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RESEARCH ARTICLE

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Analytical profile of the lysergamide 1cP-AL-LAD and detection of impurities

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Abstract

The development of novel lysergamides continues to occur, based on both the needs of psychedelic medicine and commercial interest in new recreational substances. The present study continues the authors' research on novel lysergamides and describes the analytical profile of 1-cyclopropanoyl-AL-LAD (IUPAC name: 1-(cyclopropanecarbonyl)-*N*,*N*-diethyl-6-(prop-2-en-1-yl)-9,10-didehydroergoline-8β-carboxamide; 1cP-AL-LAD), using various chromatographic, mass spectrometric, and spectroscopic methods. Analysis of a powdered sample of 1cP-AL-LAD, obtained from an online vendor, by high performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry in full scan/AutoMS/MS mode revealed the detection of 17 impurities based on high-resolution tandem mass spectral data; tentative determination of their identity was based on mass spectral grounds alone, though detection of AL-LAD and 1P-AL-LAD was confirmed using available reference standards. Other tentative compound identifications included 1-acetyl-AL-LAD and several other substances potentially reflecting oxidation of the N^6-allyl group as well as other positions on the ergoline ring system. These data may assist those interested in the chemistry of lysergamides. Finally, 1cP-AL-LAD was also detected in samples of "blotters" sold online for recreational use.

KEYWORDS
forensic, impurities, LSD, new psychoactive substances, psychedelics

1 | INTRODUCTION

The lysergamide lysergic acid diethylamide (LSD, Figure 1) is one of the prototypical hallucinogenic (psychedelic) drugs. The structure–activity relationships of LSD and other lysergamides have been explored widely by medicinal chemists over the last five decades[1,2]; these investigations have largely focused on three types of structural modifications. First, while the N^1 indole position is unsubstituted in

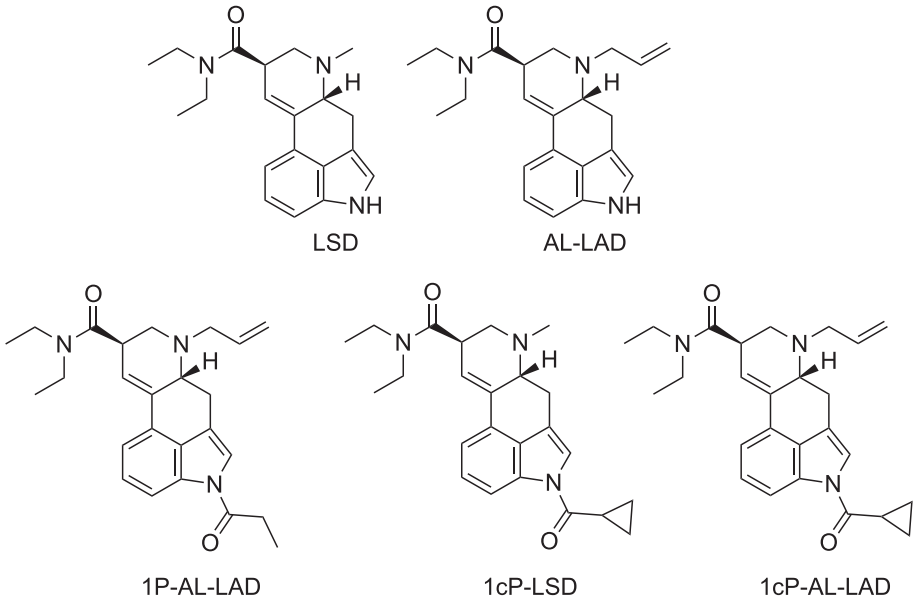


FIGURE 1 Chemical structures of lysergic acid diethylamide (LSD) along with some of its N^6-allyl and N^1-acyl derivatives previously reported.

LSD, various alkyl and acyl groups have been added to the N^1-position. Examples of N^1-substituted LSD derivatives include 1-methyl-LSD (MLD-41), which appears to retain some LSD-like psychoactive properties but with considerably lower potency, and 1-acetyl-LSD (1A-LSD, ALD-52), which has almost the same effects and potency as LSD.[3–6] LSD analogs that are modified at the N^6-position have also been synthesized. For example, the synthesis of N^6-allyl-6-norlysergic acid diethylamide (AL-LAD) was first reported in 1976[7] and was subsequently found to produce LSD-like effects in humans and rodents.[8,9] Finally, the diethylamide group in LSD has been replaced with various other alkylamide and cycloalkylamide moieties. Most other C8 amide substitution patterns yield greatly reduced hallucinogenic potency relative to LSD, although the conformationally restricted LSD analog (2′*S*,4′*S*)-lysergic acid 2,4-dimethylazetidide (LSZ) is an exception and retains high potency in vivo.[10,11]

In addition to being the focus of medical and scientific research, LSD is also a popular recreational substance. In recent years, a large number of LSD analogs have been distributed online as "research chemicals." Many of the lysergamides that have appeared as new recreational drugs were originally synthesized during the course of scientific studies, for example, ALD-52 and AL-LAD.[11–13] However, entirely novel lysergamides have also been synthesized with the specific goal of developing new designer drugs. Notable examples of this phenomenon include N^1-acylated derivatives of LSD and AL-LAD: 1-propanoyl-LSD (1P-LSD),[14] 1-butanoyl-LSD (1B-LSD),[15–17] 1-valeroyl-LSD (1V-LSD),[18] 1-cyclopropanoyl-LSD (1cP-LSD),[17,19] and 1-propanoyl-AL-LAD (1P-AL-LAD).[20] Very recently, the 1-cyclopropanoyl derivative of AL-LAD (1cP-AL-LAD, Figure 1) (IUPAC name: 1-(cyclopropanecarbonyl)-*N*,*N*-diethyl-6-(prop-2-en-1-yl)-9,10-didehydroergoline-8β-carboxamide) was detected as a new designer drug. 1cP-AL-LAD was first reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2021, based on a seizure made by law enforcement in April of the same year.[21]

The present study continues the authors' research on the properties and effects of novel lysergamides and reports the analytical profile of 1-cyclopropanoyl-AL-LAD based on various chromatographic, mass spectrometric, and spectroscopic methods. The present investigation also involved the detection of impurities in a powdered 1cP-AL-LAD sample that were characterized by liquid chromatography electrospray ionization quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-QTOF-MS). Extracts prepared from blotter paper samples purportedly containing 1cP-AL-LAD were also analyzed to confirm the presence of this lysergamide.

2 | EXPERIMENTAL

2.1 | Materials

Formic acid (HCOOH, Rotipuran® ≥ 98%, p.a.) and potassium hydrogen phosphate (≥ 99%, p.a.) were obtained from Carl Roth (Karlsruhe, Germany); acetonitrile (ACN) (LC–MS grade), ammonium formate 10 M (99.995%), and potassium hydroxide (puriss. p.a. ≥ 86% (T) pellets) from Sigma-Aldrich (Steinheim, Germany). Other chemicals and solvents were of analytical or HPLC grade and obtained from Aldrich (Dorset, UK). A powdered sample of 1cP-AL-LAD was obtained from an online retailer and 1cP-AL-LAD blotters originated from a test purchase.

2.2 | Instrumentation

2.2.1 | Gas chromatography–mass spectrometry (GC–MS)

A Finnigan TSQ 8000 triple stage quadrupole mass spectrometer coupled to a Trace GC Ultra gas chromatograph (Thermo Fisher,

Waltham, USA) equipped with a fused silica DB-1 column (30 m × 0.32 mm i.d., 0.25 μm film thickness) (Agilent Technologies, Santa Clara, USA) was used for GC-EI-MS analysis. Samples were introduced by a CTC CombiPAL (CTC Analytics, Zwingen, Switzerland) autosampler.

The following GC parameters were employed: injection volume: 0.5 μL, splitless; injector temperature: 280°C; carrier gas: helium; flow rate: 1.2 mL/min. Initially, the oven temperature was kept at 80°C for 2 min, ramped to 310°C at 20°C/min, and subsequently maintained at the final temperature of 310°C for 23 min. MS parameters were set as follows: ionization mode: EI at 70 eV; emission current: 50 μA; ion source temperature: 220°C; scan time: 1 s; scan range: *m/z* 29–600.

Data analysis was conducted using Xcalibur 4.0 Qual Browser (Thermo Fisher) and the National Institute of Standards and Technology (NIST) MS search program (version 2.3) (NIST, MD, US). EI-MS spectra were compared with EI mass spectral libraries provided by the NIST, the European Network of Forensic Science Institutes (ENFSI), Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), and the designer drug library 2021 (DigiLab, SH, DE) and libraries built in-house. Kovats retention indices (RIs) were calculated from the measurement of retention times obtained from the constituents of an *n*-alkane mixture. The temperature program is specified above. For calculation, logarithmic interpolation was applied between two consecutive *n*-alkanes.

