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EUROPEAN PROJECT RESPONSE 2 to challenges in forensic drugs analyses
Co-funded by the Internal Security Found of the European Union

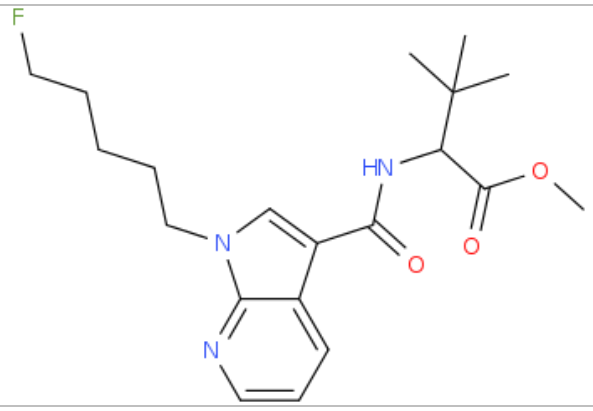
ANALYTICAL REPORT

5F-MDMB-P7AICA (C20H28FN3O3)

methyl 2-{[1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]formamido}-3,3-dimethylbutanoate

Remark – other NPS detected:

Sample ID:	1899-17
Sample description:	powder
Sample type:	test purchase /ISF projekt (NFL-SI)
Date of sample receipt (M/D/Y):	12/18/2017
Date of entry (M/D/Y) into NFL database:	2/12/2018
Report updates (if any) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure[1] (base form)	
Systematic name	methyl 2-{[1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]formamido}-3,3-dimethylbutanoate
Other names	methyl-[2-(1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)-3,3-dimethylbutanoate];
Formula (per base form)	C20H28FN3O3
M_w (g/mol)	377,46
Salt form/anions detected	base
StdInChIKey (per base form)	LIRBKFHBIDESHO-UHFFFAOYSA-N
Other NPS detected	
Additional info (purity..)	impurity observed by GC-MS, HPLC_TOF (C19H17NO3, M=187.1208); compound was purchased as 4'N 5F-ADB5

[1] Created by OPSIN free tool: http://opsin.ch.cam.ac.uk/ **DOI:** 10.1021/ci100384d

Report updates

date	comments (explanation)

Instrumental methods (if applied) in NFL

1. GC-MS (Agilent): GC-method is RT locked to tetracosane (9.258 min). Injection volume 1 ml and split mode (1:50). Injector temperature: 280 0C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickens 0.25 µm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 ^{0}C for 1 min, followed by heating up to 190 ^{0}C at rate 8 ^{0}C/min, then heating up to 293 0C at a rate of 18 ^{0}C/min, hold for 7.1 min, then heating at 50 ^{0}C/min up to 325 ^{0}C and finally 6.1 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235^{0}C, source and quadropole temperatures 280^{0}C and 180^{0}C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300 until 6 min) amu.

2. HPLC-TOF (Agilent): 6230B TOF with Agilent 1260 Infinity HPLC with binary pump, column: Zorbax Eclipse XDB-C18, 50 x 4.6 mm, 1.8 micron. Mobile phases (A) 0.1% formic acid and 1mM ammonium formate in water; (B) 0.1% formic acid in methanol (B). Gradient: starting at 5% B, changing to 40% B over 4 min, then to 70% over 2 min and in 5 min to 100%, hold 1 min and back to 5%, equilibration for 1.7 min. The flow rate: 1.0 ml/min; Injection volume 1 µl. MS parameters: 2GHz, Extended Dynamic range mode to a maximum of 1700 amu, acquisition rate 1.30 spectra/sec. Sample ionisation: by Agilent Jet Stream technology (Dual AJS ESI). Ion source: positive ion scan mode with mass scanning from 82 to 1000 amu. Other TOF parameters: drying gas (N2) and sheath temperature 325 °C; drying gas flow rate 6 l/min; sheath gas flow rate 8 l/min; nebulizer 25 psig; Vcap. 4000 V; nozzle 2000 V; skimmer 65 V; fragmentor 175 V and Octopole RF 750 V.

3.FTIR-ATR (Perkin Elmer): scan range 4000-400 cm-1; resolution 4cm-1

4. GC- (MS)-IR condensed phase (GC-MS (Agilent) & IR (Spectra analyses-Danny)
GC-method: Injection volume 1 ml and split mode (1:5). Injector temperature 280 ^{0}C. Chromatographic separation as above **(1).**
Split MS : IR = 1: 9.
MSD source EI = 70 eV. GC-MS transfer line T= 235^{0}C, source and quadropole temperatures 280^{0}C and 180^{0}C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300) amu.
IR (condesed (solid) phase): IR scan range 4000 to 650, resolution 4 cm^{-1}.

5. **IC** (anions) (Thermo Scientific, Dionex ICS 2100), Column: IonPac AS19, 2 x 250mm; Eluent: 10mM from 0 to 10 min, 10-58 mM from 10 to 40min; Flow rate: 0.25 ml/min; Temperature: 30˚C; Suppressor: AERS 500 2mm, suppressor current 13mA; Inj. Volume: 25 µl

