1   **Reporting the novel synthetic cathinone 5-PPDI through its analytical**

2   **characterization by mass spectrometry and nuclear magnetic resonance**

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15 **Abstract**

16 **Purpose** In this work, the identification and characterization of the novel synthetic

17 cathinone 5-PPDI found in a suspect drug sample was performed.

18 **Methods** The suspect sample was analyzed by gas chromatography–mass spectrometry

19 (GC–MS), Fourier-transformed infrared spectroscopy (FTIR), ultra-high performance

20 liquid chromatography–high-resolution mass spectrometry (UHPLC–HRMS) and

21 nuclear magnetic resonance (NMR).

22 **Results** The fragmentation observed in GC–MS and the identification of functional

23 groups by FTIR were not enough for compound identification. After an exhaustive

24 analysis of the accurate-mass fragmentation observed in HRMS, the compound was

25 tentatively identified as the novel cathinone 5-PPDI. Finally, five different NMR

26 experiments were used for the unequivocal identification and complete characterization

27 of the compound. In addition, the origin of this cathinone was investigated in depth.

28 **Conclusions** The analytical data provided in this work will be useful for the

29 identification of 5-PPDI by forensic laboratories. In addition, the origin of this

30 cathinone has been investigated, which could be of interest for the identification of

31 future synthetic cathinones prepared following the same synthesis route than that

32 employed for obtaining 5-PPDI.

33

34 **Keywords** 5-PPDI, Synthetic cathinones, 1-(2,3-Dihydro-1*H*-inden-5-yl)-2-(pyrrolidin-

35 1-yl)butan-1-one, High-resolution mass spectrometry, NMR spectroscopy, FTIR

36 spectroscopy

Introduction

According to the last report from the European Monitoring Centre for Drug and Drug Addiction (EMCDDA), 14 novel cathinones were reported in the European Union in 2016. Synthetic cathinones represent the second largest novel psychoactive substances (NPS) family, with 118 compounds currently being monitored by EMCDDA. These compounds were the most commonly seized NPS in 2015, representing the third part of the total number of seizures [1]. Of these, many are pyrovalerone analogs; they are cathinones that contain the pyrrolidine moiety (42 substances) [1]. The compound reported in this paper, 5-PPDI, newly appeared as a new pyrovalerone analog that produces effects in humans and is not controlled. It is the indane analog of α-PBP, a drug that is currently controlled in the USA, China, and other countries [2, 3]. It is likely that it shares the same synthetic route, albeit with different precursors, as other pyrovalerone derivatives (α-bromination of the pentan-1-one precursor to form the 2-bromopentan-1-one intermediate, and reaction with pyrrolidine to yield the substance), and therefore the synthesis is easy to carry out by a facility that has the means to manufacture α-PBP [4].

Monitoring and identification of NPS is still handicaped due to this wide range of structures along with their high turn-out rate. For this reason, it is essential to keep developing analytical approaches for their characterization [5–7].

The most commonly used analytical techniques in toxicological routine laboratories are Fourier-transformed infrared (FTIR) spectroscopy and gas chromatography–mass spectrometry (GC–MS), with the predominating ionization source being electron ionization (EI) [8]. FTIR is especially useful for NPS analysis when attenuated total reflectance (ATR) is used, allowing a direct analysis with a small amount of recoverable sample. The use of ATR-FTIR has recently demonstrated its potential for direct classification of NPS in seizures through the use of multivariate discriminant

62	analysis, allowing compound identification with a cost-effective and rapid analysis (2 min

63	per sample) [9, 10]. Nevertheless, this methodology can only be applied if the compound

64	spectrum has been previously acquired, which limits its suitability for monitoring

65	emerging NPS. GC-MS is probably the most frequently used instrumental technique in

66	the field of toxicology, where its applicability for cathinone analysis has been widely

67	reported [11–14]. Although GC–MS provides a way to quickly identify a compound by

68	the use of EI spectrum libraries, the frequent emergence of novel cathinone derivatives

69	proves a serious drawback. First of all, most of the novel cathinones that have been

70	detected recently are not listed in spectral libraries. Additionally, these cathinone

71	derivatives tend to produce very similar (or identical) fragmentation patterns, and the

72	identification of the molecular ion is commonly difficult due to the high fragmentation

73	produced by an EI source [13].

74	Recent studies dealing with the analysis of synthetic cathinones have been carried

75	out by ultra-high performance liquid chromatography (UHPLC) coupled to high

76	resolution mass spectrometry (HRMS), using electrospray ionization (ESI) interface as

77	the ionization source. These studies have demonstrated the potential of this technique for

78	cathinone identification in legal high samples, usually employing a hybrid quadrupole

79	time-of-flight (QTOF) mass analyzer [15, 16]. The QTOF instrument allows for a

80	tentative compound identification even without the use of reference standards. Moreover,

81	the applicability of the "non-target" approach for unknown compounds present in these

82	samples has also been demonstrated [17].

83	When no reference standard is available, the use of UHPLC–HRMS is not enough

84	for compound identification, and thus, additional spectroscopic techniques must be used.

85	Nuclear magnetic resonance (NMR) is one of the most useful techniques for structural

86	elucidation (including synthetic cathinones), allowing the differentiation of the

87   substitutional isomerism without the use of reference standards [18–20]. Thus, the

88   combination of UHPLC–HRMS and NMR allows the identification and complete

89   characterization of unknown (or unreported) NPS [17, 21–24].

90   In this work, an unknown white powder (suspected to contain a synthetic

91   cathinone) was received in our laboratory. After analysis by GC–MS and ATR-FTIR, the

92   compound could not be identified. Analysis by UHPLC-HRMS allowed a tentative

93   compound identification of the unreported synthetic cathinone 1-(2,3-dihydro-1*H*-inden-

94   5-yl)-2-(pyrrolidin-1-yl)butan-1-one, sold in several webpages as 5-PPDI. The analysis

95   of this cathinone by NMR in combination with HRMS data provided enough information

96   for the unequivocal compound identification.

97

Materials and methods

Drug sample

100   The suspect sample was submitted by an anonymous user to Energy Control's

101   drop-in service for its analysis. Additional information about Energy Control can be seen

102   elsewhere [25].