2.2.2 | GC-solid-phase-infrared analysis (GC-sIR)

A GC-solid phase-IR-system consisting of an Agilent GC 7890B (Agilent Technologies) equipped with a fused silica capillary DB-1 column (30 m × 0.32 mm i.d., 0.25 μm film thickness), an Agilent G4567A probe sampler (Agilent Technologies), and a DiscovIR-GC (Spectra Analysis, Marlborough, MA, US) were used for the acquisition of solid transmission IR spectra. The eluting substances were cryogenically accumulated on a spirally rotating ZnSe disk cooled by liquid nitrogen to −40°C. IR spectra were recorded through the IR-transparent ZnSe disk using a nitrogen-cooled mercury cadmium telluride (MCT) detector.

The GC parameters were as follows: injection volume 1 μL; splitless mode; injection port temperature 240°C; carrier gas: helium; flow rate 2.5 mL/min. Chromatographic conditions were as follows: oven temperature program: 80°C for 2 min, ramped to 290°C at 20°C/min, and maintained for 20 min; transfer line: 280°C. IR conditions: oven temperature 280°C; restrictor temperature 280°C; disk temperature −40°C; Dewar cap temperature 35°C; vacuum 0.2 mTorr; disk speed 3 mm/min; spiral separation 1 mm; wavelength resolution 4 cm^{-1}; IR range 650–4000 cm^{-1}; acquisition time: 0.6 s/file and 64 scans per spectrum. Data were processed using GRAMS/AI Ver. 9.1 (Grams Spectroscopy Software Suite, Thermo Fisher) followed by OMNIC Software, Ver. 7.4.127 (Thermo Fisher).

2.2.3 | HPLC-ESI-QTOF-MS

HPLC-ESI-QTOF-MS analysis was performed on an impact II™ QToF instrument coupled with an Elute HPLC system (both from Bruker Daltonik, Bremen, Germany). Chromatographic separation was achieved on a Kinetex® Biphenyl column (100 × 2.1 mm, 2.6 μm particle size, Phenomenex, Aschaffenburg, Germany) equipped with a corresponding guard column (SecurityGuard™ ULTRA Cartridges UHPLC Biphenyl for 2.1 mm ID columns, Phenomenex, Aschaffenburg, Germany). Mobile phases A (1% v/v ACN, 0.1% v/v HCOOH, 2 mM ammonium formate in water) and B (0.1% v/v HCOOH, 2 mM ammonium formate in ACN) were freshly prepared prior to analysis and varied in a linear program (T_{min}/A:B; T_0/90:10; T_{10}/20:80; $T_{10.5-12.5}$/5:95; $T_{12.7-14}$/90:10) with LC flow set at 0.3 mL/min and column oven temperature at 40°C. The autosampler was cooled down to 5°C. The injection volume was 10 μL. HyStar™ version 3.2 and DataAnalysis version 4.2 (both from Bruker Daltonik) were used for data acquisition and processing, respectively. The QTOF-MS was operated in positive ESI mode acquiring spectra in the range of *m/z* 30–500 Da (acquisition rate of 4.0 Hz). Acquisition was performed in full scan/bbCID mode and in a second run in full scan/AutoMS/MS mode to obtain cleaner fragment spectra. The collision energy applied for bbCID and Auto-MS/MS was 30 ± 6 eV. The dry gas temperature was set to 200°C with a dry gas flow of 8.0 L/min. The nebulizer gas pressure was 200 kPa. Nitrogen was used as collision gas. The voltages for the capillary and end plate offset were 2500 and 500 V, respectively. External and internal mass calibrations were performed using sodium formate/acetate clusters and high-precision calibration (HPC) mode.

2.3 | Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra of the powdered sample were recorded in DMSO-d_6 using an Agilent DD2-700 spectrometer with an Agilent HFCN cryogenically cooled 5 mm probe (^{1}H at 700 MHz; ^{13}C at 175 MHz) (Agilent, Santa Clara). A sample of 1cP-AL-LAD subjected to preparative thin-layer chromatography (TLC) was prepared in DMSO-d_6 (^{1}H at 600 MHz; ^{13}C at 150 MHz), and spectra were recorded on a Bruker AVANCE III 600 MHz NMR spectrometer (Bruker UK Ltd, Coventry, UK). Spectra were referenced to residual solvent, and assignments were supported by both 1D and 2D experiments.

2.4 | Extraction of blotters

Ten blotters were extracted using 2 mL methanol under vigorous shaking for 1 min. The methanolic extract was evaporated down to 100 μL using a stream of nitrogen at room temperature.

3 | RESULTS AND DISCUSSION

3.1 | Analytical features

Figure 2a depicts the electron ionization (EI) mass spectrum recorded from 1cP-AL-LAD. The molecular ion was detected at *m/z* 417 and showed significant abundance. Radical loss of the N^6-substituent (allyl

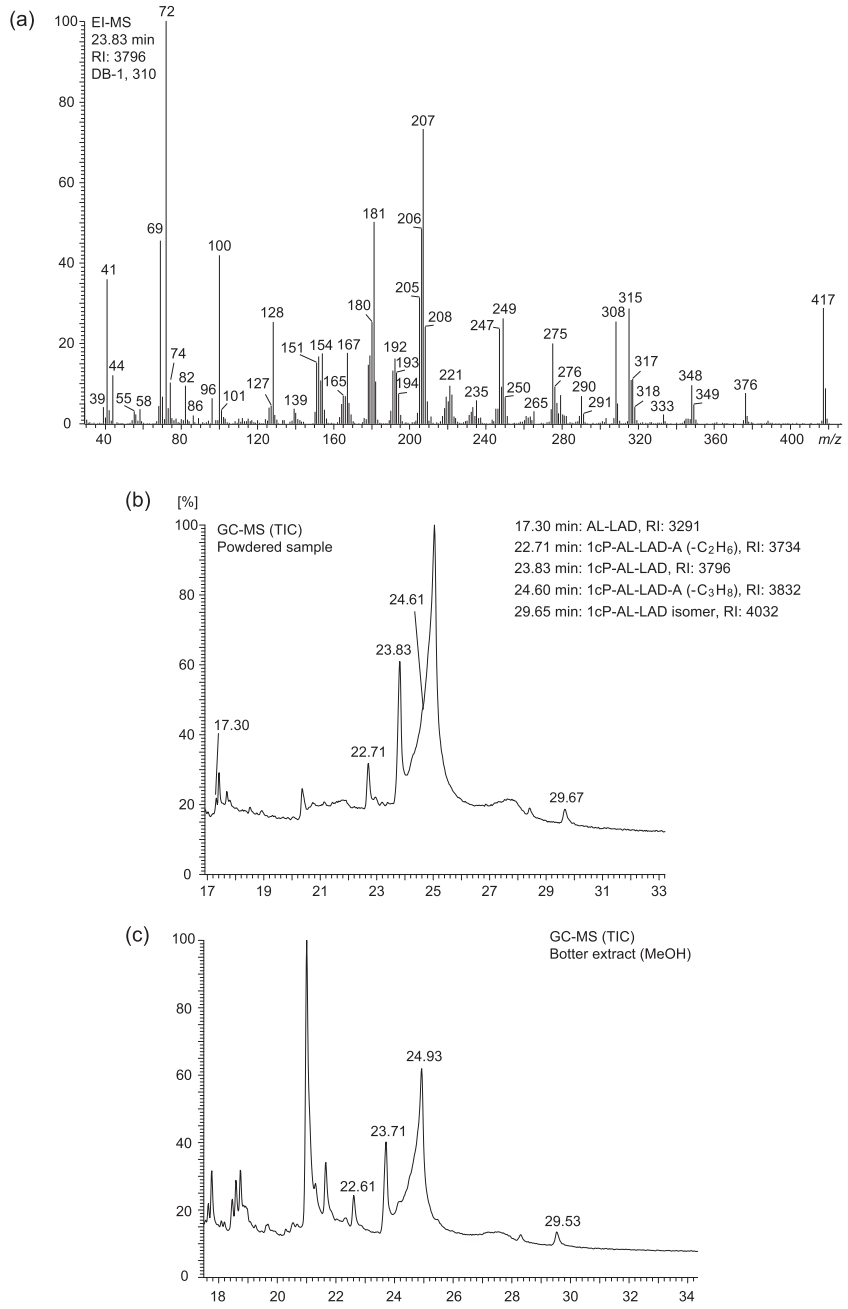


FIGURE 2 (a) Electron ionization mass spectrum of 1cP-AL-LAD. (b) Gas chromatography–mass spectrometry (GC–MS) chromatogram (TIC) recorded from a powdered sample of 1cP-AL-LAD. (c) Full scan GC–MS trace obtained from a methanolic blotter extract