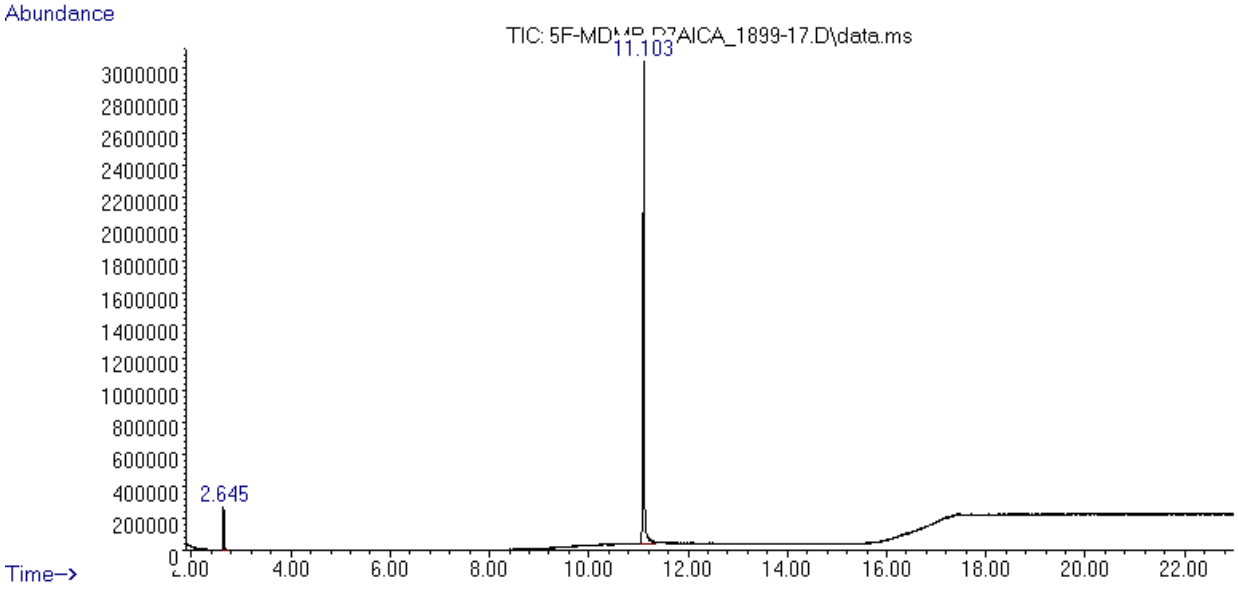
Supporting information

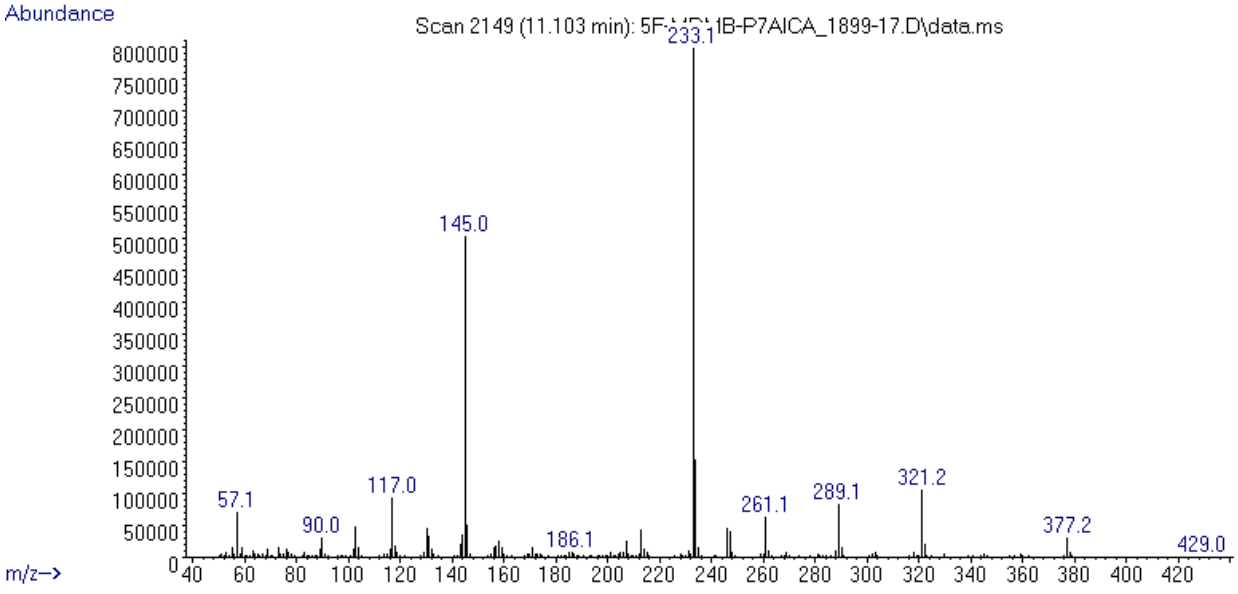
Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	partially

Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 11,1 BP(1): 233; BP(2): 145,BP(3) :234,
HPLC-TOF	+	Exact mass (theoretical): 377,2115; measured value Δppm:-0,77; formula:C20H28FN3O3
FTIR-ATR	+	direct measurement (sample as received)
FTIR (solid phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	+	extended report is given
validation		
other		

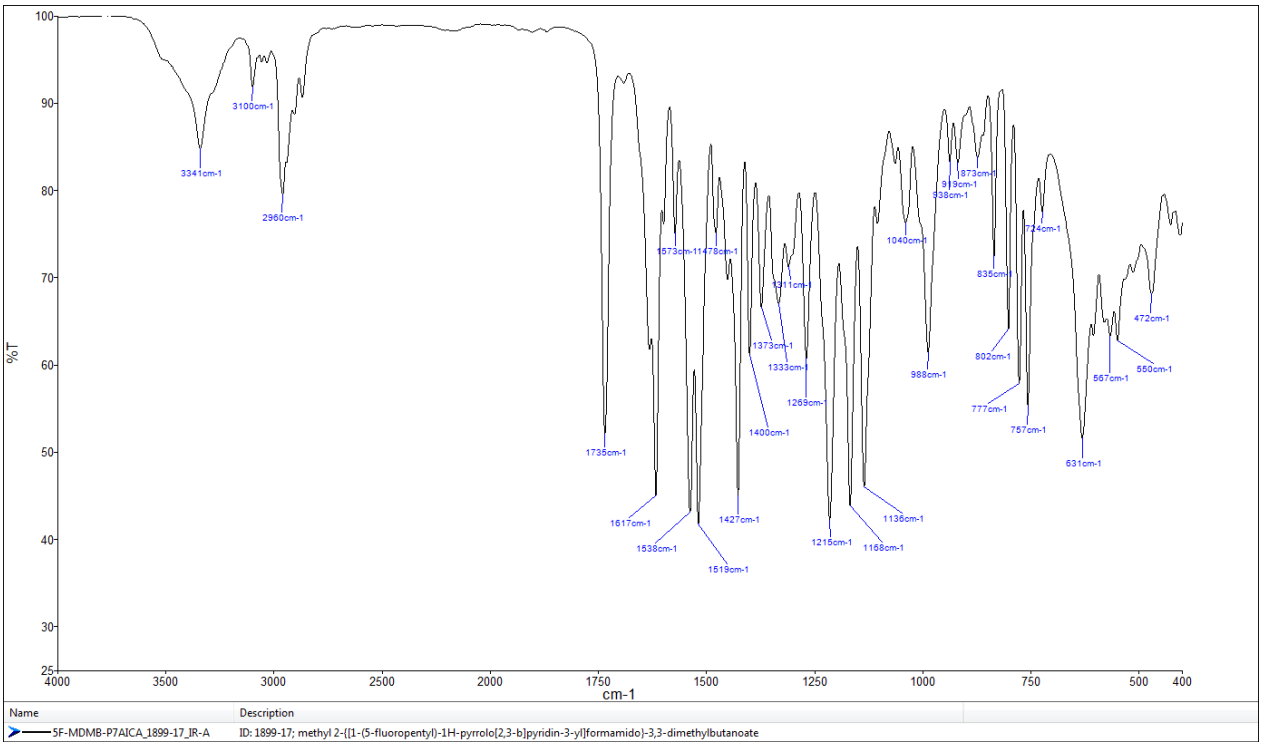
ANALYTICAL RESULTS

GC-MS (EI)