103

Reagents and chemicals

105   For GC–MS analysis, GC-grade *n*-hexane and GC-grade acetone were purchased

106   from Scharlau (Scharlab, Barcelona, Spain). For UHPLC-HRMS analysis, HPLC-grade

107   water was obtained by purifying demineralized water using a Milli-Q system from

108   Millipore (Bedford, MA, USA). HPLC-grade methanol, HPLC-grade acetonitrile, formic

109   acid, acetone, and sodium hydroxide (NaOH) were acquired from Scharlau. Leucine

110   enkephalin was purchased from Sigma-Aldrich (St. Louis, MO, USA). For NMR

111   analysis, deuterated chloroform ($CDCl_3$) was purchased from Sigma-Aldrich. For FTIR

112   analysis potassium bromide (KBr) was purchased from Scharlau.

113

114   **Sample treatment**

115   For FTIR analysis, the sample was directly analyzed by ATR-FTIR spectroscopy.

116   For GC-MS analysis, 10 mg of sample were extracted with 1 mL of acetone in an

117   ultrasonic bath for 15 min. After centrifugation, the supernatant was five thousand-fold

118   diluted with GC-grade *n*-hexane, and 1 µL of the extract were injected in the GC-MS

119   system.

120   For UHPLC-HRMS analysis, 10 mg of sample were extracted with 1 mL of

121   acetone in an ultrasonic bath for 15 min. After centrifugation, the supernatant was ten

122   thousand-fold diluted with HPLC-grade water, and 20 µL of the extract were injected in

123   the UHPLC-HRMS system.

124   For NMR analysis, approximately 15 mg of sample were dissolved in 0.6 mL of

125    $CDCl_3$.

126

127   **Instrumentation**

128   For FTIR analysis, a Jasco FT/IR-6200 FTIR spectrometer (Jasco Inc., Easton,

129   MD, USA) equipped with a Specac Silver Gate ATR accessory (Specac, Orpington, UK)

130   was used. Data acquisition was performed at 23 ºC between 4000 and 400 cm^{-1}, with a

131   resolution of 4 cm^{-1} and performing 32 acquisitions.

132   For GC–MS analysis, an Agilent 6890N gas chromatograph (Agilent

133   Technologies, Santa Clara, CA, USA) equipped with an Agilent 7683 autosampler

134   (Agilent Technologies) was coupled to a Quattro Micro GC triple quadrupole mass

135   spectrometer (Micromass, Boston, MA, USA) using an electron ionization (EI) interface.

136   The injector and the interface were operated at 250 ºC. A 1-μL aliquot of sample was

137   injected in splitless mode using deactivated liners into a 30 m x 0.25 mm i.d., 0.25 μm

138   film thickness DB-5MS column (Agilent Technologies). Helium (99.999%; Praxair,

139   Valencia, Spain) was used as carrier gas at a flow rate of 1 mL/min. The oven temperature

140   was initially maintained at 90 ºC for 1 min and programmed to reach 300 ºC at 20 ºC/min.

141   It was finally maintained at 300 ºC for 1.5 min (total run time was 12 min). The mass

142   spectrometer was operated in electronic ionization mode at 70 eV. MS system worked in

143   scan acquisition mode, acquiring from *m/z* 50 to 400 Da. Analytical data were acquired

144   and processed using MassLynx data station operation software (version 4.0; Waters,

145   Mildford, MA, USA).

146   UHPLC–HRMS analysis was performed using an ACQUITY UHPLC system

147   (Waters) coupled to a XEVO G2 QTOF hybrid QTOF mass spectrometer (Waters

148   Micromass, Manchester, UK) with an orthogonal Z-spray ESI interface operating in

149   positive ionization mode. The chromatographic separation was performed using a

150   CORTECS C18 (Waters) analytical column (100 x 2.1 mm i.d., 2.7 μm particle size;

151   Waters) at a flow rate of 0.3 mL/min. The column temperature was set to 40 ºC. The

152   mobile phases used were H_2O with 0.01% formic acid (A) and methanol with 0.01%

153   formic acid (B). The mobile phase gradient was performed as follows: 10% of B at 0 min,

154   90% B at 14 min linearly increased, 90% B at 16 min, and finally 10% B at 18 min in

155   order to return to initial conditions. The injection volume was 20 μL. Nitrogen (Praxair)

156   was used as desolvation and nebulizing gas. The desolvation gas flow was set at 1000

157   L/h. The TOF resolution was ~20000 at full width at half maximum at *m/z* 556. The range

158   acquired by the MS system was *m/z* 50 to 1000. A capillary voltage of 0.7 kV and a cone

159   voltage of 20 V were used during all the chromatographic run. Argon 99.995% (Praxair)

160   was used as a collision gas. The interface temperature was set to 650 ºC and the source

161   temperature to 120 ºC. For MS^E experiments, two acquisition functions with different

162   collision energy were created. The low energy function used a collision energy of 4 eV

163   in order to obtain information about the protonated molecule and adducts (if present),

164   while the high energy function applied a collision energy ramp from 15 to 40 eV, in order

165   to promote fragmentation of the compounds. Calibration of the mass-axis was performed

166   daily from *m/z* 50 to 1000 using 0.05 M NaOH/5% formic acid (1:1, v/v), diluted 25-fold

167   with acetonitrile/H_2O mixture (80:20, v/v). For accurate mass measurement, a 2 μg/mL

168   leucine enkephalin solution in acetonitrile/H_2O with 0.1% formic acid (50:50, v/v) was

169   used as lock-mass, and pumped at a flow rate of 20 μL/min. The leucine enkephalin

170   protonated molecule (*m/z* 556.2771) was used for recalibrating the mass axis and ensure

171   an accurate mass during all the chromatographic run. UHPLC-HRMS data were acquired

172   in continuum mode using MassLynx data station operation software (version 4.1; Waters)

173   and processed with UNIFI scientific information system (version 1.8; Waters).

174   NMR analyses were performed using a Bruker Ascend 400 MHz spectrometer

175   equipped with a SampleCase autosampler (Bruker, Etlingen, Germany), performing data

176   acquisition at 303 K using $CDCl_3$. The residual solvents signals at δ = 7.24 ppm for ^1H

177   ($CHCl_3$) and at δ = 77.23 ppm for ^{13}C ($CDCl_3$) were used as internal references.

178   Characterization of the compound was performed using 5 gradient-enhanced

179   experiments: ^1H NMR, ^{13}C NMR, correlated spectroscopy (COSY), heteronuclear single

180   quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC). NMR

181   experiment data were collected using the Bruker Icon NMR 5.0.5 software (Bruker).

