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Characterization of the synthesis of *N*,*N*-dimethyltryptamine by reductive amination using gas chromatography ion trap mass spectrometry

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The present study established an impurity profile of a synthetic route to the hallucinogenic *N*,*N*-dimethyltryptamine (DMT). The synthesis was carried out under reductive amination conditions between tryptamine and aqueous formaldehyde in the presence of acetic acid followed by reduction with sodium cyanoborohydride. Analytical characterization of this synthetic route was carried out by gas chromatography ion trap mass spectrometry using electron- and chemical-ionization modes. Methanol was employed as a liquid CI reagent and the impact of stoichiometric modifications on side-products formation was also investigated. Tryptamine 1, DMT 2, 2-methyltetrahydro-β-carboline (2-Me-THBC, 3), *N*-methyl-*N*-cyanomethyltryptamine (MCMT, 4), *N*-methyltryptamine (NMT, 5), 2-cyanomethyl-tetrahydro-β-carboline (2-CM-THBC, 6) and tetrahydro-β-carboline (THBC, 7) have been detected under a variety of conditions. Replacement of formaldehyde solution with paraformaldehyde resulted in incomplete conversion of the starting material whereas a similar replacement of sodium cyanoborohydride with sodium borohydride almost exclusively produced THBC instead of the expected DMT. Compounds 1 to 7 were quantified and the limits of detection were 28.4, 87.7, 21.5, 23.4, 41.1, 36.6, and 34.9 ng mL^{-1}, respectively. The limits of quantification for compounds 1 to 7 were 32.4, 88.3, 25.4, 24.6, 41.4, 39.9, and 37.0 μg mL^{-1}, respectively. Linearity was observed in the range of 20.8–980 μg mL^{-1} with correlation coefficients >0.99. The application holds great promise in the area of forensic chemistry where development of reliable analytical methods for the detection, identification, and quantification of DMT are crucial and also in pharmaceutical analysis where DMT might be prepared for use in human clinical studies.

Keywords: hallucinogens; clinical; forensic; pharmaceutical; profiling

Introduction

Many bioactive compounds are structurally based on the tryptamine **1** nucleus (Figure 1). Although tryptamine itself does not appear to be active in man, a variety of simple modifications give rise to a large number of psychoactive derivatives. One such derivative is *N*,*N*-dimethyltryptamine (DMT) **2** (Figure 1). DMT shows hallucinogenic properties when either inhaled as the free base[1,2] or when, for example, injected intravenously as the appropriate salt.[3,4] In order to render DMT orally active, co-administration of a suitable monoamine oxidase A inhibitor is required.[5,6] DMT is a Schedule 1 drug that is abundantly available in the plant kingdom.[7,8] It can also be easily prepared by a variety of synthetic routes.[9,10] Increased availability of drug-related information on the Internet and the fact that DMT is able to induce powerful altered states of consciousness in humans resulted in increased popularity within recreational communities and, in recent years, it also became a target for intense human clinical studies.[11–16] The pharmacology of DMT and related derivatives is complex but current knowledge points towards the involvement of 5-HT_{2A} and 5-HT_1 receptor subtypes.[17–19] Recent findings also suggested that DMT served as an agonist at the sigma-1 receptor[20] and that it displayed substrate-like properties at both the plasma membrane serotonin transporter and the vesicle monoamine transporter, respectively.[21]

A large number of synthetic routes can be used for the preparation of DMT and structurally related antimigraine triptan derivatives. A commonly used synthetic route for large-scale preparation of DMT derivatives involves the reductive amination between formaldehyde and the tryptamine starting material in acidic media, often carried out in methanol. A reducing agent, often $NaBH_3CN$, enables the reduction of an intermediate imine or iminium salt.[22] This traditionally used $CH_2O/NaBH_3CN$/acetic acid/methanol system has also been used for the synthesis of triptan-type antimigraine compounds that are also derivatives of DMT but formation of impurities was not reported.[23,24]

The identification of potentially toxic and route-specific byproducts is of fundamental importance when preparing pharmaceu-

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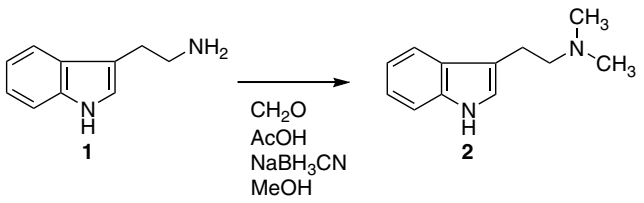

Figure 1. Structural representations of tryptamine **1** and DMT **2** obtained from a reductive amination procedure.

tical ingredients or products for human clinical studies, either by characterization of the bulk drug or by implementation of stability-indicating methodologies.[25] Gas chromatography mass spectrometry (GC-MS) has been commonly employed for the successful detection of DMT in a variety of bioanalytical or plant matrices. Examples include investigations on DMT metabolism in whole rat brain homogenates[26] and detection in DMT-containing plant, i.e. *Ayahuasca*, extracts.[27]

A method that combined ion mobility spectrometry (IMS) and GC-MS successfully determined the presence of the DMT derivatives psilocybin and psilocin in *Psilocybe subcubensis* mushrooms.[28] Additional examples include the detection of so-called designer tryptamines and phenylethylamines in urine and blood samples using GC-MS and high performance liquid chromatography electrospray MS[29] and identification of 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT, *Foxy*) in urine samples using GC-MS.[30] All these studies accentuate the need for developing accurate and reliable methods for detection and identification of hallucinogenic derivatives.

