Gas Chromatographic–Mass Spectrometric and High-Performance Liquid Chromatographic Analyses of the Bromination Products of the Regioisomeric Dimethoxyphenethylamines: Differentiation of Nexus from Five Positional Isomers

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Abstract

The brominated products from all six positional isomers of dimethoxyphenethylamine are prepared, and their analytical properties are evaluated. The major bromination product from 3,5-dimethoxyphenethylamine is the 2,6-dibromo isomer; all other regioisomers of dimethoxyphenethylamine yield a monobromo species as the major product. The mass spectra divide these compounds into two distinct groups: one group showing a strong *m/z* 180 ion via loss of bromine from the molecular ion $(M\text{-}Br)^+$ and a second group showing no significant *m/z* 180 ion. The three compounds that do not show the *m/z* 180 ion in their electron-impact mass spectra are brominated 2,4-; 2,5-; and 2,6-dimethoxyphenethylamine. These compounds are well-resolved by reversed-phase liquid chromatographic methods using a Hypersil Elite C_{18} stationary phase and a mobile phase of phosphate buffer (pH 3) and methanol–acetonitrile.

Introduction

4-Bromo-2,5-dimethoxyphenethylamine (Nexus, 2C-B) was placed in Schedule I of the United States Controlled Substances Act in 1995 due to its potential as a hazard to public safety (1). Data from human studies (1,2) indicate that Nexus produces intoxication with euphoria and sensory enhancement at doses of 0.1–0.2 mg/kg and intense, frightening hallucinations at higher doses. These effects are likely to be mediated through the affinity of central serotonin receptors for this compound (1). Nexus is structurally similar to the Schedule I hallucinogens 4-methyl-2,5-dimethoxyamphetamine (DOM) and 4-bromo-2,5-dimethoxyamphetamine (DOB). Nexus and DOB have a similar pharmacological profile and differ primarily in relative potency; DOB is approximately 10 times more potent (2). Nexus has been described as having a pharmacological profile similar to that of both Ecstasy (3,4-methylenedioxymethamphetamine) and LSD.

The most likely method for the preparation of Nexus is shown in Figure 1 and involves use of the commercially available starting material 2,5-dimethoxybenzaldehyde (3). All possible isomeric dimethoxybenzaldehydes (2,3-; 2,4-; 2,5-; 2,6-; 3,4-; and 3,5-) are commercially available and could be used to prepare the six dimethoxyphenethylamine positional isomers. Bromination of these amines would be expected to yield six distinct bromodimethoxyphenethylamine positional isomers (see Figure 2). Based on their close structural similarity, each of the dimethoxyphenethylamines and bromodimethoxyphenethylamine products would be expected to yield very similar analytical profiles by conventional forensic analytical techniques. Thus, to distinguish the Schedule I drug Nexus from its five isomeric bromodimethoxyphenethylamines would present a significant challenge to the forensic chemist. The analytical profiles of