group, 41 u) resulted in *m/z* 376 similar to the related lysergamides AL-LAD (*m/z* 308)[11] and 1P-AL-LAD (*m/z* 364).[20] The *m/z* 348 species might have reflected formation of the retro-Diels-Alder fragment, and because this included the attachment of the N^1-cyclopropanoyl group, the same fragment ion could be detected in the EI mass spectrum of 1cP-LSD.[17,19] Other ions associated with this substituent included the cyclopropanoyl ion at *m/z* 69 followed by a neutral loss of 28 u (CO) to give the cyclopropylium ion at *m/z* 41 ($C_3H_5^+$), which has also been reported for the EI mass spectrum of 1cP-LSD.[17,19] 1cP-AL-LAD, together with AL-LAD, 1P-AL-LAD, and other lysergamides with an *N*,*N*-diethylamide group, showed the characteristic formation of *m/z* 72 (iminium ion), *m/z* 100, and *m/z* 128.[11,20] Finally, the cluster of fragment ions typically observed for a number of LSD-type compounds has also been detected here, which included *m/z* 205–208, 191–197, 178–182, 161–169, and 151–156. One of the fragmentation sequences that highlighted the mass difference between 1cP-LSD (N^6-methyl) and 1cP-AL-LAD (N^6-allyl) of 26 u could be observed with *m/z* 315. This was thought to form by a consecutive neutral loss of *N*,*N*-diethylformamide (101 u) from the molecular ion at *m/z* 417 (leading to *m/z* 316) followed by a loss of a hydrogen radical to yield a fully aromatized species. In 1cP-LSD, the same sequence was thought to give *m/z* 290 and *m/z* 289, respectively.[19] Suggested fragmentation pathways have been added as Supporting Information.

Analysis of the powdered 1cP-AL-LAD sample by GC–MS in full scan mode (Figure 2b) revealed noticeable degradation and detection of three additional peaks. Because these were not observed under

LC–MS conditions, it was thought that these might have formed artificially during GC analysis, potentially including the injection port. In addition, the detection of AL-LAD was noted (17.30 min; RI = 3291), which was consistent with its detection by LC–MS (see below), indicating that this was not formed by degradation during GC analysis.

The EI mass spectra recorded for the three impurities are shown in Figure 3. One artifact was detected at 22.71 min. The EI mass spectrum (Figure 3a) suggested a molecular ion at *m/z* 387, and the detection of ions related to the acyl group (*m/z* 69 and 41) indicated that the N^1-cyclopropanoyl group was still attached and that the loss of the cyclopropanoyl radical (69 u) would be consistent with the formation of *m/z* 318. The *m/z* 318 ion might have also been formed because of a retro-Diels-Alder mechanism (RDA). One potential identity of this degradant was thought to be an *N*-ethylideneacetamide-type compound reflecting a modification of the *N*,*N*-diethylamide functionality. For this reason, this degradant was labeled as 1cP-AL-LAD-A (-C_2H_6) to indicate that one *N*-ethyl group was absent. Another degradant was identified in the chromatogram at about

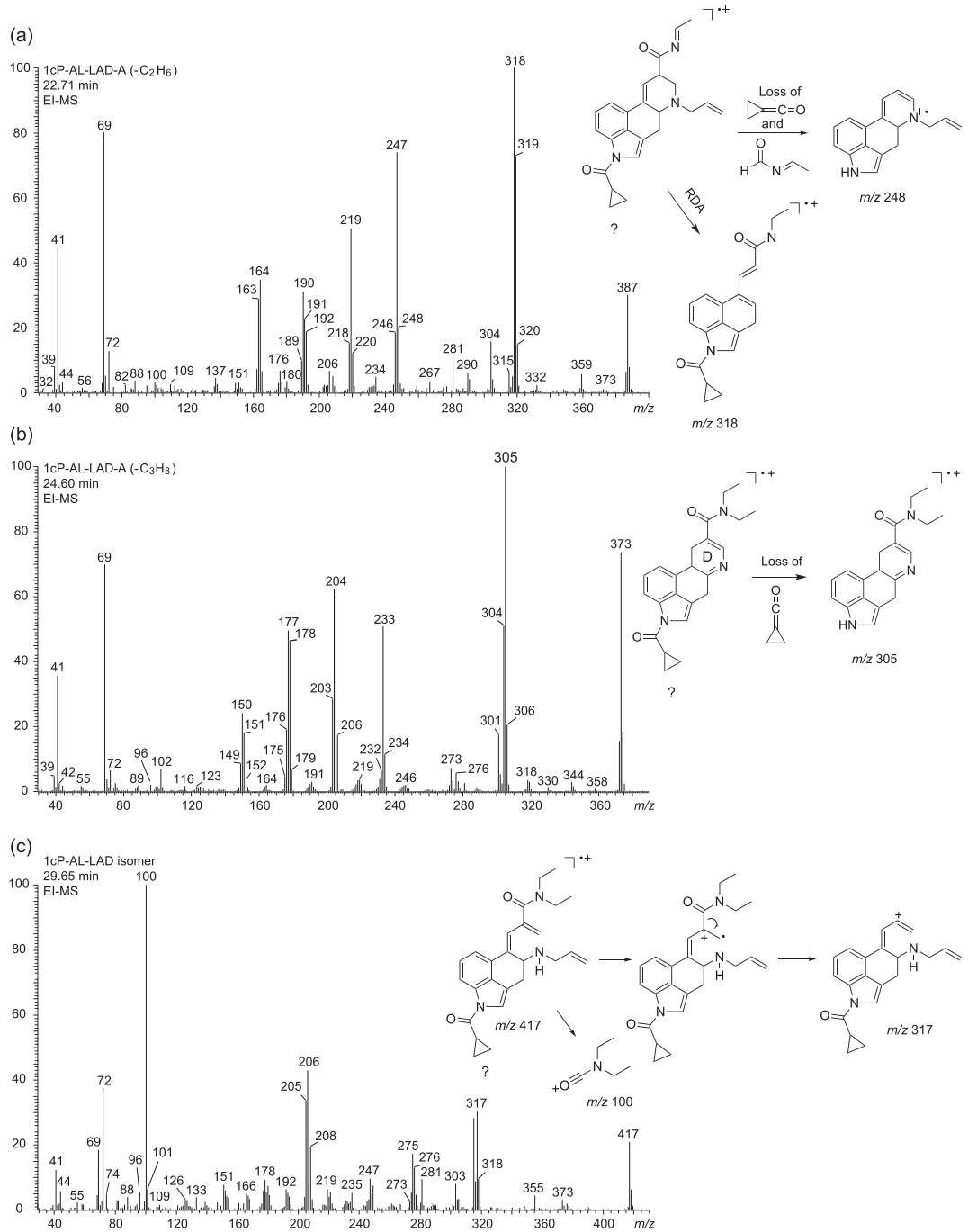


FIGURE 3 Tentative identification of gas chromatography (GC)-induced degradation products. (a) 1cP-AL-LAD-A (-C_2H_6). (b) 1cPAL-LAD-A (-C_3H_8). (c) Ring-opened 1cP-AL-LAD isomer

25 min that showed some fronting. The corresponding mass spectrum (Figure 3b) revealed the molecular ion to be recorded at *m/z* 373 and that this spectrum was very similar to one degradant reported previously during the GC–MS analysis of 1cP-LSD.[19] The spectrum would be consistent with the loss of the N^6-allyl group and full aromatization of the D-ring. Therefore, the suggested compound was labeled as 1cP-AL-LAD-A (-C_3H_8).

1cP-AL-LAD eluted at 23.83 min, and another isomer was detected at 29.67 min. Given that the EI mass spectrum differed significantly from 1cP-AL-LAD, the putative presence of the C8-epimer (iso-1cP-AL-LAD) was excluded. Instead, the detection of a ring-opened species was considered, which would be consistent with the detection of *m/z* 317 (Figure 3c). This was consistent with a related degradant suggested previously during the analysis of 1P-AL-LAD.[20] The mass difference of 12 u between 1P-AL-LAD and 1cP-AL-LAD was seen in some of the fragment ions, for example, *m/z* 305 for 1P-AL-LAD[20] versus *m/z* 317 for 1cP-AL-LAD. Suggested fragmentation pathways for the three degradants have been supplied as Supporting Information together with extracted ion chromatograms created from the EI mass spectra recorded for 1cP-AL-LAD and the artificially induced degradants and AL-LAD.

