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Dehydration-fragmentation mechanism of cathinones and their metabolites in ESI-CID

Short Tittle: Dehydration-fragmentation mechanism of cathinones in ESI-CID

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Abstract

Various cathinone-derived designer drugs (CATs) have recently appeared on the drug market. This study examined the mechanism for the generation of dehydrated ions for CATs during electrospray ionization collision-induced dissociation (ESI-CID). The generation mechanism of dehydrated ions is dependent on the amine classification in the cathinone skeleton, which is used in the identification of CATs. The two hydrogen atoms eliminated during the dehydration of cathinone (primary amine) and methcathinone (secondary amine) were determined, and the reaction mechanism was elucidated through the deuterium labeling experiments. The hydrogen atom bonded to the amine nitrogen was eliminated with the proton added during ESI, in both of the tested compounds. This provided evidence that CATs with tertiary amine structures (such as dimethylcathinone and α-pyrrolidinophenones (α-PPs)) do not undergo dehydration. However, it was shown that the two major tertiary amine metabolites (1-OH and 2″-oxo) of CATs generate dehydrated ions in ESI-CID. The dehydration mechanisms of the metabolites of α-pyrrolidinobutiophenone (α-PBP) belongs to α-PPs were also investigated. Stable-isotope labeling showed the dehydration of the 1-OH metabolite following a simple mechanism where the hydroxy group was eliminated together with the proton added during ESI. In contrast, the dehydration mechanism of the 2″-oxo metabolite involved hydrogen atoms in three or more locations along with the carbonyl group oxygen, indicating that dehydration occurred via multiple mechanisms likely including the rearrangement reaction of hydrogen atoms. These findings presented herein indicate that the dehydrated ions in ESI-CID can be used for the structural identification of CATs.

Keywords: cathinones; fragmentation mechanism; ESI-CID-MS/MS; dehydration; designer drugs

1 Introduction

Cathinone-derived designer drugs (CATs) are structural analogs of cathinone (Figure 1a), which is an active component of Khat (evergreen tree leaves). CATs, when consumed, exert a central nervous system excitatory effect, which is similar to typical stimulants [1–4]. Since the year 2000, many CATs containing modified chemical structures have been distributed to evade legal regulations. These changes to the CAT structure usually consist of modifications to the type of amine group, substituents added on the benzene ring, and alteration to the length of the alkyl chain. Therefore, it has become essential in the field of forensic science to identify the slightly modified structures of CATs.

We have previously reported on analytical techniques to identify the structures of CATs in powder, solution, and biological samples using gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) [5–10]. In particular, the use of the LC-ESI-MS/MS can target a wide range of compounds from low to high polarity, and the structures of compounds can be identified based on the product ion spectra generated in the electrospray ionization collision-induced dissociation (ESI-CID) following LC separation.

In this study, the generation mechanism of dehydrated product ions in ESI-CID was examined. Dehydrated ion generation appears to be dependent on the partial structure of the cathinone skeleton. Dehydrated ions have been observed from both primary and secondary amine structures such as 3,4-dimethylcathinone, methcathinone, 3,4-dimethylmethcathinone, *N*-propylcathinone, hexedrone and ethylone [10–18], while tertiary amine structures such as 4-chloro-*N,N*-dimethylcathinone and α-pyrrolidinophenones (α-PPs), do not generate the dehydration ion [6–9,14–20]. Thus, the dehydrated ions can possibly be used for the structural identification of CATs, regardless of the substituents on the benzene ring and/or the alkyl chain length. Cathinone (primary amine) and methcathinone (secondary amine) were selected as model drugs (Figure 1a), and the two hydrogen atoms eliminated during dehydration were identified using their deuterium (D)-substituted compounds to elucidate the dehydrated ion generation mechanism.

The two main metabolites of CATs with a tertiary amine structure, the reductive metabolites (1-OH metabolite) and oxidative metabolites (2″-oxo metabolite) (Figure 1b), generate dehydrated ions in ESI-CID. These metabolites lose the cathinone skeleton by conversion from a carbonyl group to a hydroxy group and an amine group to an amide group, which leads to the generation of dehydrated ions [6–10,19,20]. In this study, the two

metabolites of α-pyrrolidinobutiophenone (α-PBP) selected as a model drug of α-PPs, had hydrogen and oxygen atoms eliminated during dehydration and these were identified using stable isotope (D or oxygen-18 (^{18}O))-labeled metabolites synthesized in our laboratory.

2 Experimental

2.1 Reagents

Cathinone, methcathinone, dimethylcathinone, α-PBP, 1-OH-α-PBP and 2″-oxo-α-PBP were synthesized according to the previously-reported methods [5,7]. Six stable isotope labeled compounds (Figure 2) were also synthesized in our laboratory according to the methods described below. All synthesized standards were confirmed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-high-resolution mass spectrometry. Deuterium oxide (99.9 %, for NMR) and methanol-d_4 (≥99.8 atom % D) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma-Aldrich Japan (Tokyo, Japan), respectively. 2-Pyrrolidinone-3,3,4,4,5,5-d_6 (99.6 %-d_6) and water-^{18}O (≥98 %-^{18}O) were obtained from C/D/N Isotopes (Pointe Claire, QC, Canada) and Taiyo Nippon Sanso (Tokyo, Japan), respectively. All other chemicals and reagents were of analytical grade and were obtained from Wako. Distilled water and LC/MS-grade methanol were used throughout the experiments.

2.2 Synthesis of stable isotope-labeled compounds

2.2.1 Synthesis of deuterium labeled compounds by H/D exchange method

The D-labeled cathinone (cathinone-d_2, Figure 2a), D-labeled methcathinone (methcathinone-*d*, Figure 2b) and D-labeled 1-OH-α-PBP (1-OD-α-PBP, Figure 2c) were simply synthesized using a following H/D exchange method: One milligram of each compound (cathinone, methcathinone, and 1-OH-α-PBP) was dissolve in one milliliter of methanol-d_4–deuterium oxide (1:4) solvent to substitute hydrogen atoms bonded nitrogen atom and oxygen atom with D atoms.