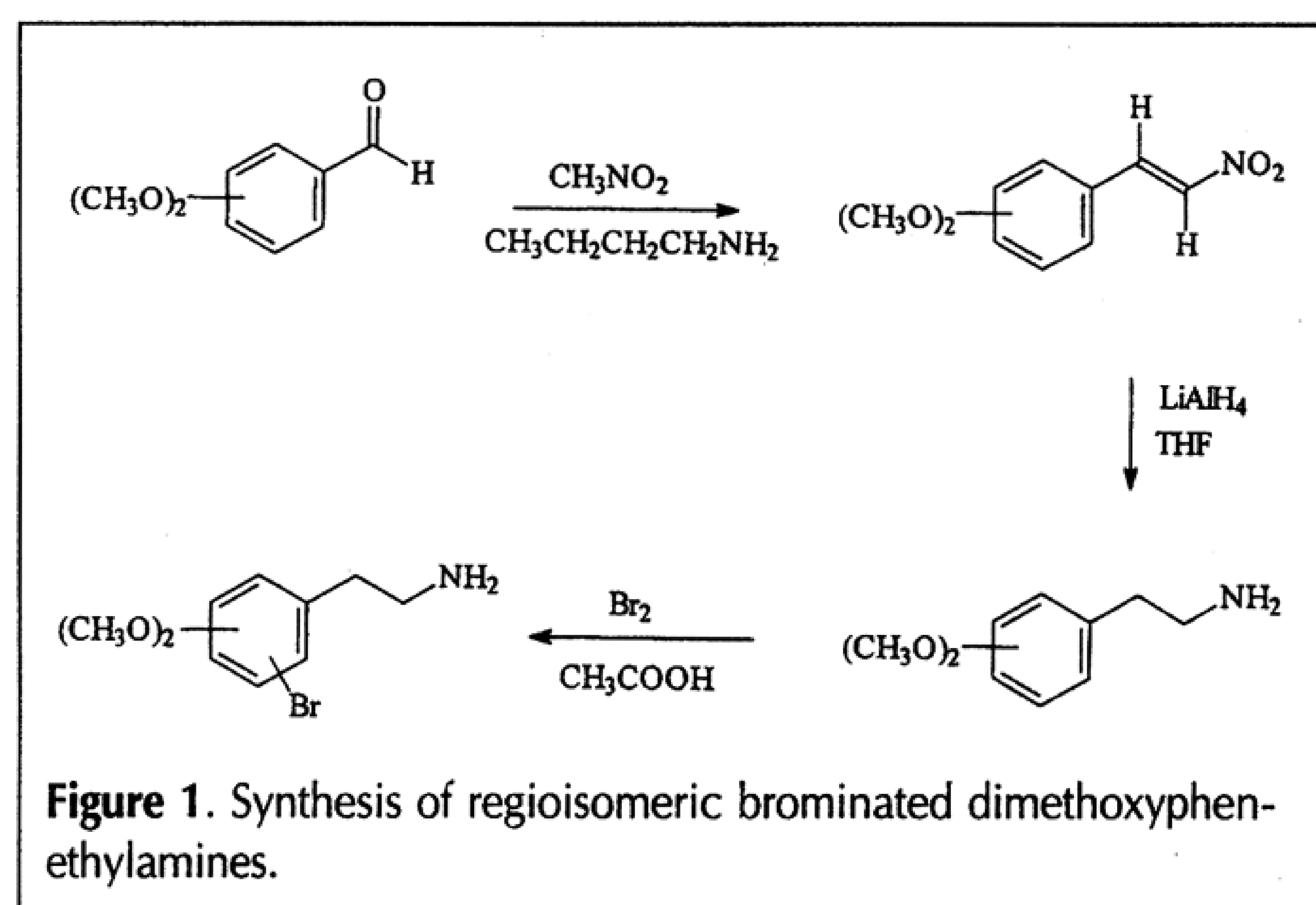

Figure 1. Synthesis of regioisomeric brominated dimethoxyphenethylamines.

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Nexus and its precursor chemicals were compared to those of DOM and DOB in a previous report (4). In this paper, the synthesis of all six isomeric bromodimethoxyphenethylamine products and methods for the specific identification of Nexus from the other positional isomers are described.

Experimental

Instrumentation and methods

Gas chromatographic–mass spectrometric (GC–MS) analyses were performed using a Hewlett-Packard 5970B mass selective detector (Palo Alto, CA). The MS was operated in the electron-impact (EI) mode with an ionization voltage of 70 eV and a source temperature of 220°C. The samples were dissolved in methanol (1 mg/mL), and 0.5 µL was introduced into the MS via a GC equipped with a 12-m × 0.20-mm-i.d. fused-silica column with a 0.33-µm-thick methylsilicone film (HP-1). The column temperature was held at 70°C for 2.5 min and programmed to 170°C at a rate of 25°C/min and from 170 to 275°C at a rate of 12°C/min with a hold time of 6 min. The split ratio for the GC was 10:1 and the injector port temperature was 230°C. The carrier gas was ultra pure helium.

High-performance liquid chromatographic (HPLC) analyses were conducted using a Waters model 590 pump (Milford, MA), a Laboratory Data Control 3000 Spectromonitor ultraviolet (UV) detector (Riveria Beach, FL), a Rheodyne 7125 injector (Cotati, CA), and a Linear Model LR 93125 recorder. The analytical column was 15 cm × 4.6-mm i.d. Hypersil Elite C_{18} (Shandon HPLC, Cheshire, UK). The mobile phase consisted of phosphate buffer (pH 3.0) and methanol (75:25) for Figure 3 and phosphate buffer (pH 3), methanol, and acetonitrile (75:20:5) for Figure 4. The pH 3.0 phosphate buffer was prepared by mixing 9.2 g of monobasic sodium phosphate in 1 L of double-distilled water and adjusting the pH to 3.0 with H_3PO_4. The UV absorbance detector was operated at 280 nm and 0.05 absorbance units full scale (AUFS). The mobile phase flow rate was 1 mL/min, and the compounds were prepared as methanol solutions, and volumes of 2–10 µL were injected.

