Research article

Drug Testing and Analysis

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Identification and characterization of *N-tert*-butoxycarbonyl-MDMA: a new MDMA precursor

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In September 2015, 80 litres of a viscous, light-red liquid, described as hair product, was seized by the Australian Border Force (ABF). Initial testing by ABF indicated that the liquid was the 3,4-methylenedioxymethamphetamine (MDMA) precursor chemical safrole and custody of the material was transferred to the Australian Federal Police (AFP) who coordinated all subsequent investigations. Initial gas chromatography–mass spectrometry (GC-MS) analysis by the AFP indicated that the material was not safrole and samples of the liquid were transferred to the National Measurement Institute Australia (NMIA) for identification. Using a combination of nuclear magnetic resonance spectroscopy (NMR), GC-MS, infrared spectroscopy, and synthesis, the unknown substance was identified as *N-tert*.-butoxycarbonyl-MDMA (*t*-BOC-MDMA). The substance was also converted in high yield to MDMA (aqueous HCl, 80 °C, 30 min). The possibility that the *t*-BOC-MDMA may act as a pro-drug following ingestion was explored by exposure to simulated gastric juice (pH 1.5) and monitored by NMR (37 °C) at various intervals. The majority of *t*-BOC-MDMA was converted to MDMA after 305 min, which suggested that this derivatized form might serve as a pro-drug *in vivo*. An investigation into the chemistry of potential pro-drugs showed that *t*-BOC derivatives of methamphetamine, pseudoephedrine and 4-methylmethcahtinone (mephedrone) could also be prepared using di-*tert*.-butyl dicarbonate. The appearance of *t*-BOC-derivatives on the drug market requires further monitoring.

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Keywords: MDMA; Ecstasy; precursor chemicals; NMR; *t*-BOC-MDMA

Introduction

Most 3,4-methylenedioxymethylamphetamine (MDMA) is clandestinely produced *via* reductive amination of 3,4-methylenedioxyphenyl-2-propanone (MDP-2-P) with methylamine. The reduction step is usually achieved by either (1) catalytic hydrogenation, (2) a hydride such as sodium borohydride, or (3) an amalgam reaction and the MDP-2-P itself is most often prepared from either safrole or piperonal.[1–4] All three chemicals are border-controlled precursors in Australia.[5] Historically, most MDMA used in Australia, either as powder or in the form of Ecstasy tablets, has been trafficked across the Australian border. However, domestic production of MDMA has occurred and according to the 2012/2013 Illicit Drug Data Report released by the Australian Crime Commission,[5] there were 12 seizures of MDMA precursors during that period although the total weight seized was less than 12 kg. During 2012/2013, seven MDMA clandestine laboratories were detected in Australia. Because clandestine MDMA production requires controlled precursor chemicals, attempts have been made in the past to chemically mask these substances to evade detection at the border. A major instance of this occurred in 2004 when approximately one metric ton of a precursor to MDP-2-P was seized at the Australian border. The substance, methyl 3-[3′,4′-(methylenedioxy)phenyl]-2-methyl glycidate, had never been encountered anywhere in the world before but was easily prepared in a custom synthesis in China from piperonal. This precursor was converted in high yield to MDP-2-P. However, unlike piperonal and MDP-2-P, it was not a controlled precursor chemical but rather a 'precursor to a precursor'.[6]

In September 2015, a consignment from China of eight crates each containing three bottles were seized by the Australian Border Force (ABF). The consignment was described as a hair product and contained approximately 80 litres of a viscous light-red liquid. Initial testing by the ABF indicated that the contents of the bottles were safrole and custody of the material was transferred to the Australian Federal Police (AFP) who coordinated all subsequent investigations. The AFP's National Forensic Rapid Laboratory (NFRL) undertook gas chromatography–mass spectrometry (GC-MS) analysis of the liquid, which revealed that the substance was not safrole. Unfortunately, retrospective searching of mass spectral libraries revealed no matches. However, GC-MS screening revealed two minor components

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as3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyphenyl-2-propanol (MDP-2-P-ol). NFRL scientists took a sample of the unknown liquid and treated it with dilute hydrochloric acid for 30 min at 80 °C resulting in a high yield of MDMA confirming that the seized substance was an MDMA precursor. Samples of this material were sent to the Australian Forensic Drug Laboratory (AFDL) at the National Measurement Institute (NMIA) for further analysis.