2.2.2 1-(1-oxo-1-phenylbutan-2-yl)pyrrolidin-2-one-3,3,4,4,5,5-d_6 (2″-Oxo-α-PBP-d_6)

2″-Oxo-α-PBP-d_6 was synthesized according to the methods used in our previous study [7] (Figure 3a): One drop of bromine in dichloromethane was added to a dichloromethane solution of butyrophenone (Wako). This solution was stirred for 5 min for the reaction to initiate. An equimolar amount of bromine solution was added over a period of a further 10 min. The solvent was removed under vacuum to yield 2-bromo-butyrophenone. To a solution of 2-bromobutyrophenone in tetrahydrofuran (THF; Wako) at 0 °C, a suspension of 2-pyrrolidinone-3,3,4,4,5,5-d_6 and sodium hydride (60 %, dispersion in paraffin liquid) in THF was added dropwise. The mixture was stirred at room temperature for 48 h. The reaction mixture was treated with 10 % hydrochloride aqueous solution to make it acidic and was then washed with diethyl ether. The aqueous layer was then made basic with 10 % sodium carbonate and extracted with ethyl acetate. The organic extract was washed with brine, dried over anhydrous sodium sulfate, and evaporated under vacuum. The resultant residue was subjected to column chromatography using a silica gel column and an ethyl acetate/*n*-hexane mixture (1:1, *v*/*v*) as an eluent to isolate 2″-oxo-α-PBP-d_6 as pale yellow oil.

2.2.3 1-(1-oxo-1-phenylbutan-2-yl-2-d)pyrrolidin-2-one (2″-Oxo-α-PBP-d)

2″-Oxo-α-PBP-*d* was synthesized by reference to the methods of Harbeson [21] as follows (Figure 3b): To a solution of butyrophenone in methanol-d_4, deuterium oxide was added slowly under nitrogen gas, followed potassium carbonate. The mixture was stirred at room temperature for 20 h. The mixture was concentrated *in vacuo* nearly to dryness and then partitioned between ethyl acetate and deuterium oxide. The organic layer was washed with saturated sodium chloride deuterium oxide solution, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain a colorless oil of butyrophenone-2,2-d_2.

One drop of bromine in dichloromethane was added to a dichloromethane solution of butyrophenone-2,2-d_2. This solution was stirred for 5 min for the reaction to initiate. An equimolar amount of bromine solution was added over a period of a further 10 min. The solvent was removed under vacuum to yield 2-bromobutyrophenone-2-*d*.

To a solution of 2-bromobutyrophenone-2-*d* in THF at 0 °C, a suspension of 2-pyrrolidinone and sodium hydride (60 %, dispersion in paraffin liquid) in THF was added dropwise. The mixture was stirred at room temperature for 48 h. The reaction mixture was treated with 10 % hydrochloride aqueous solution to make it acidic and was then washed with diethyl ether. The aqueous layer was then made basic with 10 % sodium carbonate and

extracted with ethyl acetate. The organic extract was washed with brine, dried over anhydrous sodium sulfate, and evaporated under vacuum. The resultant residue was subjected to column chromatography using a silica gel column and an ethyl acetate/*n*-hexane mixture (1:1, *v*/*v*) as an eluent to isolate 2″-oxo-α-PBP-*d* as pale yellow oil.

2.2.4 1-(1-(oxo-^{18}O)-1-phenylbutan-2-yl)pyrrolidin-2-one (2″-Oxo-α-PBP-^{18}O)

2″-Oxo-α-PBP-^{18}O was synthesized by modifying the methods of Attygalle et al. [22] as follows (Figure 3c): To a solution of 2″-oxo-α-PBP in THF, water-^{18}O and concentrated hydrosulfate were added, and the mixture was stirred at 50 °C for 12 h. The mixture was then made basic with potassium carbonate and extracted with ethyl acetate. The organic extract was washed with brine, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain a pale yellow oil of 2″-oxo-α-PBP-^{18}O.

2.3 Sample preparation

The stock standard solutions (1 mg/mL) of CATs and metabolites, and their stable isotope labeled compounds were stored in methanol or methanol-d_4 at −20 °C until analysis, and were diluted with water-methanol (4:1) or deuterium oxide-methanol-d_4 (4:1) solution to appropriate concentrations as needed for analysis with the mass spectrometer. The D-labeled compounds synthesized using an H/D exchange method were invariably stored in deuterium oxide and methanol-d_4, and were diluted with them as needed.

2.4 Instruments

Analysis was performed on an LTQ XL linear ion-trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an ESI interface. The single scan (MS) and product ion scan (MS^2 and MS^3) analysis was conducted in the positive mode, and the protonated and deuterated molecule of each compound were selected as precursor ions. MS^3 spectra of 2″-oxo-α-PBP and 2″-oxo-α-PBP-d_6 were obtained by selecting their dehydrated ions ($[M+H-H_2O]^+$; $[M-17]^+$) as precursor ions. Other conditions were as follows: sheath gas, nitrogen at flow rate of 35 arbitrary unit; source voltage, 4.0 kV; ion transfer capillary temperature, 270 °C; capillary voltage, 31 V; tube lens voltage, 100 V; isolation width, 1.0 Da; damping gas, helium; activation Q, 0.25; activation time 30 ms; and normalized collision energy, 35 %; wideband activation, disabled; infusion rate, 10 μL/min.

3 Results and Discussion

3.1 Generation of dehydrated ions from CATs with primary or secondary amines

In an ion trap instrument, such as the one used in this study, CID is accomplished by exciting the precursor ions at their resonant frequency. As the product ions have different mass-to-charge-ratio (*m/z*) from the precursor ion, they are not in resonance with the excitation frequency, and are not subjected to further ion fragmentation (single-step fragmentation). Therefore, the fragment pattern (product ion mass spectrum) obtained is hardly influenced by collision energy in CID. Under such analytical condition, we examined the generation mechanism of the dehydrated product ion ($[M+H-H_2O]^+$) from the protonated CATs ($[M+H]^+$). As shown in Figures 4a–4c, cathinone (primary amine) and methcathinone (secondary amine) both show dehydrated ions (*m*/*z* 132 and 146, respectively) as base peaks. As expected, the tertiary amine, dimethylcathinone, did not produce a dehydrated ion as a product ion. Considering that only primary and secondary amine-containing structures produced dehydrated ions, it was predicted, that one of two hydrogen atoms eliminated during dehydration is bonded to nitrogen (tertiary amines have no hydrogen bonded to nitrogen). Therefore, the synthesized *N*-deuterated compounds (Figures 2a and 2b) were analyzed under the condition of methanol-d_4-deuterium oxide. As shown in Figures 4d and 4e, each compound produced a product ion (*m*/*z* 133 or 146) corresponding to $[M+D-D_2O]^+$ as a base peak, while ions corresponding to $[M+D-HDO]^+$ or $[M+D-H_2O]^+$ were not detected. These results indicate that the hydrogen atom bonded to nitrogen was eliminated along with the protons added to the compound during ESI and suggest that CATs with primary and secondary amines undergo water elimination according to the reaction mechanism shown in Scheme 1. This finding demonstrates that dehydration does not occur in CATs with a tertiary amine structure and also shows that the presence or absence of dehydrated ions can be used to identify the amine structures of CATs.