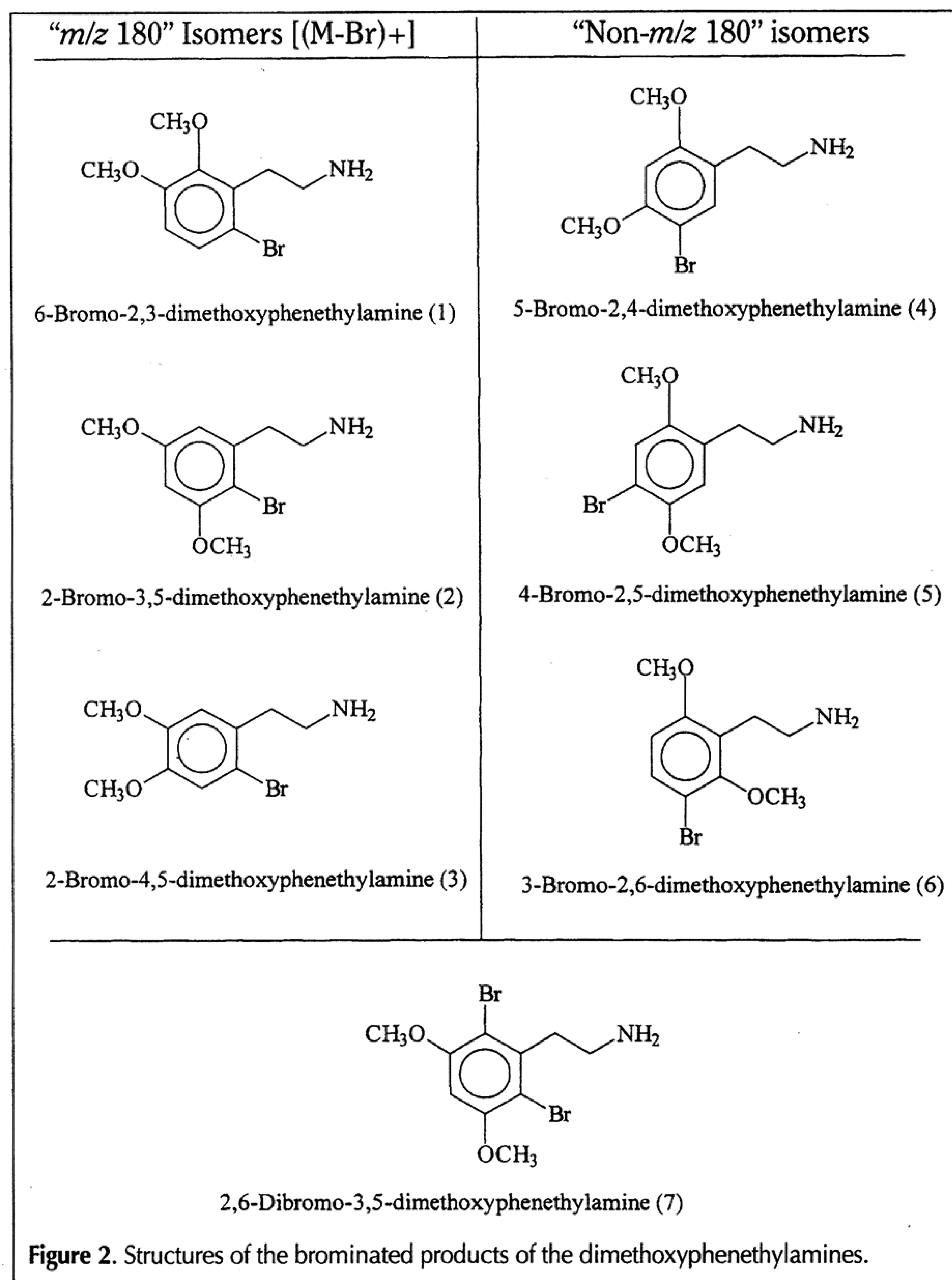

Figure 2. Structures of the brominated products of the dimethoxyphenethylamines.

Synthesis of dimethoxyphenyl-2-nitroethenes

A mixture of the appropriate dimethoxybenzaldehyde, butylamine, and benzene was stirred at reflux for several hours using a Dean-Starke trap to remove water. The reaction mixture was evaporated under reduced pressure, and the remaining oil was dissolved in a mixture of glacial acetic acid and nitromethane. After this solution was stirred at reflux for several hours, it was cooled, and ice was added to precipitate the product. Concentrated HCl was added to adjust the pH to 2. The product was isolated by filtration, washed with water, and recrystallized from 2-propanol to yield the nitroethenes as highly colored needles.

Synthesis of brominated dimethoxyphenethylamines

A solution of the nitroethene intermediate in dry tetrahydrofuran (THF) was added to a suspension of lithium aluminum hydride (LAH) in THF stirred at room temperature. After the addition was complete, the reaction mixture was stirred at reflux for several hours, then cooled in an ice bath. The excess LAH and LAH salts were decomposed by the successive addition of water, 2N NaOH, and additional water. The mixture was then filtered, and the filtrate was evaporated under reduced pressure. The remaining oil was suspended in water and acidified to pH 1. The resulting aqueous solution was washed with benzene, then made basic by the addition of NaOH pellets and extracted twice with methylene chloride. The combined methylene chloride extracts were washed with water and dried over anhydrous sodium sulfate. Filtration followed by evaporation of the filtrate solvent yielded the product amines as oils. The amines were converted to their corresponding HCl salts by treatment with ethereal HCl.