182   MestreNova program was used for raw data processing (Mestrelab Research, Santiago de

183   Compostela, Spain).

184

185   **Results and discussion**

186   **Infrared spectroscopy and gas chromatography –mass spectrometry**

187   Preliminary analyses were performed by ATR-FTIR and GC–MS. In the case of

188   FTIR analysis, no spectra databases were available at our laboratory, and therefore only

189   functional groups could be identified. No significant information could be obtained, and

190   only aliphatic (<3000 cm^{-1}) and aromatic (>3000 cm^{-1}) C-H stretching signals, and

191   carbonyl stretching signal (1675 cm^{-1}) were present in the FTIR spectrum. The FTIR

192   spectrum and the identification of the observed bands can be found in supplementary

193   material Fig. S1.

194   Analysis by GC–MS revealed the presence of only one organic compound,

195   detectable by this equipment, which presented a chromatographic peak at 9.45 min. When

196   the mass spectrum of this chromatographic peak was extracted (Fig. 1), no matches were

197   obtained after applying the spectra libraries available at the laboratory (NIST, Cayman

198   Chemical, and a home-made library). The fragmentation spectrum showed only an

199   intense fragment ion at *m/z* 112. No information about the molecular ion could be

200   obtained from the EI spectrum.

201   The combination of the information provided by FTIR and GC–MS was not

202   enough for compound identification, requiring analysis by HRMS (and NMR) for

203   compound identification.

204

205   **High-resolution mass spectrometry**

206 The analysis by UHPLC–HRMS confirmed the high purity of the sample, and only a

207 chromatographic peak was observed in the total ion current chromatogram. The low

208 energy function spectrum of this chromatographic peak showed an ion at m/z 258.1845,

209 corresponding to the protonated molecule of the compound ($C_{17}H_{24}NO^+$, -2.9 ppm) (Fig.

210 2a). The fragmentation observed in the high energy function spectrum suggested the

211 compound to be a synthetic cathinone (Fig. 2b). The product ion 2 observed at m/z

212 187.1111 ($C_{13}H_{15}O^+$, -3.4 ppm) suggested the presence of a pyrrolidine moiety (neutral

213 loss of C_4H_9N, 71.0735 Da). This neutral loss has been described for several synthetic

214 cathinones with a pyrrolidine moiety [15, 20, 22, 23]. The product ion 4(at m/z 145.0642,

215 $C_{10}H_9O^+$, -4.5 ppm) indicated that the alkyl chain in the α-carbon of the cathinone should

216 be an ethyl moiety. This fact was in accordance to product ion 1at m/z 229.1448

217 ($C_{15}H_{19}NO^{+\cdot}$, -5.8 ppm), corresponding to a radical loss of 29.0391 Da ($C_2H_5^\cdot$). Finally,

218 product ion 6 at m/z 117.0692 ($C_9H_9^+$, -5.8 ppm) was obtained after a CO loss (27.9949

219 Da) from product ion 4. The double bond equivalence for product ion 6 indicated the

220 presence of 5 insaturations, 4 of them corresponding to the aromatic ring. The remaining

221 one, and the presence of 3 carbon atoms, could be related with the presence of a 2,3-

222 dihydroindene moiety.

223   Thus, a pyrrolidine, an ethyl and a 2,3-dihydroindene moieties would be the three

224 parts of the cathinone structure, being proposed as 1-(2,3-dihydro-1H-inden-5-yl)-2-

225 (pyrrolidin-1-yl)butan-1-one. Searching for this systematic name on different websites

226 which sell research chemicals, our putative cathinone was found under the name of 5-

227 PPDI. To the best of our knowledge, this synthetic cathinone has not been reported yet.

228 Once the compound was tentatively identified as 5-PPDI, the fragmentation pathways for

229 this synthetic cathinone were proposed. As it is shown in Fig. 3, all the observed product

230   ions could be justified based on the structure of this cathinone. The base peak at m/z 112

231   observed in the EI mass spectrum (Fig. 1) corresponds to the product ion 7.

232   Nevertheless, the information obtained by HRMS allowed only a tentative

233   identification. The complete characterization and unequivocal identification of the

234   compound was performed by the combination of different NMR experiments.

235

Nuclear magnetic resonance

237   Fig. 4 shows the ^{1}H NMR spectrum and the ^{13}C NMR spectrum for the tentatively

238   identified 5-PPDI, and Table 1 presents signal assignment for ^{1}H and ^{13}C NMR signals.

239   For ^{1}H NMR spectrum, all the observed signals could be justified based on the

240   structure of 5-PPDI without major problems. Nevertheless, some signals presented certain

241   curiosities that should be discussed in more detail. Resonances of hydrogen atoms 4 and

242   5 presented broad signals, as usual in aliphatic rings with heteroatoms (for example, the

243   pyrrolidine moiety) [20, 22, 23]. Methylene hydrogens signals which present resonance

244   between δ 1.75 and 2.25 presented overlapping, making the assignation of these signals

245   difficult. These signals were finally assigned after an accurate evaluation of the COSY

246   and HSQC spectra, which can be found in supplementary material (Fig. S2). The study

247   of HSQC spectra also allowed a direct assignation of ^{13}C NMR signals.

248   The combination of the NMR experiments and the fragmentation observed in

249   HRMS, allowed the complete characterization of the compound and thus, its

250   identification. Nevertheless, in order to enhance the confidence of compound structure,

251   an additional bidimensional NMR experiment was performed. Fig. 5 shows the HMBC

252   spectrum of 5-PPDI. The multiple bond correlations observed in this experiment

253   confirmed the structure initially proposed. Thus, the compound was unequivocally

254   identified as the synthetic cathinone 5-PPDI.

255

Reasons behind synthesizing 5-PPDI

Structure-activity relationship (SAR) is very difficult to deduce from theoretical data. There is some available information on the SAR of amphetamines, but less for the SAR of synthetic cathinones.

Although SARs of amphetamines and synthetic are not the same, some inferences can be made from modifications in one family to the other. It has been shown that the phenyl ring in pyrovalerone derivatives can be substituted with a benzodioxole and the compound will retain similar activity (α-PVP to MDPV, Fig. 6). Similarly, the benzodioxole moiety in MDA can be substituted with an indane and also will retain similar properties (MDA to 5-APDI). It is therefore a reasonable assumption that the benzodioxole and phenyl moieties are interchangeable in pyrovalerone derivatives and amphetamine analogs. Then, the benzodioxole could be replaced by an indane, substituting the phenyl moiety of α-PBP with an indane moiety which will yield to the active compound 5-PPDI. Replacing the phenyl moiety of α-PBP with a benzodioxole leads to MDPBP, a compound that is, at least, active; it is a logical next step to see if something similar happens with 5-PPDI (Fig. 6).

