



Accepted manuscript before final correction

Return of the lysergamides. Part I: Analytical and behavioral characterization of 1-propionyl-d-lysergic acid diethylamide (1P-LSD)

Journal:	<i>Drug Testing and Analysis</i>
Manuscript ID:	DTA-15-0251.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Brandt, Simon; School of Pharmacy & Biomolecular Sciences , Liverpool John Moores University Kavanagh, Pierce; Trinity Centre for Health Sciences, Department of Pharmacology and Therapeutics Westphal, Folker; State Bureau of Criminal Investigation Schleswig-Holstein, Section Narcotics/Toxicology Stratford, Alexander; Synex Ltd, Elliott, Simon; ROAR Forensics, Hoang, Khoa; University of the Sciences, Department of Chemistry and Biochemistry Wallach, Jason; University of the Sciences, Department of Pharmaceutical Sciences Halberstadt, Adam; University of California San Diego, Department of Psychiatry
Keywords:	New psychoactive substances, LSD, psychedelics, 5-HT2A receptor, head-twitch response
Abstract:	1-Propionyl-d-lysergic acid diethylamide hemitartrate (1P-LSD) has become available as a 'research chemical' in form of blotters and powdered material. This non-controlled derivative of d-lysergic acid diethylamide (LSD) has previously not been described in the published literature despite being closely related to 1-acetyl-LSD (ALD-52), which was developed in the 1950s. This study describes the characterization of 1P-LSD in comparison with LSD using various chromatographic, mass spectrometric methods and nuclear magnetic resonance spectroscopy. An important feature common to LSD and other serotonergic hallucinogens is that they produce 5-HT2A-receptor activation and induce the head-twitch response (HTR) in rats and mice. In order to assess whether 1P-LSD displays LSD-like properties and activates the 5-HT2A receptor, male C57BL/6J mice were injected with vehicle (saline) or 1P-LSD (0.025–0.8 mg/kg, IP) and HTR assessed for 30 min using magnetometer coil recordings. It was found that 1P-LSD produced a dose-dependent increase in HTR counts, and that it had ~38% (ED ₅₀ = 349.6 nmol/kg) of the potency of LSD (ED ₅₀ = 132.8 nmol/kg). Furthermore, the HTR was abolished when 1P-LSD administration followed pre-treatment with the selective 5-HT2A receptor antagonist M100907 (0.1 mg/kg, SC), which confirms that the behavioral response is mediated by

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	activation of the 5-HT _{2A} receptor. These results indicate that 1P-LSD produces LSD-like effects in mice, consistent with its classification as a serotonergic hallucinogen. Nevertheless, the extent to which 1P-LSD might show psychoactive effects in humans similar to LSD remains to be investigated.

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3 **Return of the lysergamides. Part I: Analytical and behavioral**
4 **characterization of 1-propionyl-*d*-lysergic acid diethylamide (1P-**
5 **LSD)**
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8 Simon D. Brandt,^{a,*} Pierce V. Kavanagh,^b Folker Westphal,^c Alexander
9 Stratford,^d Simon P. Elliott,^e Khoa Hoang,^f Jason Wallach,^g Adam L.
10 Halberstadt,^h
11

12
13
14 ^a *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom*
15 *Street, Liverpool L3 3AF, UK*
16

17 ^b *Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for*
18 *Health Sciences, St. James Hospital, Dublin 8, Ireland*
19

20 ^c *State Bureau of Criminal Investigation Schleswig-Holstein, Section Narcotics/Toxicology,*
21 *Mühlenweg 166, D-24116 Kiel, Germany*
22

23 ^d *Synex Ltd, 67-68 Hatton Garden, N13 4BS, London, UK*
24

25 ^e *ROAR Forensics, Malvern Hills Science Park, Geraldine Road, WR14 3SZ, UK*
26

27 ^f *Department of Chemistry and Biochemistry, University of the Sciences, Philadelphia, PA*
28 *19104, USA*
29

30 ^g *Department of Pharmaceutical Sciences, Philadelphia College of Pharmacy, University of*
31 *the Sciences, Philadelphia, PA 19104, USA*
32

33 ^h *Department of Psychiatry, University of California San Diego, La Jolla, CA 92093-0804,*
34 *USA*
35

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38
39 * Correspondence to: Simon D. Brandt, School of Pharmacy and Biomolecular Sciences,
40 Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK. E-Mail:
41 s.brandt@ljmu.ac.uk
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45 **Running title:** Analytical and behavioral characterization of 1-propionyl-LSD
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48 **Keywords:** New psychoactive substances; LSD; 5-HT_{2A} receptor; lysergamides;
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Abstract

1-Propionyl-*d*-lysergic acid diethylamide hemitartrate (1P-LSD) has become available as a 'research chemical' in form of blotters and powdered material. This non-controlled derivative of *d*-lysergic acid diethylamide (LSD) has previously not been described in the published literature despite being closely related to 1-acetyl-LSD (ALD-52), which was developed in the 1950s. This study describes the characterization of 1P-LSD in comparison with LSD using various chromatographic, mass spectrometric methods and nuclear magnetic resonance spectroscopy. An important feature common to LSD and other serotonergic hallucinogens is that they produce 5-HT_{2A}-receptor activation and induce the head-twitch response (HTR) in rats and mice. In order to assess whether 1P-LSD displays LSD-like properties and activates the 5-HT_{2A} receptor, male C57BL/6J mice were injected with vehicle (saline) or 1P-LSD (0.025–0.8 mg/kg, IP) and HTR assessed for 30 min using magnetometer coil recordings. It was found that 1P-LSD produced a dose-dependent increase in HTR counts, and that it had ~38% (ED₅₀ = 349.6 nmol/kg) of the potency of LSD (ED₅₀ = 132.8 nmol/kg). Furthermore, the HTR was abolished when 1P-LSD administration followed pre-treatment with the selective 5-HT_{2A} receptor antagonist M100907 (0.1 mg/kg, SC), which confirms that the behavioral response is mediated by activation of the 5-HT_{2A} receptor. These results indicate that 1P-LSD produces LSD-like effects in mice, consistent with its classification as a serotonergic hallucinogen. Nevertheless, the extent to which 1P-LSD might show psychoactive effects in humans similar to LSD remains to be investigated.

Introduction

It is perhaps fair to consider that the synthesis^[1] and discovery of the psychoactive properties of *d*-lysergic acid diethylamide (LSD)^[2] (Figure 1) in 1943 triggered an avalanche of investigations that continue to capture the imagination of researchers across all disciplines.^[3-10] Although the pharmacology and properties of LSD have been investigated in many studies, major questions still remain unanswered and are expected to occupy the attention of researchers in the future.^[11-16]

Reports have been published indicating LSD may possess therapeutic efficacy in patients suffering from disorders such as anxiety, alcoholism, cluster headaches, and autism, but unfortunately most of this evidence is anecdotal in nature or confounded by methodological shortcomings.^[17-19] Importantly, although most clinical work with LSD ceased in the late 1960s, human trials have cautiously resumed during the last few years.^[20-23]

A range of lysergamide derivatives have been prepared to explore their molecular pharmacology (e.g.^[24-32]) but the extent to which these show psychoactive properties in humans is not always clear.^[7] In recent years, several lysergamide derivatives have been distributed as new psychoactive substances or "research chemicals" in the UK and Europe.^[33] For example, lysergic acid 2,4-dimethylazetidide (LSZ)^[31] and *N*₆-allyl-6-norlysergic acid diethylamide (AL-LAD)^[24,30] are two lysergamide derivatives with LSD-like effects in animals that originated from academic research

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3 and have been available for purchase in powdered and blotter form. Another closely
4 related derivative with modification at the indole nitrogen is 1-acetyl-LSD (ALD-52)
5 (Figure 1). Synthesis of ALD-52 was first reported in 1957 (e.g.^[34]) and it was found
6 to be psychoactive in humans^[35-38] but it is not clear whether ALD-52 was also sold in
7 the UK. Recent changes in UK legislation, however, precluded the open sale of
8 several lysergamides, including ALD-52, LSZ and AL-LAD.^[39] In response to those
9 legal restrictions, 1-propionyl-LSD (Figure 1), also known as 1P-LSD, became
10 available as a “research chemical” either as powdered material or on blotters.
11 Although an assortment of LSD derivatives substituted at the 1-position have been
12 described (e.g.^[34,37,40-42]), it is noteworthy that chemical, analytical or pharmacological
13 data related to 1-propionyl-LSD appear to be absent from the literature.
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17 Because of the difficulty associated with studying hallucinogens in humans, animal
18 behavioral models are often used to investigate the pharmacology of hallucinogenic
19 drugs. One behavioral model that has been widely adopted is the head twitch
20 response (HTR), a paroxysmal side-to-side head movement induced by 5-HT_{2A}
21 agonists in rats and mice. The HTR is considered to be a rodent behavioral proxy for
22 human hallucinogenic effects because it can distinguish between hallucinogenic and
23 non-hallucinogenic 5-HT_{2A} agonists.^[43-46] Although the HTR has traditionally been
24 assessed using direct observation, new methods have been developed to detect the
25 HTR in a semi-automated fashion using a head-mounted magnet and a
26 magnetometer coil.^[46,47]
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30 The present report details the analytical characterization of 1P-LSD using various
31 chromatographic, mass spectrometric and spectroscopic methods relevant to clinical
32 and forensic investigations. Supplementary analytical data derived from the
33 characterization of LSD have also been included for comparative purposes. In
34 addition, *in vivo* studies were conducted to assess the potential similarity between
35 1P-LSD and LSD in regards to their 5-HT_{2A} receptor pharmacology and behavioral
36 effects. Previous investigations using magnetometer-based measurements have
37 shown that LSD and other serotonergic hallucinogens induce the HTR in a dose-
38 dependent manner in male C57BL/6J mice^[47] consistent with the mechanism of 5-
39 HT_{2A} receptor activation involved in this behavior.^[45,47] In the present study, it was
40 hypothesized that 1P-LSD would induce the HTR in mice if it had LSD-like
41 properties. Furthermore, we predicted that pretreatment with a selective 5-HT_{2A}
42 receptor antagonist, such as M100907, would block the HTR induced by 1P-LSD,
43 confirming the involvement of 5-HT_{2A} receptor activation.
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47 **Experimental**