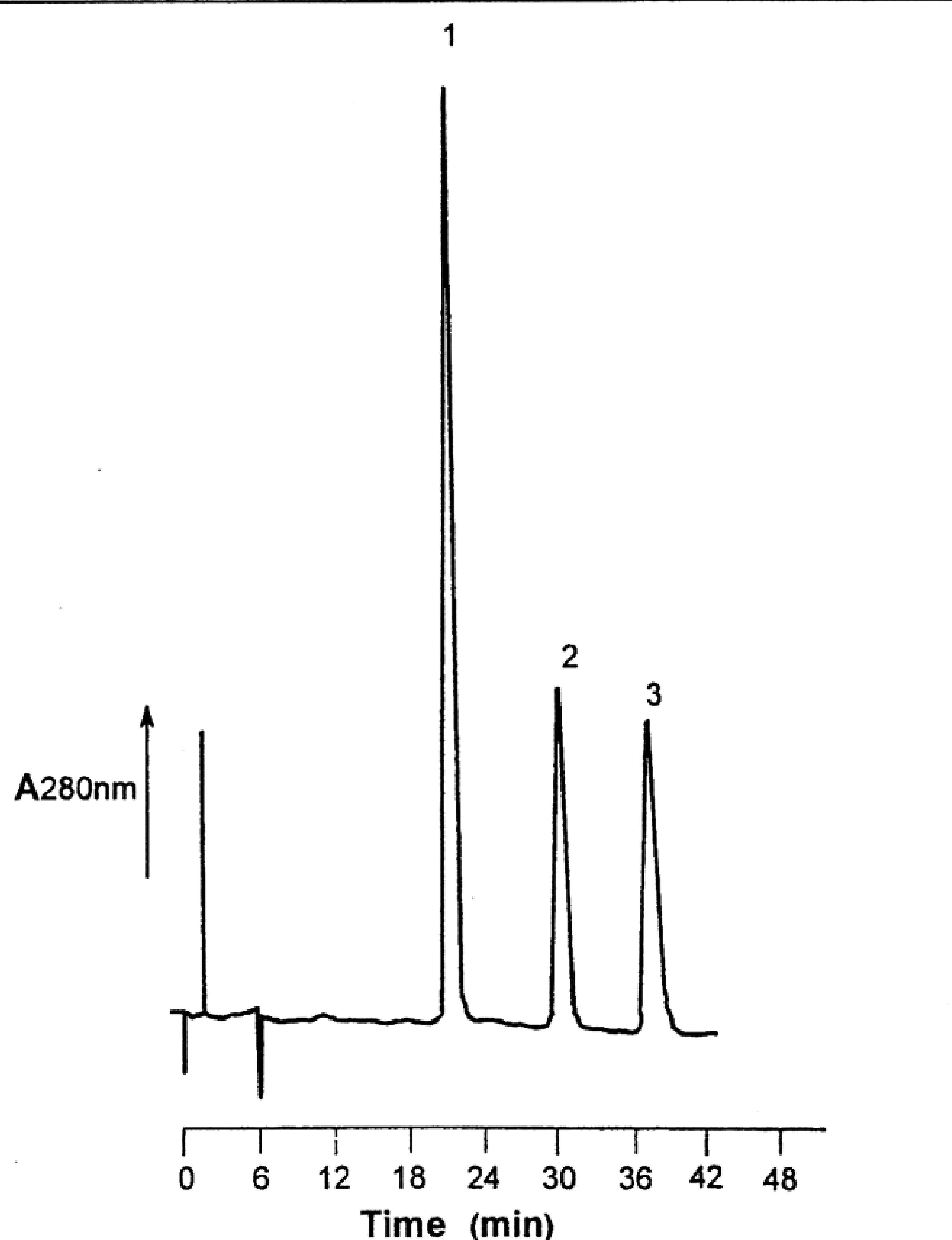

Figure 3. HPLC separation of the regioisomeric bromodimethoxyphenethylamines not showing the *m/z* 180 fragment ion. Peaks: 1, 5-bromo-2,4-dimethoxyphenethylamine; 2, 4-bromo-2,5-dimethoxyphenethylamine; 3, 3-bromo-2,6-dimethoxyphenethylamine.

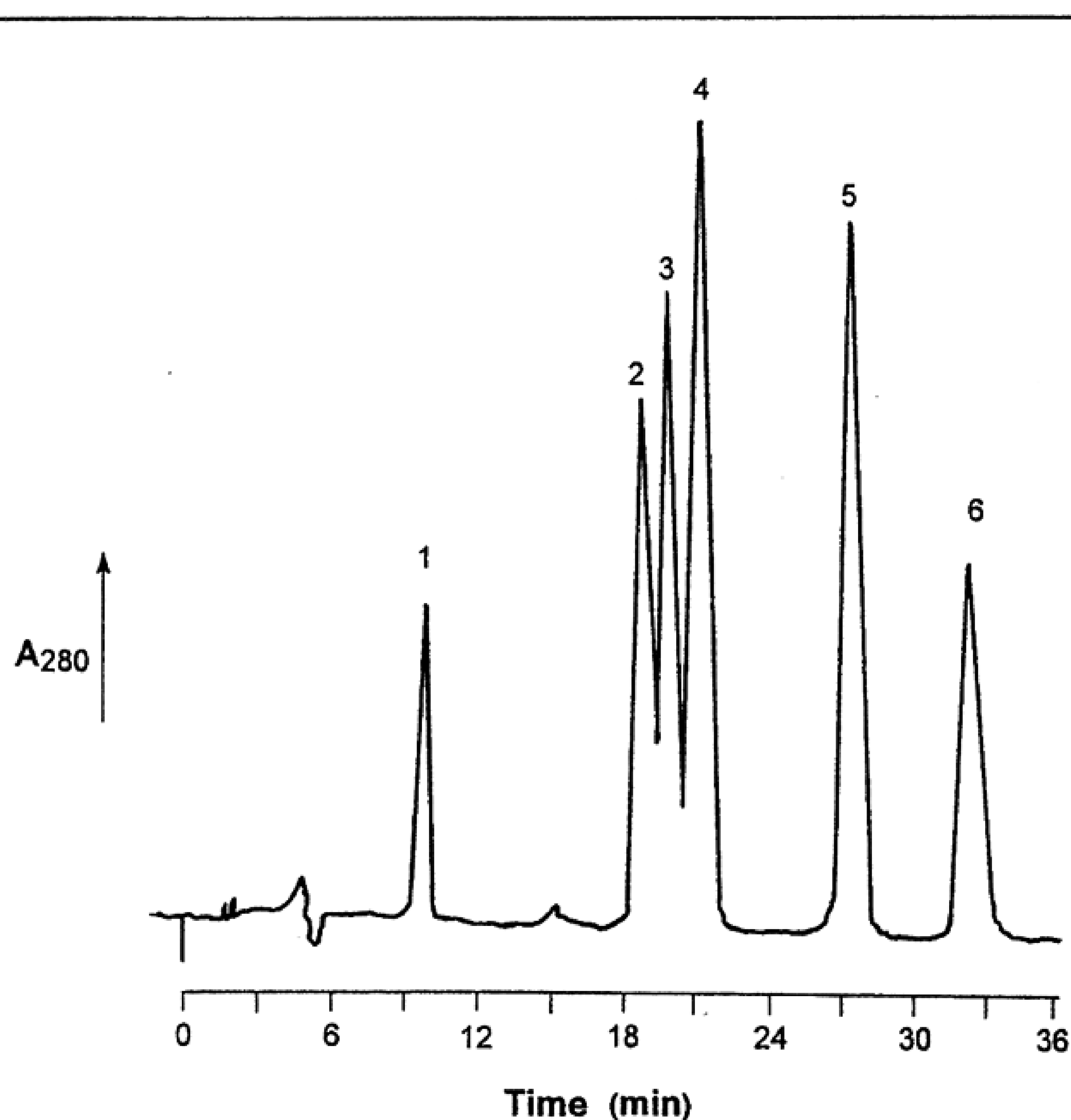

Figure 4. HPLC separation of all six brominated products. Peaks: 1, 2-bromo-4,5-dimethoxyphenethylamine; 2, 6-bromo-2,3-dimethoxyphenethylamine; 3, 5-bromo-2,4-dimethoxyphenethylamine; 4, 2,6-dibromo-3,5-dimethoxyphenethylamine; 5, 4-bromo-2,5-dimethoxyphenethylamine; 6, 3-bromo-2,6-dimethoxyphenethylamine.

