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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

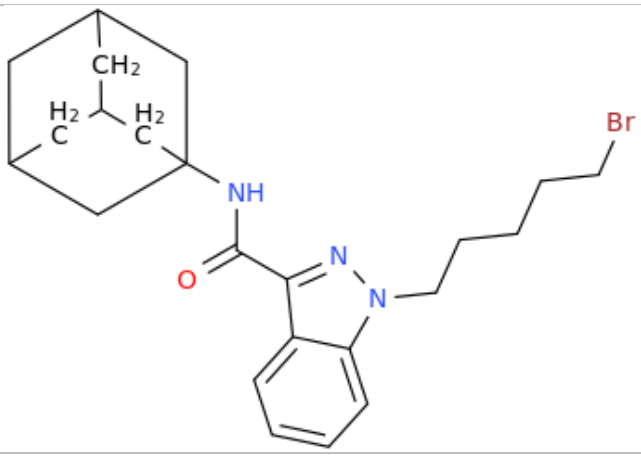
ANALYTICAL REPORT

5B-AKB-48 (C23H30BrN3O)

N-(adamantan-1-yl)-1-(5-bromopentyl)-1H-indazole-3-carboxamide

Remark – other NPS detected: 5C-AKB-48 (as major compound)

Sample ID:	2190-20
Sample description:	powder
Sample type:	test purchase /ISF projekt (NFL-SI)
Date of entry (DD/MM/YYYY) into NFL database:	20/10/2020
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	N-(adamantan-1-yl)-1-(5-bromopentyl)-1H-indazole-3-carboxamide
Other names	5Br-APINACA; 5-Bromo AKB48, 5Br-AKB-48
Formula (per base form)	C23H30BrN3O
M_w (g/mol)	444,42
Salt form/anions detected	base
StdInChIKey (per base form)	JKYXWDCDKGVNGP-UHFFFAOYSA-N
Other NPS detected	5C-AKB-48 (as major compound)
Additional info (purity..)	major and minor compounds in molar ratio of 1 : 0.15 based on 1H NMR spectrum
For 5C-AKB-48 analytical data (2015) are published here:	https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/5C-AKB48-ID-1186-15-report_final.pdf

[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 µl 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 µl 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 KOH 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