Recently, there has been renewed interest in human clinical studies and increased availability of drug-related information on the internet that is attracting great interests from recreational, clandestine and scientific communities. In addition, recent additions of several designer tryptamines to the Drug Enforcement Administration (DEA) schedule of controlled substances necessitate the development of analytical procedures for the detection and quantification of these compounds. Increased availability of DMT-containing plant products from internet websites, and the fact that illegally manufactured preparations of DMT and analogues have not yet been analytically characterized in much detail[31], makes it necessary to provide these data in the attempt to serve clinical, forensic and public health communities.

The work reported in this study was designed to provide an analytical characterization of a synthetic route to DMT **2** based on reductive amination combined with gas chromatography ion trap mass spectrometry (GC-ITMS). Ionization methods used involved electron ionization (EI-MS) and low-pressure chemical ionization (CI-MS) with internal ionization using methanol as the CI reagent. Furthermore, it was intended to gain further insights into the extent of side product formation under a variety of stoichiometric modifications. Identification and quantification of byproducts were supported by organic synthesis of the target molecules. For the first time the characterization of this synthetic route included the determination of limit of detection (LOD) and limit of quantification (LOQ) for starting material, product and byproducts, i.e. tryptamine (**1**), DMT (**2**), 2-methyltetrahydro-β-carboline (2-Me-THBC, **3**), *N*-methyl-*N*-cyanomethyltryptamine (MCMT, **4**), *N*-methyltryptamine (NMT, **5**), 2-cyanomethyl-THBC (2-CM-THBC, **6**) and tetrahydro-β-carboline (THBC, **7**), respectively.

Experimental

Materials

All solvents and reagents were either of high performance liquid chromatography (HPLC) grade, analytical grade, or equivalent. Tryptamine **1** (98%), sodium cyanoborohydride ($\geq$95%), sodium borohydride ($\geq$98%), tetrahydro-β-carboline (THBC,**7**) (98%) and all other solvents and reagents used for the synthesis of standards were purchased from Aldrich (Dorset, UK) and were of the highest grade available. Formaldehyde solution (39% w/v) was obtained from VWR (Leicestershire, UK). *N*,*N*-dimethyltryptamine (DMT, **2**), 2-methyltetrahydro-β-carboline (2-Me-THBC, **3**), and *N*-methyltryptamine (NMT, **5**) have been previously synthesized and were available as standards from previous work.[32,33]

Instrumentation

Samples were subjected to both electron ionization (EI) and chemical ionization (CI) modes. Both EI and CI mass spectra (scan range m/z 40–m/z 500) were obtained on a Varian 220-MS ion trap MS equipped with a Varian 450-GC gas chromatograph and a Varian 8400 AutoSampler. Data handling was carried out with the workstation, Version 6.91 software. The carrier gas was helium at a flow rate of 1 mL min^{-1} using the EFC constant flow mode. A CP-1177 injector (275 °C) was used in split mode (1 : 50). Transfer line, manifold, and ion-trap temperatures were set at 280, 80 and 220 °C, respectively. HPLC-grade methanol was used as the liquid CI reagent. CI ionization parameters (0.5 s/scan): CI storage level 19.0 *m/z*; ejection amplitude 15.0 *m/z*; background mass 55 *m/z*; maximum ionization time 2000 µs; maximum reaction time 40 ms; target TIC 5000 counts. The number of ions in the trap was controlled by an automatic gain control function. Separations were carried out using 30 m $\times$ 0.25 mm (0.25 µm film thickness) DB1ms column (J&W). The column temperature was programmed as follows: 150 °C held for 1 min, then heated at 10 °C min^{-1} to 280 °C and held constant for 6 min; the total run time was 20 min.

NMR spectra were recorded using a Bruker Avance 300 spectrometer at 300.1 MHz (^{1}H NMR) or 75.5 MHz (^{13}C NMR). NMR spectra were recorded in $CDCl_3$ and obtained by ^{1}H, proton decoupled ^{13}C, DEPT-135, HSQC and HMBC experiments. Chemical shifts are reported relative to TMS at $\delta = 0$ ppm. When d_6-DMSO was used, chemical shifts were determined relative to the residual solvent peak at $\delta = 2.51$ (^{1}H NMR) and $\delta = 39.6$ ppm (^{13}C NMR).

Synthesis procedures

Standard reductive amination conditions used for synthesis of N,N-dimethyltryptamine (DMT) **2**

Tryptamine **1** (1 g, 6.24 mmol, 1.0 eq) was dissolved in 40 mL ice-cold methanol, followed by the addition of glacial acetic acid (1.26 g, 21.0 mmol, 1.20 mL, 3.4 eq), sodium cyanoborohydride (629 mg, 10.0 mmol, 1.6 eq), and left to stir on ice for 5 min. An aqueous solution of 39% (w/v) formaldehyde (429 mg, 14.3 mmol, 1.1 mL, 2.3 eq) in 10 mL methanol was added dropwise to the reaction mixture over a period of 20 min. The ice was removed and the reaction was left to stir for 2.5 h at room temperature. NaOH (3 mL, 20%) was added and the solvent was removed under reduced pressure. This was followed by the addition of 20 mL distilled water and three extractions with 20 mL chloroform. The combined organic layers were washed once with 20 mL water and 20 mL brine, dried with $MgSO_4$, and filtered. The filter cake was washed twice with 20 mL chloroform with the filtrate

Table 1. Percentage yields of DMT **2** and byproducts **3**–**7** formed from tryptamine **1** (6.24 mmol, 1 equivalent) under reductive amination conditions using differing molar equivalents of formaldehyde, FA, acetic acid, A, and $NaBH_3CN$ as the reducing agent, RA[a]