Synthesis of brominated dimethoxyphenethylamines

A solution of bromine in glacial acetic acid was added over a few minutes to a solution of the amine hydrochloride in glacial acetic acid. After the addition was complete, the reaction mixture was stirred at room temperature for an hour, resulting in the formation of a yellow precipitate. The precipitate was isolated by filtration and suspended in aqueous NaOH. The aqueous basic suspension was extracted with methylene chloride, and the combined organic extracts were washed with water and dried over potassium carbonate. Filtration followed by evaporation of the filtrate solvent yielded the product amines as oils. The amines were converted to their corresponding hydrochloride salts by treatment with ethereal HCl.

Results and Discussion

The synthesis of substituted phenethylamines can be accomplished through several methods. Many of the common methods begin with precursor chemicals which are now controlled under the U. S. Precursor and Essential Chemical Act of 1989. A significant exception involves the use of readily available and uncontrolled substituted benzaldehydes. These compounds are converted to the versatile 2-nitroalkene, which is reduced to the corresponding primary amine or, in some cases, subjected to reduction hydrolysis. This general method of preparing phenethylamines is illustrated in Figure 1, which shows the synthesis of the substituted phenethylamine, 4-bromo-2,5-dimethoxyphenethylamine. Commercially available 2,5-dimethoxybenzaldehyde is treated with butylamine and nitromethane to yield the nitroethene intermediate. Upon reduction with lithium aluminum hydride (LAH), the phenethylamine is obtained in high yield. Bromination of the amine hydrochloride produces the 4-bromo-substituted phenethylamine, which has the street name Nexus. This analogous procedure using the appropriately substituted dimethoxybenzaldehyde was used to prepare all the isomeric amines included in this study. The structures of all products were confirmed by standard spectroscopic techniques (nuclear magnetic resonance [NMR], infrared [IR], and MS).

The position of bromination for each final product was confirmed by the coupling patterns of the aromatic protons by proton NMR (^{1}H–NMR), and the structures of these products are shown in Figure 2. The position of bromination of the dimethoxyphenethylamines is dependent upon the pattern of the methoxy group substitutions. Though each of the dimethoxyphenethylamines have three-ring carbons available for substitution, bromination typically occurs at only one of these positions. This reaction proceeds via an electrophilic mechanism, thus substitution occurs preferentially at ring carbons that are in direct conjugation with the electron-donating methoxy groups: the so-called *"ortho-"* or *"para-"* positions. In cases where there are multiple-ring carbons in conjugation, bromination usually occurs at the least sterically hindered site. Thus, while there are three distinct ring positions available for electrophilic substitution in 2,5-dimethoxyphenethylamine, bromination occurs only at the 4-position (Figure 2, structure

5). In this compound, the 4-position is in direct conjugation with the 5-methoxy (*ortho-*) group and is not as sterically crowded as it would be at the 6-position. Similarly with 2,4-dimethoxyphenethylamine, bromination occurs at the 5-position because this carbon is in direct conjugation with the 2-methoxy (*para-*) and 4-methoxy (*ortho-*) groups, and is not as sterically crowded as the other position in conjugation, the 3-carbon (Figure 2, structure 4). With 2,6-dimethoxyphenethylamine, only two bromination products are possible as a result of the ring symmetry imparted by the 2,6-dimethoxy substitution. In this case, only the 3-position is brominated because it is "*ortho-*" to one ring methoxy group and "*para-*" to the other (Figure 2, structure 6). With 2,3-dimethoxyphenethylamine, bromine substitution can occur at both positions 5- and 6- because both of these carbon atoms are "*para-*" to at least one electron-rich methoxy group. The predominant product isolated in this study was the 6-bromo derivative (Figure 2, structure 1). Similarly, bromination of 3,4-dimethoxyphenethylamine yielded only the 6-bromination product. In this case, it should be noted that the addition of bromine changes the priority for ring numbering (to obtain the lowest number for substituents), thus this product is named 2-bromo-4,5-dimethoxyphenethylamine instead of 6-bromo-3,4-dimethoxyphenethylamine (Figure 2, structure 3). Bromination of 3,5-dimethoxyphenethylamine primarily yielded a mixture of the 2-bromo- (Figure 2, structure 2) and 2,6-dibromo- (Figure 2, structure 7) substitution products. In this case, the dibromination predominated, even when minimal amounts of bromine were used in the reaction. The preferential formation of the 2,6-dibromo product in this reaction is consistent with results reported for the bromination of 3,5-dimethoxyamphetamine (2).

The mass spectra for the brominated dimethoxyphenethylamines showed similar fragmentation patterns and are summarized in Figure 5. Those fragment ions containing bromine were easily identified based on the nearly equal isotopic ratios of ^{79}Br:^{81}Br. The substituted benzyl radical cation containing bromine was a major fragment for many of the compounds at *m/z* 230/232. The loss of the entire ethylamine side chain to yield the substituted benzene occurred at *m/z* 215/217. The *m/z* 199/201 ion was likely the result of a loss of CH_2O from the brominated dimethoxybenzyl cation at *m/z* 229/231. The loss of bromine from the molecular ion is a major fragment for those compounds in which the position of the bromine substitution is "*ortho-*" to the alkylamine side chain. This ion at *m/z* 180 is a prominent fragment in brominated 2,3-; 3,5-; and 3,4-dimethoxyphenethylamines (Figures 6A–6C). Thus, the $(M\text{-}Br)^+$ ion at *m/z* 180 is a major diagnostic reference point for differentiating the "*ortho-*" brominated positional isomers from the "non-*ortho-*" brominated isomers. It should be pointed out that the *m/z* 180 ion will be the base peak in these spectra when the MS scan range excludes the *m/z* 30 ion. Although the mass spectra for 2-bromo-3,5-dimethoxyphenethylamine is shown in Figure 6B, the bromination of 3,5-dimethoxyphenethylamine yielded primarily the dibromo product. This dibromo product showed the loss of one bromine from the molecular ion $(M\text{-}Br)^+$ as the major peak at *m/z* 258/260 (Figure 6D).