Because 5-PPDI has not previously appeared in the literature and little is known about it, vendors tend to send it for free with other orders, or even to send a sample at no cost to the consumer in an attempt to get users to describe its effects and generate interest in the substance [26]. It appears that the compound is inactive at dosages similar to other pyrovalerone derivatives, and users tend to not push the dose above which they perceive as safe. Some users report light activity, especially with administration through vaporization of the compound, which is reported to be more potent than oral or nasal use. Reports are mixed however, likely due to factors such as purity, personal tolerance, route

280 of administration, etc., leading to conflicting reports such as one user reporting 20 mg

281 vaporized to be an active dose, and another reporting that 32 mg vaporized to yield no

282 effects. It is also possible that some vendors claim to ship 5-PPDI, but in reality they ship

283 other compounds, leading to the disparity in effects reported [27, 28].

284
285
286 **Conclusions**

287 This work presents the detection and characterization of the novel cathinone 1-

288 (2,3-dihydro-1*H*-inden-5-yl)-2-(pyrrolidin-1-yl)butan-1-one, better known as 5-PPDI.

289 The results obtained in this study remark the limitations of the routine analysis techniques

290 used in forensic laboratories for NPS detection and identification. Thus, FTIR

291 spectroscopy and GC–MS allow a rapid identification of the sample only if the

292 corresponding spectrum has been previously recorded.

293 In this work, GC–MS revealed that the compound was highly pure, without any

294 other organic compound being detected. Nevertheless, no matches were obtained for the

295 acquired spectrum using commercial libraries, illustrating that this technique is not

296 efficient for structure elucidation of unknown substances or unanalyzed compounds;

297 therefore, advanced techniques are required for that aim.

298 The analysis by UHPLC–HRMS allowed a tentative identification of the

299 compound, based on the accurate-mass fragmentation observed. Nevertheless, due to the

300 lack of an analytical reference standard at the moment of developing this work, the

301 compound could not be unequivocally identified based only on HRMS data. The use of

302 different NMR experiments (^{1}H, ^{13}C, COSY, HSQC and HMBC) confirmed its structure,

303 and after combining this information with that obtained by HRMS, the substance was

304 unequivocally characterized as 5-PPDI.

305   The analytical data provided in this work will facilitate the detection and
306 identification of this novel synthetic cathinone by forensic and toxicological laboratories,
307 even if they use routine techniques.

308   Although this compound does not appear to be very potent, and it will be unlikely
309 to see its widespread use, it is interesting to consider that it was synthesized with a clear
310 objective to produce a viable alternative to compounds like α-PBP. Its structure
311 demonstrates some knowledge on pharmacology and SAR of synthetic cathinones, and
312 contributes to clarifying the theory, by which manufacturers of NPS are proficient at
313 finding alternatives to banned compounds. Such a theory casts a doubt on the efficacy of
314 systematically scheduling NPS, because manufacturers have been able to provide
315 alternatives that not only evade legislation, but also are usually active compounds.

316

317 **Acknowledgements**

331

332 **Compliance with ethical standards**

333 **Conflict of interest**

334 There are no financial or other relations that could lead to a conflict of interest.

335

336 **Ethical approval**

337 This article does not contain any studies with human participants or animals performed
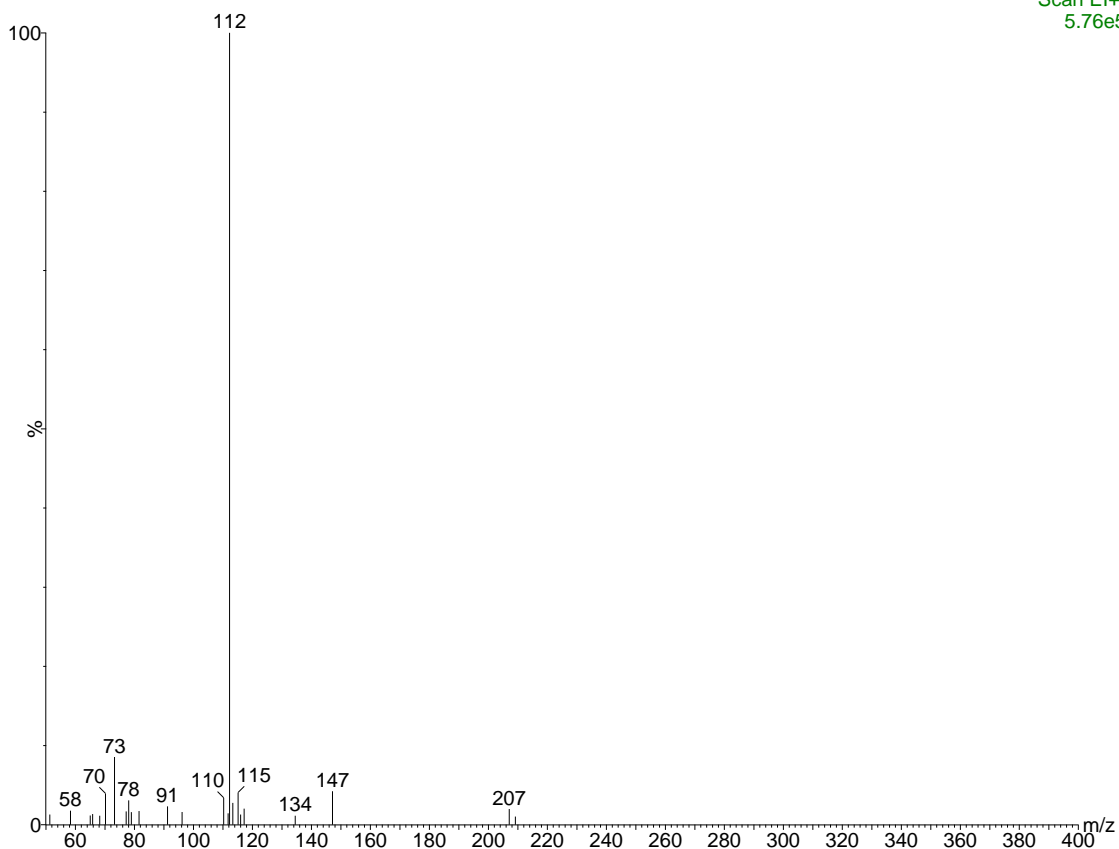
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91