This paper describes the identification of the seized material as *N-tert.*-butoxycarbonyl-3,4-methylenedioxymethylamphetamine (*t*-BOC-MDMA) (Figure 1) by proton and carbon nuclear magnetic resonance spectroscopy (NMR), ATR-infrared analysis (IR), and GC-MS. The identification was confirmed by organic synthesis of *t*-BOC-MDMA from MDMA and di-*tert.*-butyl dicarbonate to facilitate the analytical comparison of the synthetic material with the seized material. In addition, exposure of *t*-BOC-MDMA to simulated gastric juice (pH 1.5) was monitored by NMR to consider the existence of a pro-drug and formation of MDMA. Analytical data obtained from the preparation of *t*-BOC-methamphetamine, *t*-BOC-pseudoephedrine and *t*-BOC-4-methylmethcathinone (mephedrone) have also been included.

Experimental section

Reagents and standards

All reference materials, internal standards, and surrogate standards used in the analysis of samples were obtained from the reference material collection of the NMIA (North Ryde, NSW, Australia). Di-*tert*-butyldicarbonate (Product #34660; ≥98%) was purchased from Fluka (Steinheim, Germany). 1,4-Dioxane (Product #: 360481), silica gel (200–400 mesh 60 Å) and octacosane (Product #O504-25G, 99%) were purchased from Sigma Aldrich (Castle Hill, NSW, Australia). Analytical grade ethyl acetate, chloroform, hexane, methanol and toluene were all obtained from Merck (Kilsyth, Vic, Australia). Sodium hydroxide pellets were purchased from UNIVAR Ajax Finechem (Seven Hills, NSW, Australia). Deuterochloroform (Product #DLM-7-100S, Lot #12 L-417) and dimethylsulfoxide-d_6 (Product #DLM-10-25, Lot #13B-030) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

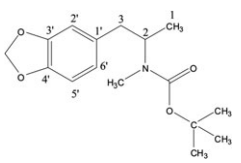
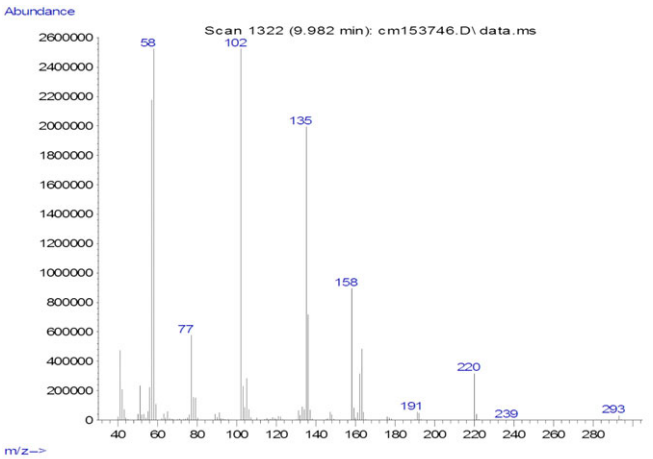


Figure 1. Chemical structure of N-tert-butoxycarbonyl MDMA (t-BOC-MDMA).



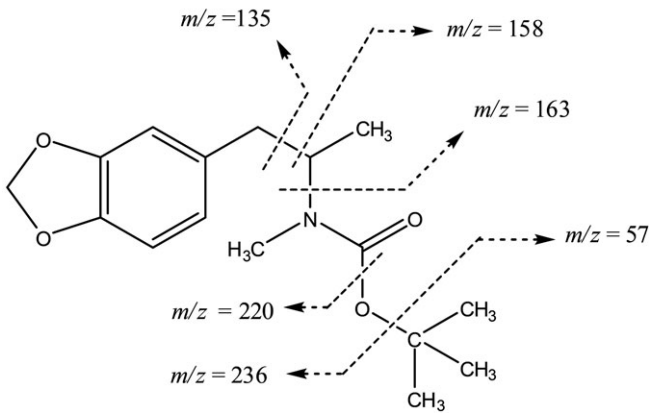


Figure 3. Mass Spectrum corresponding to the chromatographic peak at 9.98 minutes in the TIC of the seized material as obtained by electron ionization.