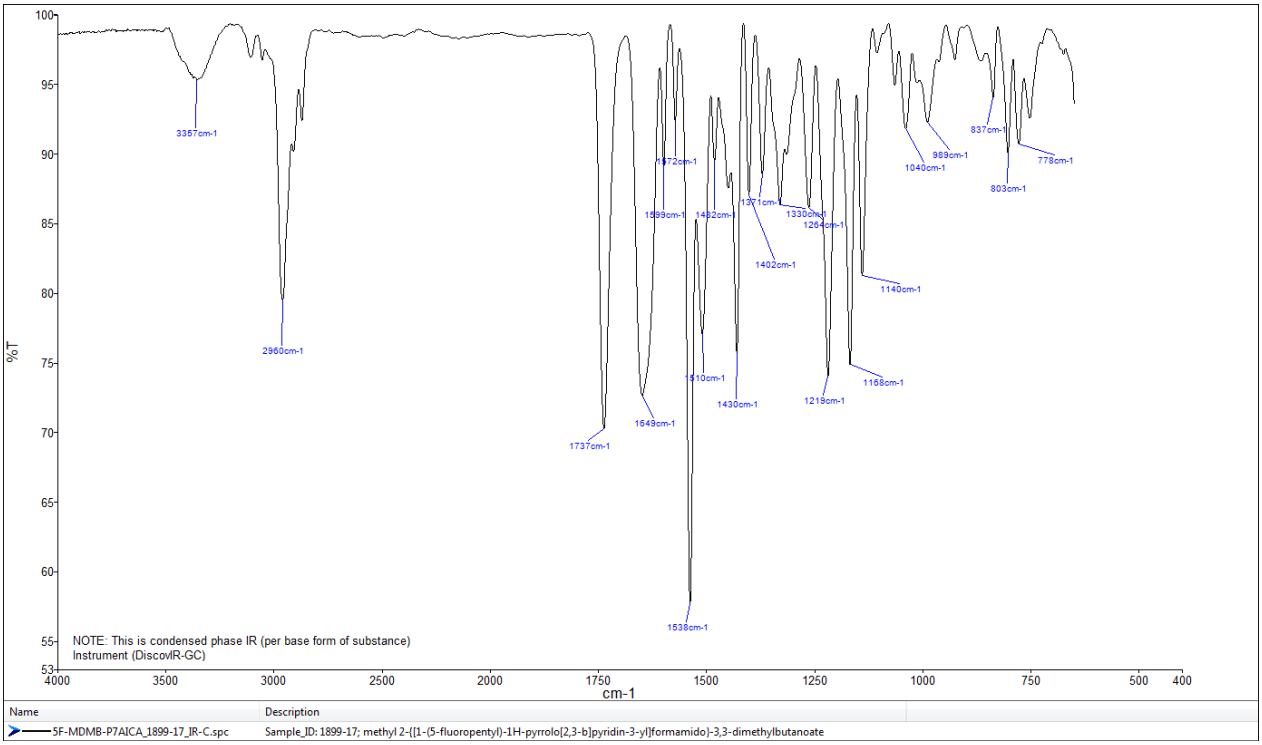




FTIR-ATR - direct measurement (sample as received)



IR (solid phase – after chromatographic separation)



TOF REPORT

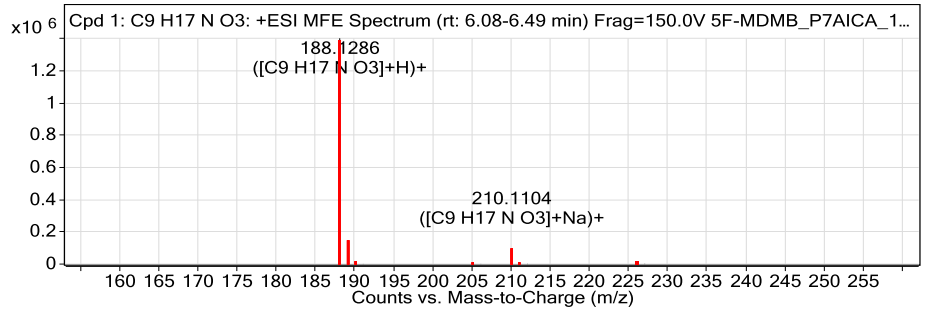
Data File	5F-MDMB_P7AICA_1899-17.d	**Sample Name**	1899-17
Sample Type	Sample	**Position**	P1-F6
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-04_12_2017-XDB-C18-ESI+.m	**Acquired Time**	12/20/2017 1:40:01 PM
IRM Calibration Status	Success	**DA Method**	a-Drugs_NFL.m
Comment	MeOH		

Compound Table

Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 1: C9 H17 N O3		C9 H17 N O3	6.14	187.1212
Cpd 2: 5F-MDMB-P7AICA	5F-MDMB-P7AICA	C20 H28 F N3 O3	8.3	377.2118

Obs. m/z	Obs. RT	Obs. Mass
188.1286	6.14	187.1212

MFE MS Zoomed Spectrum

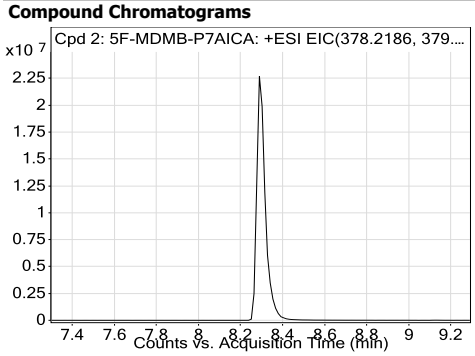


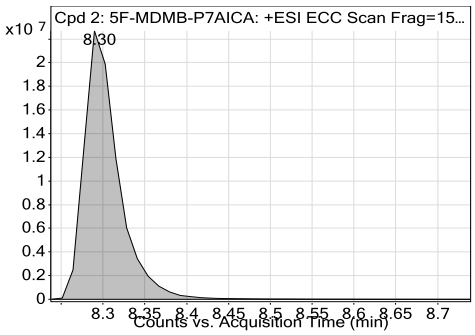
MS Spectrum Peak List

Obs. m/z	Charge	Abund	Formula	Ion/Isotope
188.1286	1	1401819.75	C9 H17 N O3	(M+H)+
189.1318	1	133750.08	C9 H17 N O3	(M+H)+
190.1336	1	13163.34	C9 H17 N O3	(M+H)+
205.1547	1	8637.67	C9 H17 N O3	(M+NH4)+
206.1559	1	940.03	C9 H17 N O3	(M+NH4)+
210.1104	1	92921.22	C9 H17 N O3	(M+Na)+
211.1138	1	9734.15	C9 H17 N O3	(M+Na)+
212.1158	1	1259.04	C9 H17 N O3	(M+Na)+
226.0842	1	10784.27	C9 H17 N O3	(M+K)+
227.0877	1	1531.69	C9 H17 N O3	(M+K)+