The IR spectrum obtained from GC solid-state analysis (GC-sIR) of 1cP-AL-LAD base is shown in Figure 4. Similar to the N^1-propanoyl lysergamide 1P-AL-LAD reported previously,[20] two carbonyl stretches were detectable. In the present study, these were detected at 1689 and 1637 cm^{-1}, whereas in 1P-AL-LAD, they were recorded at 1704 and 1639 cm^{-1}. Signals related to the N^1-H were undetectable due to substitution, whereas in the N^1-unsubstituted AL-LAD, this signal was shown to be around 3280 cm^{-1} together with a single carbonyl stretch at 1626 cm^{-1}.[11]

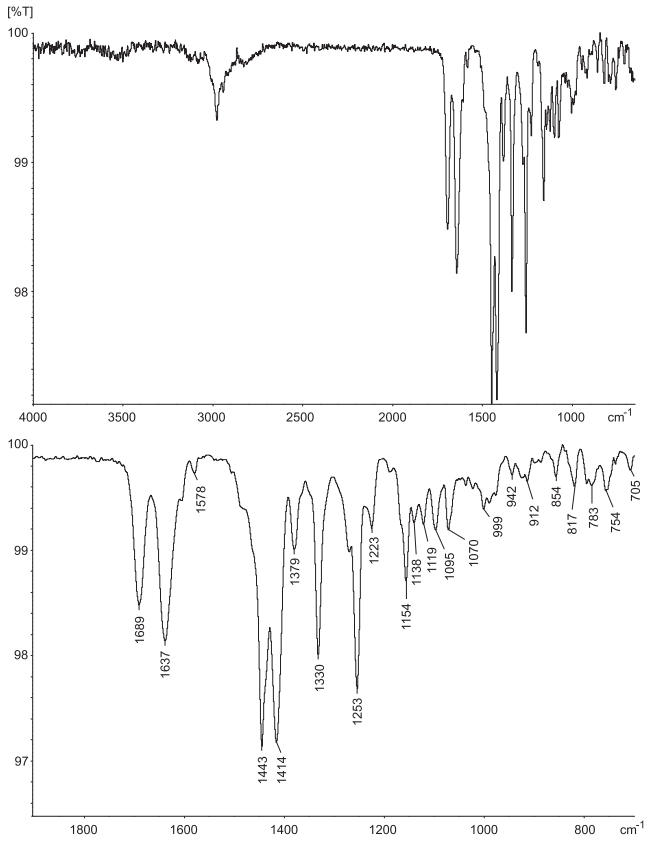
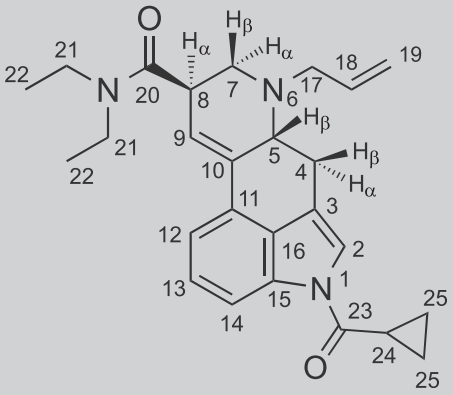


FIGURE 4 Gas chromatography-solid-phase-infrared analysis (GC-sIR) of 1cP-AL-LAD

The results from NMR spectroscopy experiments are summarized in Table 1. Full 1D/2D NMR spectra are supplied in the Supporting Information document. Inspection of the full spectra revealed that the powdered 1cP-AL-LAD sample was not pure, and further investigations (see below) showed the detection of several impurities. Overall, the resonances associated with the lysergamides could still be identified and were comparable with those reported previously for AL-LAD, 1cP-LSD, and 1P-AL-LAD.[11,19,20] One of the impurities (Supporting Information) detectable in the NMR spectrum was consistent with cyclopropanecarboxylic acid by comparison with a commercially available sample. The synthesis of 1cP-AL-LAD will have involved the use of an acylating agent; for example, cyclopropanecarbonyl chloride and the fact that the carboxylic acid was detected suggests that some residual material might have undergone hydrolysis at some stage. Investigations involving other lysergamides (for example, 1P-AL-LAD[20]) have shown that the amide-to-tartaric acid ratio was typically around 2:1 based on integration of tartaric acid protons and sometimes some slight excess of acid was observed. However, in the present sample, the integration value was around 0.4, which suggested that the lysergamide base might have been present in some excess. An attempt was made to purify the lysergamide by preparative TLC (2 mm layer thickness, chloroform/methanol, 9/1), which facilitated the removal of the cyclopropanecarboxylic acid impurity. The proton and carbon NMR spectra recorded from the isolated band has been supplied as Supporting Information for visual comparison between spectra.

Figure 5a presents the ESI-QTOF-MS/MS data for 1cP-AL-LAD ([M + H^+ at *m/z* 418.2488; $C_{26}H_{32}N_3O_2^+$), and the detected product ions were consistent with its structure and related to several lysergamides reported in the literature. For example, similar to AL-LAD[11] and 1P-AL-LAD,[20] the N^6-allyl group was lost to give a radical cation at *m/z* 377.2097 ($C_{23}H_{27}N_3O_2^{\bullet+}$, 377.2098) as the base peak. The product ion at *m/z* 349.1908 ($C_{22}H_{25}N_2O_2^+$) was also considered to represent an ion possibly formed by an RDA-type mechanism involving a neutral loss of *N*-allylmethanimine. Correspondingly, the same ion was detected in the ESI-QTOF tandem mass spectrum of 1cP-LSD, which was based on the fact that the substituent connected to the N^6-position was included in the preceding neutral loss resulting in the same product ion.[19] Two other product ions of considerable abundance included *m/z* 276.1253 and 208.0993, and it was thought that these could have formed by a similar mechanism suggested previously for 1P-AL-LAD.[20] For example, in case of the former product ion ($C_{18}H_{16}N_2O^{\bullet+}$, 276.1257), its formation could be rationalized by a loss of *N*,*N*-diethylformamide from the N^6-deallyl radical cation at *m/z* 377.2097, whereas the latter was consistent with a subsequent loss of the acyl group in the form of a neutral species (cyclopropylidenemethanone, C_4H_4O) resulting in $C_{14}H_{12}N_2^{\bullet+}$ (*m/z* 208.0993). Proposed mechanisms possibly involved in the formation of product ions detected for 1cP-AL-LAD can be found as Supporting Information.

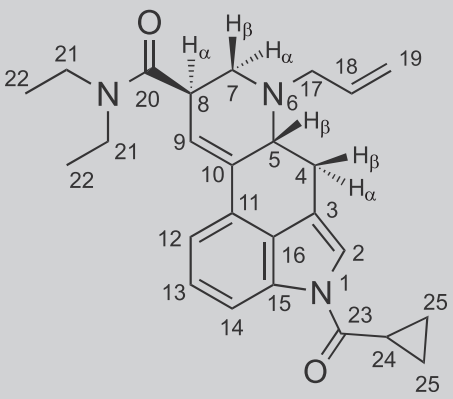
TABLE 1 ^{1}H and ^{13}C NMR data for 1cP-AL-LAD hemitartrate in DMSO-d_6 (referenced to residual solvent) at 700/175 MHz



No.	^{13}C [δ / ppm]	^{1}H [δ / ppm]
2	120.02	7.87 (d, *J* = 1.6 Hz, 1H)
3	116.53	–
4	26.20	2.49–2.45 (m, H-4α, 1H) 3.54 (dd, *J* = 15.2, 5.3 Hz, H-4β, 1H)
5	58.84	3.35–3.33 (m, H-5β, 1 H) *partially overlapping with H-21 (2H)
6	–	–
7	51.55	3.07 (dd, *J* = 11.2, 4.5 Hz, H-7α, 1H) 2.58 (t, *J* = 10.8 Hz, H-7β, 1H)
8	39.13	3.77–3.72 (m, 8α, 1H)
9	122.38	6.34 (s, 1 H)
10	134.11	–
11	128.22	–
12	116.62	7.33 (d, *J* = 7.3 Hz, 1H)
13	125.87	7.29 (t, *J* = 7.8 Hz, 1H)
14	114.86	7.99 (d, *J* = 7.9 Hz, 1H)
15	133.18	–
16	127.69	–
17	56.29	3.64 (dd, *J* = 14.6, 4.9 Hz, 1H) 3.16 (dd, *J* = 14.7, 8.0 Hz, 1H)
18	134.82	5.97 (dddd, *J* = 18.0, 10.2, 8.0, 4.9 Hz, 1H)
19	117.89	5.30 (d, *J* = 16.4 Hz, 1H; H-19 *trans* to H-18
19	117.89	5.20 (d, *J* = 10.2 Hz, 1H; H-19 *cis* to H-18
20	170.53	–
21	41.56	3.43 (AB qq, *J* = 13.9, 7.1 Hz, 2H)
21	39.72	3.32–3.27 (m, 2 H) *peaks are partially overlapping with H-5β
22	14.83	1.17 (t, *J* = 7.1 Hz, 3H)
22	13.08	1.06 (t, *J* = 7.0 Hz, 3H) *partial overlap with H-25 (4H)
23	172.17	–
24	13.22	2.65–2.61 (m, 1H)
25	9.38	1.11–1.08 (m, 2H) *peaks are overlapping with H-22 (3H)