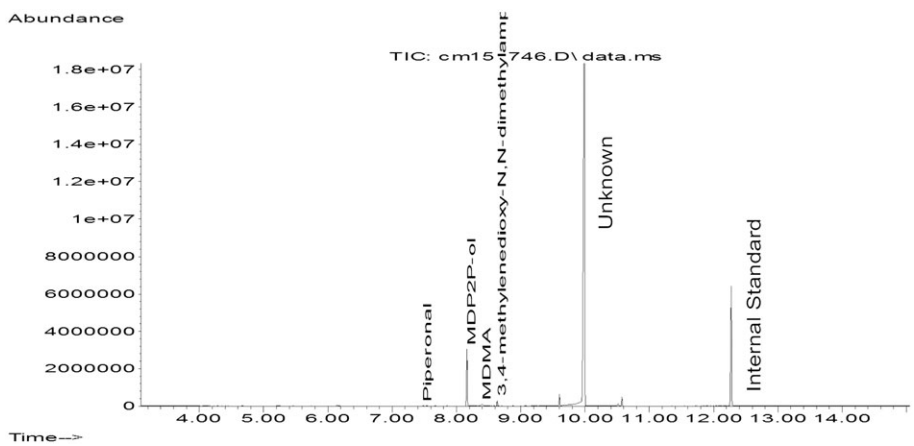


Figure 2. Total Ion Chromatogram (TIC) of seized material

Column chromatography

The seized material (600 mg) was chromatographed over silica, eluting with a mobile phase of ethylacetate:hexane (1:5). The fractions containing the compound of interest were combined and the solvent removed under vacuum to give a clear colourless oil (340 mg).

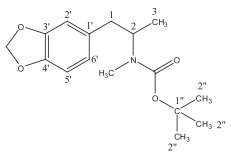
Preparation of the t-BOC derivatives of MDMA, methylamphetamine, pseudoephedrine, and 4-methylmethcathinone (mephedrone)

MDMA hydrochloride (190 mg) was dissolved in 1,4-dioxane (3 mL) and H_2O (1.5 mL) and the pH was adjusted to pH 8 with 30% aqueous NaOH solution. Di-*tert*.-butyldicarbonate (190 mg) was added and the mixture left to stir for 4 h at room temperature. The aqueous mixture was extracted with 3 x 50 mL chloroform. The combined chloroform extracts were dried over sodium sulfate and the solvent removed under vacuum, leaving behind a clear oil (195 mg, 80% yield). The oil was identified as *N-tert*.-butoxycarbonyl-MDMA (*t*-BOC-MDMA) and the mass spectral, ^{1}H NMR and ^{13}C NMR data matched that of the purified seized material. *N-tert*.-butoxycarbonyl-methylamphetamine, *N-tert*.-butoxycarbonyl-pseudoephedrine and *N-tert*.-butoxycarbonyl-4-methylmethcathinone were prepared in a similar manner to the *t*-BOC-MDMA.

Nuclear magnetic resonance spectroscopy

^{1}H NMR spectra of the seized material were acquired initially as solutions in $CDCl_3$ using a Bruker Avance 500 MHz NMR Spectrometer (11.76 T) equipped with a Bruker BBFO 5 mm Probe at a probe temperature of 295 K. Bruker TopSpin software was used to operate the NMR spectrometer and process raw data. A pulse program with a 90° pulse (13.2 μs), a relaxation delay of 5 s and an acquisition time of 4.4 s was used. Eight scans were acquired and the raw data collected over 15 ppm into 64 K data points. Phase correction and baseline correction were performed automatically and the integration width was established manually. ^{13}C NMR spectra were obtained at 125 MHz and 1024 scans were acquired with the raw data collected over 260 ppm into 64 K data points. A pulse program with a 30° pulse (3.3 μs), a relaxation delay of 2 s and an acquisition time of 4.4 s was used. Proton and carbon NMR spectra were also acquired in d_6-DMSO at 340 K using the experimental parameters described above for $CDCl_3$ solutions.

Table 1. ^{1}H NMR, ^{13}C NMR, ^{1}H/^{1}H COSY and ^{1}H/^{13}C HMBC Chemical Shifts



Position	^{13}C (ppm)	^{1}H (ppm) ^{1}H/^{13}C-HSQC Correlation	^{1}H/^{1}H COSY	^{1}H/^{13}C HMBC*
1	38.9	2.55 – 2.68, 2H, m	2	2, 3, 1′, 2′, 6′
2	51.7	4.25, 1H, m	1, 3	-
3	17.5	1.09, 3H, d, J = 6.8 Hz	2	1, 2
1′	132.7	N/A	N/A	-
2′	108.8	6.71, 1H, d, J = 1.5 Hz	6′	1, 3′, 4′, 6′
3′	146.8	N/A	N/A	-
4′	145.1	N/A	N/A	-
5′	107.5	6.77, 1H, d, J = 7.9 Hz	6′	1′, 3′, 4′ 6′
6′	121.4	6.62, 1H, dd, J =7.9 , 1.5Hz	2′, 5′	2′, 4′, 5′
N-CH_3	27.5	2.62, 3H, s	N/A	2, −C = O
2″	27.7	1.31, 9H, s	N/A	1″
1″	77.8	N/A	N/A	-
-O-CH_2-O-	100.2	5.92, 2H, s	N/A	3′, 4′
C = O	154.3	N/A*	N/A	N-CH_3

s = singlet; d-doublet; dd = doublet of doublets; m = multiplet
Proton-Carbon ^1J Correlation.
*^2J and ^3J long range coupling between protons indicated in the Position Column and correlated carbon atoms.

