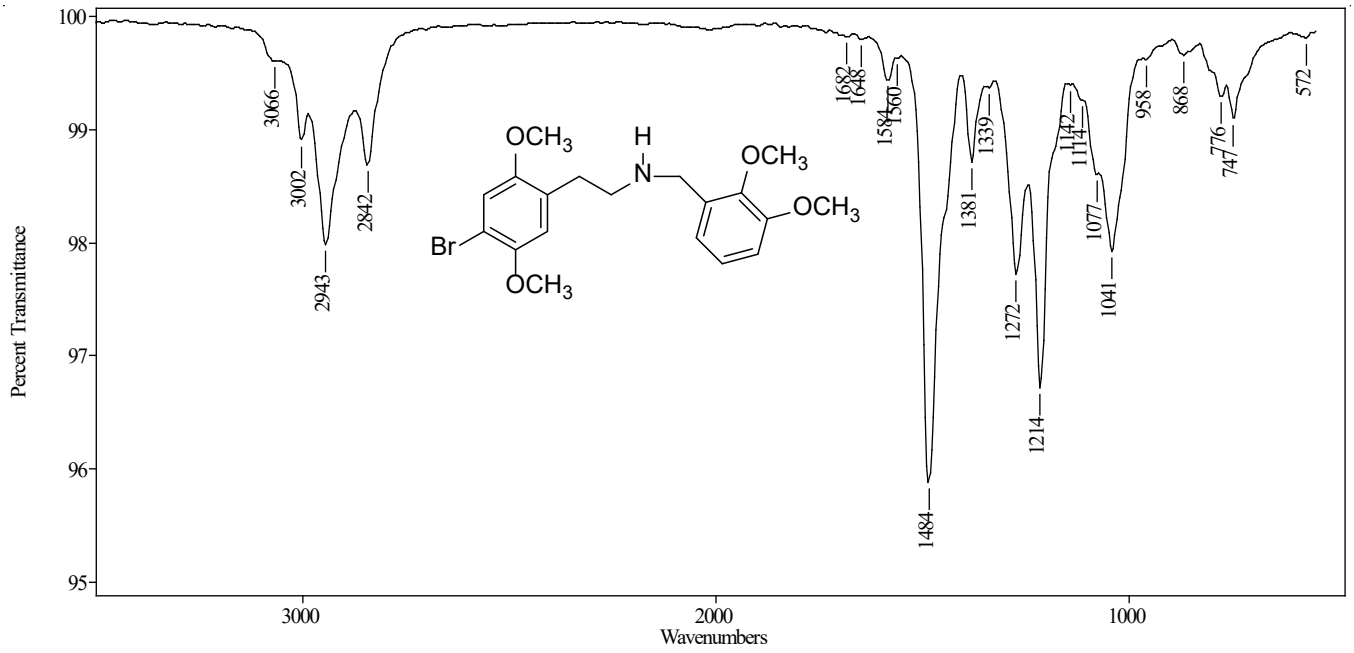
Appendix 12. Proposed EI-MS fragmentation pathway for the N-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenethyl-trifloroacetamide. *The m/z 136 ion only occurs in the N-(2,3-dimethoxybenzyl)-isomer.