3.2 Generation of dehydrated ions from the main metabolites of α-PBP (tertiary amine structure)

As described above, α-PPs have a tertiary amine structure and do not produce a dehydrated product ion [6–9,16,17,19,20]. However, dehydrated ions are generated from the two major metabolites of α-PPs, the 1-OH and 2″-oxo [6–9,19,20]. In this section, the dehydration mechanism of the metabolites of α-PBP, 1-OH-α-PBP and 2″-oxo-α-PBP (Figure

1b), were examined and discussed.

3.2.1 Dehydration of 1-OH-α-PBP

The product ion spectrum of α-PBP, shown in Figure 5a, did not contain a dehydrated ion (*m/z* 200); however, a dehydrated ion (*m/z* 202) was observed, as the base peak for 1-OH-α-PBP (diastereomers 1 and 2; Figures 5b and 5c). To identify the hydrogen atoms eliminated during dehydration in 1-OH-α-PBP, 1-OD-α-PBP (Figure 2c) was analyzed with a methanol-d_4-deuterium oxide solvent. In each case of diastereomers, the product ion corresponding to $[M+D-D_2O]^+$ (*m/z* 202) was observed as a base peak (Figures 5d and 5e), while ions corresponding to $[M+D-HDO]^+$ and $[M+D-H_2O]^+$ were not detected. These results indicate that upon dehydration of 1-OH-α-PBPs, the hydrogen atom on the hydroxy group was eliminated with the proton added during ESI. This suggests that water molecules were eliminated according to the reaction mechanism shown in Scheme 2. Therefore, the dehydration reaction of 1-OH metabolites of α-PPs proceeds without hydrogen atoms on the amine group, as the carbonyl group in the cathinone skeleton is reduced to a hydroxy group. Similarly, in dimethylcathinone with a tertiary amine structure, the reductive metabolite (1-OH metabolite, Figure 1b), methylephedrine (antitussive drug), generated a dehydrated ion ($[M+H-H_2O]^+$) in ESI-CID by the same dehydration mechanism (results not shown).

When comparing diastereomers 1 and 2, the dehydrated ion of diastereomer 1 (*m/z* 202) was observed with a strong intensity on the single scan mass spectrum (Figure 5b MS), which suggests that dehydration proceeds more readily in diastereomer 1 compared to diastereomer 2. Our previous study [7] showed that the diastereomer 1 and 2 of 1-OH-α-PBP are *syn*-form and *anti*-form isomers, respectively. The hydroxy group of diastereomer 1 (*syn* form) is therefore located in the three-dimensional position closer to the proton added to the amine group during ESI, compared to that of diastereomer 2 (*anti* form). The location of the hydroxyl group could contribute to more rapid dehydration of diastereomer 1.

3.2.2 Dehydration of 2″-oxo-α-PBP

2″-Oxo-α-PBP is an oxidative metabolite of α-PBP, and the oxidation of the 2″ position of pyrrolidine ring changes the structure from an amine to an amide (Figure 1b). In the product ion spectrum, the dehydration ion (*m/z* 214) was observed at low intensity (Figure 6a). In order to identify the oxygen and hydrogen atoms eliminated during dehydration, three

stable-isotope compounds (Figures 2d–2f) were synthesized and analyzed.

First, an ^{18}O-labeled compound (Figure 2f) was analyzed to identify the oxygen atom eliminated during dehydration. As shown in Figure 6b, the dehydrated ion corresponding to $[M+H-H_2{}^{18}O]^+$ was detected at *m/z* 214. This indicated that the oxygen atom in the alkyl carbonyl group was eliminated during dehydration, while the pyrrolidone ring was not involved in the reaction.

The pyrrolidone ring-deuterium labeled compound (Figure 2d) was dissolved in methanol-water solvent and analyzed to investigate the involvement of the pyrrolidone ring hydrogen atoms during dehydration. As shown in Figure 6c, the dehydrated ion corresponding to $[M+H-H_2O]^+$ was detected at *m/z* 220, indicating that the hydrogen atoms on the pyrrolidone ring were not involved in dehydration. In addition, the product ion spectrum produced from the dehydrated ion (*m/z* 220) included a pyrrolidone-d_6-eliminated ion (*m/z* 129), suggesting that the pyrrolidone ring was not involved in dehydration (Figure 6c MS^3).

Additionally, in order to identify which hydrogen atom becomes eliminated, an alkyl chain-deuterium labeled compound (Figure 2e) was dissolved either in methanol-water solvent or in methanol- d_4-deuterium oxide solvent (Figures 6d and 6e). As shown in Figure 6d, under methanol-water solvent condition, two dehydrated ions corresponding to $[M+H-HDO]^+$ and $[M+H-H_2O]^+$ were detected at *m/z* 214 and *m/z* 215, respectively, indicating that the hydrogen atom at position 2 of the alkyl chain was partially involved in the dehydration process. On the other hand, under methanol-d_4-deuterium oxide solvent condition, dehydrated ion corresponding to $[M+D-HDO]^+$ was detected at *m/z* 215 (Figure 6e). The fact that a hydrogen atom was pulled out of a deuterated molecule revealed that a hydrogen atom *other than* a) the hydrogen atom at position 2 of the alkyl chain and b) the proton added during ESI was eliminated during the dehydration reaction. Furthermore, to determine whether the proton added during ESI was involved in the dehydration process, 2″-oxo-α-PBP dissolved in methanol-d_4-deuterium oxide solvent was analyzed. The dehydrated ions corresponding to $[M+H-HDO]^+$ (*m/z* 214) and $[M+H-H_2O]^+$ (*m/z* 215) were detected as product ions, as shown in Figure 6f, and it indicates that the proton added during ESI is also partially eliminated during dehydration. Ratios of ion intensity of *m/z* 214 to that of *m/z* 215 shown in Figures 6d and 6f additionally suggested that the proton added during ESI is likely to be more involved in the dehydration reaction than the hydrogen atom at position 2 of the alkyl chain. Besides the dehydrated ions, product ions corresponding to