Gas chromatography–mass spectrometry

Analyses were performed on an Agilent 6890 N GC interfaced with an Agilent 5973 MSD. A 30 m x 0.25 mm x 0.25 μm HP-5MS column was employed using helium carrier gas in the constant flow rate mode. Injection port temperature was 240 °C and the MS interface temperature was 300 °C. The oven temperature program was 55 °C (hold for 3 min), ramped at 30 °C/min to 300 °C (no hold), and ramped at 20 °C/min to 325 °C (hold for 3 min). Injections (1 μL) were made in pulsed splitless mode and a mass range of *m/z* 40 to 500 was scanned.

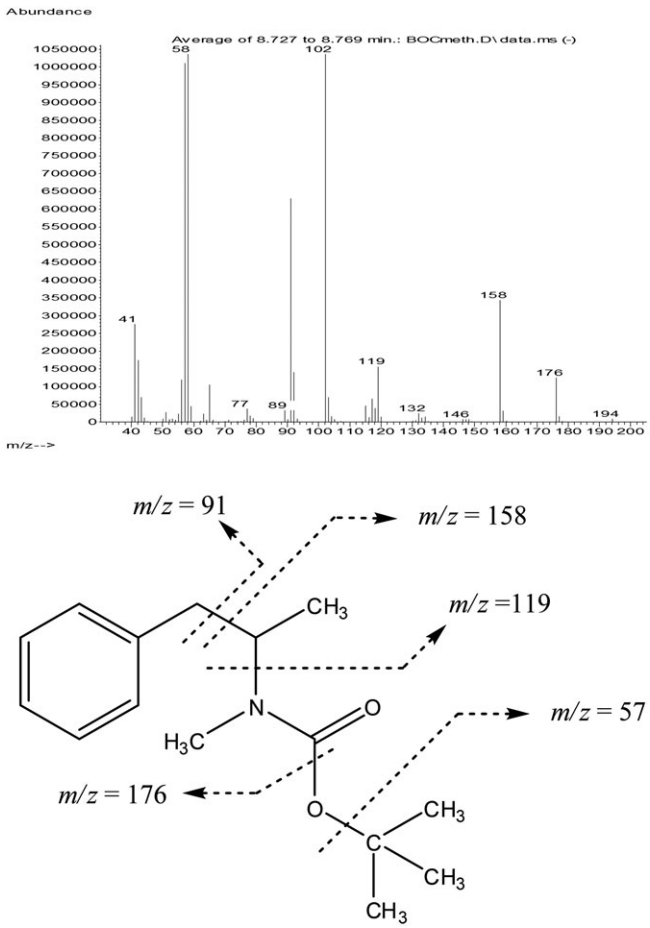


Figure 4. Mass Spectrum of the methylamphetamine BOC derivative as obtained by electron ionization.

Infra-red spectroscopy

All FTIR spectra were obtained on a Bruker ALPHA ATR Platinum Diamond instrument. Thirty-two background scans were acquired and the scan truncation range was 400–4000 cm^{-1}. Thirty-two scans of the sample were acquired in transmittance mode. A small amount of sample (~2 mg) was placed on the diamond ATR and the clamp engaged. FTIR spectra of *t*-BOC-MDMA, *t*-BOC-methylamphetamine, *t*-BOC-pseudoephedrine and *t*-BOC-4-methylmethcathinone are shown in the Supporting Information (Figures S1 to S4, respectively).