110 115

134

147

207

60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400

m/z

Scan EI+
5.76e5

444
445 **Fig. 1** Electron ionization mass spectrum of a chromatographic peak at 9.54 min,
446 corresponding to the unknown compound, obtained by gas chromatography–mass
447 spectrometry
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449
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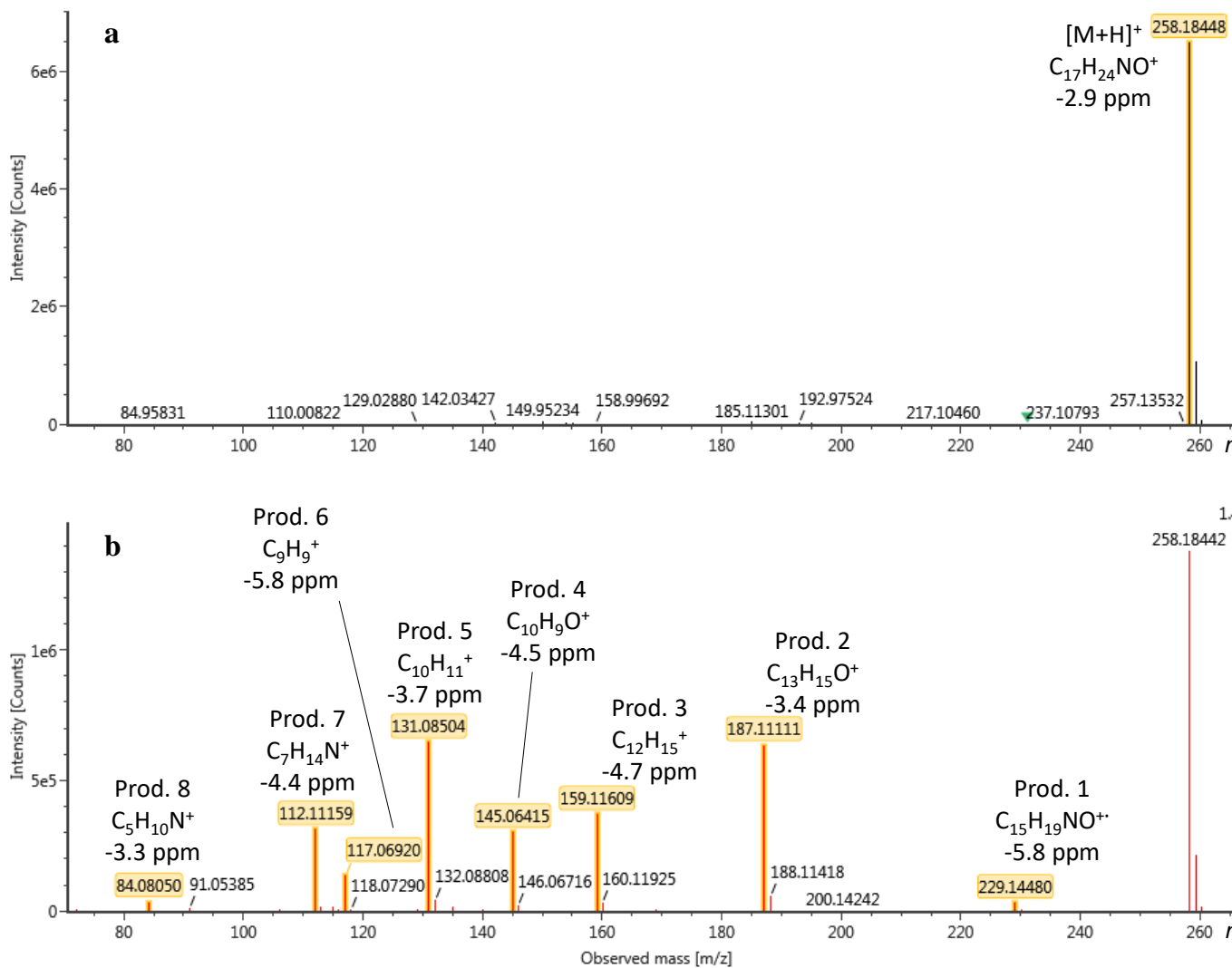