$[M+D-C_4H_7NO]^+$, loss of a pyrrolidine ring, were abundantly detected at *m/z* 149 and *m/z* 148, shown in Figures 6e and 6f, respectively. It is worth noting that these ions still contain deuterium atoms after losing the pyrrolidone ring from the deuterated molecule; this suggests that the deuterium ion added during ESI is exchanged from its initial position (most likely at the nitrogen atom), presumably moving to the cathinone backbone.

In summary, results from this study demonstrated that three or more hydrogen atoms (the hydrogen atom at position 2 of the alkyl chain, the proton added during ESI, and others) were eliminated with an oxygen atom in the alkyl carbonyl group during dehydration, while likely involving the rearrangement reaction of hydrogen atoms.

4 Conclusion

In this study, the generation mechanism of dehydrated ions for CATs during ESI-CID was investigated using synthesized stable-isotope labeled compounds. For CATs that have a primary or secondary amine structure, the hydrogen atom bonded to the nitrogen of the amine was found to be eliminated with the proton added during ESI and the carbonyl oxygen atom, which demonstrates that dehydration does not occur in CATs with a tertiary amine structure. This result provides evidence that dehydrated ions can be used to distinguish amine structures of CATs. However, the two major tertiary amine metabolites of CATs (1-OH and 2″-oxo) produce dehydrated ions as product ions. The dehydration of the 1-OH metabolite occurs by a simple mechanism involving the elimination of a hydroxyl group, while that of the 2″-oxo metabolite involves hydrogen atoms in three or more locations via multiple mechanisms. Further research on the intricate dehydration mechanism for 2″-oxo metabolites is in progress. The findings from this study will assist in the elucidation of reaction mechanisms in ESI-CID and provide significant contributions to the structural identification of abused drugs, including CATs.

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Fig. 1

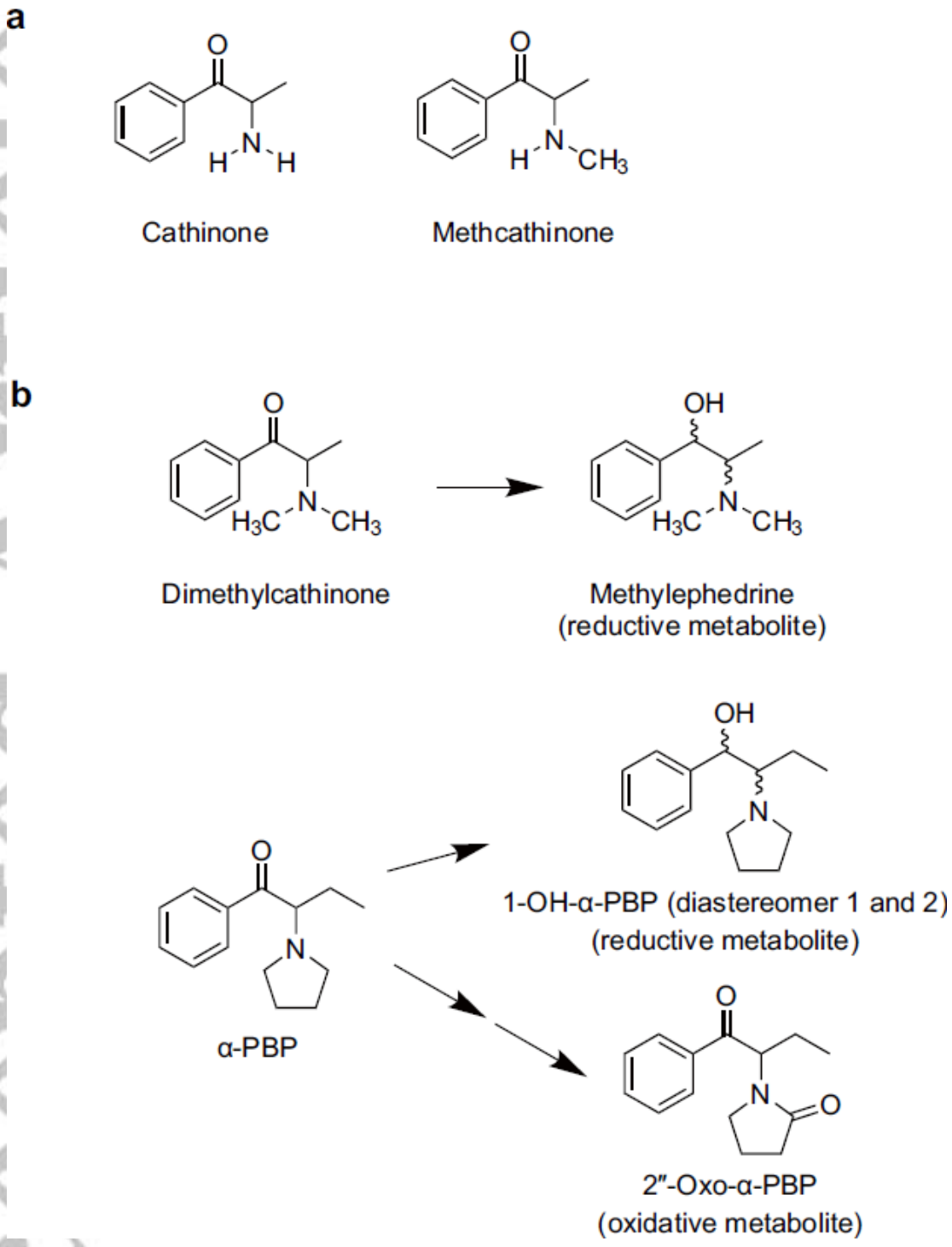


Figure 1 Chemical structures of (a) cathinone (primary amine) and methcathinone (secondary amine), and (b) dimethylcathinone (tertiary amine), α-PBP (tertiary amine) and their metabolites.