48 **Materials**

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50 All chemicals used were of analytical and HPLC grade and obtained from Aldrich
51 (Dorset, UK). d₆-DMSO (99.8% D) was from VWR (Leicestershire, UK). 1-Propionyl-
52 d-lysergic acid diethylamide hemitartrate powder (1P-LSD) was supplied by Synex
53 Ltd (London, UK), M100907 was from Hoechst Marion Roussel (Kansas City, MO,
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USA), and LSD tartrate was from Ultrafine Chemicals (Manchester, UK). One blotter labelled to contain 100 µg 1P-LSD was purchased from an Internet vendor.

Instrumentation

Nuclear magnetic resonance spectroscopy

NMR spectra were recorded in d_6 -DMSO using a Bruker Avance 300 spectrometer (^1H at 300.1 MHz; ^{13}C at 75 MHz) and suggested assignments were aided by 1-D and 2-D experiments. Internal chemical shift references were based on residual solvent peaks.

Gas chromatography mass spectrometry

Electron ionization (EI) mass spectra (70 eV) were obtained with a Finnigan TSQ 7000 triple stage quadrupole mass spectrometer coupled to a gas chromatograph (Trace GC Ultra, Thermo Electron) using a CTC CombiPAL (CTC Analytics, Switzerland) autosampler. The emission current was 200 µA and the scan time was 1 s spanning a scan range between m/z 29 – m/z 600. The ion source temperature was maintained at 175 °C. Samples were introduced via gas chromatography with splitless injection using a fused silica capillary DB-1 column (30 m x 0.25 mm, film thickness 0.25 µm). The temperature program consisted of an initial temperature of 80 °C, held for 1 min, followed by a ramp to 280 °C at 15 °C/min. The final temperature was held for 21 min. The injector temperature was 220 °C. The transfer line temperature was maintained at 280 °C and the carrier gas was helium in constant flow mode at a flow rate of 1.0 mL/min. Approximately 2 mg were dissolved in 1.5 mL methanol. For analysis, 1 µL sample solutions were injected into the GC-MS system.

High-resolution electrospray mass spectrometry

The ultrahigh-performance liquid chromatography quadrupole time of flight single and tandem mass spectrometry (UHPLC-QTOF-MS/MS) conditions were used as described previously.^[48,49] Mobile phases used for UHPLC separation consisted of 100% acetonitrile (1% formic acid) and an aqueous solution of 1% formic acid. The column temperature was set at 40 °C (0.6 mL/min) and data were acquired for 5.5 min. The elution was a 5–70% acetonitrile gradient ramp over 3.5 min, then increased to 95% acetonitrile in 1 min and held for 0.5 min before returning to 5% acetonitrile in 0.5 min. QTOF-MS data were acquired in positive mode scanning from m/z 100 – m/z 1000 with and without auto MS/MS fragmentation. Ionization was achieved with an Agilent JetStream electrospray source and infused internal reference masses. Agilent 6540 QTOF-MS parameters: gas temperature 325 °C, drying gas 10 L/min and sheath gas temperature 400 °C. Internal reference ions at m/z 121.05087 and m/z 922.00979 were used.

Liquid chromatography electrospray mass spectrometry

LC-MS analyses were performed on an Agilent 1100 system. Separation was

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3 obtained on a Restek (Bellefonte, PA, USA) Allure PFPP column (5 μm , 50 x 2.1mm).
4 Mobile phase A consisted of 0.1% formic acid in water, whereas, mobile phase B
5 consisted of 0.1% formic acid in acetonitrile. The Agilent LC-MSD settings were as
6 follows: positive electrospray mode, capillary voltage 3500 V, drying gas (N_2) 12
7 L/min at 350 $^\circ\text{C}$, nebulizer gas (N_2) pressure 50 psi, Scan mode m/z 70 – 500,
8 fragmentor voltage 150 V. The sample for LC-MS analysis was dissolved in
9 acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 10 $\mu\text{g}/\text{mL}$.
10 The injection volume was 1 μL , flow rate was 0.80 mL/min and the column
11 temperature was 30 $^\circ\text{C}$. The total run time was 25 min. The following gradient elution
12 program was used: 0–2 min 2% B, followed by an increase to 60% within 15 min,
13 then up to 80% within 20 min, returning to 2% within 25 min.
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16 17 *Gas chromatography solid-state infrared analysis*

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19 The methanolic solution was measured on a GC-solid phase-IR-system consisting of
20 an Agilent GC 7890B (Waldbronn, Germany) with probe sampler Agilent G4567A
21 and a DiscovIR-GCTM (Spectra Analysis, Marlborough, Massachusetts, USA). The
22 column eluent was cryogenically accumulated on a rotating ZnSe disk that was
23 cooled by liquid nitrogen. The IR spectra were directly recorded through the IR-
24 transparent ZnSe disk using a nitrogen cooled MCT detector. GC parameters: the
25 injection was carried out in splitless mode with an injection port temperature of
26 240 $^\circ\text{C}$ and a DB-1 fused silica capillary column (30 m x 0.32 mm i.d., 0.25 μm film
27 thickness). The carrier gas was helium with a flow rate of 2.5 mL/min; oven
28 temperature program: 80 $^\circ\text{C}$ for 2 min, ramped to 290 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$, and held at
29 the final temperature for 25 min. The transfer line heater was set at 280 $^\circ\text{C}$. IR
30 conditions: oven temperature, restrictor temperature, disc temperature, and dewar
31 cap temperatures were 280 $^\circ\text{C}$, 280 $^\circ\text{C}$, -40 $^\circ\text{C}$, and 35 $^\circ\text{C}$, respectively. The vacuum
32 was 0.2 mTorr, disc speed 3 mm/s, spiral separation was 1 mm, wavelength
33 resolution 4 cm^{-1} and IR range 650–4000 cm^{-1} . Acquisition time was 6s/file with 64
34 scans/spectrum. Data were processed using GRAMS/AI Ver. 9.1 (Grams
35 Spectroscopy Software Suite, Thermo Fischer Scientific) followed by implementation
36 of the OMNIC Software, Ver. 7.4.127 (Thermo Electron Corporation).
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42 **Animal pharmacology**

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44 Male C57BL/6J mice (6–8 weeks old) were obtained from Jackson Laboratories (Bar
45 Harbor, ME, USA) and housed up to four per cage with a reversed light-cycle (lights
46 on at 1900 h, off at 0700 h). Food and water were provided *ad libitum*, except during
47 behavioral testing. Testing was performed between 1000 and 1830 h. The head
48 twitch response (HTR) was detected using a head mounted magnet and a
49 magnetometer coil.^[47] Mice received intraperitoneal (IP) vehicle (saline) or 1P-LSD
50 (0.025, 0.05, 0.1, 0.2, 0.4, or 0.8 mg/kg), and then HTR was assessed for 30 min.
51 For the antagonist study, mice received subcutaneous (SC) vehicle (water containing
52 5% Tween-80) or 0.1 mg/kg M100907 20 min before IP vehicle (saline) or 0.4 mg/kg
53 1P-LSD, and then HTR was assessed for 20 min. The injection volume was 5 mL/kg.
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Analysis

For the 1P-LSD dose-response study, HTR counts were analyzed using one-way ANOVAs, with time as a repeated measure (when appropriate); *post-hoc* comparisons were made using Tukey's studentized range method. For the antagonist blockade study, HTR counts were analyzed using the Kruskal-Wallis test; *post-hoc* comparisons were made using the Dwass-Steel-Christchlow-Fligner test. Significance was demonstrated by surpassing an α -level of 0.05. ED₅₀ values and 95% confidence limits were calculated using nonlinear regression.