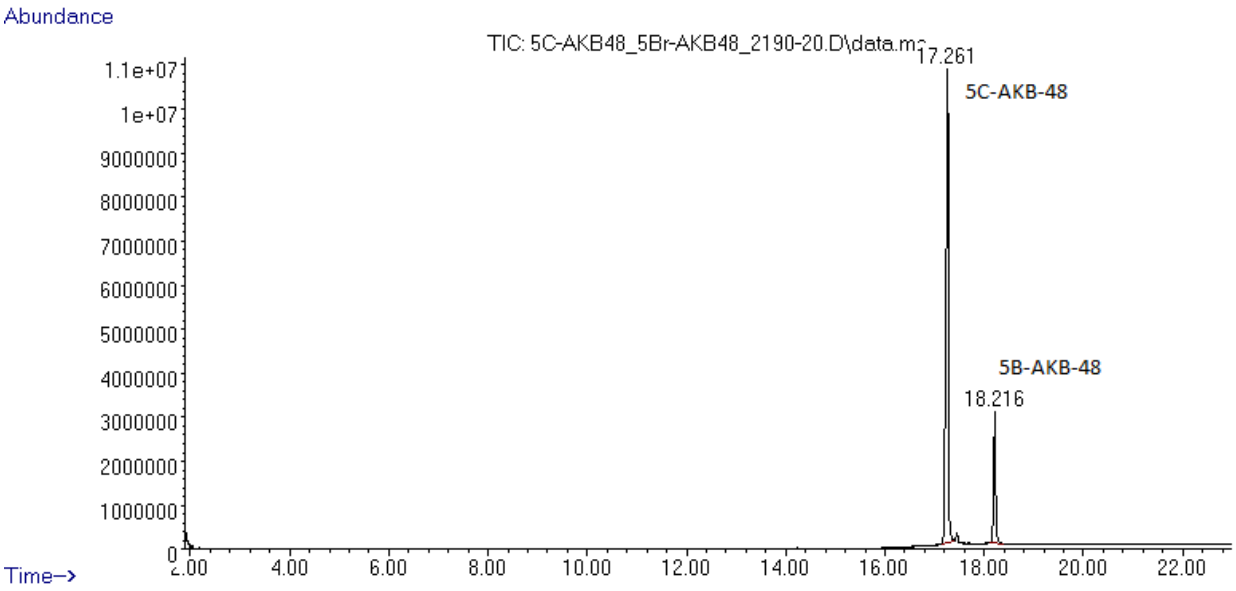
Supporting information

Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	not soluble

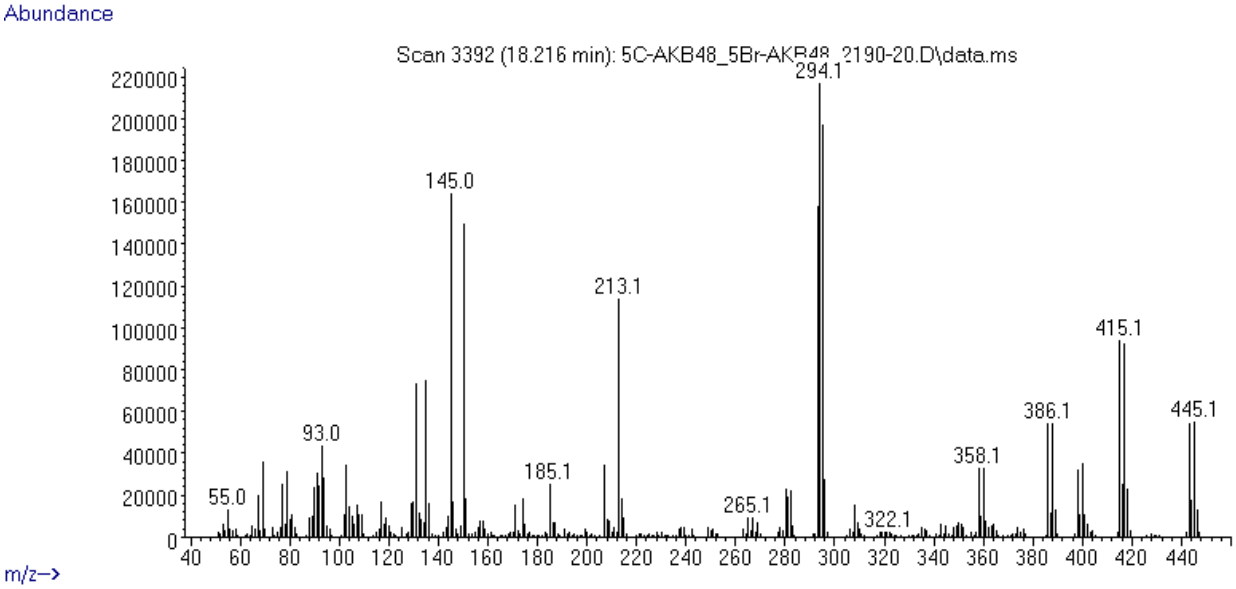
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 18,22 BP(1): 294; BP(2): 295,BP(3) :145,
HPLC-TOF	+	Exact mass (theoretical): 443,1572; measured value Δppm:-1,5; formula:C23H30BrN3O
FTIR-ATR	-	direct measurement (sample as received)
FTIR (solid phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	+	
validation		
other		