Fig. 2

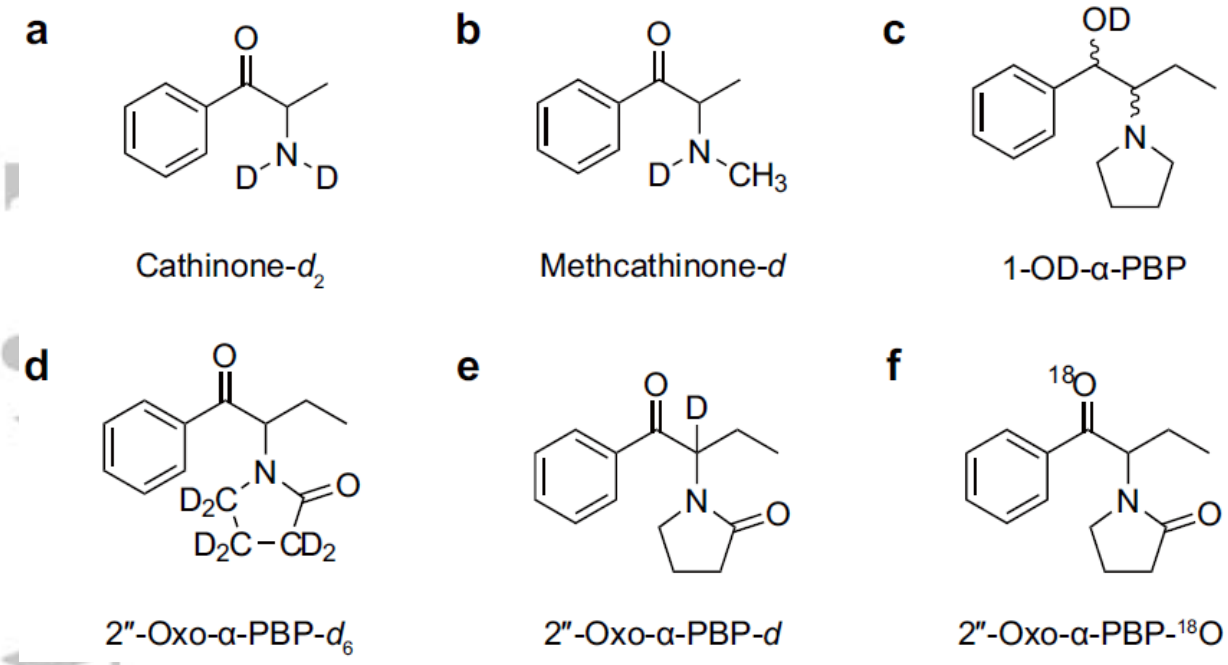


Figure 2 Stable isotope (D or ^{18}O)-labeled compounds synthesized in this study.

Fig. 3

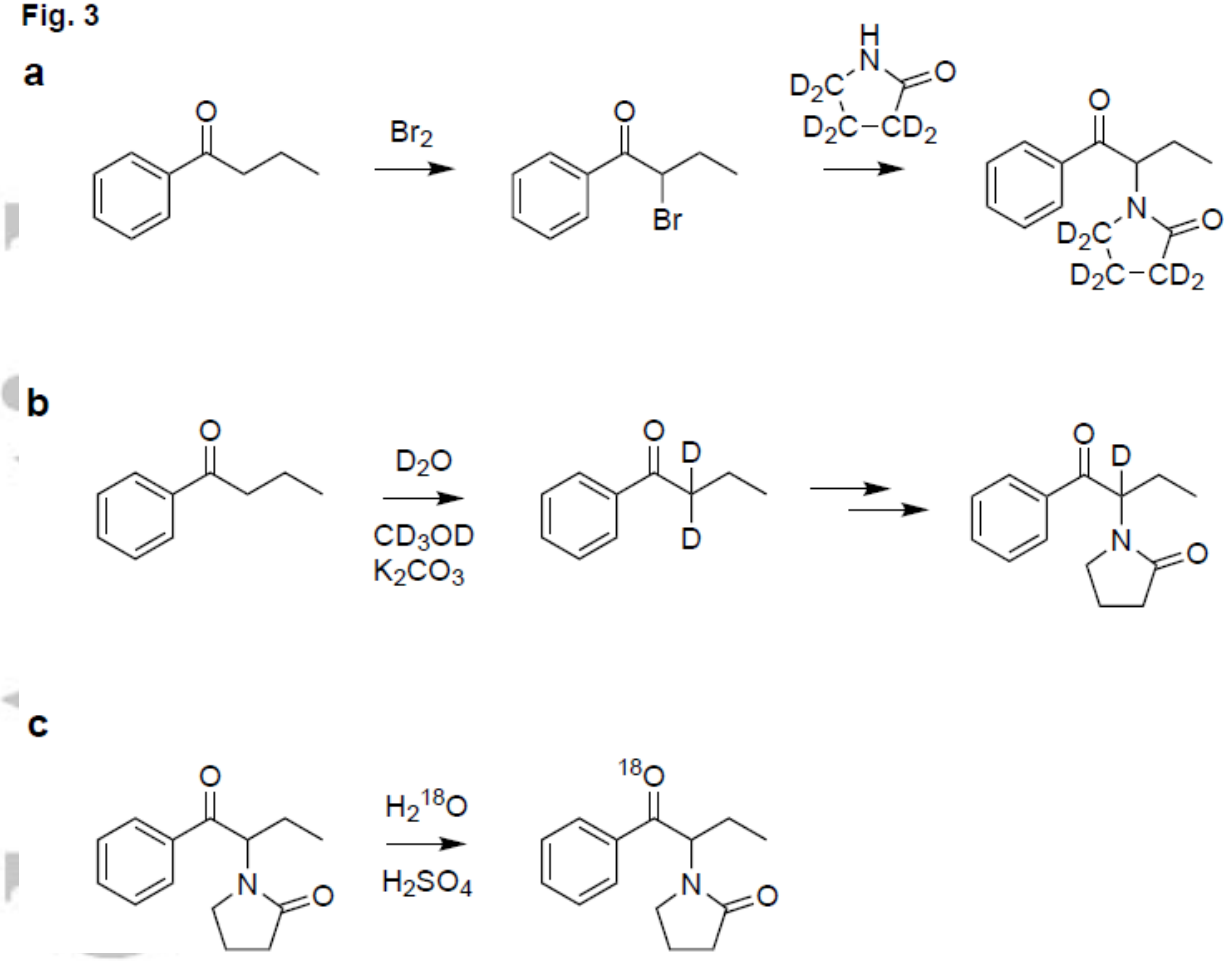


Figure 3 Synthetic pathways of stable isotope labeled compounds. (a) 2″-Oxo-α-PBP-d_6, (b) 2″-Oxo-α-PBP-*d*, (c) 2″-Oxo-α-PBP-^{18}O.