Results and discussion

The ability to characterize and correctly identify new psychoactive substances constitutes an important part of forensic and clinical work. Aside from LSD, the analysis and analytical characterizations of lysergamide derivatives have been featured in investigations carried out in previous decades, possibly due to wider availability of LSD and greater interest at the time; examples include ALD-52, *d*-lysergic acid *N*-methyl-*N*-propylamide (LAMPA) and a range of other *N,N*-dialkylated derivatives.^[50-58] The analytical and pharmacological characterization of 1-propionyl-*d*-lysergic acid diethylamide (1P-LSD) reported in the present study represents the newest addition to the field of lysergamide analysis within the field of clinical study and the topic of new psychoactive substances that are available for purchase from Internet retailers.^[33]

Analytical features

Gas chromatography electron ionization (EI) mass spectrometry (GC-MS) and retention time data for 1P-LSD and LSD are shown in **Figure 2**. A comparison of both EI mass spectra showed a number of common fragment ions with similar *m/z* values whereas others represented the corresponding mass shifts associated with the propionyl group in the 1-position. An example of this substituent-related shift may be seen in the fragment cluster at *m/z* 277, *m/z* 278 and *m/z* 279 (1P-LSD, Figure 2A) compared to the corresponding ions at *m/z* 221, *m/z* 222 and *m/z* 223 (LSD, Figure 2B), respectively. A typical set of fragments observed in both mass spectra, for example, were represented by the cluster of ions at *m/z* 151 – *m/z* 154 which indicated that 1P-LSD might have shown some converging fragmentation behavior. Intense molecular ions were detected in both cases (1P-LSD at *m/z* 379; LSD at *m/z* 323).

A possible fragmentation pattern for 1P-LSD is suggested in **Figure 3** and earlier literature on EI mass spectrometry of LSD was particularly helpful for comparison and mechanistic considerations.^[50,55,56,59-61] Similar to what was observed with LSD, iminium ion formation at *m/z* 72 was thought to be associated with α -cleavage induced mechanisms depending on the site of ionization, i.e. either directly or via *m/z* 100, respectively (Figure 3A).^[58] Figures 3B1 – 3B4 depict a number of either radical cleavages or neutral losses to account for the detection of *m/z* 336, *m/z* 322, *m/z* 279

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3 and m/z 252 (EI mass spectrum in Figure 2A), respectively. With the exception of
4 m/z 322 (Figure 3B2), which represented the loss of the acyl radical from 1P-LSD,
5 the remaining three species might have represented the propionyl substituted
6 counterparts of those species previously described for LSD detected at m/z 280
7 (retro-Diels Alder), m/z 223 and m/z 196 (loss of *N,N*-diethylacrylamide) (Figure
8 2B).^[50] Neutral loss of *N,N*-diethylformamide from the M^{*+} might have led to the
9 formation of the m/z 278 ion (Figures 2A and 3C), presumably in alignment with the
10 m/z 222 counterpart observed in the EI mass spectrum of LSD (Figure 2B).^[50] In
11 comparison, the published EI mass spectrum of 1-acetyl LSD (ALD-52) revealed the
12 detection of the retro-Diels Alder fragment at m/z 322 and the cluster of m/z 263, m/z
13 264 and m/z 265 that signified the difference in mass compared to 1P-LSD. In
14 addition to the M^{*+} of ALD-52 at m/z 365, further key ions reported for ALD-52
15 included m/z 249, m/z 238, the m/z 221 – m/z 223 cluster, m/z 207, m/z 181, m/z
16 167, the m/z 151 – m/z 154 cluster, m/z 128 and m/z 100, respectively.^[50] The
17 remaining fragments and suggested mechanisms shown in Figures 3B4 and 3C
18 exemplify a chain of radical and neutral losses frequently encountered during mass
19 spectral analysis under EI conditions.^[62] GC ion trap mass spectra of 1P-LSD
20 recorded under EI chemical ionization conditions are provided as supplemental
21 information. Under CI ion trap MS conditions, dissociation of the protonated molecule
22 at m/z 380 gave rise to an abundant species at m/z 337, consistent with a neutral
23 loss of *N*-methylmethanimine (supplemental data).
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29 The implementation of ultra high-performance liquid chromatography electrospray
30 ionization accurate mass quadrupole time of flight tandem mass spectrometry
31 (UHPLC-ESI-QTOF-MS/MS) for the detection of 1P-LSD and LSD is shown in **Figure**
32 **4**. Separation between the two analytes was obtained leading to retention times of
33 2.355 min for 1P-LSD (Figure 4A) and 2.029 min for LSD (Figure 4B), respectively.
34 Furthermore, some of the mass spectral features were consistent with product ions
35 specific for 1P-LSD due to additional mass units of 56 amu compared to LSD.
36 Prominent examples included m/z 337 vs. m/z 281 and m/z 279 vs. m/z 223,
37 respectively, and suggested structural representations and their possible
38 mechanisms of formation are summarized in **Figure 5**. The main product ions
39 observed for LSD under QTOF-MS/MS conditions essentially agreed with those
40 reported previously using Fourier transform-ion cyclotron resonance mass
41 spectrometry.^[63] The presence of LSD in the 1P-LSD powdered product was not
42 detected.
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46 As shown as supplemental data, analysis by diode array detection confirmed that 1P-
47 LSD and LSD gave distinct UV spectra that facilitated their differentiation. In the case
48 of 1P-LSD, three absorption peaks were detected at 226 nm, 250 nm and 294 nm
49 whereas two peaks were visible for LSD at 222 nm and 314 nm, respectively. The
50 implementation of GC solid-state infrared analysis is shown in **Figure 6**. The eluting
51 analyte was deposited cryogenically onto an IR-transparent zinc selenide disk that
52 enabled the recording of the IR spectrum of the solidified eluent. Key features in the
53 IR spectrum were reminiscent of those reported for ALD-52 where, for example, the
54 absence of the N-H stretch was noted as a consequence of substitution at the indole
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3 nitrogen. The appearance of two strong carbonyl bands at 1703.7 cm^{-1} (1-acetyl) and
4 1637.5 cm^{-1} (amide carbonyl) were also consistent with the structure.^[34,51]
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8 Determination of accurate mass values confirmed acceptable mass accuracy for the
9 molecular formulae linked to the protonated molecules and possible product ions.
10 Detected key product ions included m/z 337.19047 ($\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_2^+$) (Figure 5A1, loss
11 of *N*-methylmethanimine) and m/z 279.14899 ($\text{C}_{18}\text{H}_{19}\text{N}_2\text{O}^+$) that might be rationalized
12 by a loss of *N,N*-diethylformamide ($\text{C}_5\text{H}_{11}\text{NO}$) from the protonated molecule
13 ($\text{C}_{23}\text{H}_{30}\text{N}_3\text{O}_2^+$), see Figure 5A2. A product ion observed in both spectra was
14 associated with $\text{C}_{14}\text{H}_{10}\text{NO}^+$ at m/z 208.07591 (1P-LSD) and m/z 208.07578 (LSD),
15 respectively. A proposed mechanism of formation from 1P-LSD is provided in Figure
16 5A3. In such a scenario, a precursor ion at m/z 264 ($\text{C}_{17}\text{H}_{14}\text{NO}_2^+$; calculated m/z
17 264.10191) would have been required in order to continue dissociation by loss of
18 prop-1-en-1-one ($\text{C}_3\text{H}_4\text{O}$) to give the detected species at m/z 208.07591. An ion with
19 very low abundance was detected at m/z 264.10460 under QTOF-MS/MS conditions
20 although both accuracy ($\Delta + 10.19\text{ ppm}$) and isotopic match were considered
21 unsatisfactory. A potential reason for the difficulty encountered in ion detection might
22 have reflected the preferred loss of CO as the driving force for its dissociation.
23 Interestingly, a m/z 264 ion was detected cross platform when employing HPLC ESI
24 single quadrupole mass spectrometry and in-source collision-induced dissociation
25 (Figure 4C). A potential alternative for m/z 279.14899 might have involved the loss of
26 *N,N*-diethylamine ($\text{C}_4\text{H}_{11}\text{N}$) from the protonated molecule to give m/z 307.14283
27 ($\text{C}_{19}\text{H}_{19}\text{N}_2\text{O}_2^+$) followed cleavage of CO (Figure 5A4). As previously demonstrated
28 with the detection of the new psychoactive substance 2-methoxydiphenidine (MXP)
29 from a tablet surface,^[64] the analysis of a 1P-LSD blotter by matrix-assisted ionization
30 inlet Orbitrap mass spectrometry provided mass spectral information that agreed with
31 those detected under QTOF-MS/MS conditions (supplemental data). An additional
32 ESI triple quadrupole tandem mass spectrum was added as supplemental
33 information that also showed comparable product ions.
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39 Analysis of 1P-LSD by nuclear magnetic resonance spectroscopy (NMR) was aided
40 by implementation of 1-D and 2-D experiments including $^1\text{H}/^1\text{H}$ COSY, $^1\text{H}/^{13}\text{C}$ HSQC
41 and $^1\text{H}/^{13}\text{C}$ HMBC. For comparison purposes, 1-D and 2-D spectra were also
42 acquired for a LSD tartrate sample under identical conditions (d_6 -DMSO as solvent at
43 300 / 75 MHz). Table 1 summarizes the suggested assignments and representative
44 spectral data for 1P-LSD, i.e. HSQC and DEPTQ, are presented in Figure 7 for
45 illustrative purposes. All remaining NMR data for 1P-LSD and LSD are supplied in
46 form of supplemental information.
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50 The integration value measured for the tartrate singlet at 4.26 ppm was consistent
51 with the presence of 1P-LSD hemitartrate and a molar ratio of 1:2 for tartaric acid:1P-
52 LSD (Table 1, Figure 7A). In comparison, ^1H NMR analysis of a commercial sample
53 of LSD tartrate showed the presence of a molar ratio of 1:1 for tartaric acid:LSD
54 based on the integrals associated with the tartrate methine protons at 4.23 ppm
55 (supplemental data). The NMR data obtained for 1P-LSD is consistent with its
56 structure and closely related substances reported before.^[52,65-69] In the proton NMR
57 spectrum, the chemical shifts linked to the propionyl group overlapped with the
58 resonances associated with one of the methyl groups of the *N,N*-diethylamide group,
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3 H-7 α and H-5 β (Figure 7A), respectively. The singlet representing the N₆-methyl
4 group (H-17) was found to display the same chemical shift value as the solvent used
5 for analysis (d₆-DMSO), which also included an overlap with H-4 α . Interestingly, the
6 solvent overlap was not observed in the spectrum of the LSD tartrate sample
7 although H-17 still showed an overlap with H-4 α (supplemental data). Inspection of
8 the ¹H NMR spectrum also showed that one of the methylene groups belonging to
9 the diethylamide group (H-20) (restricted rotation) appeared as a multiplet rather than
10 the expected quartet as seen with the second set of H-20 at 3.45 ppm (Table 1,
11 Figure 7A). At closer inspection, it was considered that this might have been
12 consistent with two overlapping quartets of doublets (Table 1). Due to the adjacent
13 chiral centre, one of the methylene groups may be considered prochiral. This
14 resulted in geminal coupling and coupling to the adjacent methyl group, thus,
15 resulting in the formation of a doublet of quartet for each proton of the methylene
16 group. Interestingly, this was not observed in the ¹H of the LSD tartrate sample
17 where two quartets (overlapping with H-5 β) were observed (supplemental data).