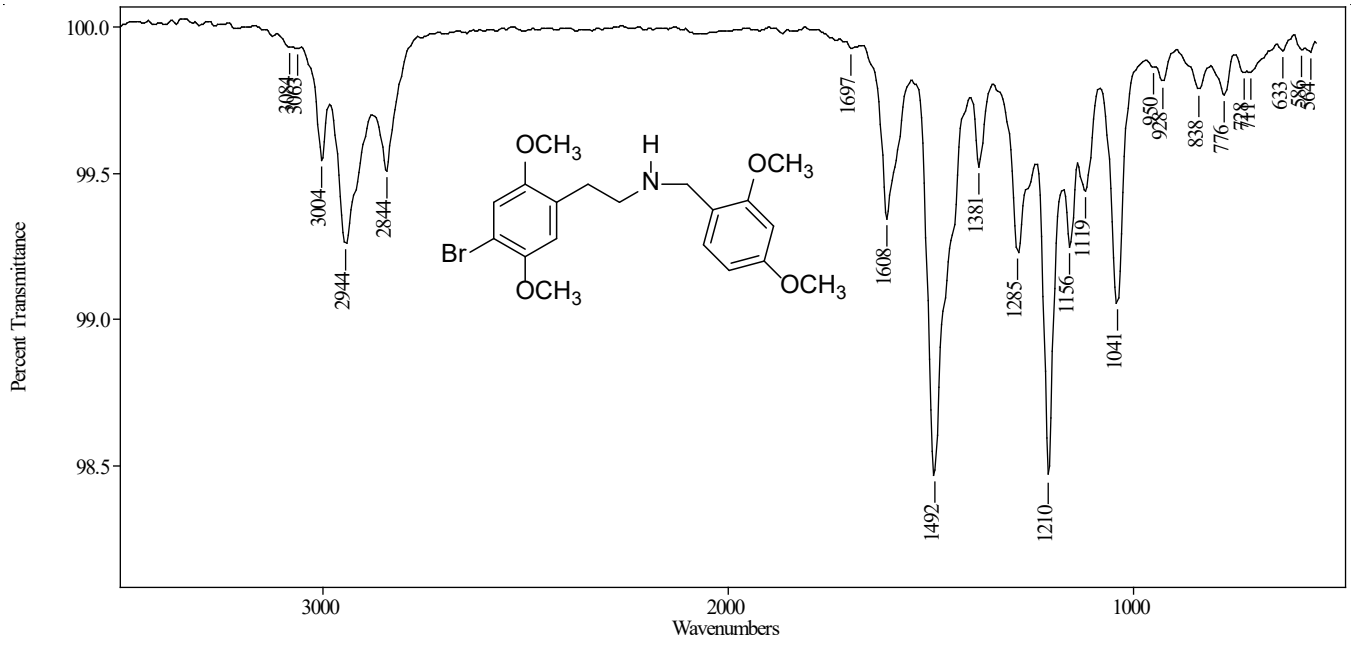
Appendix 13. Structure of the 2,5-dimethoxybenzyl-trifloroacetamide m/z 263 radical cation

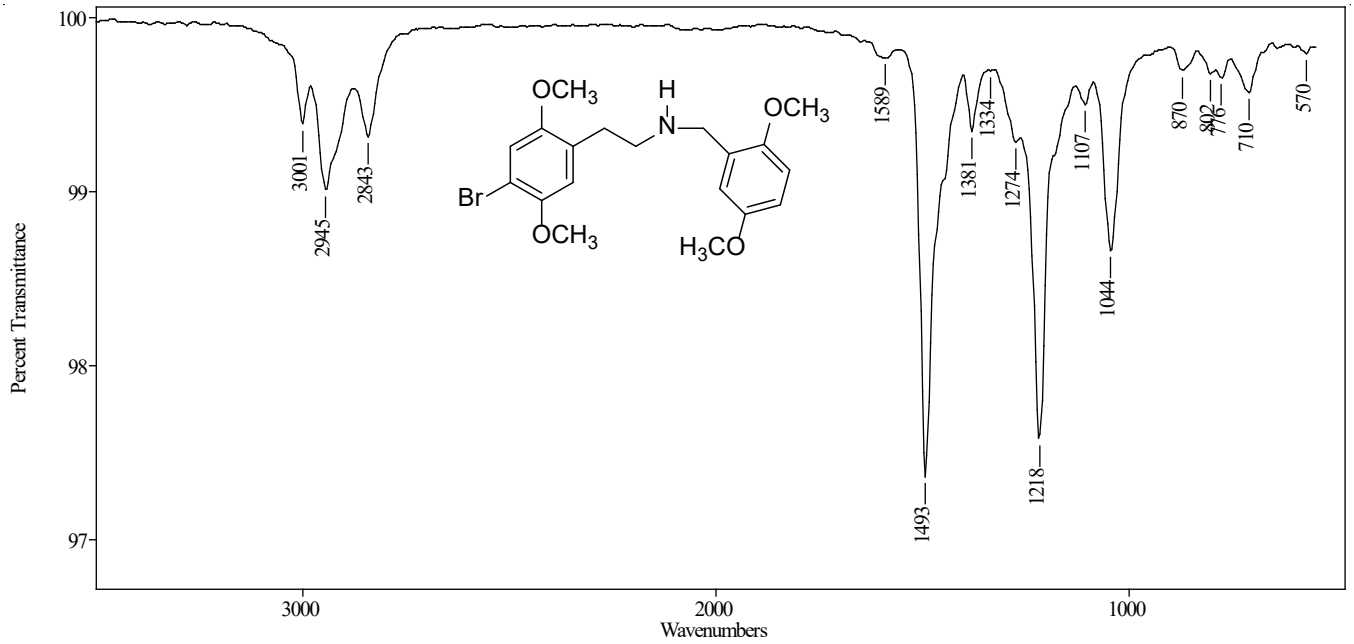