Results and discussion

When the seized material was transferred to NMI for identification, its GC-MS trace was recorded again and its total ion chromatogram (TIC) is shown in Figure 2. The TIC exhibited a major chromatographic peak at 9.98 min and three smaller chromatographic peaks at 7.47, 8.15, and 8.39 min, respectively. The mass spectrum corresponding to the chromatographic peak at 9.98 min is shown in Figure 3 and it was obvious from this spectrum that the seized material was not safrole and that this compound had a possible molecular ion at *m/z* 293. Retrospective searching of mass spectral libraries revealed no matches. The smaller peaks at 7.47, 8.15, and 8.39 min had mass spectra consistent with piperonal, MDP-2-P-ol, and MDMA, respectively. The presence of *m/z* fragments of 58 and 135 in the mass spectrum corresponding to the chromatographic peak at 9.98 min and the presence of minor amounts of piperonal and MDP-2-P in the sample was indicative that the seized material contained a 3,4-methylenedioxyphenyl moiety. The presence of a major ion at *m/z* 57 also indicated the possibility of a tertiary butyl group.

Analysis by thin layer chromatography revealed the presence of five components in the seized sample. A 600 mg portion was subjected to column chromatography over silica gel to yield a colourless oil (340 mg) followed by proton NMR analysis in $CDCl_3$ at 295 K (Table 1, Figure S5). The proton resonances in the NMR spectrum exhibited poor definition indicating the potential presence of distinct rotamers (Supporting Information). Consequently, proton and carbon NMR spectra were recorded in d_6-DMSO at 340 K (66.8 °C) (Table 1, Figures S6 and S7), which resulted in peak sharpening at elevated this temperature. All subsequent NMR spectra were obtained in d_6-DMSO at 340 K.

The three aromatic resonances (6.6–6.8 ppm) and the singlet integrating for two protons (5.9 ppm) were consistent with a 3,4-methylenedioxyphenyl moiety. The resonance at 6.7 ppm showed an 8.0 Hz coupling to the proton at 6.6 ppm consistent with these two protons being *ortho* to each other. The proton at 6.6 ppm also displayed a *meta* coupling of 2.3 Hz to the proton at 6.7 ppm. The

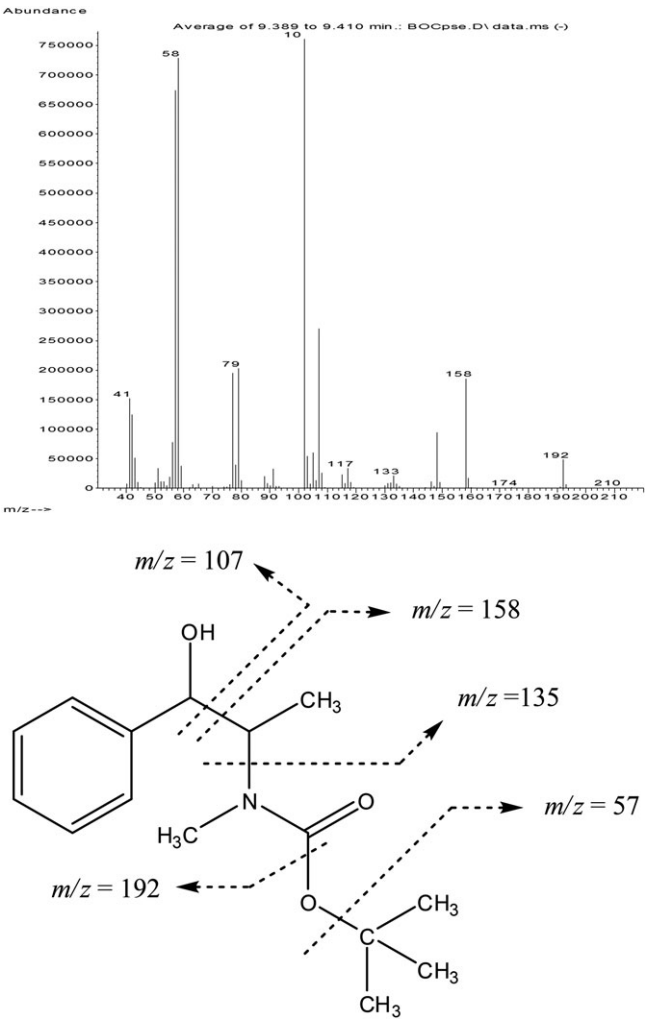


Figure 5. Mass Spectrum of the pseudoephedrine BOC derivative as obtained by electron ionization.