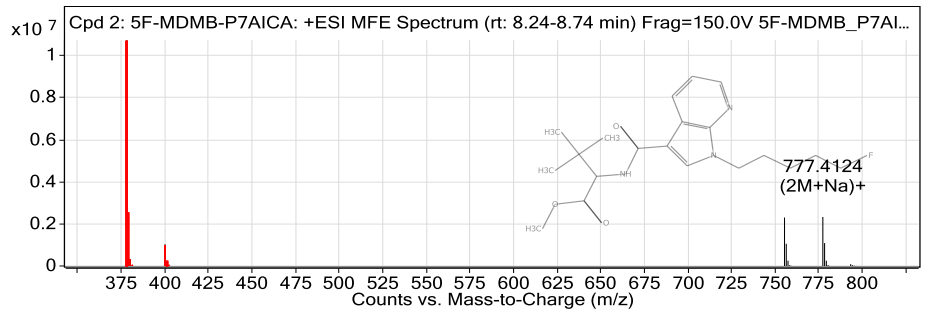
Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
5F-MDMB-P7AICA	378.2189	8.3	377.2118	8.3	C20 H28 F N3 O3	377.2115	-0.77

Compound Chromatograms



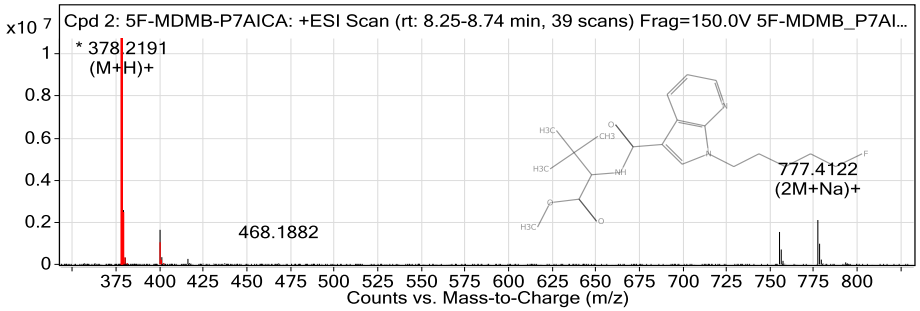


MFE MS Zoomed Spectrum



TOF REPORT

MS Zoomed Spectrum



MS Spectrum Peak List

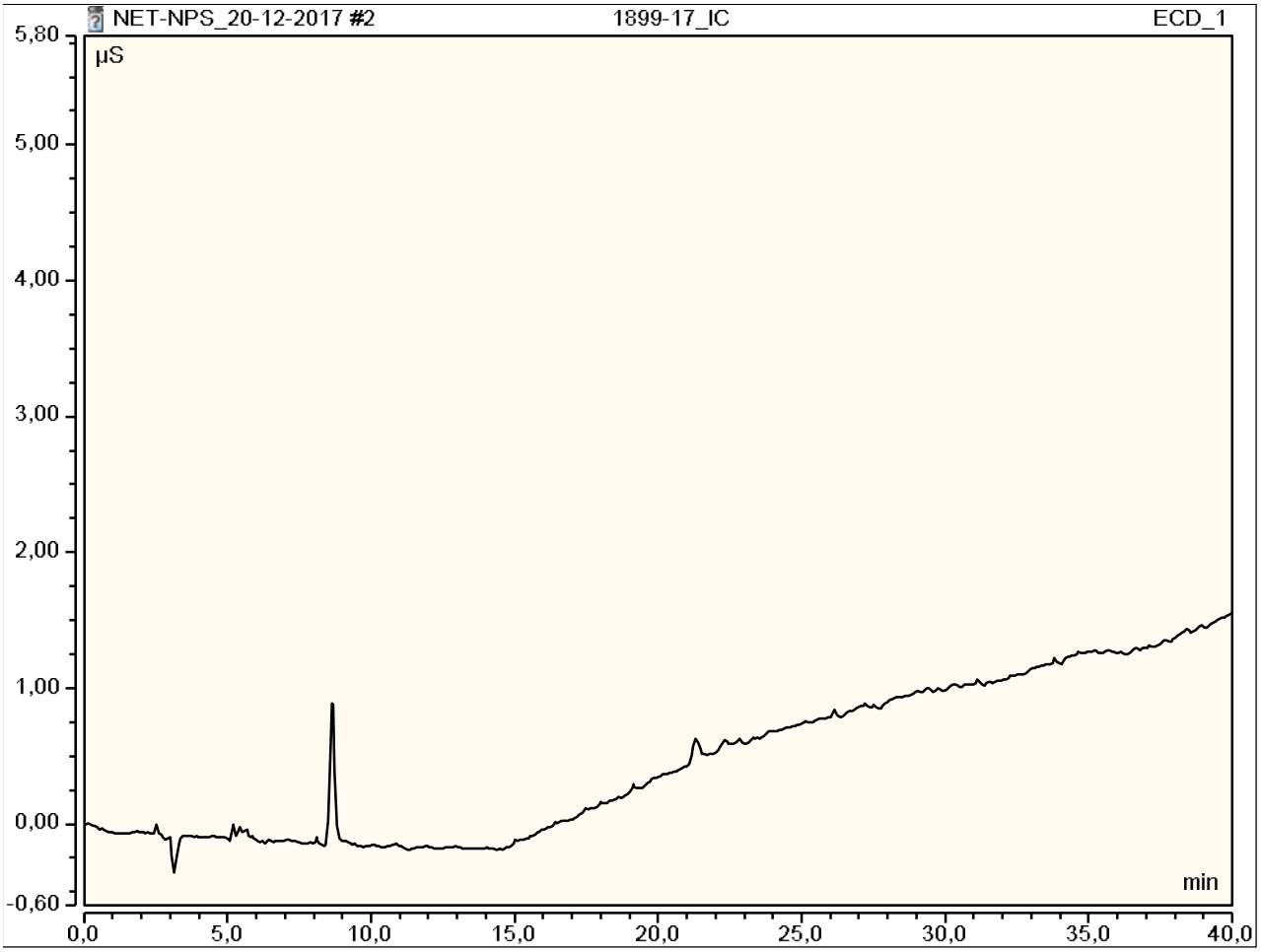
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
378.2189	1	10734712	C20 H28 F N3 O3	(M+H)+
379.2225	1	2525217.92	C20 H28 F N3 O3	(M+H)+
380.2256	1	304992.62	C20 H28 F N3 O3	(M+H)+
400.2014	1	1029663.31	C20 H28 F N3 O3	(M+Na)+
755.4305	1	2301257.25		(2M+H)+
756.4338	1	1057773.38		(2M+H)+
757.4368	1	251219.03		(2M+H)+
777.4124	1	2330047		(2M+Na)+
778.4159	1	1088133.79		(2M+Na)+
779.4188	1	250524.04		(2M+Na)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**1899-17_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**20-dec-2017 / 12:43**	*Run Time:*	**42,00**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount n.a.
		TOTAL:		0,00	0,00	0,00