Fig. 4

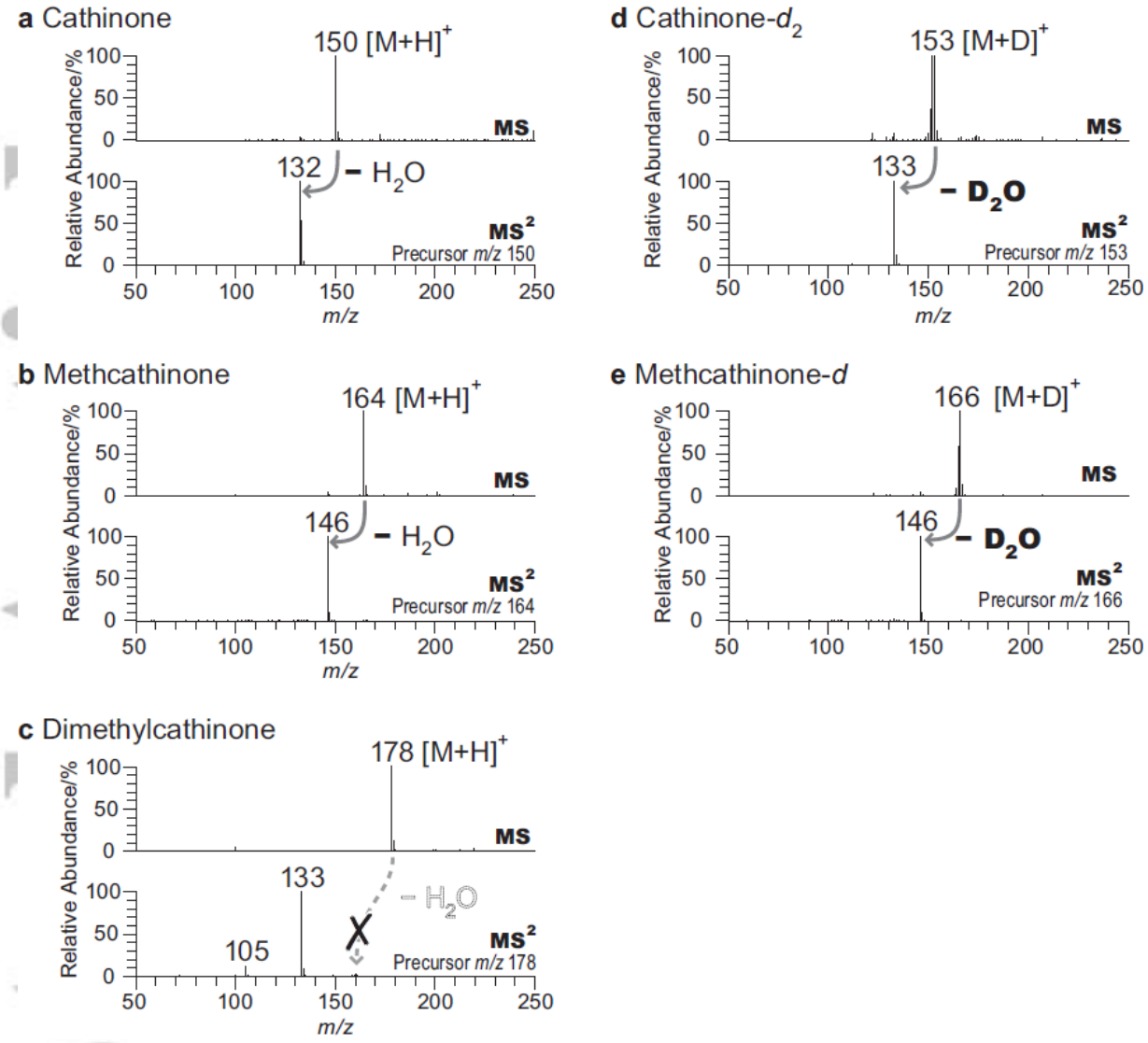


Figure 4 ESI-MS and ESI-CID-MS^2 spectra of (a) cathinone in water-methanol (4:1), (b) methcathinone in water-methanol (4:1), (c) dimethylcathinone in water-methanol (4:1), (d) cathinone-d_2 in deuterium oxide-methanol-d_4 (4:1), and (e) methcathinone-d in deuterium oxide-methanol-d_4 (4:1). Each protonated or deuterated molecule was selected as a precursor ion.