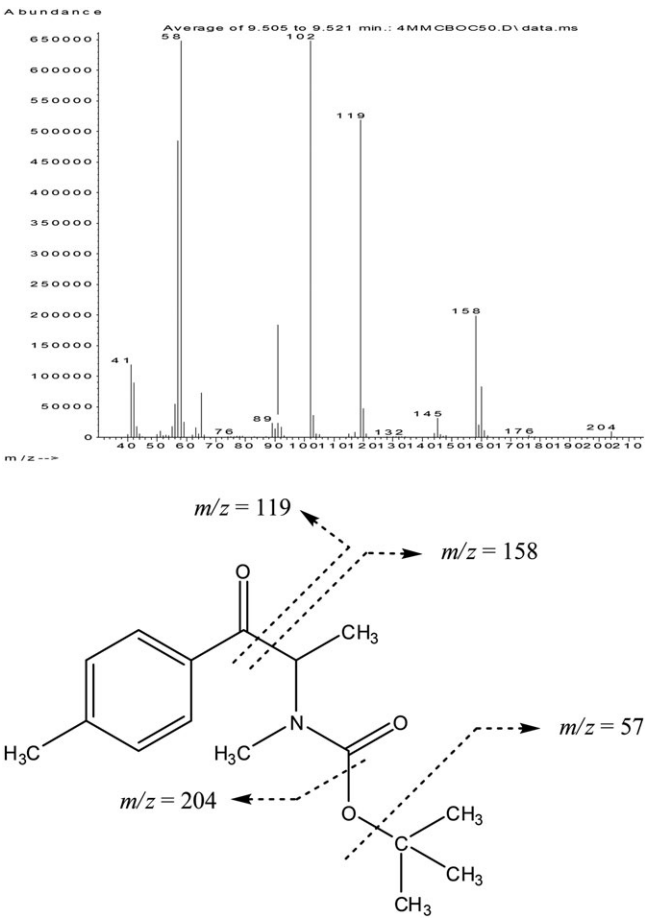


Figure 6. Mass Spectrum of the 4-methylmethcathinone BOC derivative as obtained by electron ionization.

singlet at 1.3 ppm integrating for nine protons was identified as a *tertiary*-butyl group. The doublet integrating for three protons at 1.1 ppm together with the broad multiplet integrating for one proton at 4.2 ppm was consistent with a terminal methyl group *alpha* to a methine proton. Between 2.5 and 2.7 ppm a coincidental singlet and multiplet integrating for 5 protons was observed and this was attributed to an *N*-methyl group resonating at approximately the same frequency with two benzylic protons. The carbon NMR spectrum exhibited 14 resonances including one at 154 ppm consistent with a carbonyl group belonging to an ester or an amide group. Based on the NMR data and a possible molecular ion at *m/z* 293, the structure shown in Figure 1 was postulated as the major constituent of the seized material, i.e., the *tertiary*-butoxycarbonyl derivative MDMA (*t*-BOC-MDMA). To confirm that the seized unknown was *t*-BOC-MDMA, an authentic MDMA sample was derivatized using di-*tert*-butyl dicarbonate and the mass spectra and NMR spectra of the resulting product were compared with the corresponding spectra of the seized unknown material. The mass spectrum and ^{1}H NMR spectrum of the product prepared in the author's laboratory matched the corresponding spectra of the unknown seized material and confirmed that the seized material was *t*-BOC-MDMA as shown in Figure 1.

It is conceivable that similar protecting group chemistry might be employed to conceal other drugs or their precursors. To illustrate this facile conversion, the preparations of the *t*-BOC derivatives of methylamphetamine, its precursor pseudoephedrine and 4-methylmethcathinone were carried out. The mass spectral data for each of these compounds is provided in Figures 4 to 6, respectively. Most of the observed fragments are easily explicable with the exception of the base peak *m/z* 102. As this ion is present in the mass spectra of all derivatives investigated, it was considered reasonable that must have arisen from *tertiary* butoxycarbonyl moiety. A possible explanation for the *m/z* 102 is given in Figure 7.

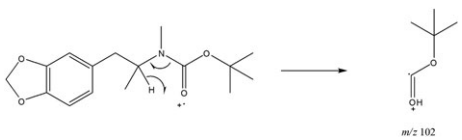


Figure 7. Proposed fragmentation leading to the m/z 102.

A series of two-dimensional (2D) NMR experiments including heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC), and distortionless enhancement by polarization transfer (DEPT) were performed to assign all carbon chemical shifts and provide further confirmation of identity. All the experiments were performed on samples of the seized material following chromatographic clean-up, in d_6-DMSO at 340 K. Proton and carbon chemicals shifts complete with long range correlations are presented in Table 1. The raw 2D NMR data is provided in the supplementary material.