451
Fig. 2 MS^E spectra of the unknown compound. Low energy function (**a**) and high
energy function (**b**) spectra of the tentatively identified 5-PPDI
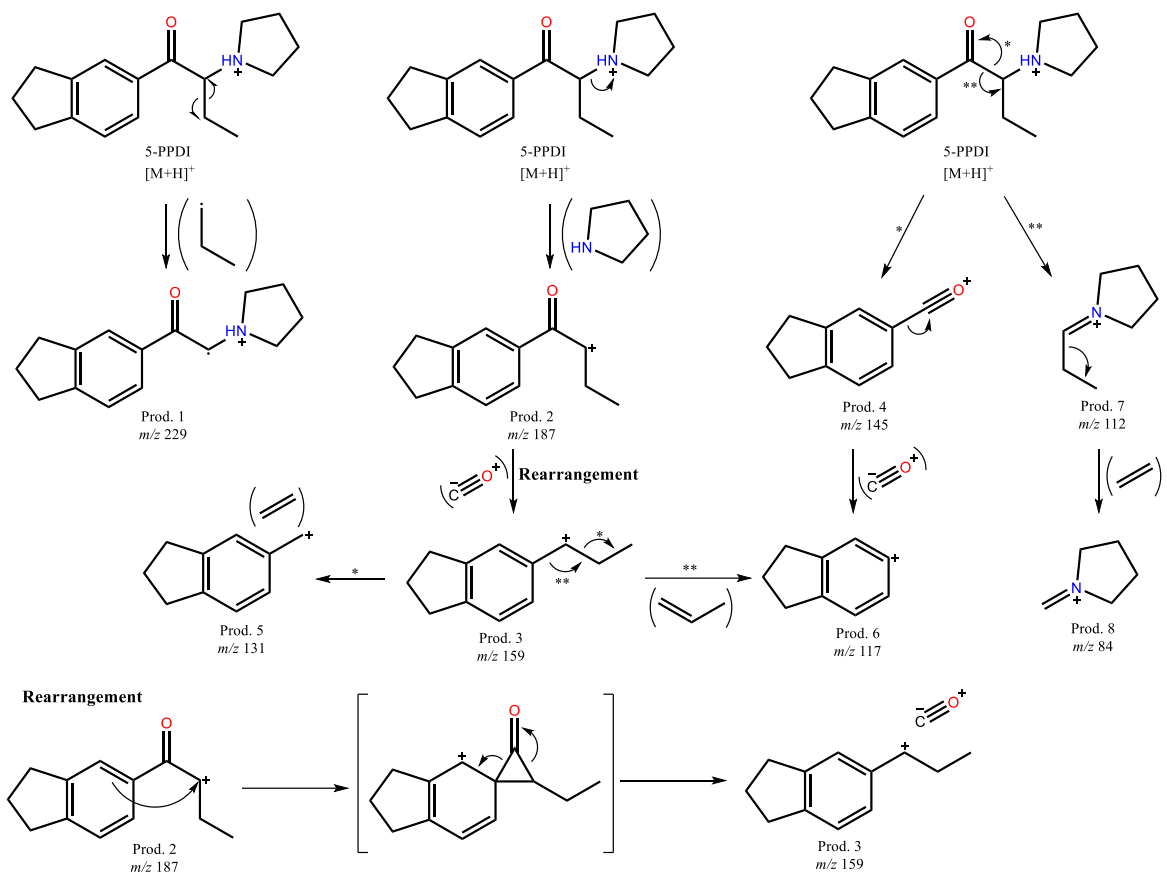
455
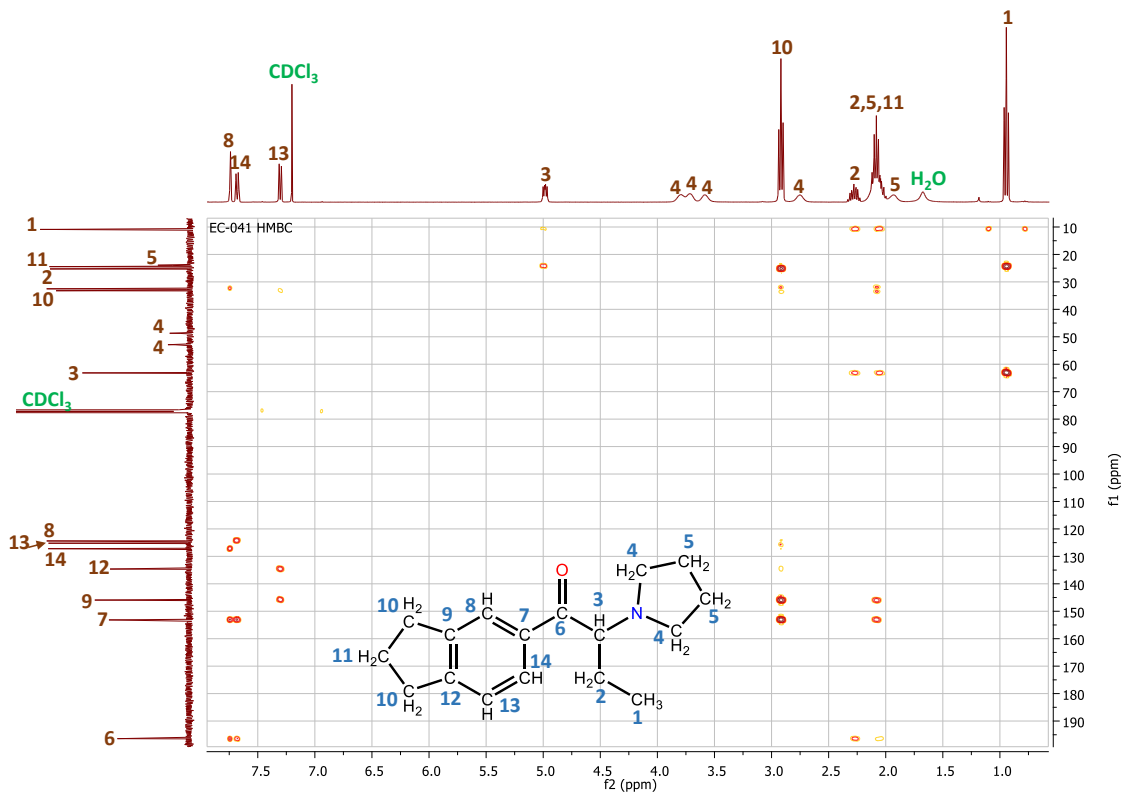456 **Fig. 3** Proposed collision induced dissociation (CID) fragmentation pathways for the 5-
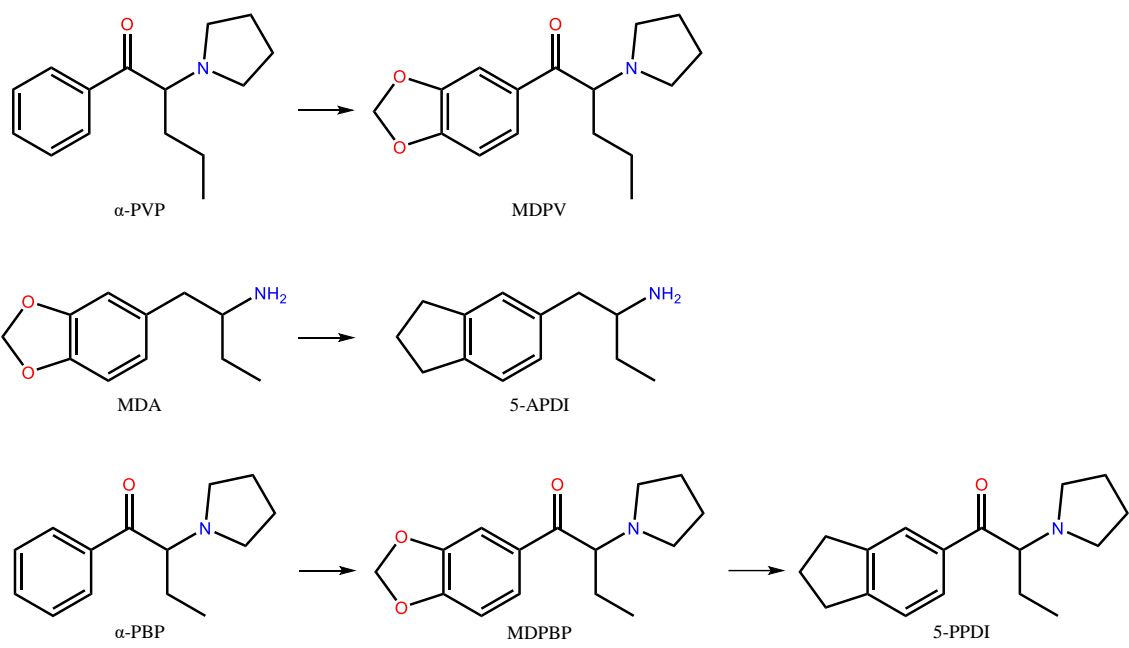457   PPDI