Reaction No.	S (eq)	FA (eq)	A (eq)	RA (eq)	**1** [%]	**2** [%]	**3** [%]	**4** [%]	**5** [%]	**6** [%]	**7** [%]
1	1	2.30	3.40	1.60	–	77.8 ± 3.90	2.10 ± 0.20	6.60 ± 0.20	–	–	–
2	1	1.15	3.40	1.60	21.2 ± 15.8	43.9 ± 3.70	–	0.80 ± 0.10	7.10 ± 2.40	–	–
3	1	4.60	3.40	1.60	–	78.8 ± 4.80	0.90 ± 1.20	2.90 ± 0.10	–	–	–
4	1	2.30	1.70	1.60	–	67.8 ± 2.60	2.20 ± 0.60	29.2 ± 1.40	–	–	–
5	1	2.30	6.80	1.60	–	74.7 ± 0.70	2.30 ± 0.30	3.60 ± 0.10	–	–	–
6	1	2.30	3.40	0.80	–	46.7 ± 8.80	2.30 ± 0.10	22.0 ± 2.40	–	9.20 ± 6.60	–
7	1	2.30	3.40	3.20	–	61.2 ± 1.50	–	1.90 ± 0.30	–	–	–
8	1	2.30[b]	3.40	1.60	11.5 ± 5.30	11.1 ± 6.00	0.80 ± 0.10	5.40 ± 4.80	2.60 ± 0.10	–	–
9	1	2.30	3.40	1.60[c]	3.00 ± 0.50	2.90 ± 4.10	2.40 ± 0.20	–	–	–	71.5 ± 10.4

[a] Each reaction was carried out in duplicate. For structures see Figures 1 and 2F.
[b] Paraformaldehyde (431 mg, 14.35 mmol, 2.3 eq) was used instead of formaldehyde solution.
[c] $NaBH_4$ (379 mg, 10 mmol, 1.6 eq) was used instead of $NaBH_3CN$.

evaporated under reduced pressure. The oily residue was dried over P_2O_5 overnight to yield 1.169 g of a pale brown waxy solid and characterized by GC-MS analysis. The standard conditions were then modified to study the impact of varied reagent stoichiometry (Table 1). All reactions were carried out in duplicate.

Synthesis of N-methyl-N-cyanomethyltryptamine (MCMT) **4**

NMT **5** (1.0 g, 5.74 mmol, 1.0 eq) was added to a solution of benzene (50 mL) and potassium carbonate (820 mg, 5.93 mmol, 1.0 eq). Chloroacetonitrile (433 mg, 5.74 mmol, 363 µL, 1.0 eq) was then added and the resulting mixture was heated at reflux overnight. The solvent was removed under reduced pressure to give a pale brown solid that was purified by flash chromatography ($CHCl_3$/MeOH, 95 : 5, v/v). Yield: 967 mg (4.53 mmol, 79%). ^{1}H-NMR ($CDCl_3$): 8.02 (NH-1, br s), 7.59 (1H, d, H-4, J 7.5 Hz,), 7.32 (1H, d, H-7, J 7.9 Hz), 7.19 (1H, td, H-6, J 7.6, 1.3 Hz), 7.11 (1H, td, H-5, J 7.6, 1.3 Hz), 6.98 (1H, d, H-2, J 2.4 Hz), 3.56 (2H, s, CH_2CN), 2.94-2.88 (2H, m, CH_2-α), 2.83-2.77 (2H, m, CH_2-β), 2.43 (3H, s, *N*-CH_3). ^{13}C-NMR ($CDCl_3$): 136.3 (C-7a), 127.3 (C-3a), 122.1 (C-6), 121.7 (C-2), 119.4 (C-5), 118.7 (C-4), 114.8 (CN), 113.4 (C-3), 111.3 (C-7), 56.3 (CH_2-α), 45.2 (*N*-$\underline{C}H_2CN$), 42.1 (N-CH_3), 23.6 (CH_2-β). HRESIMS theory $[M+H]^+$: 214.1344; observed: 214.1355. This procedure was adapted from a previously published method.[34]

Synthesis of 2-cyanomethyltetrahydro-β-carboline (2-CM-THBC) **6**

Tetrahydro-β-carboline **7** (860 mg, 5.0 mmol, 1 eq) was added to a solution of benzene (100 mL) and sodium carbonate (1.50 g, 14.2 mmol, 2.8 eq). Chloroacetonitrile (1.19 g, 15.8 mmol, 1.0 mL, 3.1 eq) was then added and the resulting mixture was heated at reflux for two days. After solvent evaporation ethanol was added to the residue and heated to boiling point temperature. After filtration the ethanol filtrate was reduced in volume under reduced pressure and left to crystallize at 4 °C to give a white solid. Yield: 412 mg (1.95 mmol, 39%). ^{1}H NMR (d_6-DMSO): 10.80 (1H, br s, N-9H), 7.38 (1H, d, H-5, *J* 7.5 Hz), 7.30 (1H, d, H-8, *J* 7.9 Hz), 7.04 (1H, td, H-7, *J* 7.4, 1.2 Hz), 6.96 (1H, td, H-6, *J* 7.3, 1.1 Hz), 3.98 (2H, s, *N*-CH_2CN), 3.74 (2H, s, CH_2-1), 2.85 (2H, t, CH_2-3, *J* 5.5 Hz), 2.75 (2H, t, CH_2-4, *J* 5.1 Hz). ^{13}C NMR (d_6-DMSO): 135.9 (C-8a), 131.6 (C-9a), 126.5 (C-4b), 120.5 (C-7), 118.3 (C-6), 117.4 (C-5), 116.1 CN), 110.9 (C-8), 105.9 (C-4a), 49.6 (CH_2-3), 48.6 (CH_2-1), 44.9 (*N*-$\underline{C}H_2CN$), 21.1 (CH_2-4). HRESIMS theory $[M+H]^+$: 212.1188; observed: 212.1177. This procedure was adapted from a previously published method.[34]