ANALYTICAL RESULTS

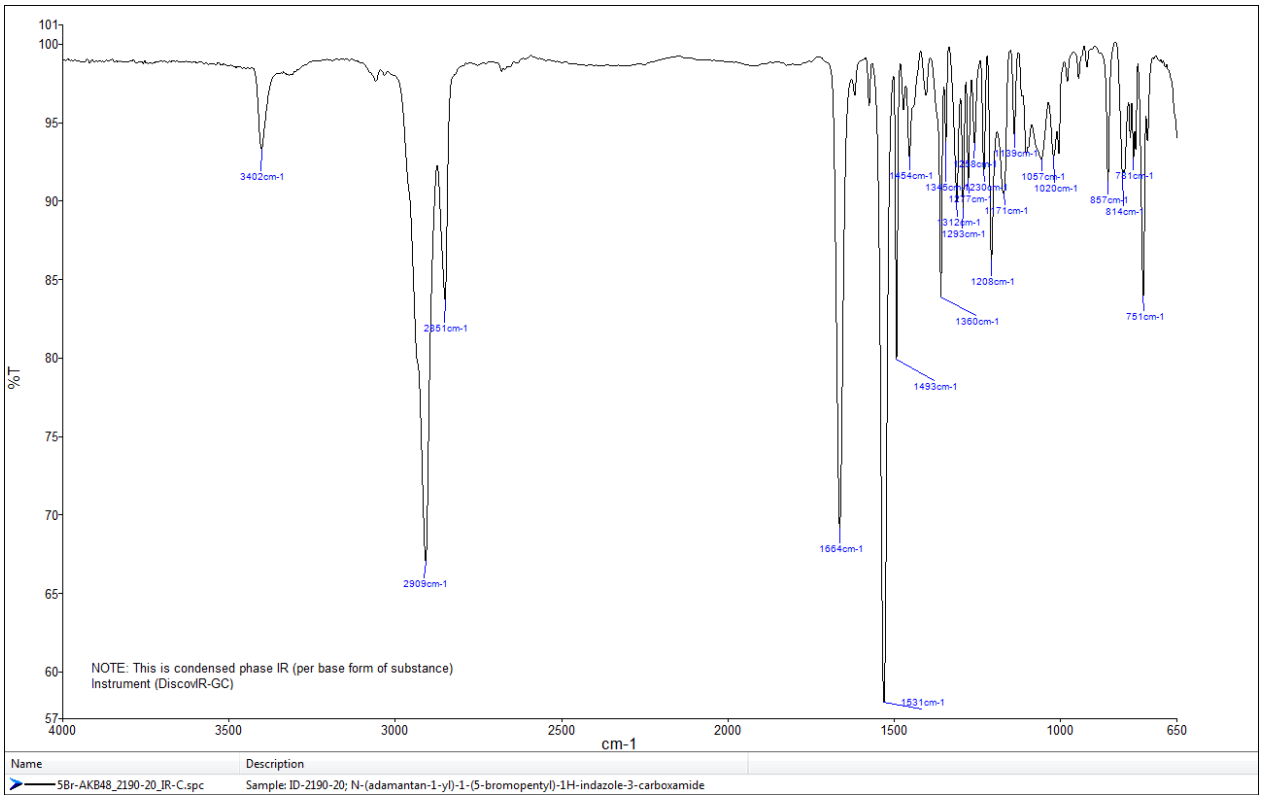
GC –chromatogram



MS (EI) at RT 12.216 min (5B-AKB-48)



IR (solid phase for 5B-AKB-48 – after chromatographic separation)



TOF REPORT

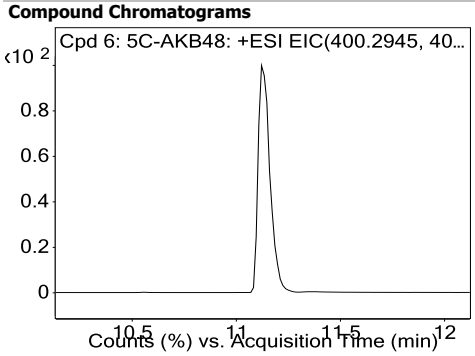
Data File	_2190-20.d	**Sample Name**	ID-2190-20
Sample Type	Sample	**Position**	P2-A7
Instrument Name	6230B TOF LC-MS	**User Name**	
Acq Method	general-15_01_2020-XDB-C18-ESI+.m	**Acquired Time**	9/2/2020 9:04:03 PM
IRM Calibration Status	Success	**DA Method**	a-Drugs_NFL.m
Comment			