The use of di-*tert*.-butyl dicarbonate to form *tert*-butoxycarbonyl amide (*t*-BOC) derivatives is commonplace in organic synthesis particularly in amino acid chemistry.[7–9] *t*-BOC derivatives of amines are easily formed and are stable thereby allowing further chemical modifications to be performed on other parts of the molecule without damaging the amino moiety. The *t*-BOC group is also easily removed in high yield by treatment with dilute acid at room temperature.[7–9] This seizure in 2015 was the first time that the *t*-BOC derivative of MDMA has been detected by law enforcement in a drug seizure in Australia. The *t*-BOC derivative of MDMA has not been seen in a seizure since then in Australia and it is possible that this was a one-off custom synthesis. However, Westphal *et al.* reported in 2016 a seizure by police authorities in North-Rhine Westphalia, Germany of the *t*-BOC-MDMA impregnated on silica.[10]

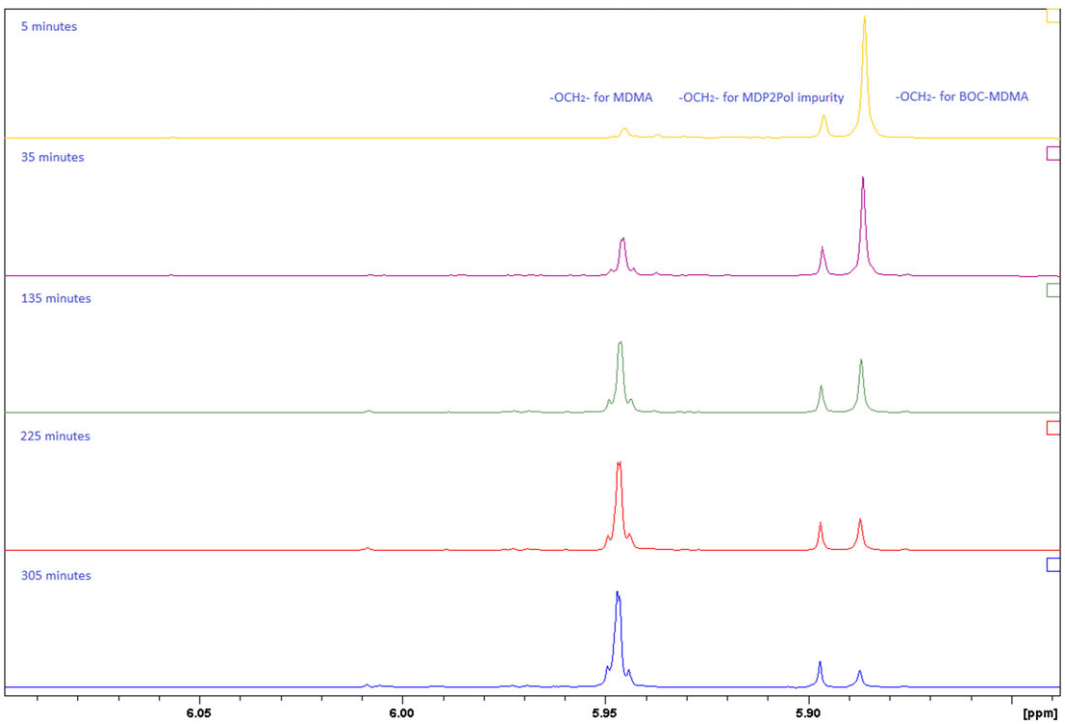


Figure 8. NMR observation of N-tert-butoxycarbonyl MDMA at pH1.5, T = 37 °C, over time.

Previous studies[11–13] have shown that carbamate derivatives of some phenethylamines can act as pro-drugs, thereby altering the levels of these amines in the brain. The possibility that the *t*-BOC derivative of MDMA might act as a pro-drug following ingestion was explored. A simulated gastric juice at pH 1.5 was prepared from d_4-methanol and D_2O chloride solution. The *t*-BOC MDMA was added to 1 mL of the 'gastric juice', vortex mixed, and 0.65 mL was transferred to a NMR tube which was immediately placed into the NMR probe at 310 K (37 °C). A spectrum was recorded as soon as the sample had been locked and shimmed which was 5 min after dissolution. Additional spectra were recorded at various intervals for the following 5 h. Figure 8 shows the methylenedioxy region of the proton NMR spectrum. It can be seen that the -O-CH_2-O- resonance corresponding to *t*-BOC MDMA decreased in size while the -O-CH_2-O- resonance of the MDMA product increased during the same period. By t = 305 min at pH 1.5 and T = 37 °C the majority of the *t*-BOC-MDMA had undergone conversion to MDMA. While these conditions do not reproduce the biochemical state of a human stomach during digestion it seems conceivable that ingestion of the *t*-BOC MDMA might result in a slow release of MDMA formation *in vivo*. The toxicity or extent of psychoactivity in humans is currently unknown.