Results and Discussion

Inspection of GC-ITMS traces revealed that DMT **2** was formed under all conditions where $NaBH_3CN$ was used as the reducing agent. As a starting point, the adopted set of standard reductive amination conditions (Reaction 1, Table 1) was based on synthetic pathways found in the published literature.[23,24,35] A number of different solvents were discussed in these articles when considering the work-up. Solvents discussed include ethyl acetate and dichloromethane (DCM). In designing the present study, it was decided to use chloroform instead of ethyl acetate or DCM because preliminary experiments revealed that the use of ethyl acetate as the extraction solvent gave poor product yields. The use of DCM was discarded because it has been shown previously that DCM react with DMT during the extraction process.[32,33,36] The result would have been the formation of additional impurities unrelated to this synthetic route. Chloroform was selected because it is a common solvent that is cheap and provides a more effective extraction. This advantage might also attract the interest of the clandestine chemist in order to obtain increased yields.

Once the standard conditions were applied, it was deemed necessary to gain further insights into the extent of DMT and byproduct formation when exposed to varied reagent stoichiometry. The rationale behind this approach aimed to mimic both a clandestine-type situation where access to reagents might be limited but also to consider the pharmaceutical quality control context in which the identification of route-specific impurities present in bulk drugs is also needed. The extent of reagent variation is summarized in Table 1 where the equivalent of one of the reagents was either reduced by 50% or increased by 100%, respectively (Reactions 2–7). In addition, replacements of aqueous formaldehyde with solid paraformaldehyde (Reaction 8) and $NaBH_3CN$ with $NaBH_4$ (Reaction No. 9) were also carried out in order to gain further insights into the impact of reagent availability on product formation. Product and byproduct yields have been determined in duplicate. The results are also summarized in Table 1.

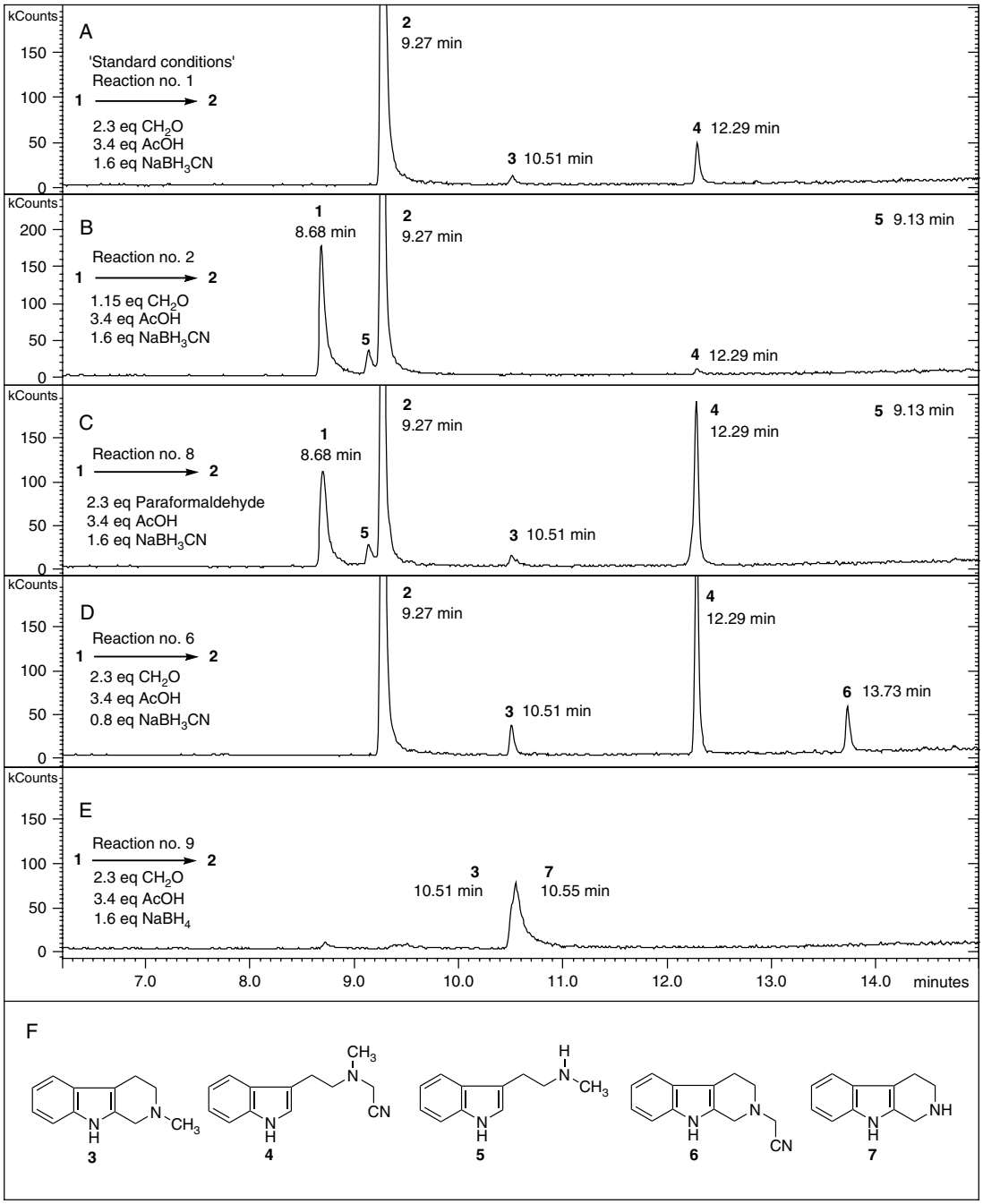

Figure 2. Representative GC-ITMS chromatograms obtained from the synthesis of DMT **2** under reductive amination conditions. (A) Standard reaction conditions. (B)–(E) Stoichiometric variations in order to assess impact on side product formation. (F) Structural representations of identified byproducts 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7**. See Table 1 for reaction yields.