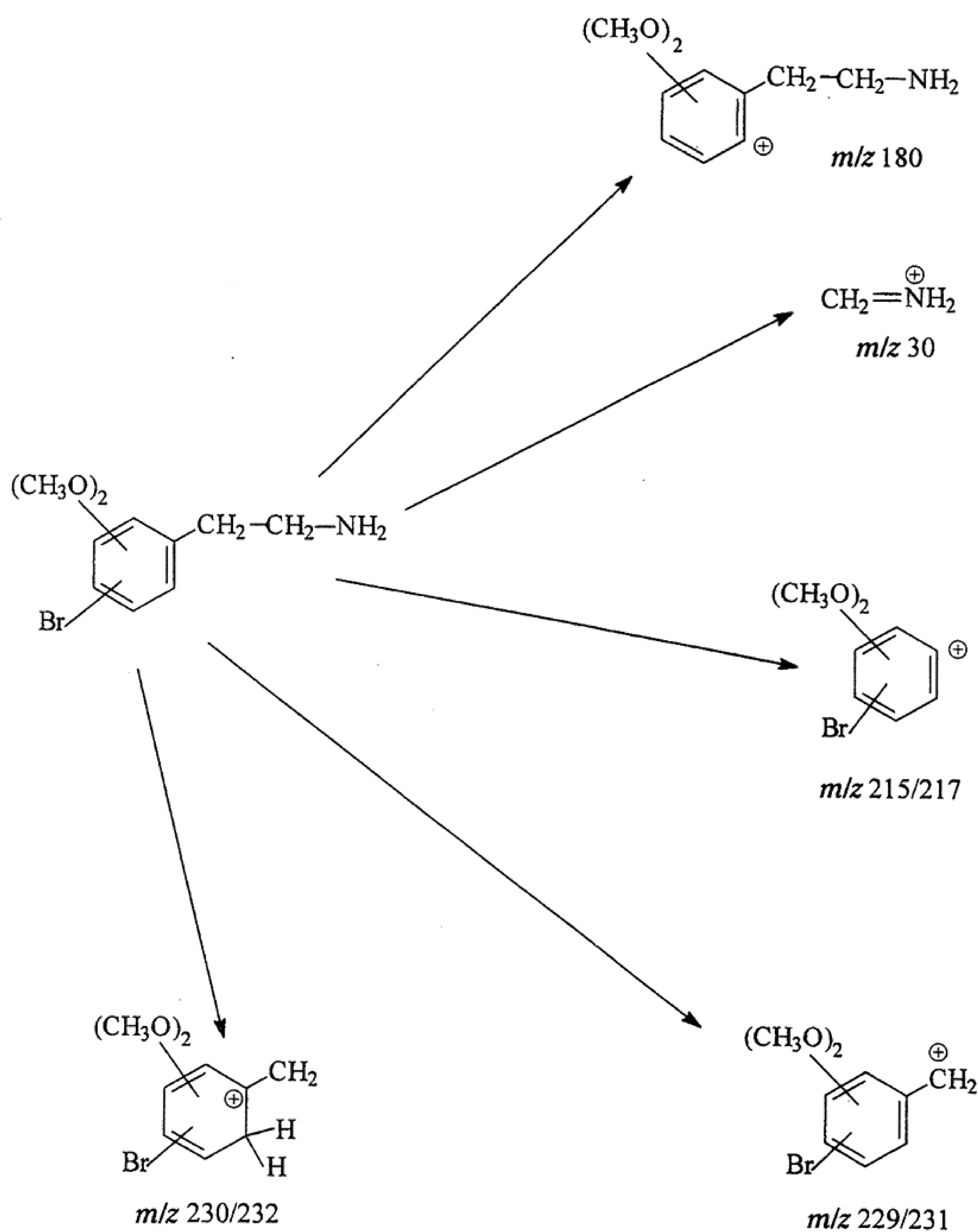

Figure 5. Major EI fragmentation reactions for the bromodimethoxyphenethylamines.

The $(M\text{-}Br)^+$ ion was absent from the mass spectra of brominated 2,4-; 2,5-; and 2,6-dimethoxyphenethylamine (Figure 7). The *m/z* 180 fragment ion then divided the six regioisomeric phenethylamines into two groups of three compounds. This subdivision, based on the presence or absence of the *m/z* 180 ion, simplified the challenge of distinguishing among the six isomeric phenethylamines and, more specifically, the identification of Nexus to the exclusion of the other five possible isomeric brominated dimethoxyphenethylamines.

The mass spectra for the three positional isomers not showing the *m/z* 180 ion had some differences in the relative abundance of the high-mass fragment clusters at *m/z* 199/201, 215/217, and 230/232 (Figure 7). The brominated 2,6-dimethoxyphenethylamine (Figure 7C) showed a high relative concentration of the *m/z* 230/232 fragment cluster with a very low abundance of the *m/z* 199/201 and 215/217 clusters. The brominated 2,4- (Figure 7A) and 2,5-dimethoxyphenethylamines (Figure 7B) showed

extremely similar mass spectra; the most notable differences were the relative abundances of the *m/z* 199/201 and 215/217 clusters.