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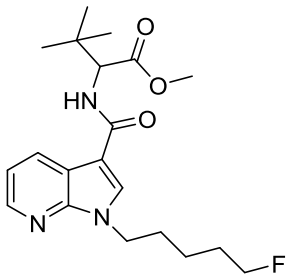
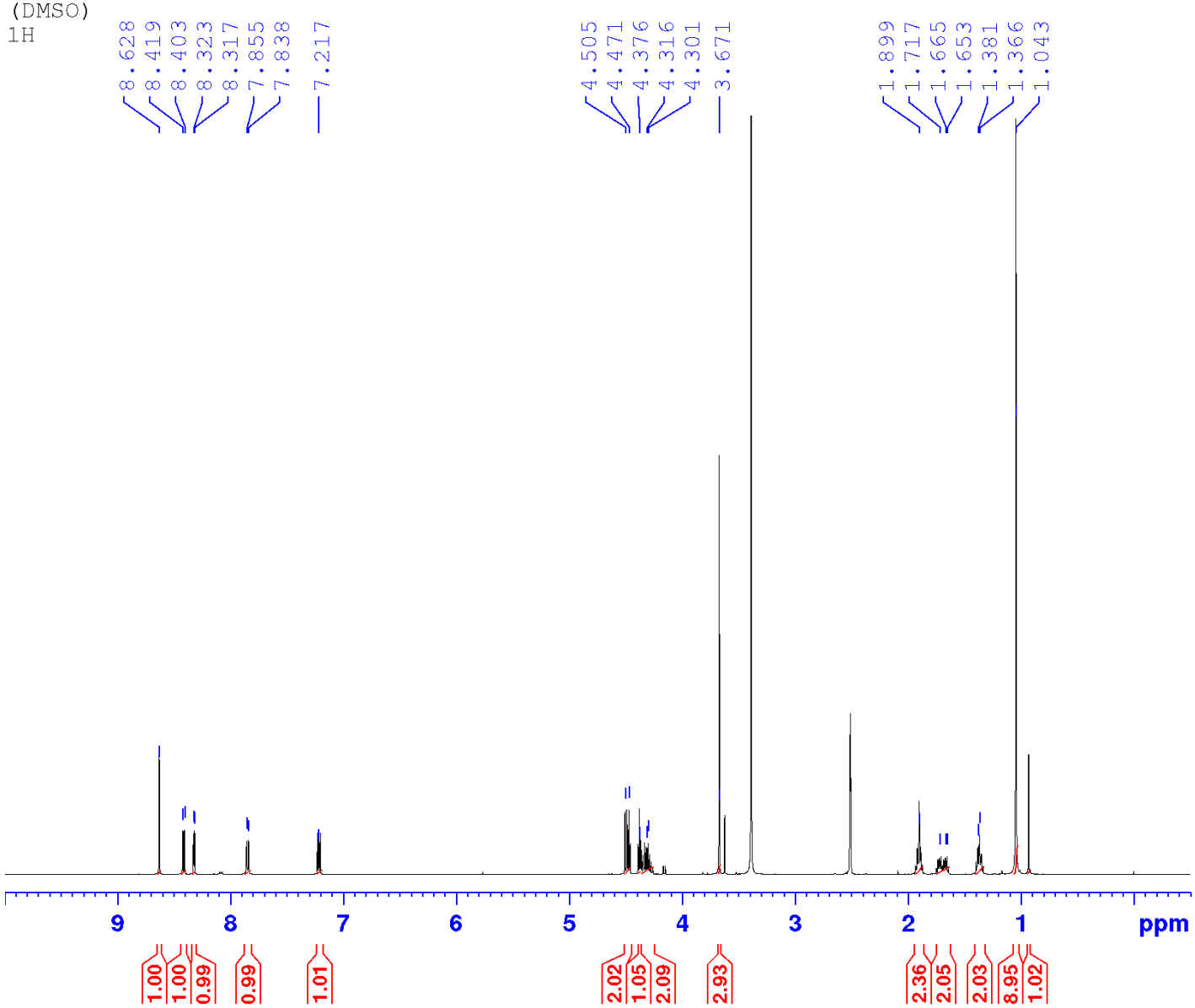
R E P O R T

Contract No.	C1714-17-460078 (Republic of Slovenia, Ministry of the Interior, POLICE)
Sample ID:	**1899-17**
Received date:	January 11, 2018
Our notebook code:	NFL-1899-17
NMR sample preparation:	21.6 mg (10.0 mg for NOESY) dissolved in 0.7 mL DMSO-d_6
NMR experiments:	^{1}H, ^{13}C, ^{19}F, ^{1}H–^{1}H *gs*-COSY, ^{1}H–^{13}C *gs*-HSQC, ^{1}H–^{13}C *gs*-HMBC, ^{1}H–^{15}N *gs*-HMBC, NOESY
Proposed structure with formula, exact mass, molecular weight:	 Chemical Formula: $C_{20}H_{28}FN_3O_3$ Exact Mass: 377,2115 Molecular Weight: 377,4604
Chemical name:	Methyl 2-(1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamido)-3,3-dimethylbutanoate
Comments:	- The structure was elucidated by analysis of the above 1D and 2D NMR spectra and HRMS. The result is supported by the literature reports. Please, see below. - >96% purity of a sample based on ^{1}H NMR spectrum.
Supporting information:	Copies of ^{1}H and ^{13}C NMR spectra, ^{1}H and ^{13}C FIDs.
Principal investigator:	Prof. Dr. Janez Košmrlj
Date of report:	February 5, 2018 Revised on February 11, 2018