Identification of Byproducts

A representative GC-ITMS trace obtained after application of the standard reaction conditions (1 eq tryptamine, 2.3 eq formaldehyde, 3.4 eq acetic acid and 1.6 eq $NaBH_3CN$) is shown in Figure 2A (Reaction No. 1). The main product peak at 9.27 min reflected the presence of DMT as judged by chromatographic comparison with a DMT standard. Typical mass spectral features included iminium ion formation at *m/z* 58, $CH_2{=}N^+(CH_3)_2$, both under EI- and CI-MS conditions[37,38] (Figures 3C and 3D). A second peak appeared at 10.51 min that was identified as 2-Me-THBC **3** where, under EI conditions, a characteristic *m/z* 143 base peak pointed towards *retro*-Diels-Alder fragmentation[39] (Figure 3E). Under single stage CI-MS conditions 2-Me-THBC **3** showed a characteristic $[M+H]^+$ at *m/z* 187 where the use of methanol as the liquid CI reagent appeared to cause significant dissociation into *m/z* 144 which was in agreement with previous work[32,33] (Figure 3F). Final confirmation arose from chromatographic and mass spectral comparison with a 2-Me-THBC standard. The extent of 2-Me-THBC formation appeared to be relatively consistent under the conditions investigated as the yields obtained from Reactions 1–9 ranged from zero to ~2.4%.

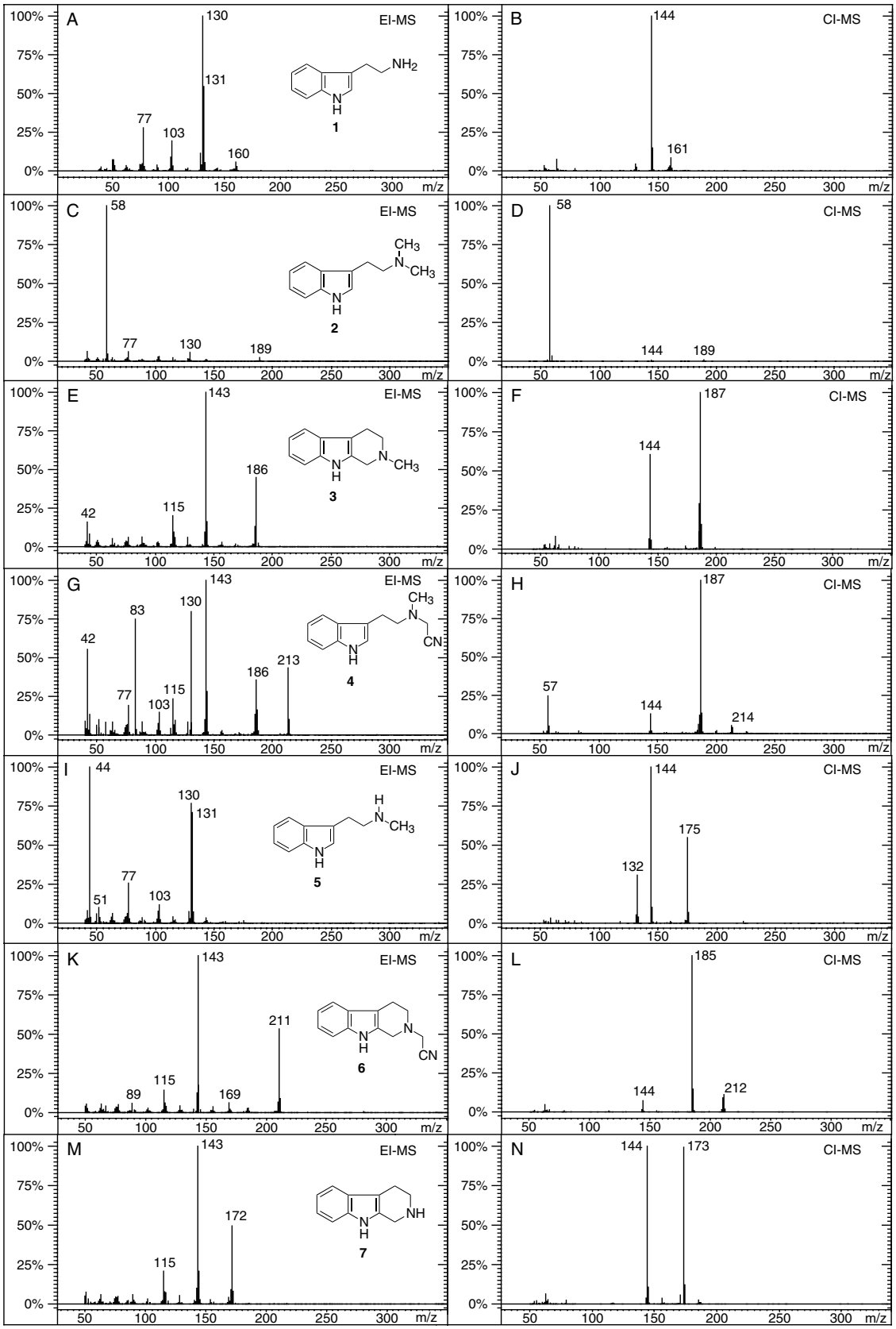

Figure 3. Electron- and chemical ionization ion trap mass spectra obtained from starting material tryptamine **1**, product DMT **2** and byproducts 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** THBC **7**.