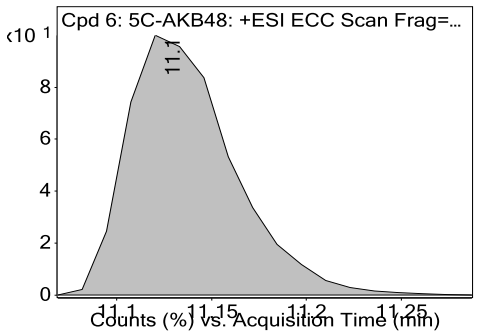
Compound Table

Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 6: 5C-AKB48	5C-AKB48	C23 H30 Cl N3 O	11.1	399.2084
Cpd 8: 5Br-AKB48	5Br-AKB48	C23 H30 Br N3 O	11.3	443.1579

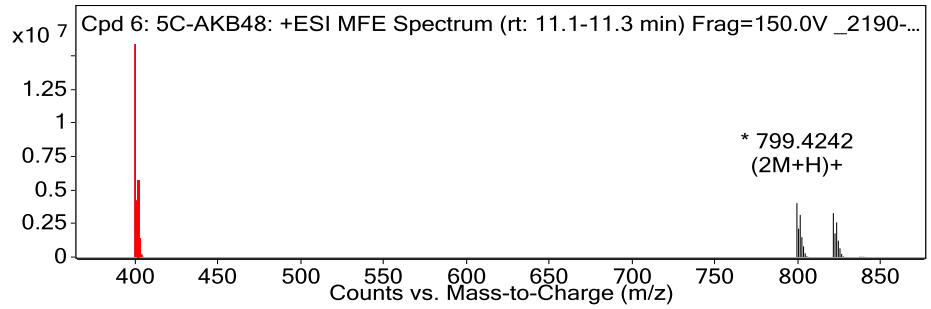
Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
5C-AKB48	400.2156	11.1	399.2084	11.1	C23 H30 Cl N3 O	399.2077	-1.55