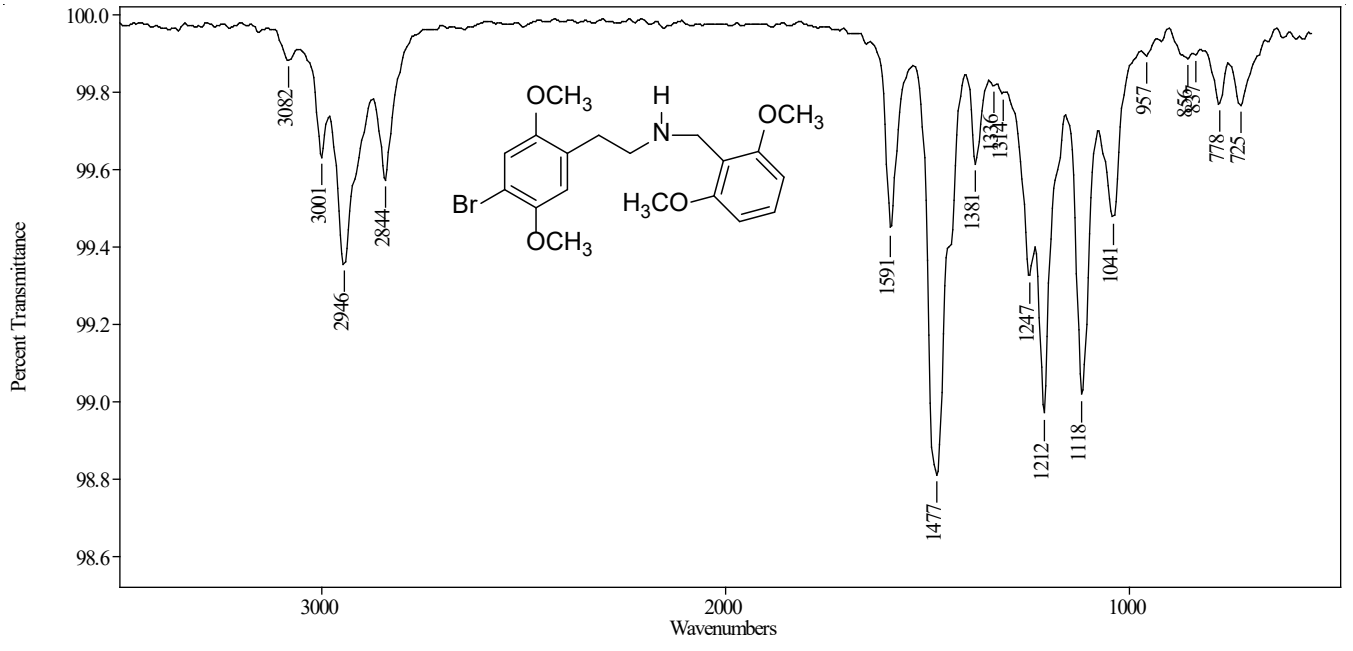
Appendix 13. Structure of the N-(2,5-dimethoxybenzyl)trifloroacetamide m/z 263 radical cation.