An additional peak was visible at 12.29 min (Figure 2A) and the corresponding EI- and CI-MS spectra are shown in Figures 3G and 3H, respectively. The comparison of both mass spectra proved to be surprising when considering the notion that the use of chemical ionization would be expected to be much softer in comparison to electron ionization. In this particular case, however, EI-MS appeared to yield the crucial information that aided identification. The CI-MS spectrum of this byproduct showed the presence of a base peak at *m/z* 187 and a *m/z* 144 fragment which would be indicative of a THBC-type candidate as mentioned above. Consequently, as far as EI-MS was concerned the $M^{\bullet+}$ ion was expected to appear at *m/z* 186. Figure 3G shows that this was not the case and that a species at *m/z* 213 was observed instead. The appearance of *m/z* 130 provided some first indication that this candidate reflected the presence of an ethylindole moiety resulting in the corresponding quinolinium fragment.[37,40] The mass difference of 27 Da between $M^{\bullet+}$ at *m/z* 213 and *m/z* 186 then pointed towards a potential neutral loss of HCN and the presence of a cyanide group. Additional indication about the nature of the candidate arose from *m/z* 83 that appeared to be consistent with a tryptamine-based $CH_2{=}N^+(CH_3)CH_2CN$ iminium ion. The structure was confirmed as MCMT **4** by organic synthesis and proved to be identical when comparing both mass spectral and chromatographic behaviour. The formation of MCMT **4** was detected under all conditions studied but appeared to be particularly pronounced in reaction numbers 4 (~29%, reduced equivalent of acetic acid) and 6 (~22%, reduced equivalent of reducing agent).

Reduction of formaldehyde equivalents from 2.3 to 1.15 (Reaction 2) reduced the yield of DMT and led to the detection of the tryptamine starting material **1** (EI and CI-MS in Figures 3A and 3B) and NMT **5** (Figure 2B) which reflected the fact that quantitative formation of DMT would require the use of at least two equivalents of formaldehyde. When solid paraformaldehyde (2.3 equivalents) was used instead of formaldehyde solution (Reaction 8) the resulting GC-MS chromatogram appeared to show a similar distribution of product formation and indicated that under the conditions used the utilization of paraformaldehyde was a less favourable option as far as tryptamine conversion to DMT was concerned (Figure 2C). The corresponding EI- and CI-MS data for NMT are displayed in Figures 3I and 3J, respectively. Under EI conditions the appearance of a monomethylated tryptamine was indicated by the characteristic $CH_2{=}NH^+CH_3$ iminium ion at *m/z* 44 and the presence of an intense combination of ions at *m/z* 130 (base peak) and *m/z* 131 (Figure 3I). The CI-MS showed the expected $[M+H]^+$ at *m/z* 175 and comparisons between mass spectral and chromatographic behaviour with an available standard provided final confirmation.

When $NaBH_3CN$ was reduced to 0.8 equivalents (Reaction 6) a new byproduct, that has not been detected in any of the other reactions, appeared at 13.73 min (Figure 2D). The corresponding EI-MS (Figure 3K) revealed a potential molecular ion at *m/z* 211 and a base peak at *m/z* 143. Under CI-MS conditions the $[M+H]^+$ at *m/z* 212 was detected with minor intensity and the entire spectrum was dominated by a base peak at *m/z* 185 (Figure 3L), again indicating a possible loss of HCN as described above for MCMT **4**. Two mass spectral features guided the identification process. The molecular weight of this new candidate was found to be two mass units below MCMT **4** which was a first indicator of potential cyclisation. A second factor was based on the presence of the base peak at *m/z* 143 under EI-MS conditions (Figure 3K) that is a characteristic feature of THBC derivatives that are unsubstituted on the carbon-1 position.[32,33] Structural confirmation was finally obtained by comparison with the pure compound which supported the identification of 2-CM-THBC **6**.

From a clinical point of view, the use of toxic reagents should be avoided as much as possible when considering the preparation of bulk drugs. Because $NaBH_3CN$ is toxic, it may not be an ideal choice for the preparation of bulk drugs. Under clandestine conditions, access to reagents and solvents guide the preparation process which can then impact product purity and the presence of route-specific byproducts. Sodium borohydride ($NaBH_4$) is a commonly used alternative for the implementation of reductive amination procedures. One such example was provided by Bosch and co-workers who also employed the acidified formaldehyde/$NaBH_4$ system for the synthesis of the antimigraine almotriptan.[41] Interestingly, when $NaBH_4$ was employed for the reaction between tryptamine **1** and formaldehyde in this study (Reaction 9, Table 1) DMT **2** formation was not observed to a significant extent (~3%). Instead, yellow crystals with reduced solubility in methanol were obtained and subsequently identified as THBC **7** by comparison with a standard using NMR and GC-ITMS. The corresponding GC-ITMS trace is shown in Figure 2E and upon closer inspection revealed that traces of 2-Me-THBC **3** were also present. However, attempts to obtain resolution between both compounds were not successful. Chromatographic separation was observed when using a 30 m × 0.25 mm Factor Four capillary column (VF-5 ms) (film thickness 0.25 μm) but this resulted in co-elution between NMT **5** and DMT **2**. The corresponding EI and CI-MS are shown in Figs. 3M and 3N, respectively.

Internal (*in situ*) ionization was based on the direct introduction of the GC effluent into the ion trap. This allowed for the use of a liquid CI reagent. Methanol was chosen as the liquid CI reagent and its vapour pressure was sufficient to provide a constant flow of reagent gas to fill the ion trap. This low-pressure approach did not result in any adduct formation as is commonly found with traditionally used CI reagent gases due to the high pressures involved. The fact that methanol was known to have a moderate proton affinity of 761.1 kJ/mol[42] proved helpful since it generated a sufficient number of product ions in addition to the protonated molecule $[M+H]^+$ in order to retain molecular weight information (Figure 3). Consequently, the implementation of a tandem MS stage was not required which meant that it was found suitable for the detection of unknown species using the full-scan approach.