The "non-*m/z* 180" group of brominated 2,4-; 2,5-; and 2,6-dimethoxyphenethylamine compounds could best be individually identified by chromatographic methods of analysis. The LC separation of these three regioisomers is shown in Figure 3. The compounds were well-resolved in this reversed-phase chromatographic system; the brominated 2,4-dimethoxyphenethylamine showed the lowest capacity factor, followed by the brominated 2,5-dimethoxyphenethylamine (Nexus), and brominated 2,6-dimethoxyphenethylamine displayed the highest affinity for the stationary phase. The chromatographic system consisted of a methanol–phosphate buffer (pH 3) mobile phase and a stationary phase of Hypersil Elite C_{18}. The Hypersil Elite C_{18} is a highly surface-deactivated material that does not require silanol masking agents such as triethylamine for successful analysis of basic compounds. The more conventional reversed-phase materials often require dynamic saturation of active silanol sites to prevent peak tailing by strong silanophilic substances such as aliphatic amines. The three regioisomers separated in Figure 3 showed excellent peak shape and eluted over an approximate 20-min time window, showing retention times from about 20 to 40 min. The total analysis time could be reduced with retention of baseline separation for these three compounds by altering the chromatographic conditions. This chromatogram clearly demonstrates that Nexus can be differentiated from the other two positional isomers by LC techniques.

The chromatogram in Figure 4 shows the HPLC separation of all six of the products obtained in the bromination of the six regioisomeric dimethoxyphenethylamines. This isocratic separation was obtained on the Hypersil Elite C_{18} stationary phase using a mobile phase of pH 3 buffer, methanol, and acetonitrile (75:20:5) at a flow rate of 1 mL/min. This ternary mobile phase system produced the best resolution observed among the many isocratic systems evaluated in this study. The first compound to elute in this chromatographic system was the brominated 3,4-dimethoxyphenethylamine (peak 1); this compound has a relatively low capacity factor compared with the other brominated amines. The lack of baseline resolution for peaks 2, 3, and 4 in

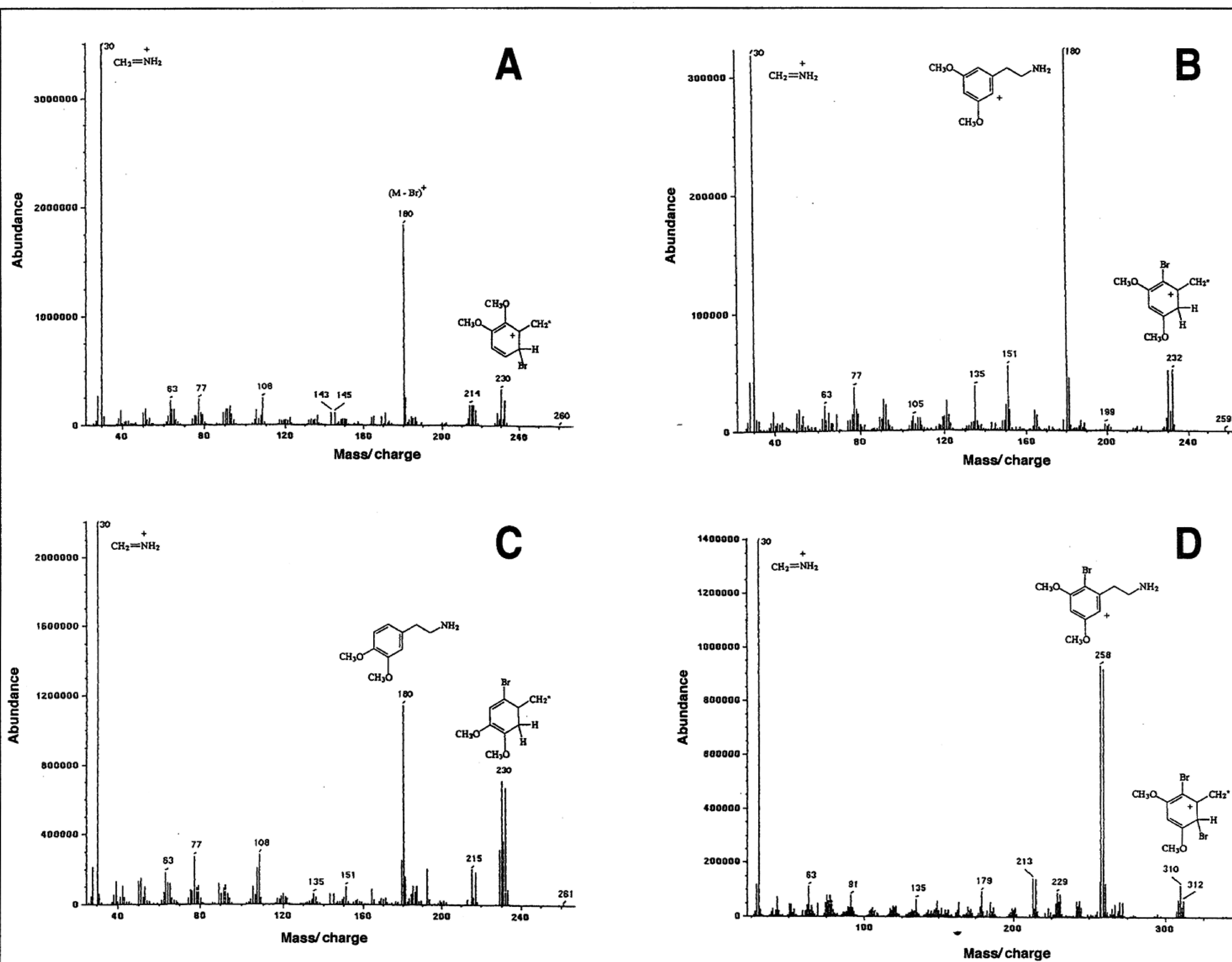

Figure 6. Mass spectra of those regioisomeric bromodimethoxyphenethylamines showing the $(M-Br)^+$ major fragment. (A) 6-bromo-2,3-dimethoxyphenethylamine, (B) 2-bromo-3,5-dimethoxyphenethylamine, (C) 2-bromo-4,5-dimethoxyphenethylamine, and (D) 2,6-dibromo-3,5-dimethoxyphenethylamine.