21
22 The ¹³C NMR spectrum of LSD recorded in this study was in agreement with one
23 report published in 1981 where the same solvent was used as well.^[65] For 1P-LSD,
24 the ¹³C chemical shift differences recorded for C-3 was particularly pronounced (LSD:
25 108.06; 1P-LSD: 115.96) whereas the chemical shift values for the non-aromatic
26 carbons were not affected by the presence of the propionyl group (Figure 7B and
27 supplemental information). Interestingly, when Kidrič and Kocjan compared the ¹³C
28 NMR spectrum of LSD with iso-LSD, one major shift difference was encountered with
29 C-7 where the 55.8 ppm value (LSD) moved upfield to 50.7 ppm (iso-LSD).^[65]
30 Although the iso-1P-LSD epimer was not available in the present study, it appears
31 tempting to consider the possibility of a similar epimeric upfield shift change for the
32 8 α -epimer. As typically observed with closely related LSD derivatives, the four
33 protons linked to the two methylene groups at the 4- and 7-position can be observed
34 as distinct resonances due to their axial and equatorial positions. The ¹H/¹³C
35 correlations observed in the HSQC experiment were helpful for the confirmation of
36 assignments (Figure 7A). More detailed spectral representations, e.g. ¹H, HSQC,
37 COSY and HMBC data, may be obtained from the supplemental data collection.
38 Following the approach taken by Bailey and Grey, who reported a conformational
39 NMR study comparing *d*-lysergic acid dimethylamide (DAM-57^[36]) and iso-lysergic
40 acid dimethylamide, α - and β -assignments were made relative to the rigidly fixed
41 position of the methine proton 5 β -H that is placed above the ring plane of rings A and
42 B (Table 1).^[52]

47 Head-twitch response

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49 To determine whether 1P-LSD produces LSD-like behavioral effects *in vivo*, we
50 assessed whether it induced the HTR in mice. Administration of 1P-LSD produced a
51 dose-dependent increase in HTR counts ($F(2,28)=13.16$, $p<0.0001$; see Figure 8).
52 1P-LSD stimulated the HTR with an ED₅₀ of 158.9 (95% CI 65.0–388.8) μ g/kg.
53 Based on molar mass, 1P-LSD (ED₅₀ = 349.6 nmol/kg) has ~38% of the potency of
54 LSD (ED₅₀ = 132.8 nmol/kg^[47]). Extra sum-of-squares *F* tests showed the responses
55 induced by LSD and 1P-LSD cannot be fit using a single regression model
56 ($F(4,47)=30.68$, $p<0.0001$), and confirmed that 1P-LSD is significantly less potent
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3 than LSD ($F(1,47)=4.84, p<0.04$). As illustrated in **Figure 9**, the response to 1P-LSD
4 is time-dependent (drug \times time: $F(30,140)=3.78, p<0.0001$). The interval between
5 drug treatment and peak behavioral response is inversely proportional to the dose of
6 1P-LSD, with the maximal response occurring 15-20 min after administration of 0.2
7 mg/kg, 10-20 min after 0.4 mg/kg, and 5-10 min after 0.8 mg/kg. In summary, 1P-
8 LSD produced a LSD-like behavioral response in mice.
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11 In addition, a blockade experiment with the selective 5-HT_{2A} antagonist M100907
12 was carried out to confirm that 1P-LSD induces the HTR by activating the 5-HT_{2A}
13 receptor. M100907 has subnanomolar affinity for 5-HT_{2A} and is at least 100-fold
14 selective over 5-HT_{2B} and 5-HT_{2C} receptors.^[70,71] As anticipated, 0.4 mg/kg 1P-LSD
15 did not induce the HTR in mice treated with 0.1 mg/kg M100907 ($H(2)=13.01,$
16 $p=0.001$; **Figure 10**), demonstrating that the response to 1P-LSD is mediated by 5-
17 HT_{2A}.
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21 The present findings show that there was a 3-fold reduction of potency in mice when
22 the *N*₁-hydrogen in LSD is replaced with a propionyl group. Although 1P-LSD is less
23 potent than LSD, it is still an extremely potent drug. Indeed, for most hallucinogens,
24 $\mu\text{mol/kg}$ doses are required to induce behavioral responses in mice and rats. For
25 example, the HTR induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine
26 (DOI) peaks at 4.47 $\mu\text{mol/kg}$ in C57BL/6J mice.^[72]
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29 Other *N*₁-substituted lysergamides undergo extensive *N*₁-dealkylation *in vivo*,^[73-75]
30 and it is possible that 1P-LSD is hydrolyzed to LSD. Indeed, ALD-52 is equipotent
31 with LSD^[76] and is considered to serve as a pro-drug, although this does not seem to
32 have ever been confirmed empirically. In order to gain initial insights into the potential
33 for hydrolysis, 1P-LSD was exposed to incubation in human serum at 37 °C followed
34 by LC-MS analysis in selective ion monitoring mode. As shown in the supplemental
35 information, LSD detection was observed under a variety of exposure times. Follow-
36 up studies are currently being conducted to compare the affinity and selectivity of
37 LSD and 1P-LSD at 5-HT receptors, and to determine whether 1P-LSD is hydrolyzed
38 to LSD *in vivo*.
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41 Conclusion

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44 The present studies confirmed that 1P-LSD, a novel LSD derivative, is currently
45 available as a 'research chemical'. To the best of the authors' knowledge, 1P-LSD
46 has never been described in the chemical literature and was an unknown compound
47 prior to its appearance as a new psychoactive substance (NPS). The emergence of
48 NPS pose challenges to researchers and health care professionals and the analytical
49 characterization of 1P-LSD presented in this study provides key data that will be of
50 interest to forensic investigators and clinicians. The fact that 1P-LSD was found to
51 induce LSD-like behavioral effects in mice highlights the usefulness of a
52 multidisciplinary approach to address the NPS phenomenon, which includes the
53 need for further studies in this expanding field.
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Acknowledgement

The authors are grateful to Dr Sally Freeman (University of Manchester, UK) for helpful comments on NMR analysis. This work was supported by NIDA (R01 DA002925) and NIMH (K01 MH100644).

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FIGURE CAPTIONS

Figure 1. Chemical structures of lysergamides *d*-LSD, 1-*d*-acetyl-LSD (ALD-52) and 1-propionyl-*d*-LSD (1P-LSD).

Figure 2. Electron ionization quadrupole mass spectra. A: 1-Propionyl-*d*-LSD (1P-LSD). B: LSD

Figure 3. Suggested electron ionization mass spectrometry fragmentation patterns for 1-propionyl-*d*-LSD (1P-LSD).

Figure 4. Comparison of electrospray ionization single and tandem mass spectra. A: quadrupole time of flight tandem mass spectrum (QTOF-MS/MS) of 1-propionyl-*d*-LSD (1P-LSD). B: ESI-QTOF-MS/MS of LSD. C: in-source CID spectrum of 1P-LSD under single quadrupole mass spectrometry conditions.

Figure 5. Suggested formation of product ions of 1-propionyl-*d*-LSD (1P-LSD) following analysis by UHPLC-ESI-QTOF-MS/MS.

Figure 6. GC-solid state-IR spectrum of 1P-LSD. The retention time was recorded at 32.61 min.

Figure 7. 1-Propionyl-*d*-LSD (1P-LSD) hemitartrate spectra. A: Heteronuclear single quantum coherence spectroscopy (HSQC) analysis. B: Distorsionless enhancement by polarization transfer spectrum with retention of quaternary carbons (DEPTQ).