(Continues)

TABLE 1 (Continued)



25	9.31	1.11–1.08 (m, 2H) *partial overlap with H-22 (3H)
TA [a]	173.16	–
TA [a]	71.98	4.23 (s, ~0.4H)

[a]TA: Tartaric acid

3.2 | Detection of impurities by LC-ESI-QTOF-MS/MS

As described above, GC–MS analysis showed the detection of AL-LAD as a potentially viable (i.e., not artificially induced) impurity. When the powdered sample was subjected to further analysis by LC-ESI-QTOF-MS/MS, a complex chromatogram was obtained that revealed the detection of more peaks in addition to the one representing 1cP-AL-LAD detected at 7.4 min (also labeled as **1**) (Figure 5b). Further inspections also showed that AL-LAD (5.6 min, **2**) and 1P-AL-LAD (7.0 min, **3**) could readily be identified by comparing tandem mass spectral data and retention times with reference material available from previous work (Supporting Information for chromatographic traces).[11,20] For the remaining peaks **4**–**11**, several potential identities have been suggested based on tandem mass spectral data though it has to be noted that they had to remain speculative until reference material becomes available. The fragmentation pathways informing these proposals were based on those suggested during previous work including those found in the Supporting Information involving 1cP-AL-LAD.

The tandem mass spectrum for compound **4** (6.0 min) (Figure 6a) suggested a protonated molecule at *m/z* 392.2327 ($C_{24}H_{30}N_3O_2^+$). The radical loss of 41 u suggested that the N^6-allyl group remained unchanged, and *m/z* 128.1071 ($C_7H_{14}NO^+$) indicated the presence of an intact *N*,*N*-diethylamide moiety. The *m/z* 323.1752 ($C_{20}H_{23}N_2O_2^+$) might have been consistent with the formation of the RDA fragment typically observed with lysergamides. Taken together, this impurity was thought to be 1-acetyl-AL-LAD (1A-AL-LAD).

The tandem mass spectrum of compound **5** ($[M + H]^+$ at *m/z* 434.2466, $C_{26}H_{32}N_3O_3^+$, 8.6 min, Figure 6b) suggested the addition of one oxygen atom to the structure of 1cP-AL-LAD. The product ion at *m/z* 317.1644 ($C_{21}H_{21}N_2O^+$) suggested that the N^1-acyl and N^6-allyl remained unchanged. However, these might have been subsequently lost during formation of *m/z* 249.1386 ($C_{17}H_{17}N_2^+$) and *m/z* 208.0995 ($C_{14}H_{12}N_2^{\bullet+}$). A potential suggestion for the impurity that included the additional oxygen atom was therefore hypothesized to be the ethyl-2-hydroxyethylamide (**5**) (Figure 6b). The loss of 117 u (from the protonated molecule) was also reported to occur with the lysergic acid ethyl-2-hydroxyethylamide, a known LSD metabolite also known as LEO,[22] which pointed toward the ethyl-2-hydroxyethylamide derivative of 1cP-AL-LAD as a potential candidate.

The peak at 6.1 min labeled as compound **6** revealed a protonated molecule at *m/z* 378.2176 ($C_{23}H_{28}N_3O_2^+$, Figure 6c). The ion at *m/z* 349.1911 ($C_{22}H_{25}N_2O_2^+$) represented a neutral loss of 29.0262 u corresponding to CH_3N, which suggested the involvement of methanimine being expelled following the retro-Diels-Alder mechanism (Figure 6c) in analogy to the one described above for 1cP-AL-LAD (neutral loss of *N*-allylmethanimine, C_4H_7N). Correspondingly, lysergamides with an N^6-methyl group such as 1cP-LSD[19] and LSD itself would show a corresponding loss of 43 u (*N*-methylmethanimine, C_2H_5N). The loss of methanimine observed in the tandem mass spectrum of compound **6** therefore indicated the potential presence of the *N*-unsubstituted analog 1cP-nor-AL-LAD. The *m/z* 69.0340 confirmed that the 1-cyclopropanoyl group was still attached as it was detectable in the form of the oxonium ion ($C_4H_5O^+$). Detection of *m/z* 74.0967 ($C_4H_{12}N^+$) also indicated that the *N*,*N*-diethylamide moiety remained unaltered. The detection of this impurity might have been a residue from the procedure used to demethylate a lysergamide at N^6. One approach involves the use of cyanogen bromide to generate the N^6-CN intermediate, conversion to the N^6-H species,[7,8,23] and finally the N^6-allyl product.

The protonated molecule of impurity **7** (6.3 min, Figure 7a) was detected at *m/z* 414.2175 ($C_{26}H_{28}N_3O_2^+$) and suggested the loss of

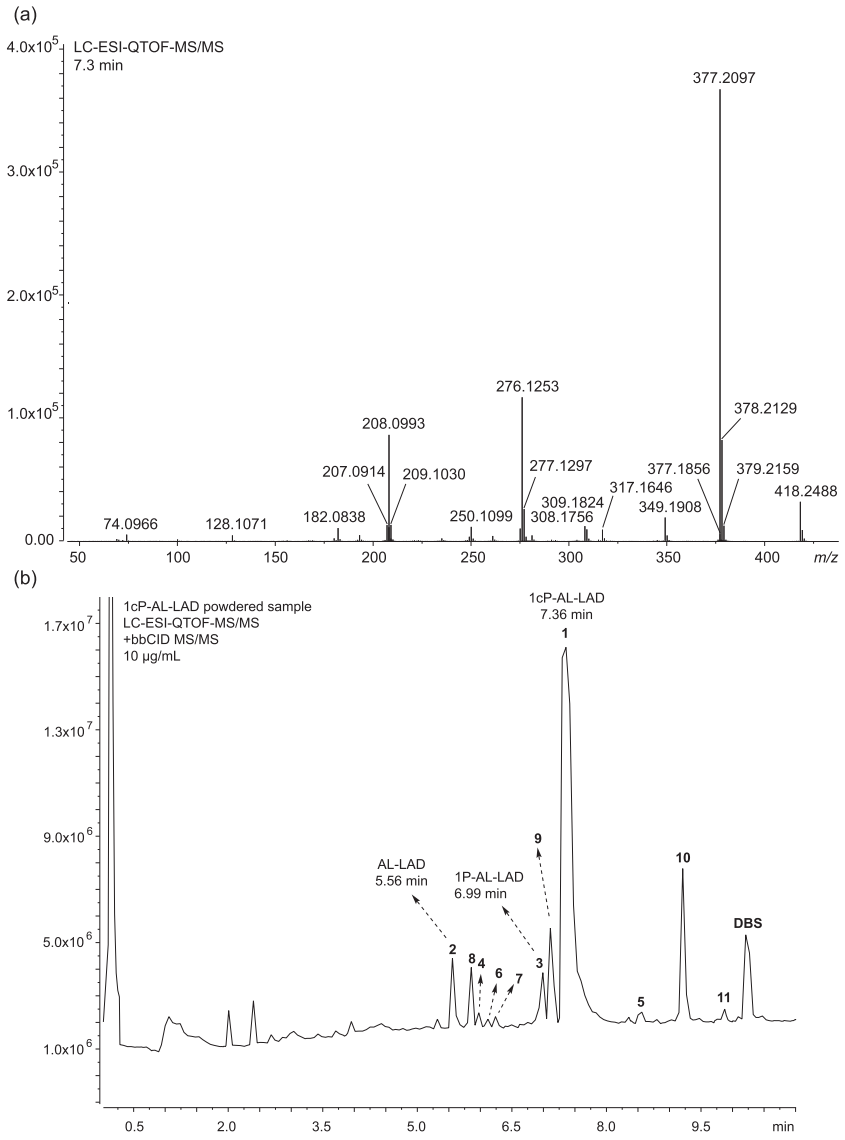


FIGURE 5 (a) Electrospray ionization (ESI) quadrupole time-of-flight (QTOF) tandem mass spectrum of 1cP-AL-LAD. (b) Detection of 1cP-AL-LAD and impurities in a powdered sample using LC-ESI-QTOF-MS/MS. DBS: dimethyldibenzylidene sorbitol, a low-molecular-weight gelator of organic solvents and plastics additive

four hydrogen atoms compared with 1cP-AL-LAD **1**. Similar to what was observed with 1cP-AL-LAD (Figure 5a), a loss of what correlated to an allyl radical led to the formation of a radical cation at *m/z* 373.1784 ($C_{23}H_{23}N_3O_2^{\bullet+}$), which underwent a subsequent neutral loss of the N^1-acyl group (cyclopropylidenemethanone), resulting in *m/z* 305.1519 ($C_{19}H_{19}N_3O^{\bullet+}$). A further cleavage of a diethylamino radical ($\bullet Et_2N$) could have been consistent with the formation of another even-electron species at *m/z* 233.0710 ($C_{15}H_9N_2O^+$). The mass difference of 4 u thus indicated that compound **7** represented a potential oxidation product of 1cP-AL-LAD resulting in the presence of two more double bonds, possibly located between C4/C5 and C7/C8 (Figure 7a).