NFL-1899-17
(DMSO)
1H




```
Current Data Parameters
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EXPNO                  1
PROCNO                 1

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Time               17.07
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG                    64
DW               50.000 usec
DE                6.50 usec
TE                296.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.1330885 MHz
NUC1                  1H
P1                 8.70 usec
PLW1        26.00000000 W

F2 - Processing parameters
SI                 65536
SF          500.1299991 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
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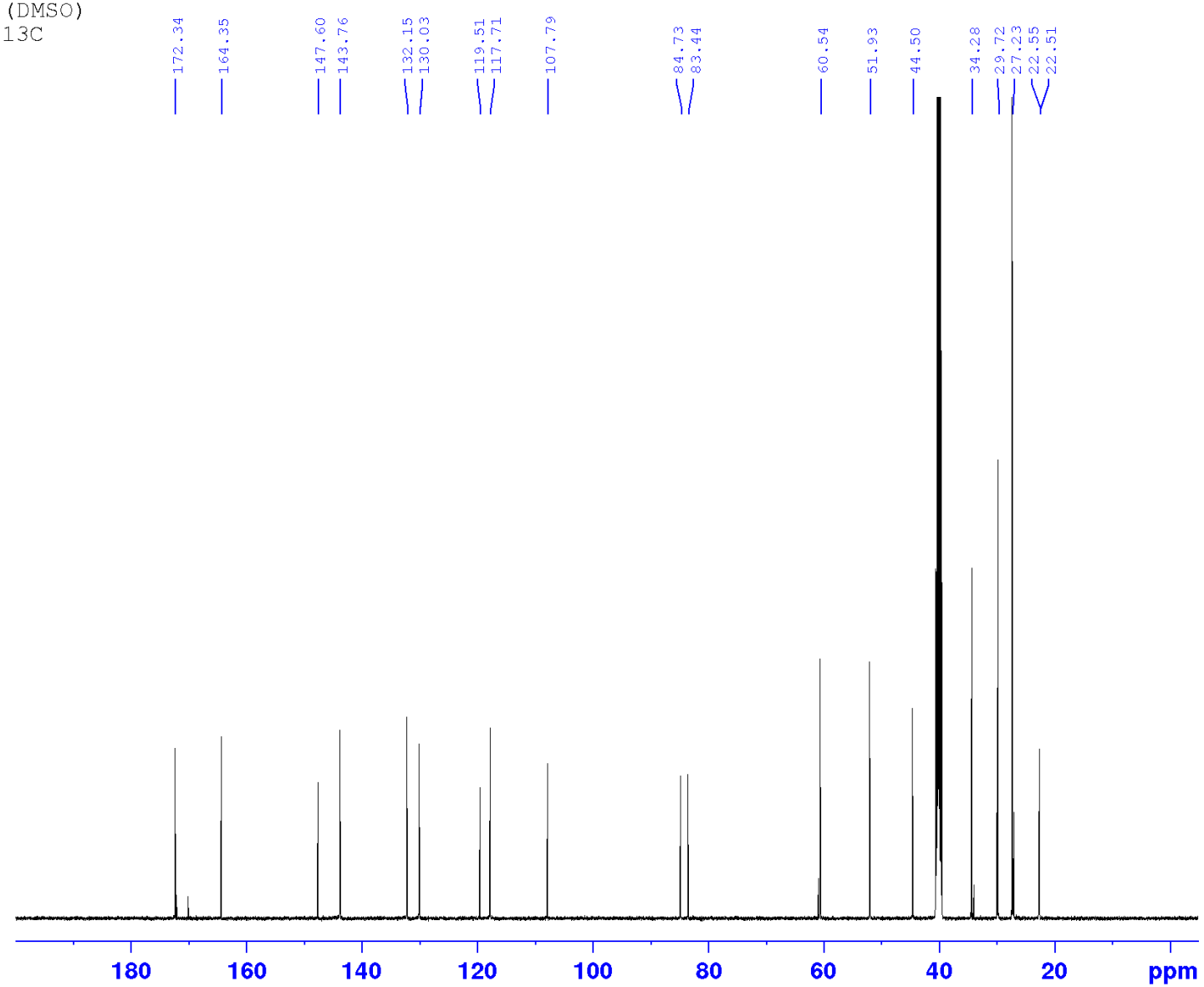
NFL-1899-17
(DMSO)
13C

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PROCNO                 1

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Time               21.49
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD                65536
SOLVENT             DMSO
NS                 5120
DS                    4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010048 sec
RG                 2050
DW               16.800 usec
DE                6.50 usec
TE               296.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.7703637 MHz
NUC1                13C
P1                 8.70 usec
PLW1       122.00000000 W

======== CHANNEL f2 ========
SFO2        500.1320005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2        26.00000000 W
PLW12        0.30046001 W
PLW13        0.15113001 W

F2 - Processing parameters
SI                 32768
SF          125.7577885 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```
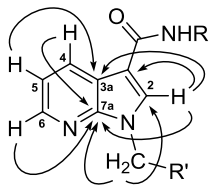

The 7-azaindole structure of the sample under investigation (NFL-1899-17) was confirmed over the 4-azaindole isomer on the basis of the following data:

The structure elucidation and the assignment of the NMR resonances were done by ^{1}H, ^{13}C, ^{19}F, $^{1}H–^{1}H$ *gs*-COSY, $^{1}H–^{13}C$ *gs*-HSQC, $^{1}H–^{13}C$ *gs*-HMBC, and $^{1}H–^{15}N$ *gs*-HMBC spectral analysis. Briefly, the analysis of the two side-chains attached to the azaindole system was trivial. In $^{1}H–^{13}C$ HMBC, the 5-fluoropentyl side-chain was the only one showing correlations with the azaindole carbon resonances, i.e., N1-CH_2/C2 and N1-CH_2/C7a (Spectrum 1). Atoms C2 and H2 were readily identified by $^{1}H–^{13}C$ HSQC. Strong HMBC correlations of H4 and H6, and a very weak correlation of H5 with C7a suggested the 7-azaindole system (Figure 1). The structure was corroborated by a strong H5/C3a cross-peak.