Figure 8. Effect of 1-propionyl-*d*-LSD (1P-LSD) on the head twitch response. Data are presented as group means \pm SEM for the entire 30-min test session. * $p < 0.01$, significant difference from vehicle control group (Tukey's test).

Figure 9. Time-course of the head twitch response induced by 1-propionyl-*d*-LSD (1P-LSD). Data are presented as group means during 5-min time blocks. * $p < 0.01$, significant difference from vehicle control group (Tukey's test).

Figure 10. Effect of pretreatment with the selective 5-HT_{2A} antagonist M100907 on the head twitch response induced by 1-propionyl-*d*-LSD (1P-LSD). Mice were pretreated with vehicle or 0.1 mg/kg M100907 and then treated with vehicle or 0.4 mg/kg 1P-LSD. Data are presented as group means \pm SEM over the entire 20-min test session. * $p < 0.01$, significant difference between groups (Dwass-Steel-Christchlow-Fligner test).

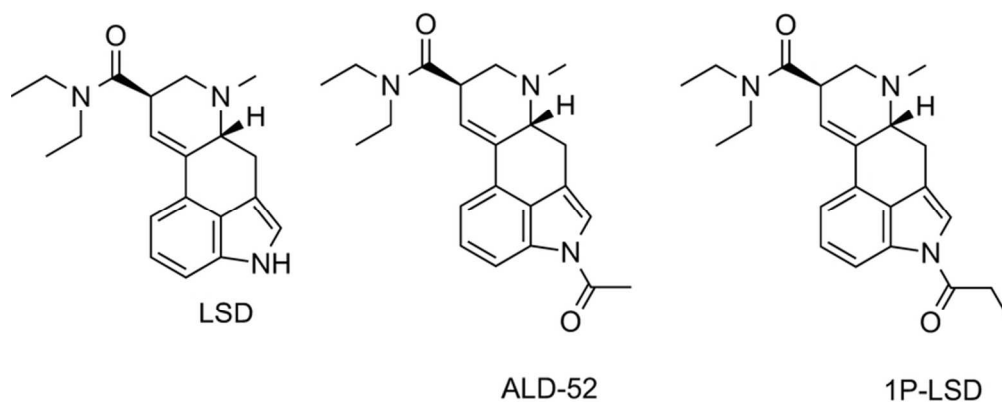


Figure 1. Chemical structures of lysergamides d-LSD, 1-d-acetyl-LSD (ALD-52) and 1-propionyl-d-LSD (1P-LSD).

74x29mm (300 x 300 DPI)

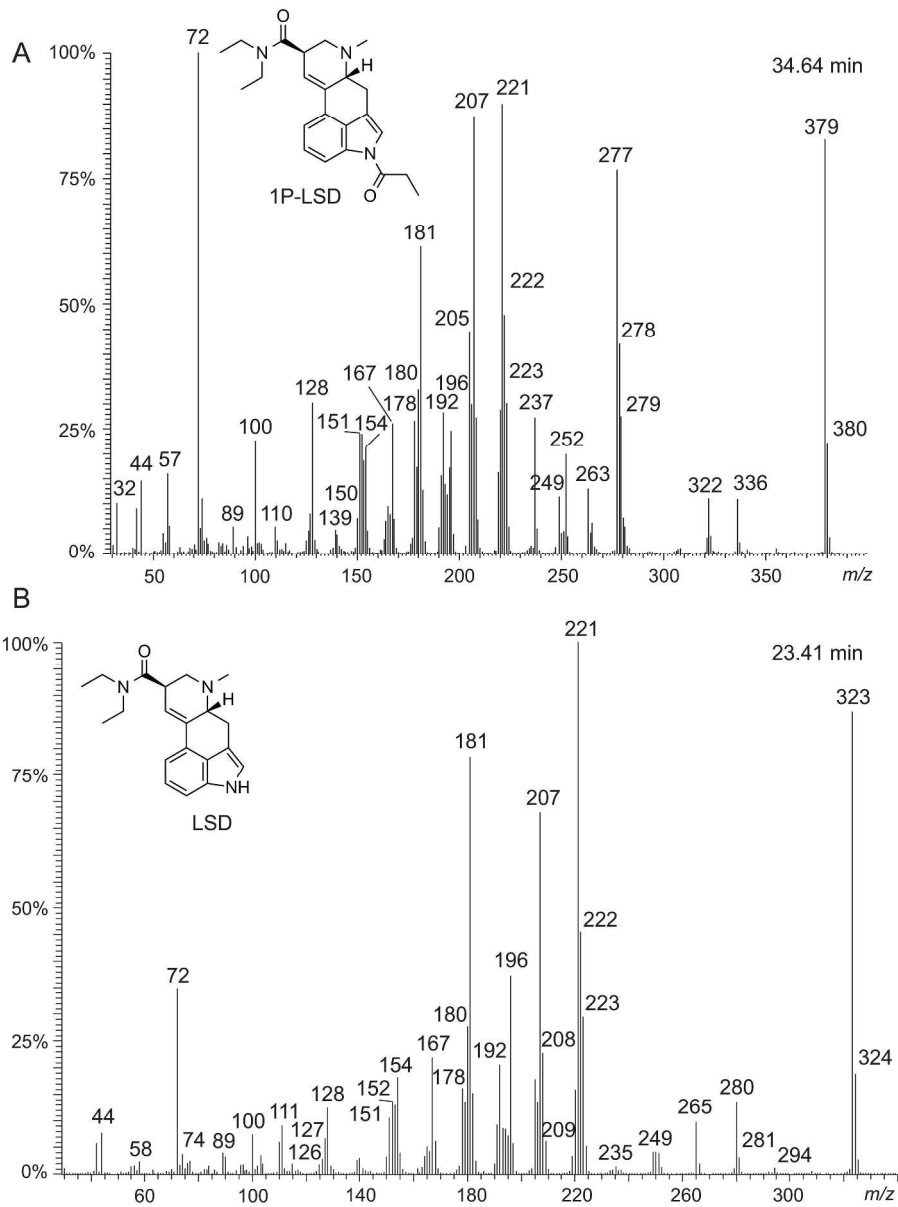


Figure 2. Electron ionization quadrupole mass spectra. A: 1P-LSD. B: LSD
266x355mm (300 x 300 DPI)