Compound **8** (*m/z* 416.2335, $C_{26}H_{32}N_3O_3^+$, Figure 7b) represented a loss of two hydrogen atoms compared to 1cP-AL-LAD that could have resulted in the formation of one new double bond. Figure 7b shows that two potential positions were considered, for example, located between C7 and C8 or between C4 and C5. The formation of *m/z* 343.1441 ($C_{22}H_{19}N_2O_2^+$) indicated a neutral loss of *N,N*-diethylamine ($C_4H_{11}N$) and formation of an oxonium ion that subsequently underwent another neutral loss of CO to give *m/z* 315.1441 ($C_{21}H_{19}N_2O^+$). Interestingly, a radical loss of the N^6-allyl group was not observed to any significant extent even though this occurred in compound **7** (Figure 7a) and 1cP-AL-LAD **1** (Figure 5a, Supporting Information). As described above, the tandem mass spectrum of 1cP-AL-LAD **1** included a neutral loss *N*-allylmethanimine (69.0578 u) suggested to reflect an RDA mechanism, and to some extent, this was also observed in the tandem mass spectrum of compound **4** tentatively identified as 1A-AL-LAD (*m/z* 323.1752, $C_{20}H_{23}N_2O_2^+$, Figure 6a). Correspondingly, the same fragment was identified in the tandem mass spectrum of 1-acetyl-LSD (1A-LSD, ALD-52).[24] In comparison, it appeared that this particular mechanism was not involved in the spectra recorded for compounds **7** and **8** and perhaps one potential explanation was the additional presence of a double bond between C7 and C8 that might have prevented this mechanism to occur (loss of *N*-allylmethanimine).

The tandem mass spectrum shown in Figure 7c (compound **9**, 7.1 min) gave rise to a protonated molecule at *m/z* 434.2433 ($C_{26}H_{32}N_3O_3^+$). Compared with 1cP-AL-LAD, the mass increase involved the additional presence of an oxygen atom. Both *m/z*

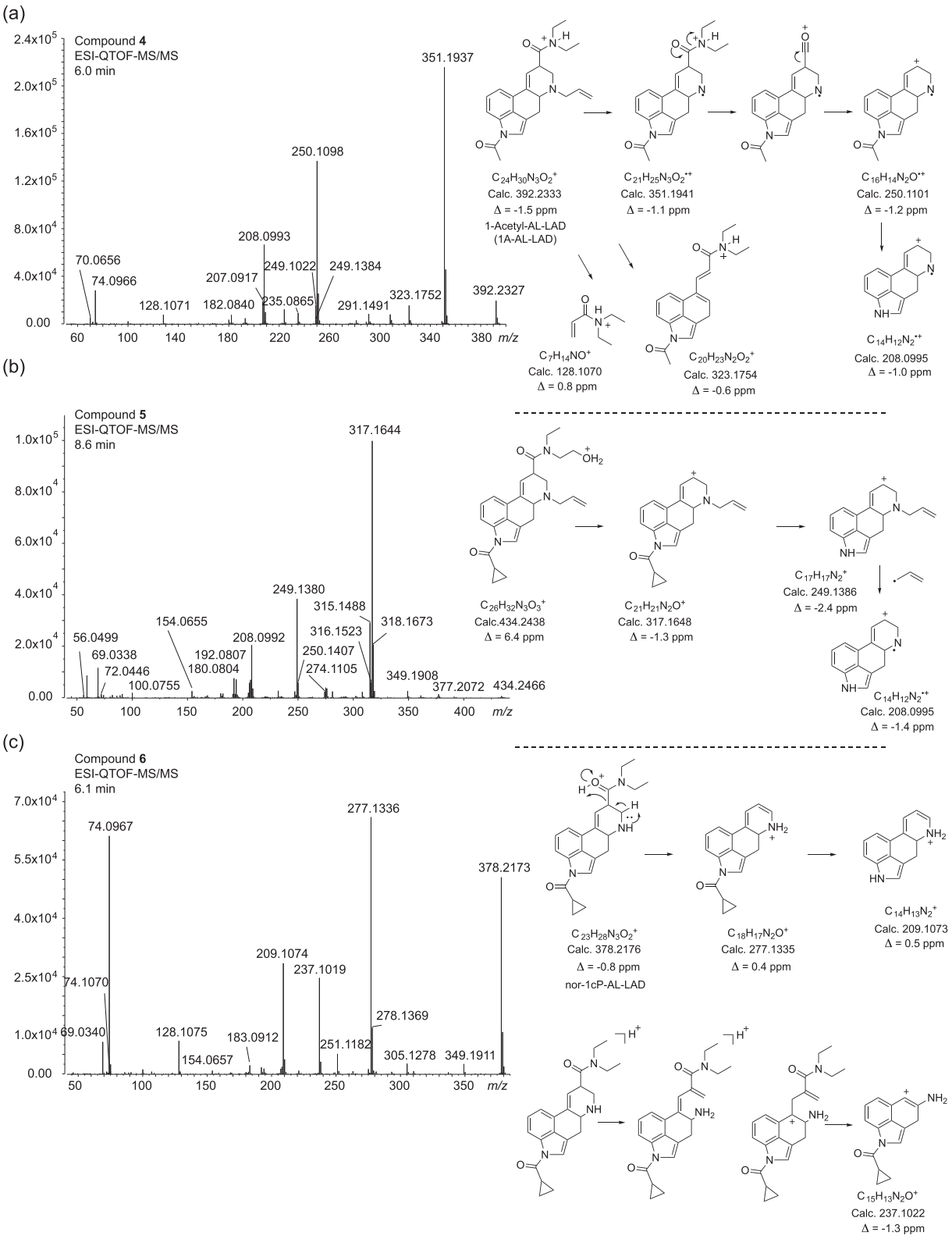


FIGURE 6 Tentative identifications of compounds **4**–**6** based on quadrupole time-of-flight (QTOF) tandem mass spectral data (a–c) and proposed fragmentations of selected product ions

69.0338 (N^1-cyclopropanoyl group in form of the oxonium ion) and *m/z* 74.0447 ($H_2NEt_2^+$) were detected and suggested that the two groups remained attached. The base peak was detected at *m/z* 349.1907 ($C_{22}H_{25}N_2O_2^+$), which was considered to be the same RDA fragment formed in the tandem spectrum of 1cP-AL-LAD (Figure 5a), which then indicated that the additional presence of the oxygen atom might have been located on the N^6-allyl substituent. As shown in Figure 7c, two potential structures (epoxide and propan-2-one-type)