As a means of masking identity to facilitate trafficking it was a good choice as the probability of it being detected using a portable device such as a hand-held Raman, mass spectral or infra-red spectrometer would be low because as with all new substances such as cathinones and synthetic cannabinoids no reference spectrum was available. Even a low-field bench-top NMR spectrometer would be of little use because the unknown was a mixture of compounds and rotamers that were observed in the ^{1}H NMR spectrum at 295 K and would therefore hinder a positive identification.

Conclusion

Through a combination of synthetic and spectroscopic techniques, the identity of the major component of the seized material in a border seizure was established as *N-tert*-butoxycarbonyl MDMA. ^{1}H NMR and ^{13}C NMR spectra and mass spectra of the synthesized material and the seized material matched each other and it is believed that this is the first time that MDMA concealed as the *tertiary*-butoxycarbonyl derivative has been detected by law enforcement agencies in the world. This is yet another example of an illicit drug being chemically 'masked' in an attempt to circumvent laws concerning the importation of border-controlled substances. In this case, those responsible relied on the use of an established protecting group, widely employed in organic chemistry in the knowledge that routine forensic testing by border authorities was unlikely to detect a substance for which no reference material existed. This case also highlights once again the issues facing forensic chemists trying to identify a novel substance, be it a drug or a precursor, for which no reference mass spectrum or certified reference material exists.

References

[1] T. A. Dal Cason. An evaluation of the potential for clandestine manufacture of 3, 4-Methylenedioxyamphetamine (MDA) analogs and homologs. *J. Forensic Sci.* **1990**, *35*, 675.
[2] A. M. A. Verweij. Clandestine manufacture of 3,4-methylenedioxymethamphetamine (MDMA) by low pressure reductive amination. A mass spectrometric study of some reaction mixtures. *Forensic Sci. Int.* **1990**, *45*, 91.
[3] M. M. van Deursen, E. R. A. Lock, A. J. Poortman-van der Meer. Organic impurity profiling of 3,4-methylenedioxymethylamphetamine (MDMA) tablets seized in the Netherlands. *Sci. Justice.* **2006**, *43*, 135.
[4] A. M. A. Verweij. Impurities in illicit drug preparations: 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine. *Forensic Sci. Rev.* **1992**, *4*, 137.
[5] Australian Crime Commission. Illicit Drug Data Report 2012–13. [Online]. ACC (**2016**). Available at: http://webarchive.nla.gov.au/gov/20160615122807/http://www.crimecommission.gov.au/publications/intelligence-products/illicit-drug-data-report/illicit-drug-data-report-2012-13 [5 August 2016]
[6] M. Collins, A. Heagney, F. Cordaro, D. Odgers, G. Tarrant, S. Stewart. Methyl 3-[3', 4'-(methylenedioxy)phenyl]-2-methyl glycidate: An ecstasy precursor seized in Sydney, Australia. *J. Forensic Sci.* **2007**, *52*, 898.
[7] L. A. Carpino. Oxidative reactions of hydrazines. II. Isophthalimides. New protective groups on nitrogen. *J. Am. Chem. Soc.* **1957**, *79*, 98.
[8] T. W. Greene, P. G. M. Wutts. *Protective Groups in Organic Synthesis*, 3rdEdnJohn Wiley & Sons, New York**1999**.
[9] M. Wakselman. in *Encyclopedia of Reagents for Organic Synthesis*, (Ed: L. Paquette)John Wiley & Sons, New York Ed:**2004**.
[10] F. Westphal, U. Girreser, K. Holz, M. Erkens. Strukturaufklärung und analytische Daten eines ungewöhnlichen MDMA-Derivates. *Toxichem Krimtech.* **2016**, *83*, 92.
[11] A. J. Verbiscar, L. G. Abood. Carbamate ester latentiation of physiologically active amines. *J. Med. Chem.* **1970**, *13*, 1176.
[12] T. Siatra-Papastaikoudi, Z. Papadopoulou-Daifoti, C. Spyraki, D. Varonos. Synthesis of carbamate esters of phenethylamines and their pharmacological action on the central nervous system. *Chim. Chron.* **1981**, *10*, 307.
[13] G. B. Baker, R. T. Coutts, A. J. Nazarali, T. J. Danielson, M. Rubens. Carbamate prodrugs of phenylethylamines: a neurochemical investigation. *Proc. West. Pharmacol. Soc.* **1984**, *27*, 523.

Supporting information

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