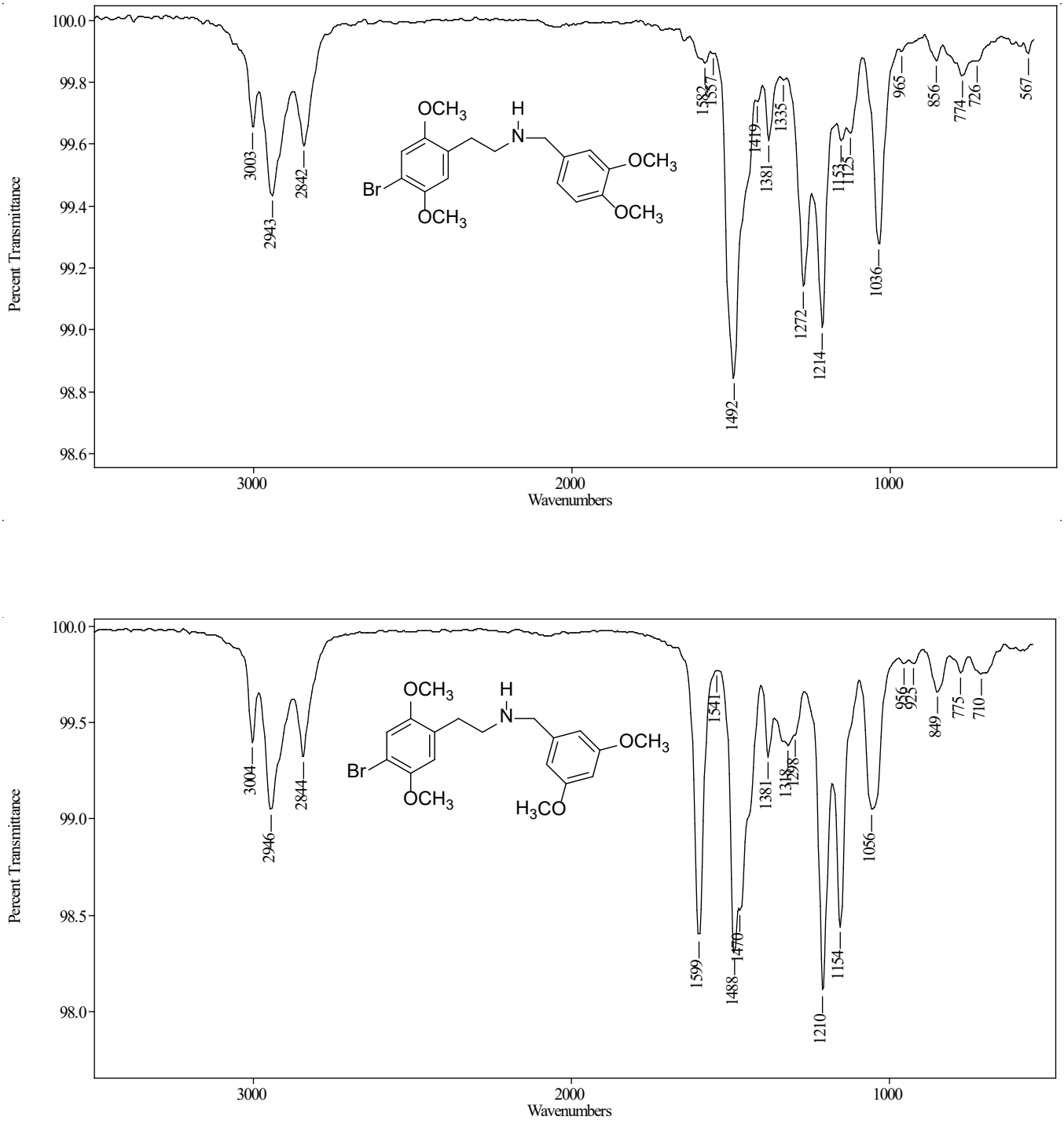
Appendix 14. Vapor phase infrared spectra for the six regioisomeric *N*-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenylamines.











Appendix 14. Vapor phase infrared spectra for the six regioisomeric *N*-(dimethoxybenzyl)-4-bromo-2,5-dimethoxyphenylamines.