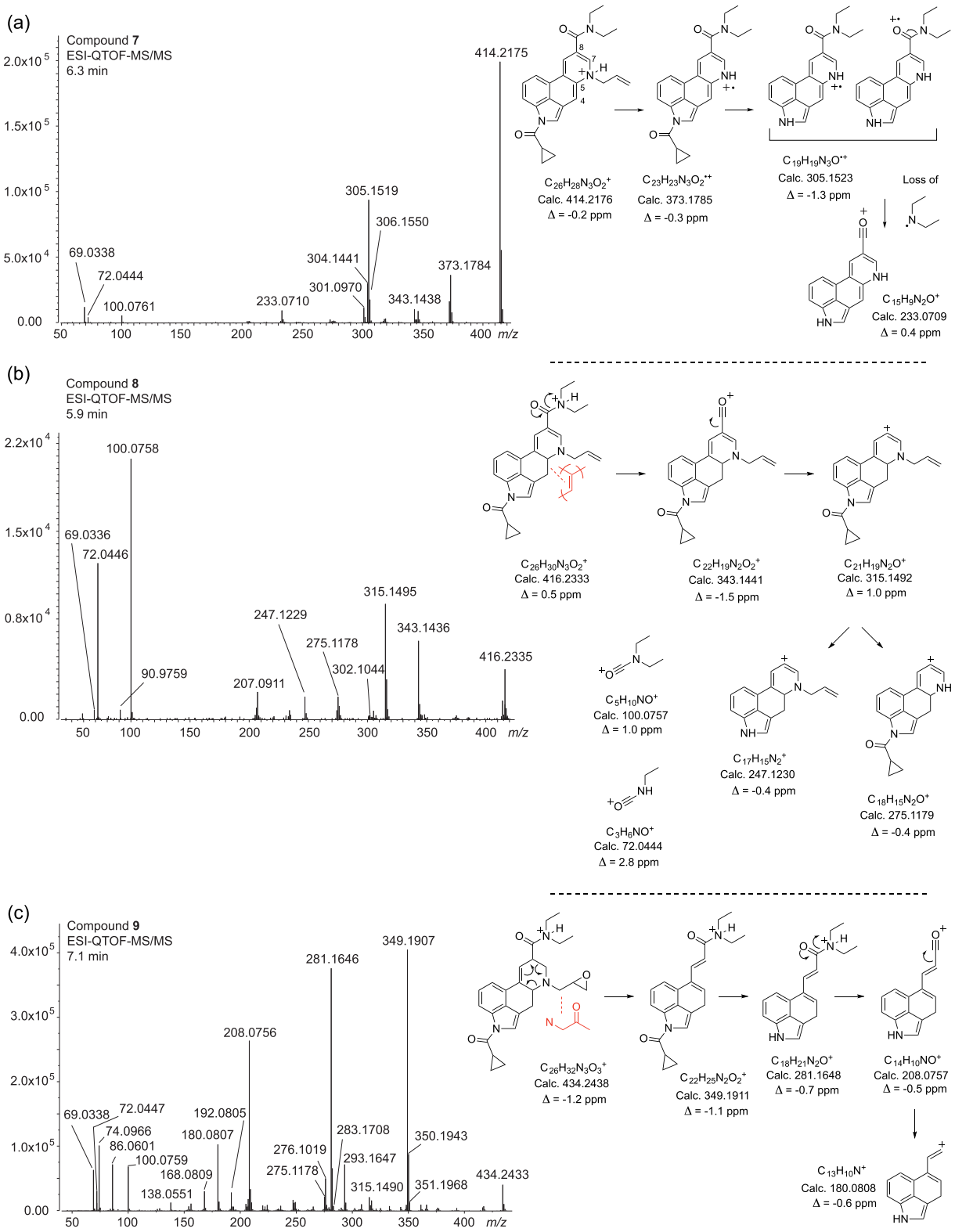


FIGURE 7 Tentative identifications of compounds **7**–**9** based on quadrupole time-of-flight (QTOF) tandem mass spectral data (a–c) and proposed fragmentations of selected product ions

were considered though it was not possible to unambiguously differentiate between them on mass spectral grounds alone as the oxidized substituent was cleaved as part of the neutral loss to form the RDA fragment at *m/z* 349.1907.

Compound **10** (*m/z* 464.2546, $C_{27}H_{34}N_3O_4^+$) eluted at 9.2 min (Figure 8a) and similar to some compounds tentatively identified above, the *m/z* 69.0338 and *m/z* 100.1057 ions suggested that the N^1-acyl substituent and the *N*,*N*-diethylamide group were not altered

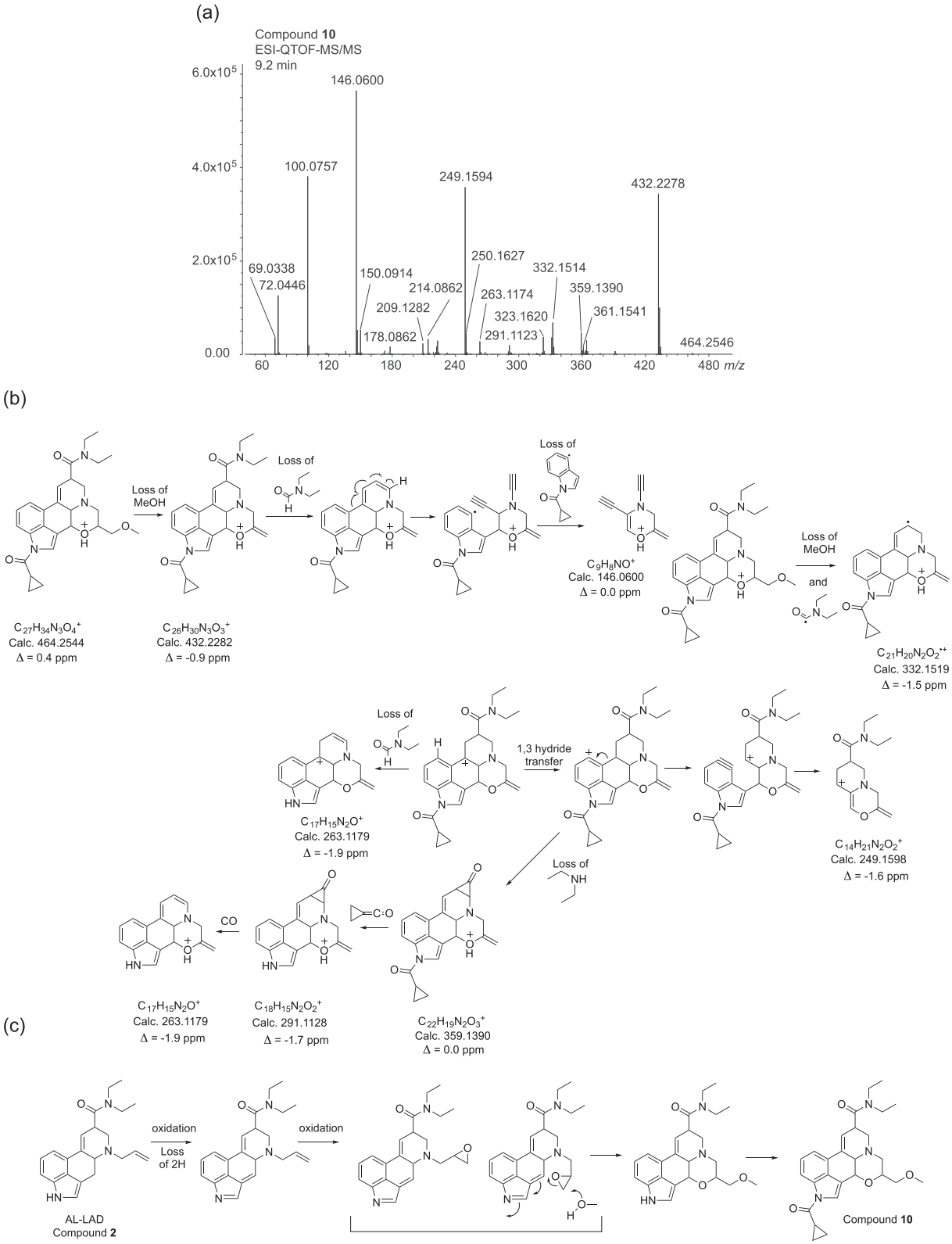


FIGURE 8 Tentative identification of compound **10** based on quadrupole time-of-flight (QTOF) tandem mass spectral data (a) and proposed fragmentation pathways (b). (c) Proposed mechanism of formation of compound **10** from the AL-LAD impurity **2**

and that modifications possibly involved the N^6-substituent. As shown in the proposed fragmentation pathway (Figure 8b), the *m/z* 432.2278 ($C_{26}H_{30}N_3O_3^+$) indicated the potential loss of methanol (32.0268 u). Compared with 1cP-AL-LAD, the mass of compound **10** appeared to involve a mass shift of 46.0055 u (CH_2O_2). One of the oxidation products tentatively identified above involved the presence of an epoxide group (compound **9**, Figure 7c), and its reactivity was considered a factor in the formation compound **10** in form of a morpholine-type