Calibration and GC-MS Method Validation

The crude reaction products obtained from Reactions 1–9 were dried under vacuum and dissolved in methanol for GC-ITMS analysis. Calibration curves for tryptamine **1**, DMT product **2** and byproducts **3**–**7** were generated by direct injection and without the need of chemical derivatization based on seven calibration levels and serial dilutions of the highest concentration of the particular solution (Table 2). The calibration curves were constructed by plotting peak area values derived from the appropriate *m/z* species *vs* concentration and a linear regression approach was found to be appropriate for correlation. Table 2 provides a summary of the calibration data and correlation coefficients (r^2) > 0.99 which indicated good linearity between 20.8 and 980 μg mL^{-1}. Accuracy was determined as recovery percentage ($[C_o/C_s] \times 100\%$) between found (C_o) and known (C_s) concentrations of the above-mentioned compounds. Standards were run in triplicate at concentration values following a 1.67-fold dilution of the maximum concentration level described in

Table 2. Calibration data for analytes **1**–**7** by GC-ITMS

Analyte	Line of best fit (r^2)	Range [μg/mL][a]	Quant Ion (EI-MS)	C_S [μg/mL][b]	C_O (± SD) [μg/mL][c]	Recovery [%][d]	RSD [%]	LOD[e] [ng/mL]	LOQ[f] [μg/mL]
1	$y = 2.89 \times 10^5 x - 7.72 \times 10^2$ (0.9989)	34.8–174	*m/z* 130	104	102 (0.001)	98.1	0.96	28.4	32.4
2	$y = 3.00 \times 10^6 x - 2.62 \times 10^5$ (0.9991)	196–980	*m/z* 58	587	602 (0.003)	99.6	0.55	87.7	88.3
3	$y = 3.99 \times 10^5 x - 7.90 \times 10^2$ (0.9995)	34.0–170	*m/z* 186	102	98.0 (0.005)	96.1	5.10	21.5	25.4
4	$y = 6.54 \times 10^5 x - 1.50 \times 10^4$ (0.9977)	29.2–146	*m/z* 143	87.0	85.0 (0.002)	97.7	2.35	23.4	24.6
5	$y = 5.53 \times 10^5 x - 2.27 \times 10^4$ (0.9962)	20.8–104	*m/z* 44	62.0	65.0 (0.004)	98.8	6.15	41.1	41.4
6	$y = 5.17 \times 10^5 x - 1.82 \times 10^4$ (0.9944)	30.8–154	*m/z* 143	92.0	90.0 (0.002)	97.8	2.22	36.6	39.9
7	$y = 5.18 \times 10^5 x - 1.76 \times 10^4$ (0.9987)	38.4–192	*m/z* 172	115	111 (0.005)	96.5	4.50	34.9	37.0

[a] Calibration solutions were obtained from serial dilutions of the highest concentration of the particular range. Dilution factors were 1.25, 1.43, 1.67, 2, 2.5 and 5 resulting in seven calibration levels. Methanol was used as the solvent with the exception of THBC **7** where chloroform was used.
[b] *Cs*: standard concentration.
[c] C_O: observed mean concentration of standard ($n = 3$).
[d] Percent recoveries were calculated by (*Co*/*Cs*) × 100%.
[e] S/N = 3.
[f] S/N = 10.

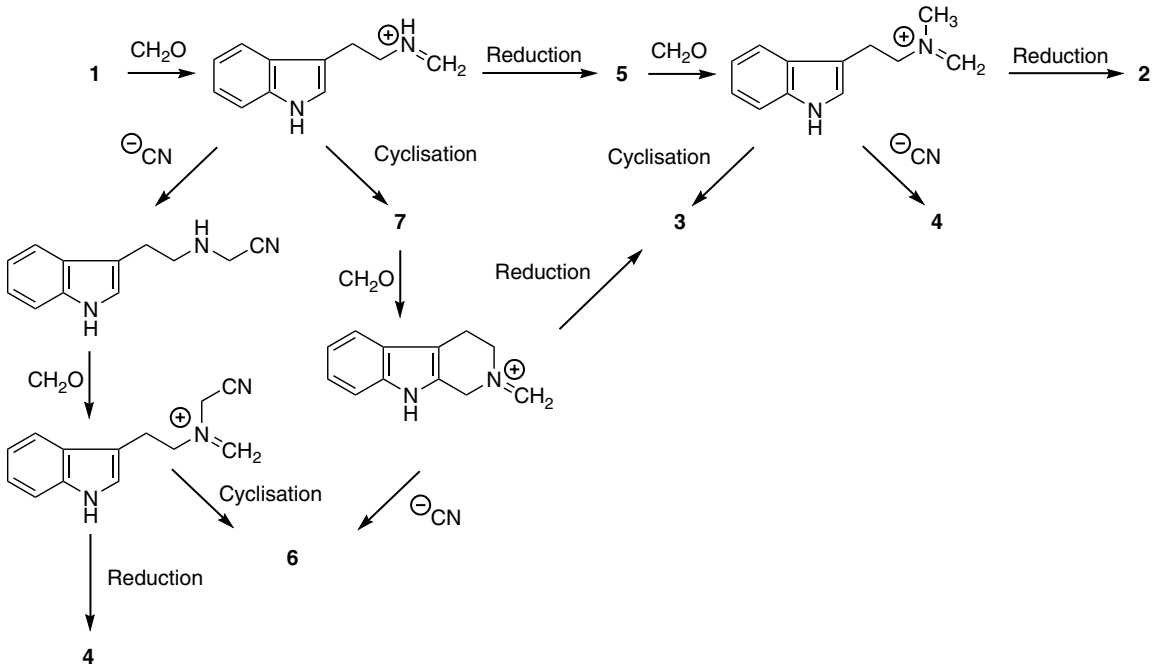

Figure 4. Proposed reaction pathways accounting for the formation of byproducts 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7** detected in the reaction sequence.