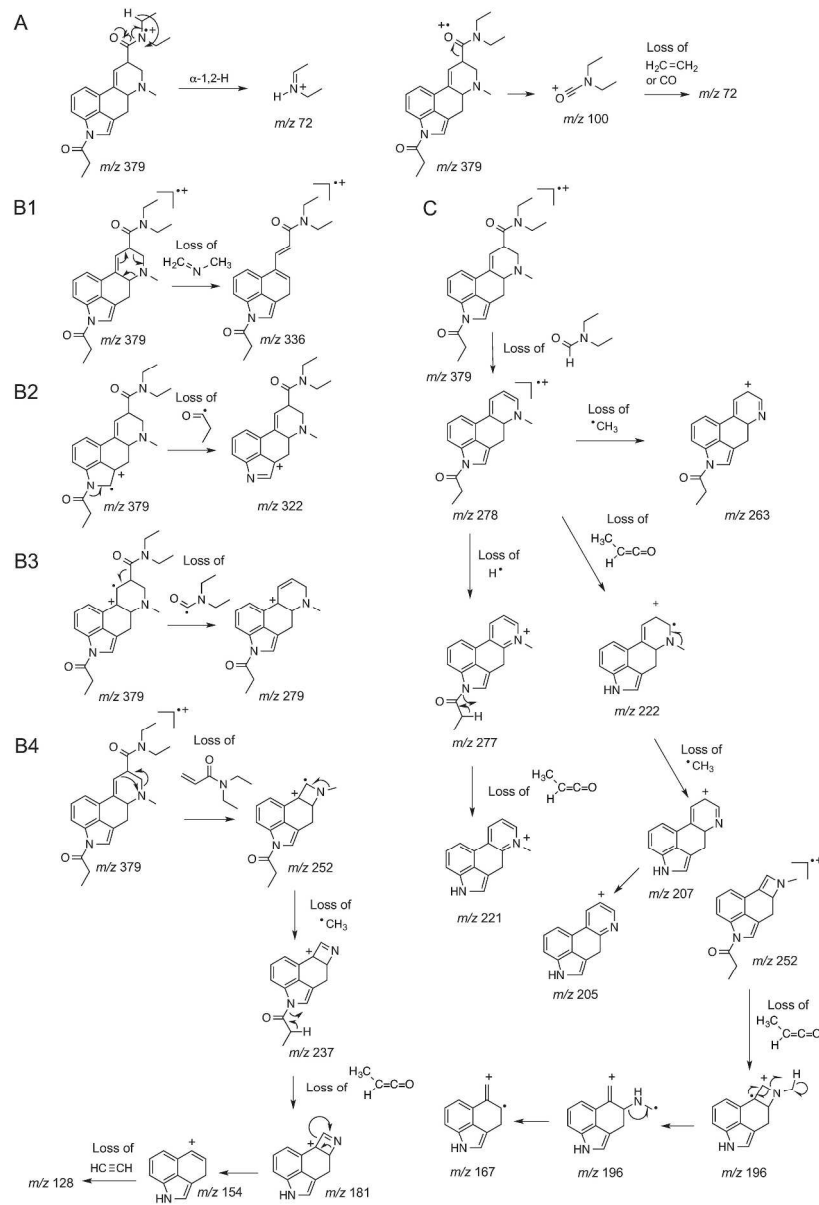
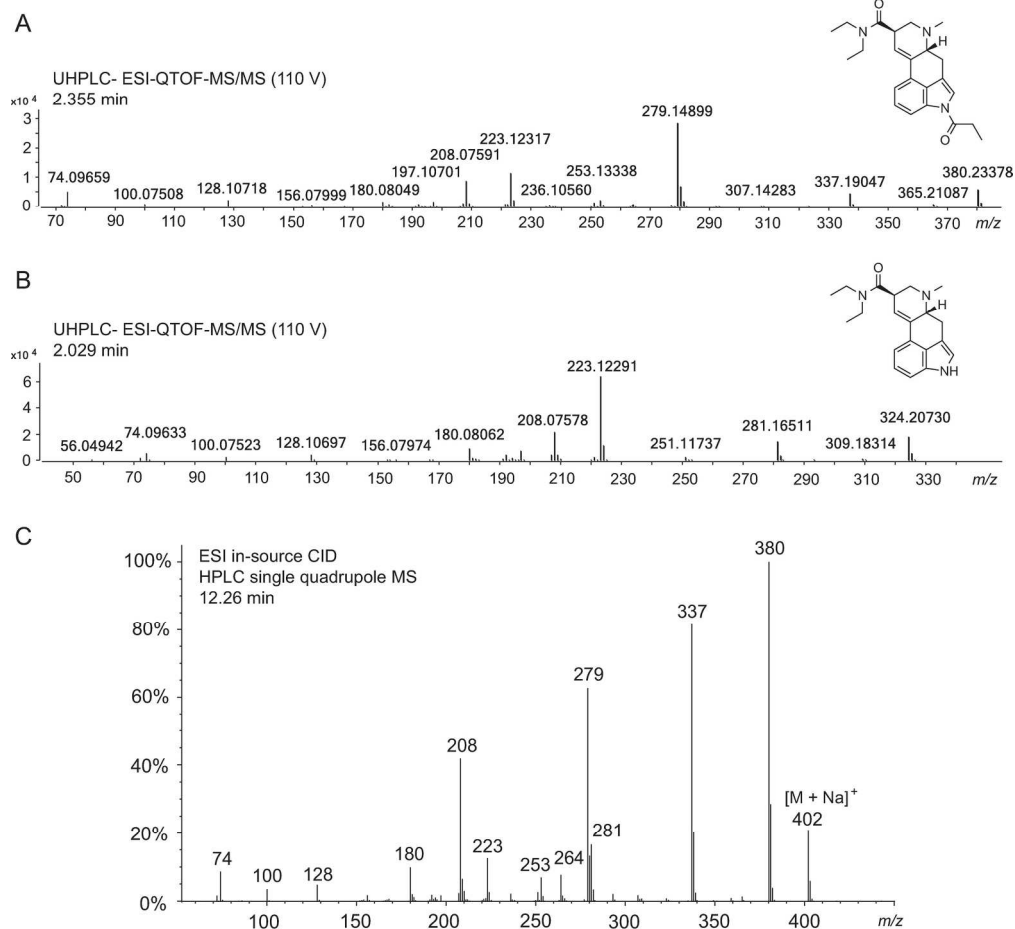


Figure 3. Suggested fragmentation patterns for 1P-LSD following analysis by electron ionization mass spectrometry.

292x433mm (300 x 300 DPI)



38 Comparison of electrospray ionization single and tandem mass spectra. A: quadrupole time of flight tandem
39 mass spectrum (QTOF-MS/MS) of 1-propionyl-d-LSD (1P-LSD). B: ESI-QTOF-MS/MS of LSD. C: in-source
40 CID spectrum of 1P-LSD under single quadrupole mass spectrometry conditions.
41 190x178mm (300 x 300 DPI)



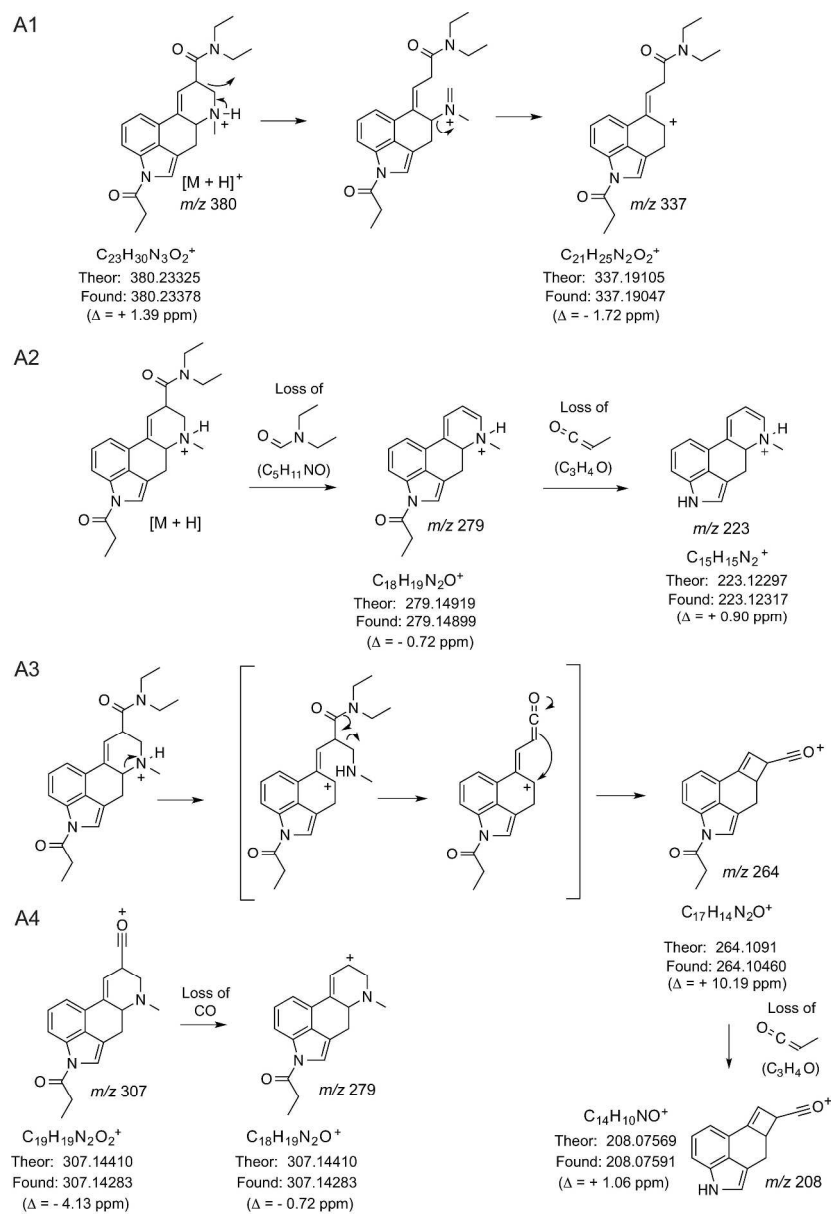


Figure 5. Suggested formation of product ions following exposure of 1P-LSD to characterization by QTOF-MS/MS.

285x417mm (300 x 300 DPI)