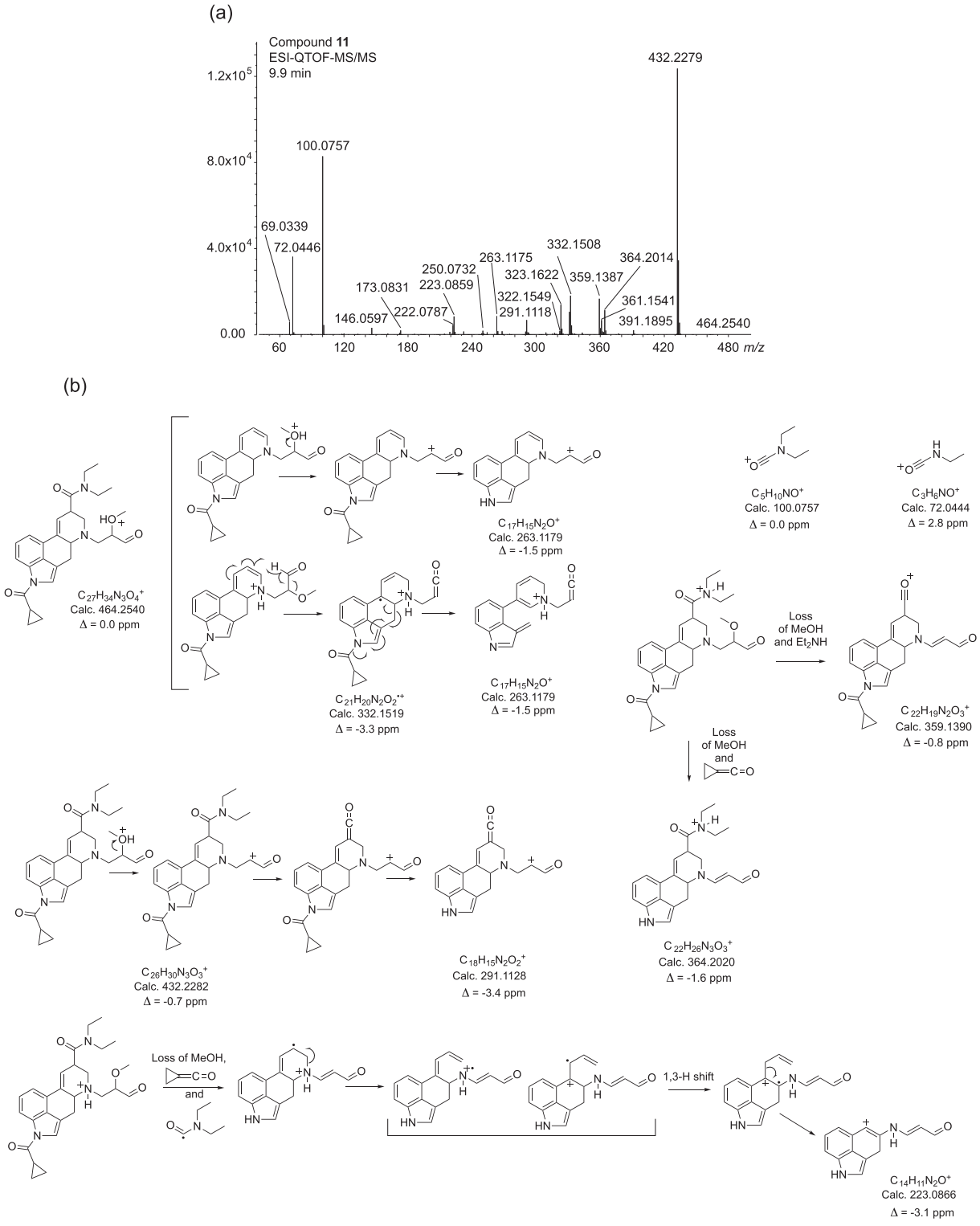


FIGURE 9 Tentative identification of compound **11** based on quadrupole time-of-flight (QTOF) tandem mass spectral data (a) and proposed fragmentation pathways (b)

candidate. In Figure 8c, a potential explanation for its formation during synthesis is presented. The presence of AL-LAD (compound **2**, Figure 5b) was described above, and this suggested that this might have been a synthesis related product. One of the steps involved in the potential oxidation of the N^6-allyl group might have involved the formation of an epoxide intermediate that could have undergone a ring opening and further reaction with C4 to form the morpholine-type ring. The reaction conditions used could have involved the use of

methanol at some stage, which could be consistent with the formation of the methoxymethyl group attached to the morpholine ring. Acylation of this morpholino AL-LAD during the synthesis of 1cP-AL-LAD could then give rise to 1-cP-morpholino AL-LAD **10**.

Another compound eluting at 9.9 min (Figure 5b) was detected in the LC–MS chromatogram (compound **11**) with a protonated molecule at *m/z* 464.2544 ($C_{27}H_{34}N_3O_4^+$), also reflecting a mass shift of 46.0055 u (CH_2O_2) comparable with the shift observed with compound **10**. Figure 9a presents the tandem mass spectrum, and proposed fragmentation pathways are shown in Figure 9b. Similar to compound **10**, the increased mass was thought to reflect a modification of the N^6-allyl group involving an oxidative transformation (Figure 9b). In this spectrum, the *m/z* 432.2279 ($C_{26}H_{30}N_3O_3^+$) formed the base peak, also possibly involving the loss of methanol 32.0265 u (CH_4O) with remaining fragmentations including those described above (Figure 9b). Seven additional tandem mass spectra were recorded in full scan/AutoMS/MS mode and provided as Supporting Information. Potential structural proposals could only be made for some spectra, which included some potential oxidation products.

The chromatogram also showed a noticeable peak at 10.2 min (labeled as DBS), and inspection of the mass spectrum recorded in single stage mode showed more than one ion, indicative of adduct formation (Supporting Information). The protonated molecule was detected at *m/z* 415.2112 ($C_{24}H_{31}O_6^+$) together with the sodiated species (*m/z* 437.1932, $C_{24}H_{30}NaO_6^+$) and potassium adduct (*m/z* 453.1670, $C_{24}H_{30}KO_6^+$). The detection of *m/z* 473.2642 ($C_{26}H_{37}O_6^+$) hypothesized to reflect the $[M + CH_3CN + NH_4]^+$ species was also observed. The tandem mass spectrum of *m/z* 415.2112 gave rise to a base peak at *m/z* 119.0856 and some others of low abundance. One other product ion however was detected at *m/z* 135.0806 ($C_9H_{11}O^+$) (Supporting Information). Taken together, the product ions indicated that the compound detected at 10.2 min was not related to a lysergamide. Instead, the identity was hypothesized to represent dimethyldibenzylidene sorbitol (DBS), a low-molecular-weight gelator of organic solvents and plastics additive.[25,26] The proposed fragmentation pathway appeared to be consistent with the structure (Supporting Information). The DBS contaminant has been observed in some other chromatographic runs previously, which suggests that it might have been introduced during analysis.

3.3 | Blotter sample analysis results

When sold for recreational use, LSD and other lysergamides are commonly sold absorbed on heavy blotter paper ("paper" or "blotters"). Analysis of methanolic extracts prepared from "blotters" purportedly containing 1cP-AL-LAD confirmed the presence of this lysergamide, as well as GC-induced impurities (Figure 2c); AL-LAD was not detected under the conditions used. Using an alternative LC–MS method, a trace of 1P-AL-LAD was noticeable in the extracted ion chromatogram, but AL-LAD was not detectable (Supporting Information). According to information disseminated on the Internet, so-called "fake blotters" have been circulating on the online recreational lysergamide market, including some samples containing 1cP-AL-LAD. Results from an LC–MS analysis performed and posted anonymously indicated significant contamination with other lysergamides, including potentially iso-1cP-AL-LAD, 1P-AL-LAD, or and/iso-1P-AL-LAD) (Supporting Information). The protonated molecule relating to one chromatographic peak was detected at *m/z* 421. Even though tandem mass spectral data were not reported, it is tempting to speculate that this might have represented the N^6-acetamide side product (N^6-(CO)NH_2) that might have been formed from the N^6-CN intermediate involved in the formation of the N^6-H precursor. Interestingly, there is precedent for the formation of the N^6-acetamide of LSD when the N^6-CN intermediate was subjected to sodium hydroxide in dioxane.[23] In comparison, the blotter extract analyzed in the present study did not reveal these types of impurities, which suggested that they might have originated from a different source.

4 | CONCLUSION

A comprehensive analytical characterization of the novel lysergamide 1cP-AL-LAD was performed involving gas- and liquid chromatography, several forms of mass spectrometry, GC-sIR, and NMR spectroscopy. The detection of 17 impurities in a powdered sample illustrates the complexities associated with the chemistry of lysergamides, especially those containing N^6-allyl substituents. Without reference standards, identification of some of the impurities is tentative. Still, it is hoped that the available information will assist others who are interested in the chemistry of these and other lysergamides.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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