Table 2. Recovery percentage values were obtained by using the calibration equations and they ranged from 96.1% to 99.6% indicating a satisfactory level of accuracy. System precision (GC response) was determined from relative standard deviation (RSD) after triplicate injections of standard solutions used for the construction of calibration curves and was found to be below 1%. The method precision was assessed after triplicate measurements of **1**–**7** standards using calibration equations. Table 2 indicates that the obtained RSD values for compounds **1**–**7** were below 6.2%. LOD and LOQ values were determined by serial dilution of **1**–**7** until a signal-to-noise ratio of 3 and 10 were obtained. Table 2 shows that the calibration data were suitable for the quantitative determinations of product and impurities. The LOD obtained for Tryptamine **1**, DMT **2**, 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7** were 28.4, 87.7, 21.5, 23.4, 41.1, 36.6, and 34.9 ng mL^{-1}, respectively. The LOQ obtained for Tryptamine **1**, DMT **2**, 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7,** were 32.4, 88.3, 25.4, 24.6, 41.4, 39.9, and 37.0 μg mL^{-1}, respectively.

Formation of Impurities

The proposed reaction pathways to account for the observed products are shown in Figure 4. Reaction of the primary or secondary amine with formaldehyde gives an aminol which dehydrates to give the iminium salt intermediates. The iminium salts are good electrophiles and can react with nucleophiles by three pathways, depending upon the precise reaction conditions. The expected reaction is a fast reduction of the iminium salts to the corresponding amines NMT **5** and DMT **2**. Lowering the equivalents of formaldehyde (1.15 equiv, Reaction 2) has the expected effect

of lowering the yield of DMT. This was because formation of DMT required at least two equivalents of formaldehyde. Lowering the equivalent did not, however, change the yield of tryptamine **1** and NMT **5**.

If the reducing agent is at a low concentration (Reaction 6) or unreactive, then a Pictet-Spengler 6-endo-trig cyclization reaction of the iminium salt at the C2 of the indole ring can occur to give the THBC functionality. The formation of THBC **7** was observed when $NaBH_4$ was used as the reducing agent (Reaction 9). This can be rationalized by reaction of the reactive $NaBH_4$ with formaldehyde to give methanol, preventing both reduction and further reaction with formaldehyde. In addition, $NaBH_4$ will also decompose under the acidic conditions of the reaction to give the less reactive sodium triacetoxyborohydride ($NaHB(OAc)_3$).

The observed cyanomethyl products **4** and **6** are formed by reaction of the iminium salt intermediates with the cyanide nucleophile. Some free cyanide may be present in the $NaBH_3CN$; however, it is also known to degrade under acidic conditions to give HCN. The cyanomethyl compounds were formed in greater amounts under low acetic acid conditions (Reaction 4) where the HCN could form the cyanide nucleophile, or under low $NaBH_3CN$ concentration (Reaction 6), where reduction would be limited. There is precedence for the reaction of cyanide with an iminium salt derived from a THBC derivative.[43]

The key synthetic routes that lead to the preparation of tryptamine derivatives can be classified into methods that create the indole nucleus by cyclization and those that start with indole and substituted indoles.[44,45] A third approach involves the modification of a commonly available starting material that already contains the tryptamine moiety and the one-step reductive amination procedure reported in the present study illustrated such an example. This apparently simple procedure revealed the need for an in-depth characterization as reflected by the number of detected side-products. A recent report described the analytical characterization of an alternative one-step synthesis to DMT based on procedures discussed on the internet. DMT was suggested to be prepared from tryptamine, methyl iodide and benzyltriethylammonium chloride/NaOH phase transfer catalyst.[46] LC-MS-MS analysis of the products did not lead to the detection of DMT but *N*,*N*,*N*-trimethyltryptammonium iodide (TMT) and 1-*N*-methyl-TMT were formed instead. A trace of NMT and tryptamine starting material were found to be present as well which indicated that the reaction did not go to completion.[46]

Conclusion

A reductive amination procedure was profiled for the detection of byproducts formed during DMT synthesis using GC-ITMS. Tryptamine **1** was conveniently, although not completely, converted into DMT **2** using formaldehyde under reductive amination conditions. The conditions used in the analytical characterization of this seemingly simple reaction, revealed the presence of five byproducts, namely, 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7**, respectively. The calibration studies revealed good linearity within the range of 20.8–980 $\mu g\ mL^{-1}$ with correlation coefficients >0.99. The LOD reported for Tryptamine **1**, DMT **2**, 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7** were 28.4, 87.7, 21.5, 23.4, 41.1, 36.6, and 34.9 $ng\ mL^{-1}$, respectively. The LOQ reported for Tryptamine **1**, DMT **2**, 2-Me-THBC **3**, MCMT **4**, NMT **5**, 2-CM-THBC **6,** and THBC **7,** were 32.4, 88.3, 25.4, 24.6, 41.4, 39.9, and 37.0 $\mu g\ mL^{-1}$, respectively. The detection of THBC **7** and the cyanomethylated derivatives revealed that the choice of the reducing agent and the extent of stoichiometric reagent variation can dramatically impact product purity. These data should be useful for forensic and law enforcement agencies that deal with exposure to illegally produced DMT. The detection of route-specific impurities should also be of interest within the pharmaceutical context where DMT might also be prepared for human clinical studies.

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