Fig. 5

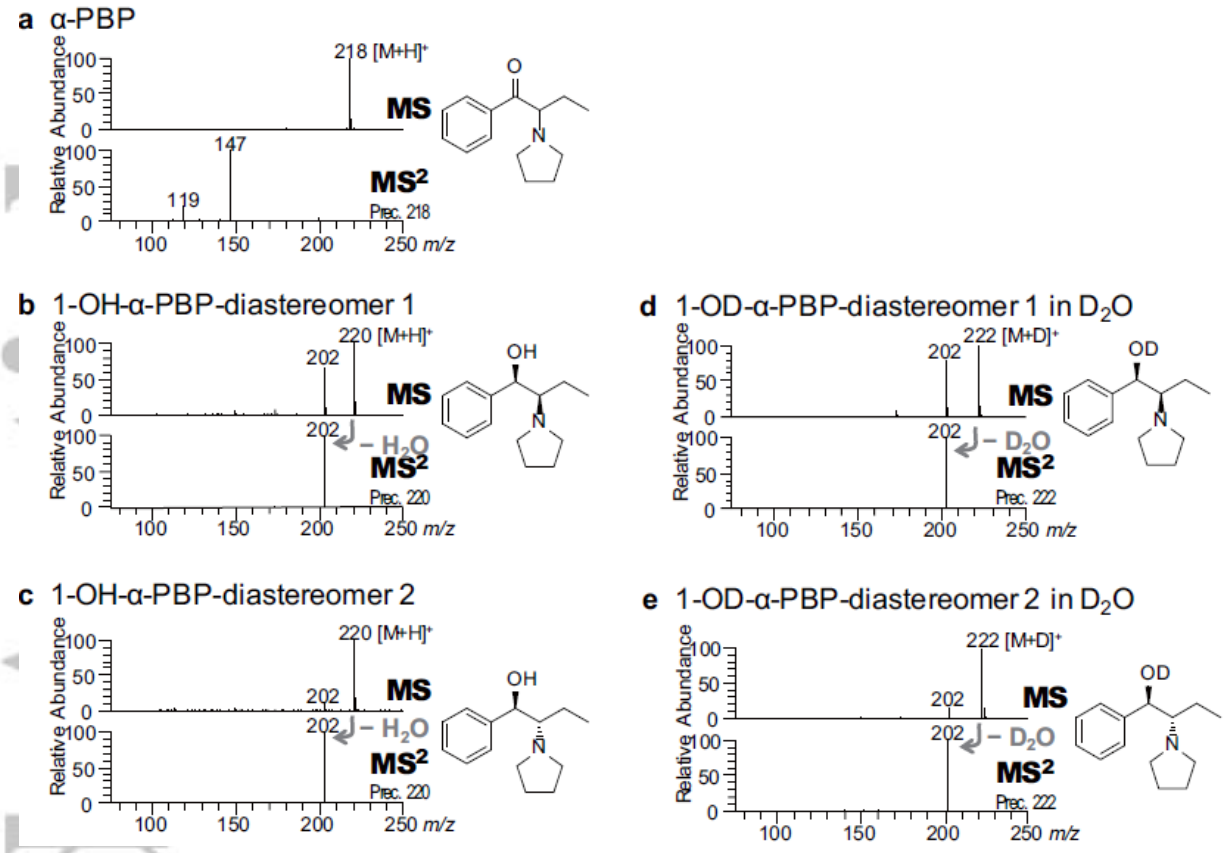


Figure 5 ESI-MS and ESI-CID-MS^2 spectra of (a) α-PBP in water-methanol (4:1), (b) 1-OH-α-PBP-diastereomer 1 in water-methanol (4:1), (c) 1-OH-α-PBP-diastereomer 2 in water-methanol (4:1), (d) 1-OD-α-PBP-diastereomer 1 in deuterium oxide-methanol-d_4 (4:1), and (e) 1-OD-α-PBP-diastereomer 2 in deuterium oxide-methanol-d_4 (4:1). Each protonated or deuterated molecule was selected as a precursor ion.