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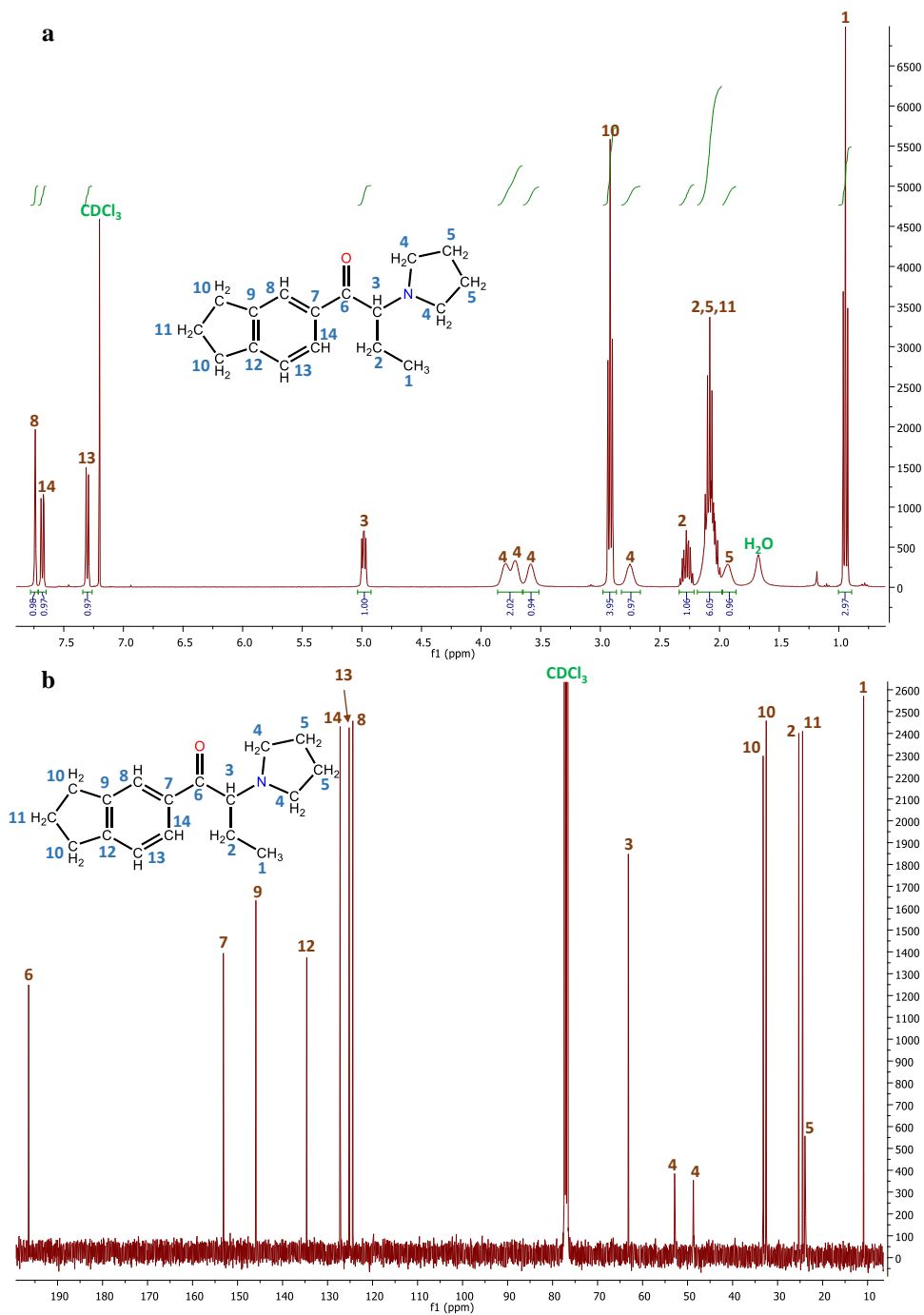
Fig. 4 Nuclear magnetic resonance spectra of the unknown substance. **a** ^{1}H spectrum, with proton-signal assignation based on the structure of 5-PPDI. **b** ^{13}C spectrum, with carbon-signal assignation based on its structure