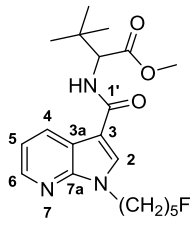
NFL-1899-17

Selected relevant ^{1}H-^{13}C HMBC correlations

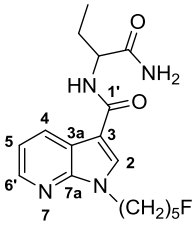
Figure 1.

The proposed 7-azaindole structure is in good agreement with the literature reported data for proton and carbon resonances in closely related analogue NNL-1 (Table 1). In addition, the ^{1}H and ^{13}C chemical shifts for the fused pyridine ring are in good agreement with the literature data for unsubstituted 7-azaindole (Table 1). In an excellent agreement with the literature data is also the ^{15}N chemical shift for N7 (269 ppm *vs.* 270 ppm) (Spectrum 2).

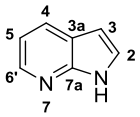
Table 1. Comparison of ^{1}H and ^{13}C chemical shifts for the heterocyclic ring system for NFL-1899-17, NNL-1, and 7-azaindole.

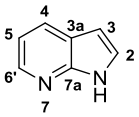


NFL-1899-17



NNL-1





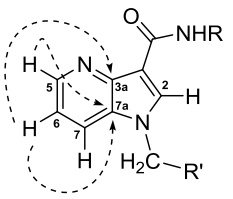
Position	δ / ppm[a,b]	δ / ppm[a,c]	δ / ppm[d,e]	δ / ppm[d,f]
H2	8.63	8.41		
H4	8.42	8.42		
H5	7.22	7.20		
H6	8.32	8.31		
C1′	164.4	163.6		
C2	132.2	131.2	125.5	125.4
C3	107.8	108.0	100.5	100.4
C3a	119.6	118.8	120.7	
C4	130.1	129.4	129.0	129.0
C5	117.8	117.1	115.6	115.6
C6	143.7	143.2	142.1	142.1
C7a	147.7	147.1	148.9	
N1	148			139.1
N7	269			270.1

[a] Recorded in DMSO-d_6.
[b] Complete assignment of ^{1}H and ^{13}C resonances was made by analysis of ^{1}H, ^{13}C, ^{19}F, $^{1}H–^{1}H$ *gs*-COSY, $^{1}H–^{13}C$ *gs*-HSQC, $^{1}H–^{13}C$ *gs*-HMBC, and $^{1}H–^{15}N$ *gs*-HMBC spectra.
[c] Data from: Z. Qian, W. Jia, T. Li, Z. Hua, C. Liu, Identification and analytical characterization of four synthetic cannabinoids ADB-BICA, NNL-1, NNL-2, and PPA(N)-2201, *Drug Test. Analysis* **2016**, DOI 10.1002/dta.1990.
[d] in $CDCl_3$.
[e] Data from: R. H. Cox, S. Sankar, ^{1}H and ^{13}C NMR Studies of 7-Azaindole and Related Compounds, *Organic Magnetic Resonance*, **1980**, *14*, 150–152.
[f] Data from: S. Minakata, S. Itoh, M. Komatsu, Y. Ohshlro, Y. Yokomichi, Multinuclear NMR and *ab initio* MO studies of 7-methyl-7H-pyrrolo [2,3-*b*]pyridine and related compounds, *Journal Of Physical Organic Chemistry*, **1993**, *6*, 139–144.