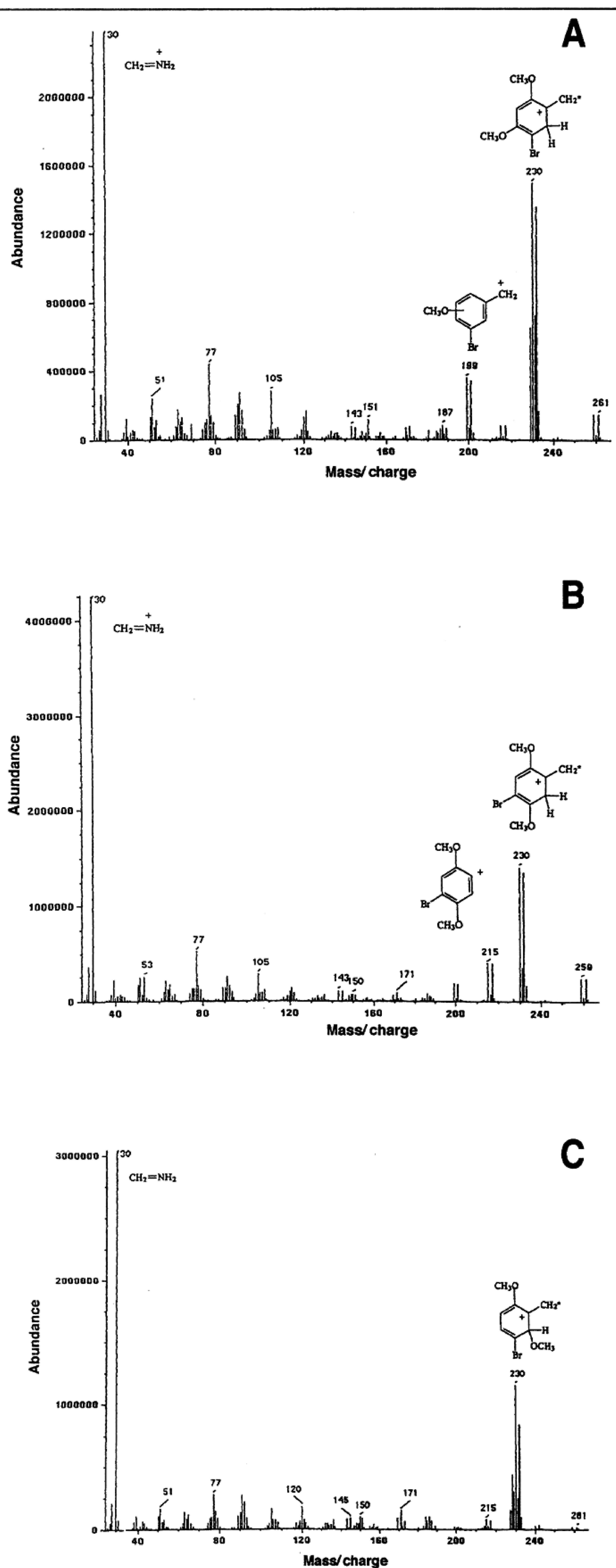

Figure 7. Mass spectra of those regioisomeric bromodimethoxyphenethylamines not showing the (M-Br)+ major fragment. (A) 5-bromo-2,4-dimethoxyphenethylamine, (B) 4-bromo-2,5-dimethoxyphenethylamine, and (C) 3-bromo-2,6-dimethoxyphenethylamine.

Figure 4 illustrates the similarity in retention properties for some of these isomeric amines. However, these three compounds can be easily differentiated by their mass spectra; peak 2 (6-bromo-2,3-dimethoxyphenethylamine) has an "*ortho*-" substituted bromine and yielded a mass spectrum showing a significant *m/z* 180 ion, peak 3 (5-bromo-2,4-dimethoxyphenethylamine) did not show a *m/z* 180 ion in its mass spectrum, and peak 4 is the dibrominated amine (2,6-dibromo-3,5-dimethoxyphenethylamine), which has a significantly different mass spectrum and molecular weight. Peaks 3, 5, and 6 in Figure 4 are the same compounds separated in Figure 3; though the compounds show the most similarity by MS, their reversed-phase retention properties yield significant chromatographic resolution. A similar situation occurred for the monobrominated compounds that showed a significant *m/z* 180 ion in their mass spectrum. Although the mass spectra for the brominated 3,4- and 2,3-dimethoxyphenethylamines are quite similar (see Figures 6C and 6A), their reversed-phase retention properties (peaks 1 and 2 in Figure 4) are quite different, producing significant chromatographic resolution.

Conclusion

In summary, the six isomeric brominated dimethoxyphenethylamines (Nexus and its five positional isomers) were prepared from the commercially available dimethoxybenzaldehydes. Those compounds in which the bromine is *ortho*-substituted to the ethylamine side chain produced a significant *m/z* 180 ion in their mass spectra. This ion arose via the loss of bromine from the molecular ion. Brominated 2,4-; 2,5- (Nexus); and 2,6-dimethoxyphenethylamine did not show a loss of bromine and did not produce the *m/z* 180 ion in their mass spectra. Thus the mass spectra of these isomers placed the compounds into two groups, depending on the presence or absence of the *m/z* 180 ion. The drug of abuse Nexus is one of three compounds that does not yield the *m/z* 180 ion, and although the mass spectra for the three "non-*m/z* 180" isomers showed some subtle differences, these compounds were best differentiated by reversed-phase LC separation.

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