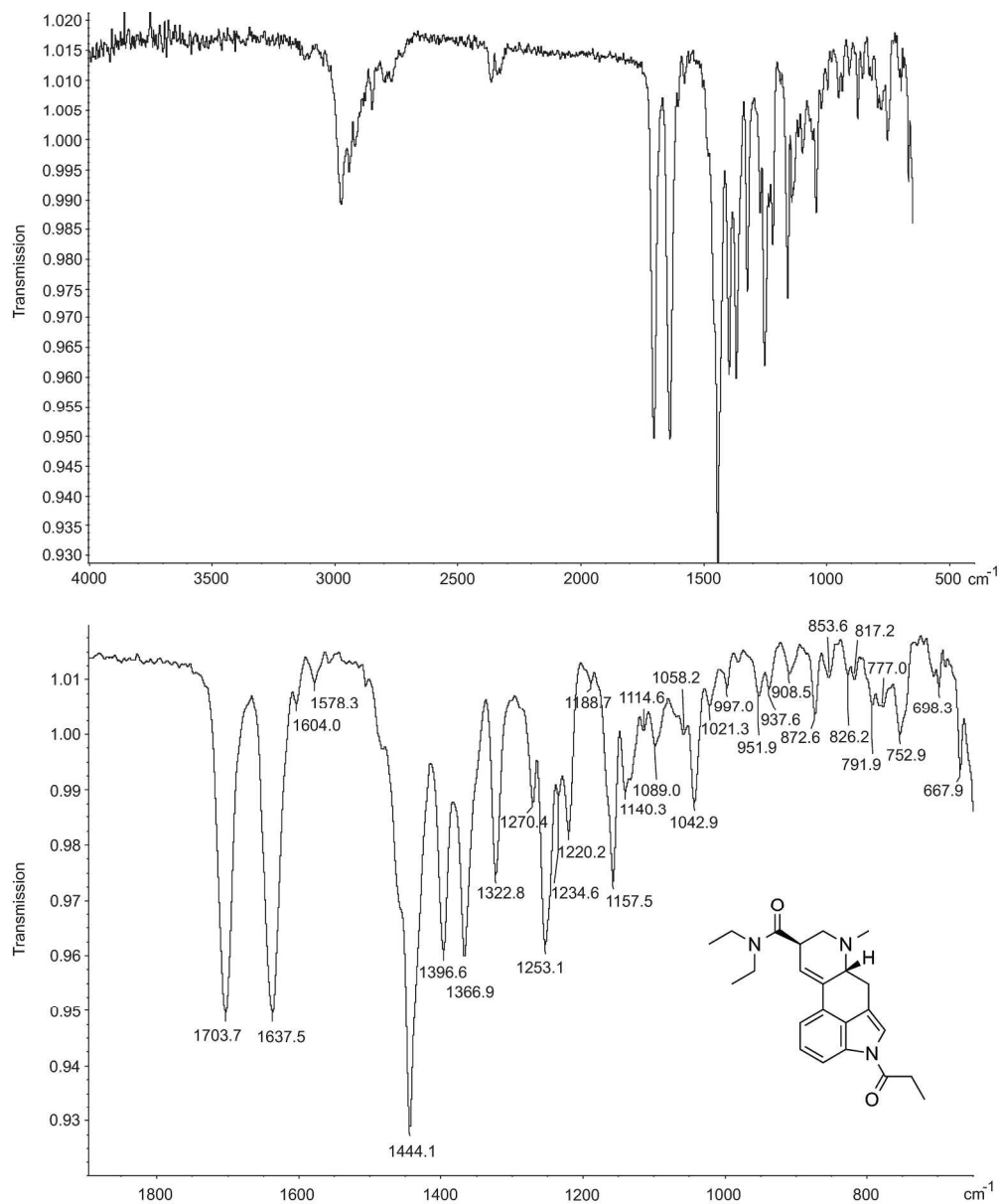
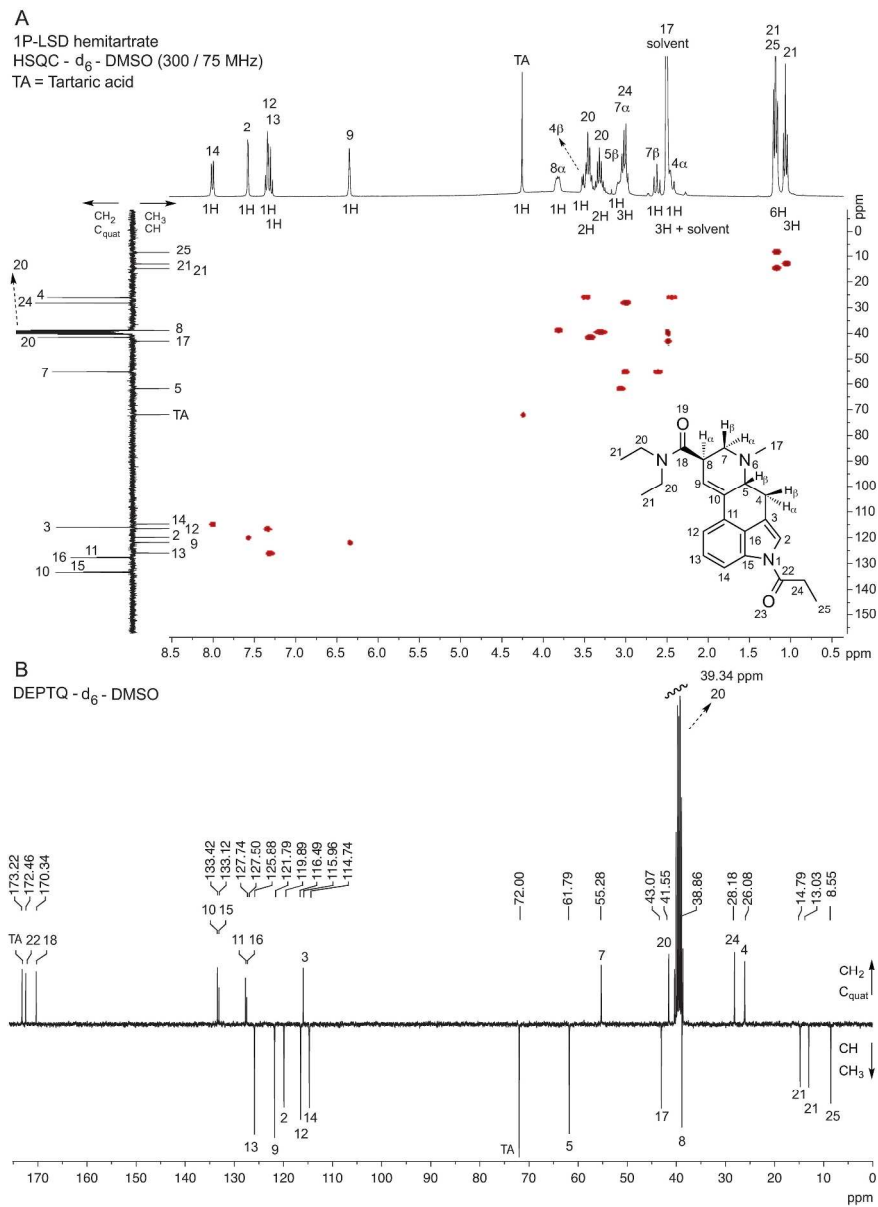
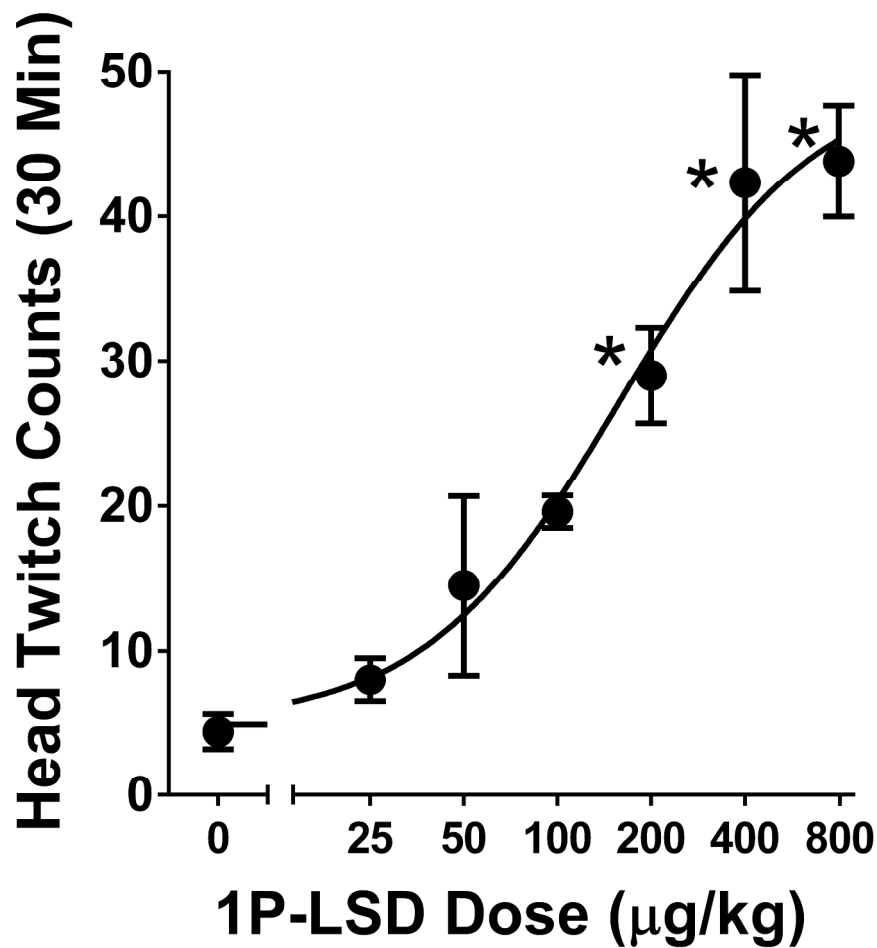


Figure 6. GC-solid state-IR spectrum of 1P-LSD. The retention time was recorded at 32.61 min.
237x286mm (300 x 300 DPI)