Should the structure of the heterocyclic system be interpreted as a 4-azaindole, the presence of one $^{1}H–^{13}C$ HMBC strong cross-peak between H5 (8.32 ppm) and C7a (147.7 ppm), and the absence of a strong cross-peak between H6 (7.22 ppm) and C7a (147.7 ppm) are less convincing (Spectrum 3). Also less likely is the presence of strong $^{1}H–^{13}C$ HMBC correlation between H6 (7.22 ppm) and C3a (119.6 ppm).

In NOESY spectrum there is a strong correlation between N1-CH_2 protons and H2, whereas the correlation between N1-CH_2 protons and H7 is missing (Spectrum 4). Although the absence of information is not a positive proof, based on the vicinity of N1-CH_2 and H7, it is unlikely that the N1-CH_2/H7 correlation is not seen if the structure of NFL-1899-17 was 4-azaindole.

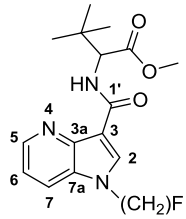
The tentatively suggested 4-azaindole structure of the sample under investigation (NFL-1899-17) is not in agreement with the literature reported data for 4-azaindole, especially in the chemical shifts for C7 and N4 (Table 2).



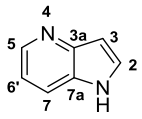
Selected issues in ^{1}H-^{13}C HMBC spectrum of tentatively assigned 4-azaindole structure

Figure 2.

Table 2. Comparison of ^{1}H and ^{13}C chemical shifts for the heterocyclic ring system for NFL-1899-17 (tentatively assigned to 4-azaindole structure) and unsubstituted 4-azaindole.

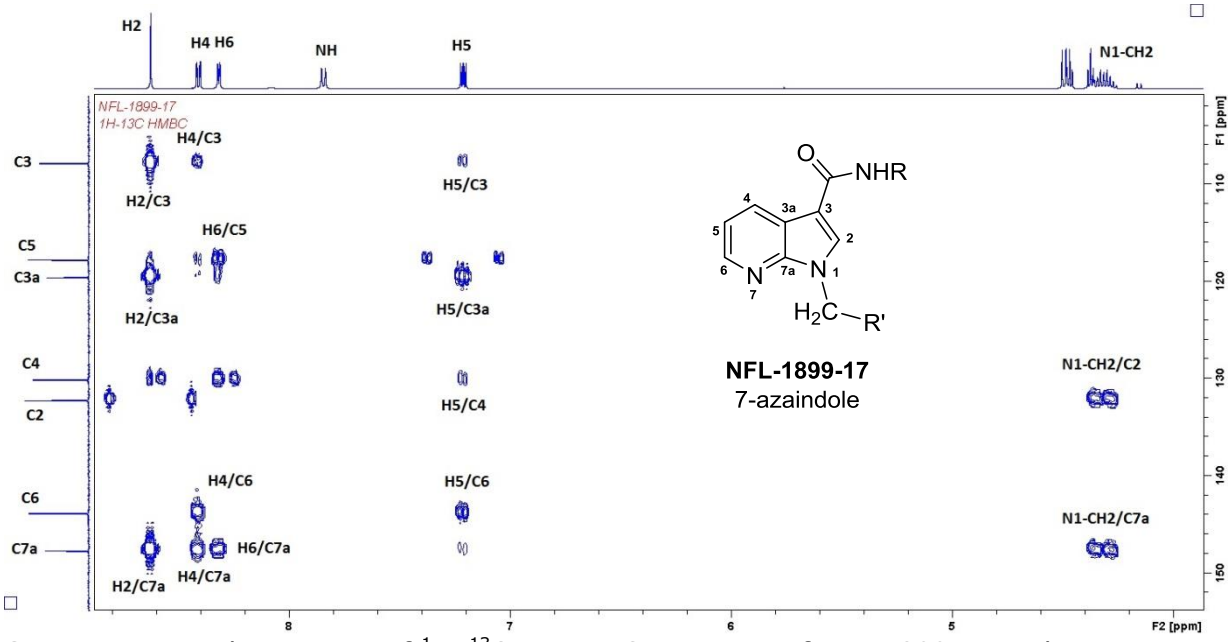


NFL-1899-17

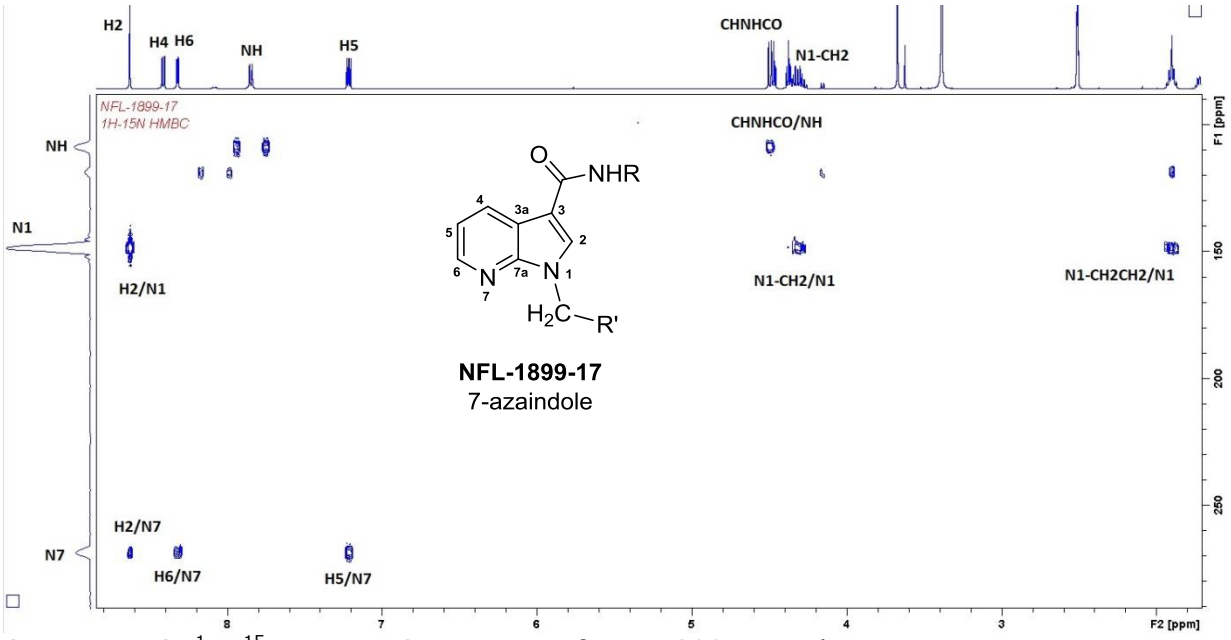


Position	δ / ppm[a,b]	δ / ppm[c,d]
H2	8.63	
H5	8.32	
H6	7.22	
H7	8.42	
C2	132.2	128.7
C3	107.8	102.3
C3a	119.6	
C5	143.7	142.6
C6	117.8	116.5
C7	130.1	119.0
C7a	147.7	
N1	148	131.5
N4	269	295.5

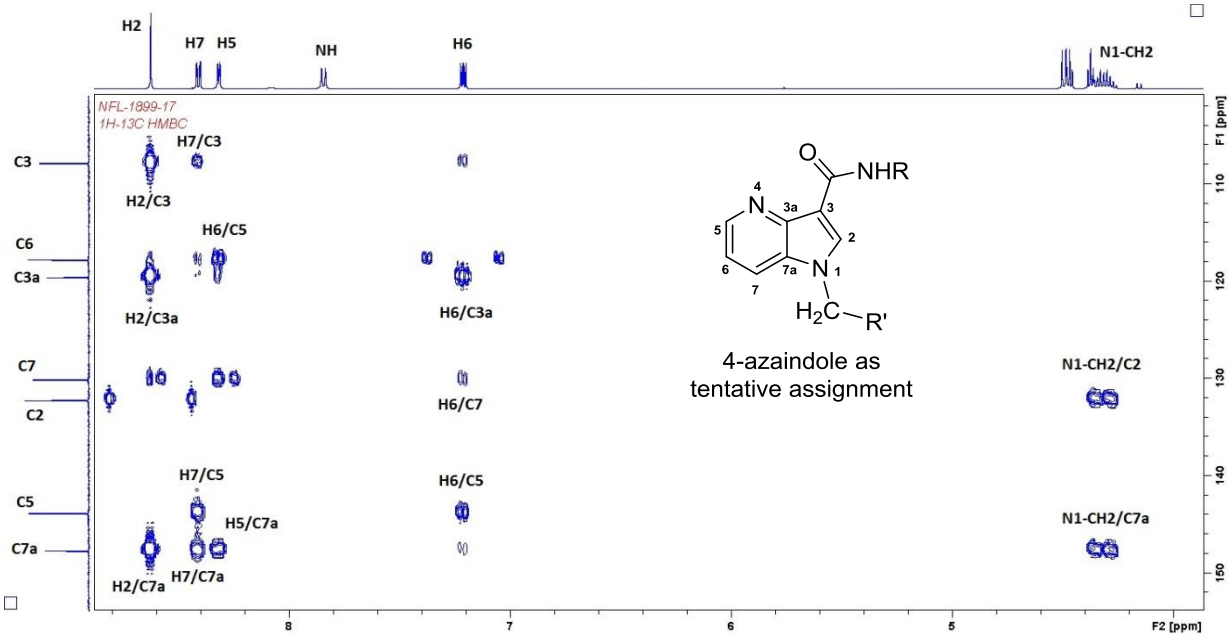
[a] Recorded in DMSO-d_6.
[b] Complete assignment of ^{1}H and ^{13}C resonances was made by analysis of ^{1}H, ^{13}C, ^{19}F, $^{1}H–^{1}H$ *gs*-COSY, $^{1}H–^{13}C$ *gs*-HSQC, $^{1}H–^{13}C$ *gs*-HMBC, and $^{1}H–^{15}N$ *gs*-HMBC spectra.
[c] in $CDCl_3$.
[d] Data from: S. Minakata, S. Itoh, M. Komatsu, Y. Ohshlro, Y. Yokomichi, Multinuclear NMR and *ab initio* MO studies of 7-methyl-7H-pyrrolo [2,3-*b*]pyridine and related compounds, *Journal Of Physical Organic Chemistry*, **1993**, *6*, 139–144.



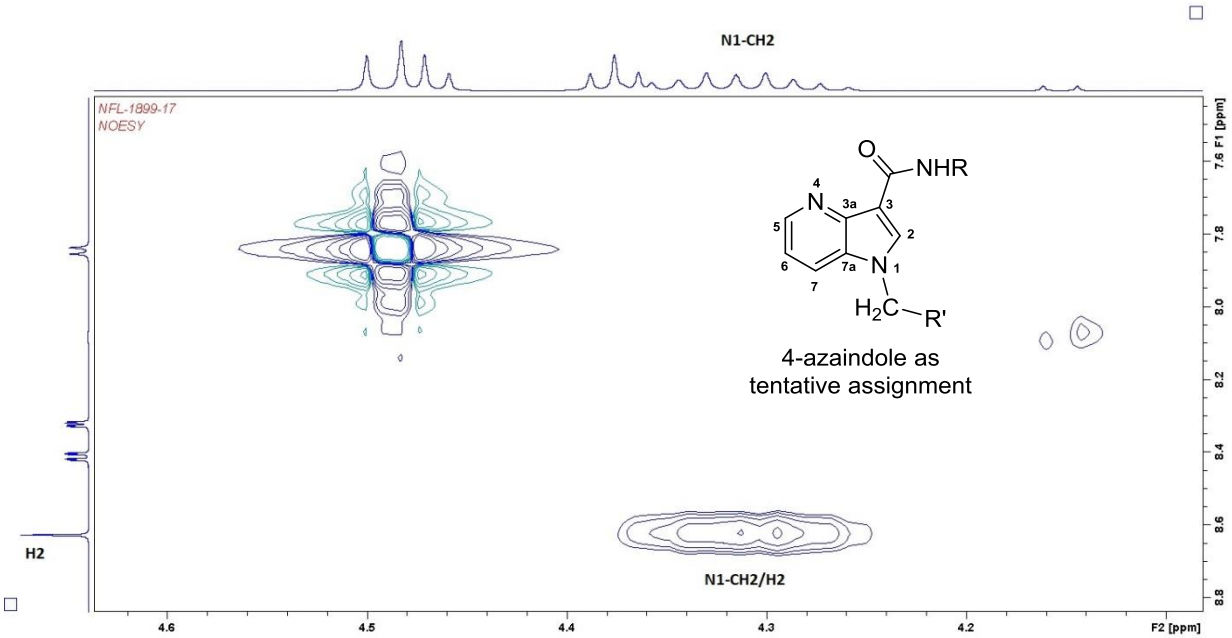
Spectrum 1. Relevant part of $^{1}H–^{13}C$ *gs*-HMBC spectrum of NFL-1899-17 with assignment.



Spectrum 2. $^{1}H–^{15}N$ *gs*-HMBC spectrum of NFL-1899-17 with assignment.



Spectrum 3. Relevant part of $^{1}H-^{13}C$ *gs*-HMBC spectrum of NFL-1899-17 with tentatively assigned 4-azaindole structure.



Spectrum 4. Relevant part of NOESY spectrum of NFL-1899-17 with tentatively assigned 4-azaindole structure.