Fig. 6

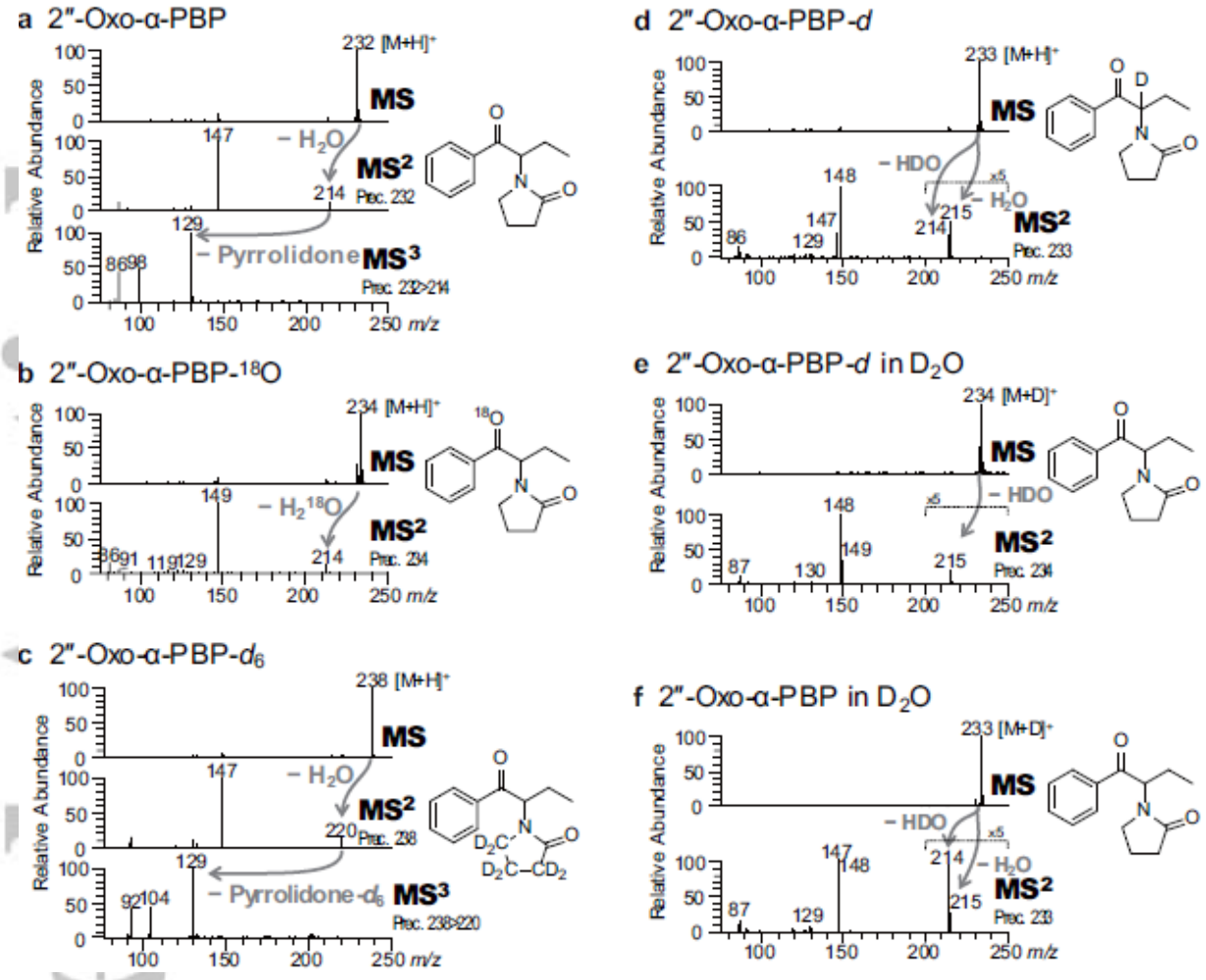
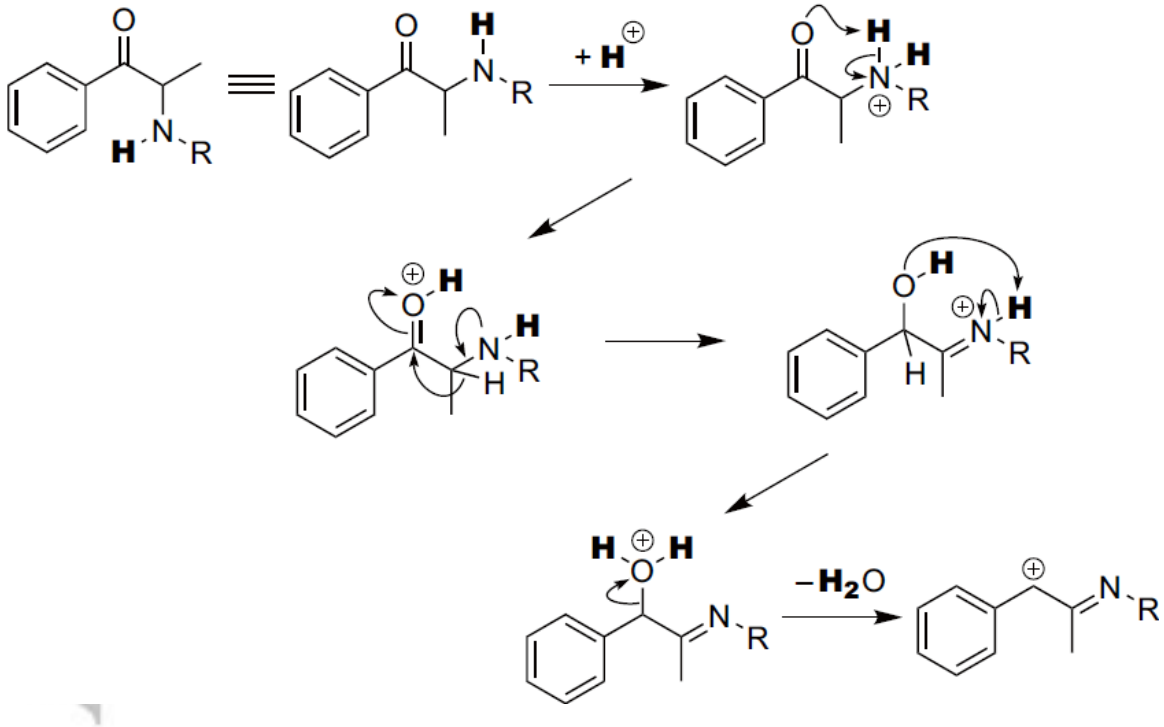


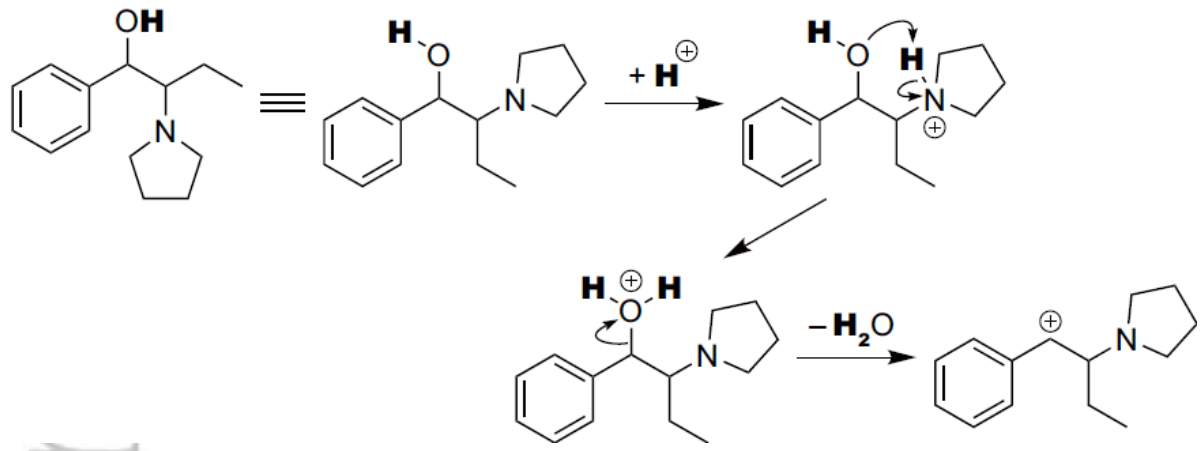
Figure 6 ESI-MS, ESI-CID-MS^2 and -MS^3 spectra of (a) 2″-oxo-α-PBP in water-methanol (4:1), (b) 2″-oxo-α-PBP-^{18}O in water-methanol (4:1), (c) 2″-oxo-α-PBP-d_6 in water-methanol (4:1), (d) 2″-oxo-α-PBP-*d* in water-methanol (4:1), (e) 2″-oxo-α-PBP-*d* in deuterium oxide-methanol-d_4 (4:1), and (f) 2″-oxo-α-PBP in deuterium oxide-methanol-d_4 (4:1). Each protonated molecule was selected as a precursor ion for MS^2, and the dehydrated ions $[M+H-H_2O]^+$ were selected as precursor ions for MS^3.

Scheme 1



Scheme 1 Proposed mechanism for generation of the dehydrated ion during ESI-CID for CATs with primary and secondary amines.

Scheme 2



Scheme 2 Proposed mechanism for generation of the dehydrated ion during ESI-CID for 1-OH-α-PBP (1-OH-α-PPs).