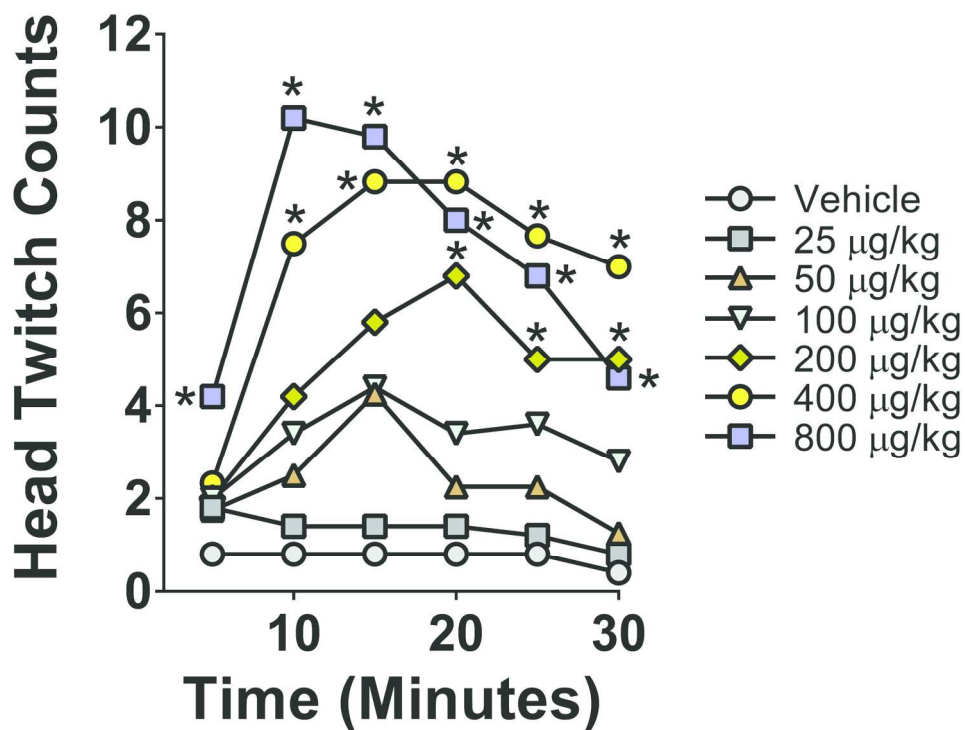


1-Propionyl-d-LSD (1P-LSD) hemitartrate spectra. A: Heteronuclear single quantum coherence spectroscopy (HSQC) analysis. B: Distorsionless enhancement by polarization transfer spectrum with retention of quaternary carbons (DEPTQ).
 286x395mm (300 x 300 DPI)



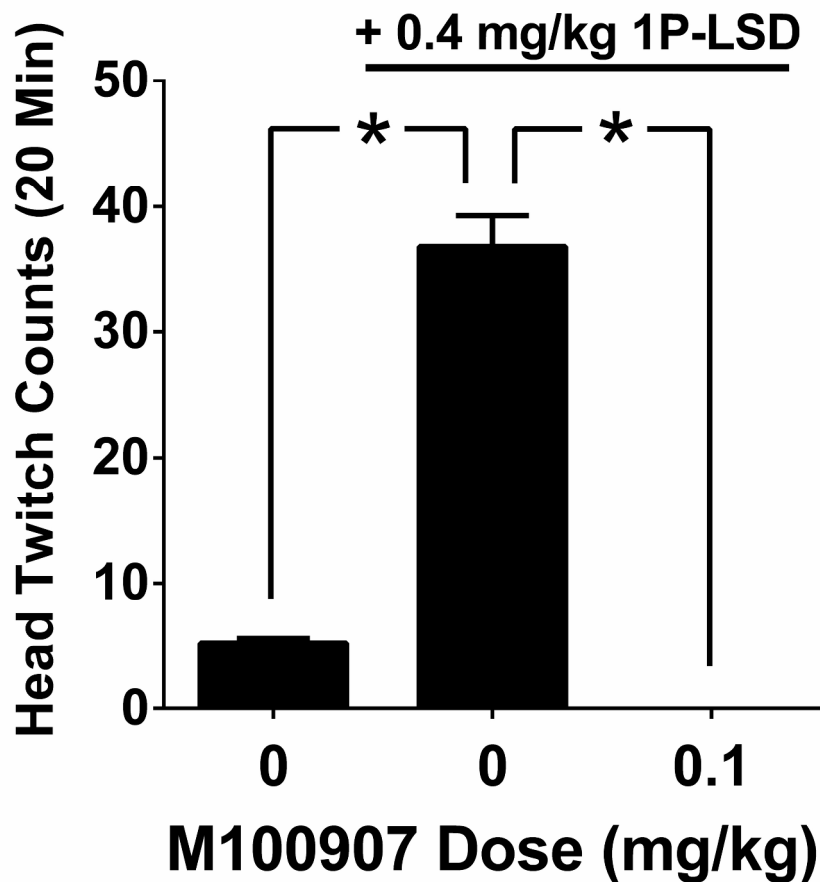
Effect of 1-propionyl-d-LSD (1P-LSD) on the head twitch response. Data are presented as group means \pm SEM for the entire 30-min test session. * $p < 0.01$, significant difference from vehicle control group (Tukey's test).

129x128mm (600 x 600 DPI)



Time-course of the head twitch response induced by 1-propionyl-d-LSD (1P-LSD). Data are presented as group means during 5-min time blocks. * $p < 0.01$, significant difference from vehicle control group (Tukey's test).

167x133mm (300 x 300 DPI)



38 Effect of pretreatment with the selective 5-HT_{2A} antagonist M100907 on the head twitch response induced
39 by 1-propionyl-d-LSD (1P-LSD). Mice were pretreated with vehicle or 0.1 mg/kg M100907 and then treated
40 with vehicle or 0.4 mg/kg 1P-LSD. Data are presented as group means \pm SEM over the entire 20-min test
41 session. * $p < 0.01$, significant difference between groups (Dwass-Steel-Critchlow-Fligner test).

127x118mm (600 x 600 DPI)

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Table 1. ^1H and ^{13}C NMR data for 1P-LSD hemitartrate in d_6 -DMSO at 300 / 75 MHz

No.	^{13}C [δ / ppm]	^1H [δ / ppm]
1	–	–
2	119.89	7.58 (d, $J_{\text{H-2/H-4}\alpha} = 1.5$ Hz, 1H)
3	115.96	–
4	26.08	2.44 (m, 4 α -H) ^{a,b} 3.50 (dd, $J_{\text{H-4}\beta/\text{H-4}\alpha} = 15.3$ Hz, $J_{\text{H-4}\beta/\text{H-5}\beta} = 5.7$ Hz, 1H, 4 β -H) ^c
5	61.79	3.13 – 3.06 (m, 1H, H-5 β) ^d
6	–	–
7	55.28	3.06 – 2.95 (m, 1H, H-7 α) ^d 2.62 ($J = 10.8$ Hz, 1H, H-7 β) ^e
8	38.86	3.98 – 3.77 (m, 1H, H-8 α)
9	121.79	6.35 (s, 1H)
10	133.42	–
11	127.74	–
12	116.49	7.35 (d, $J_{\text{H-12}/\text{H-13}} = 6.5$ Hz, 1H) ^f
13	125.88	7.31 (t, $J = 7.5$ Hz, 1H) ^g
14	114.74	8.01 (d, $J_{\text{H-14}/\text{H-13}} = 7.4$ Hz, 1H)
15	133.12	–
16	127.50	–
17	43.07	2.56 – 2.35 (m, 3H) ^a
18	170.34	–
19	–	–
20	41.55	3.45 (q, $J_{\text{H-20}/\text{H-21}} = 6.8$ Hz, 2H) ^c
20	39.34	3.33 (1H, dq, $J_{\text{gem}} 13.4$ Hz, $J_{\text{HH}} 6.8$ Hz) ^h 3.30 (1H, dq, $J_{\text{gem}} 13.4$ Hz, $J_{\text{HH}} 6.7$ Hz) ^h
21	14.79	1.18 (t, $J_{\text{H-21}/\text{H-20}} = 7.3$ Hz, 3H)
21	13.03	1.06 (t, $J_{\text{H-21}/\text{H-20}} = 7.0$ Hz, 3H)
22	172.46	–
23	–	–
24	28.18	3.06 – 2.95 (m, 2H) ^d
25	8.55	1.18 ($J_{\text{H-25}/\text{H-24}}, J = 7.3$ Hz, 3H)
TA ^f	173.22	–
TA ^f	72.00	4.26 (s, 0.5 H) ^k

^a Similar shift range for H-17 and DMSO and overlap with H-4 α between 2.55 – 2.39 ppm.

^b Indication of potential overlapping doublet of doublets for H-4 α .

^c Overlap of H-4 β and H-20 between 3.56 – 3.39 ppm.

^d Similar shift range for H-5 β , H-7 α and H-24 between 3.13 – 2.95 ppm.

^e Apparent triplet instead of doublet of doublets possibly due to $J_{gem} = J_{HH}$.

^f Indication of potential doublet of doublets ($J_{H-12/H-14} = 0.8$ Hz).

^g Apparent triplet instead of doublet of doublets possibly due to $J_{H-13/H-14} = J_{H-13/H-12}$.

^h Two overlapping doublets of quartets. Alternatively, multiplet between 3.39 – 3.24 ppm for H-20 methylene protons.

ⁱ H-21 and H25 are overlapping triplets that appear to impact on accurate determination of J values for coupling between H-20 and H-21.

^j TA: tartaric acid.

^k Reflecting molar ratio 1:2 for TA:1P-LSD (hemitartrate salt). Relative to the aromatic protons or H-9, the measured integration value was equivalent to 1H. In case of the LSD tartrate sample (suppl. information), the corresponding tartaric acid singlet gave an integral of 2H.