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465 **Fig. 5** Heteronuclear multiple bond correlation (HMBC) spectra of the compound
466 identified as 5-PPDI

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469   **Fig. 6** Structures of synthetic cathinones with moiety changes to 5-PPDI

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471 **Table 1** ^{1}H and ^{13}C nuclear magnetic resonance signal assignment

| ^{1}H NMR signal assignment			^{13}C NMR signal assignment	
Hydrogen	δ (ppm)	Multiplicity	Carbon	δ (ppm)
1	0.94	triplet	1	10.88
2	2.08, 2.29	multiplet	2	25.27
3	4.98	triplet	3	63.16
4	2.75, 3.59, 3.71, 3.79	a	4	48.72, 52.88
5	1.93, 2.08	a	5	23.92
6	-	-	6	196.43
7	-	-	7	153.16
8	7.74	singlet	8	124.39
9	-	-	9	145.93
10	2.92	triplet	10	32.52, 33.21
11	2.08	a	11	24.44
12	-	-	12	134.62
13	7.31	doublet	13	125.21
14	7.67	doublet	14	127.20
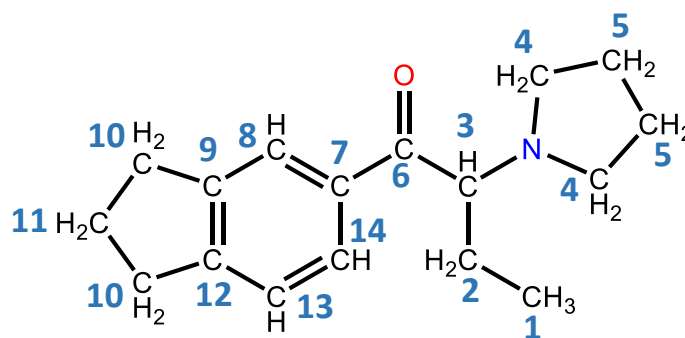
472   δ chemical shift.
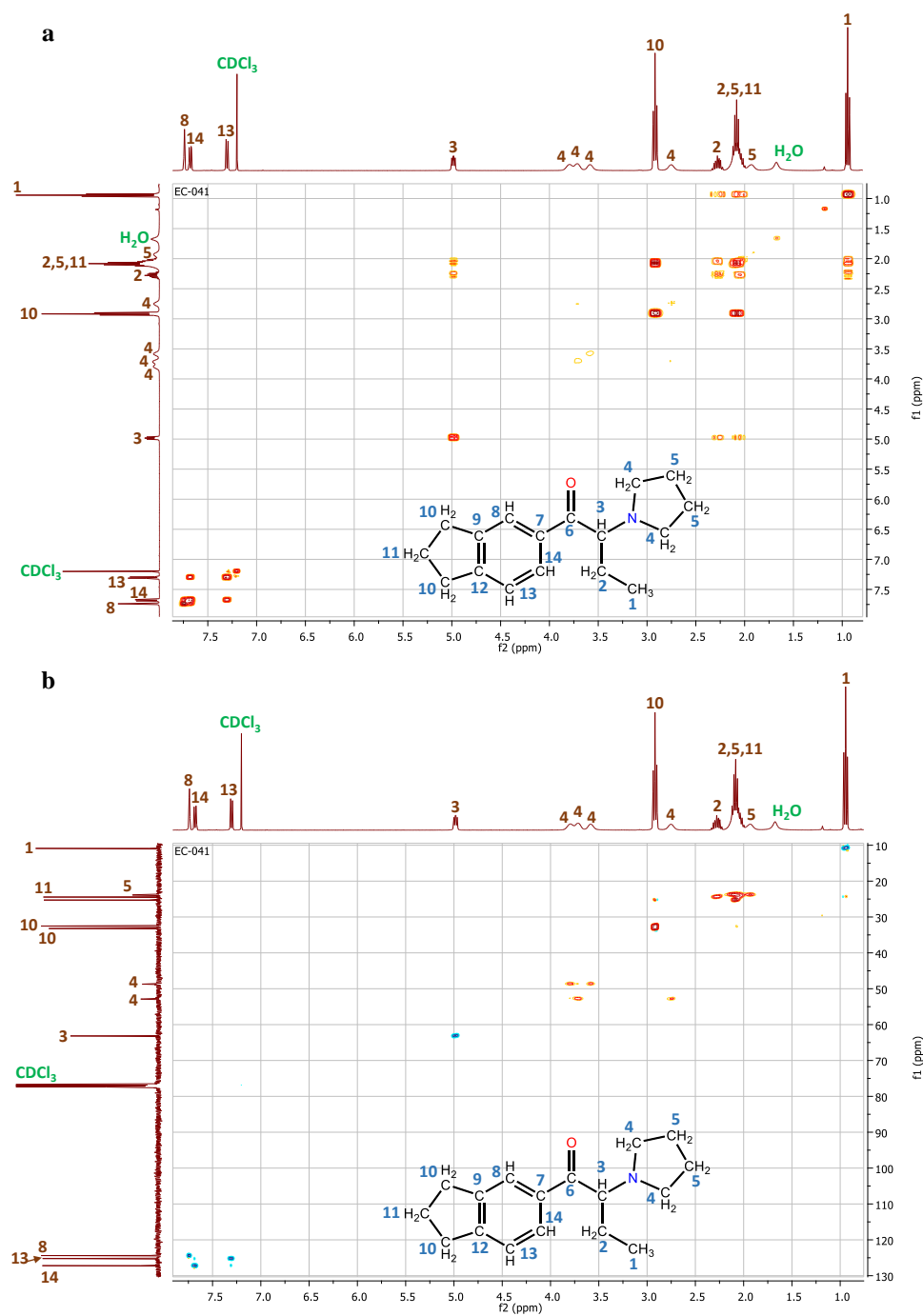473 a multiplicity of these signals could not be stablished
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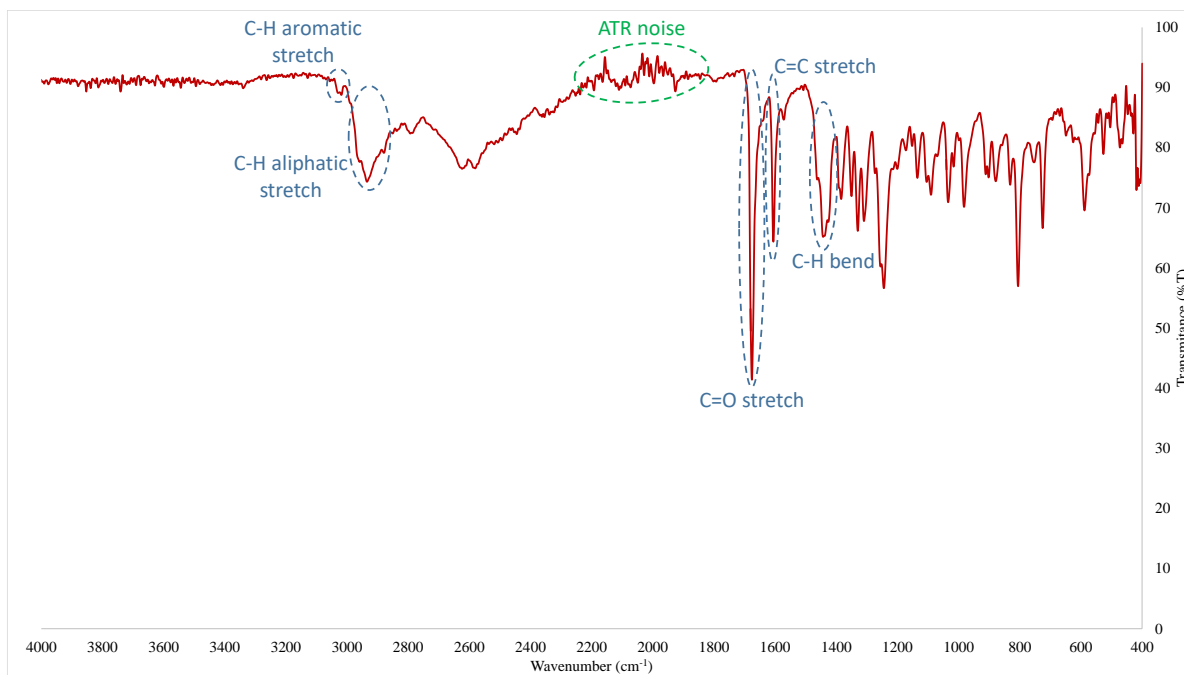
Fig. S1 FTIR spectrum of the unknown compound. Characteristic bands are highlighted
in blue. ATR noise are highlighted in green

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Fig. S2 a COSY spectrum of 5-PPDI, showing the correlation between hydrogens. **b** HSQC spectrum of 5-PPDI, linking ^{1}H and ^{13}C NMR signals. CH_3 and CH groups are represented by blue spots, and CH_2 groups are represented by red/yellow spots.

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