Compound Chromatograms

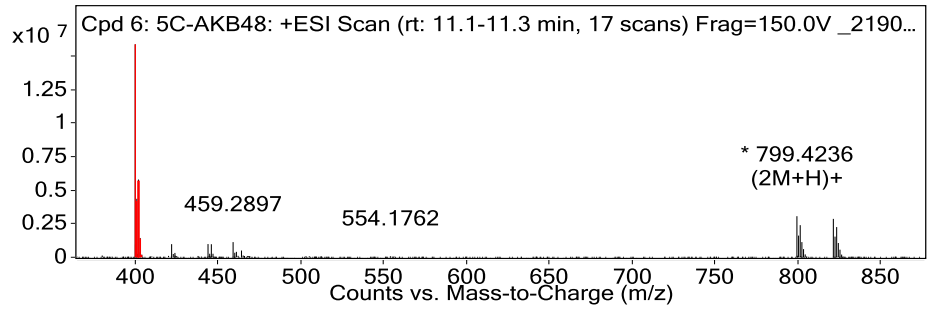




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



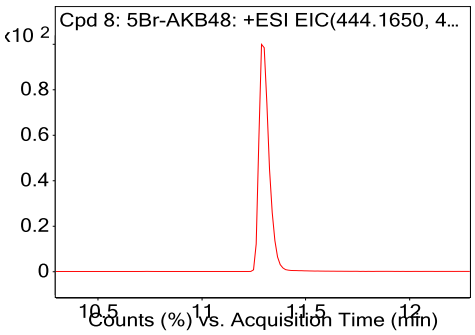
MS Spectrum Peak List

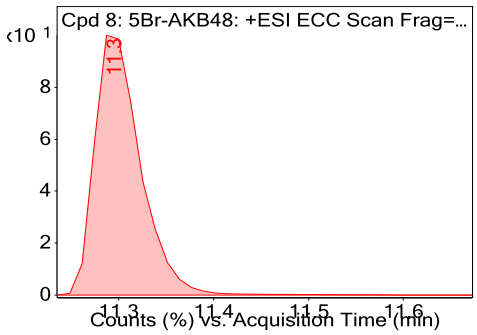
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
400.2156	1	15877104	C23 H30 Cl N3 O	(M+H)+
401.2188	1	4235377.39	C23 H30 Cl N3 O	(M+H)+
402.2136	1	5601048.82	C23 H30 Cl N3 O	(M+H)+
799.4242	1	4022652.25		(2M+H)+
800.4271	1	2123856.42		(2M+H)+
801.4226	1	3145124.33		(2M+H)+
802.4252	1	1486837.74		(2M+H)+
821.4056	1	3276910		(2M+Na)+
822.4092	1	1765231.98		(2M+Na)+
823.4045	1	2580658.07		(2M+Na)+

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
5Br-AKB48	444.1651	11.3	443.1579	11.3	C23 H30 Br N3 O	443.1572	-1.5

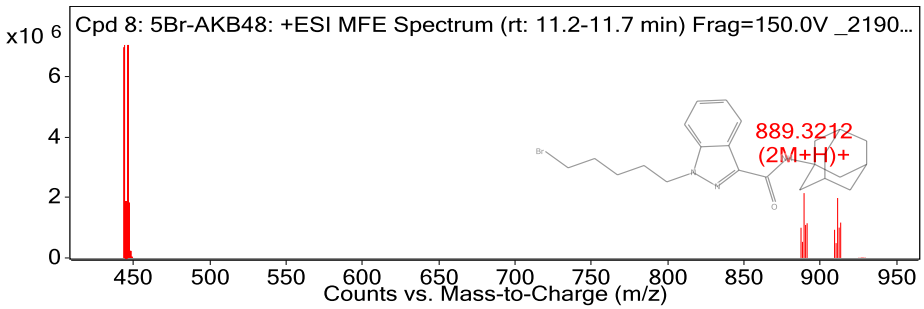
Compound Chromatograms

TOF REPORT

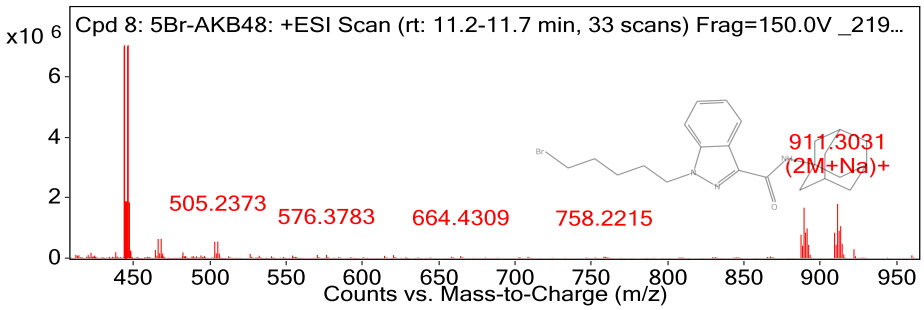




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Charge	Abund	Formula	Ion/Isotope
444.1651	1	7062752	C23 H30 Br N3 O	(M+H)+
445.1686	1	1832563.84	C23 H30 Br N3 O	(M+H)+
446.1633	1	6994609.04	C23 H30 Br N3 O	(M+H)+
447.1668	1	1843261.71	C23 H30 Br N3 O	(M+H)+
889.3212	1	2150383		(2M+H)+
890.3251	1	1094630.17		(2M+H)+
891.3211	1	1150687.79		(2M+H)+
911.3032	1	1987934.75		(2M+Na)+
912.3069	1	1006693.61		(2M+Na)+
913.3033	1	1173251.12		(2M+Na)+

--- End Of Report ---

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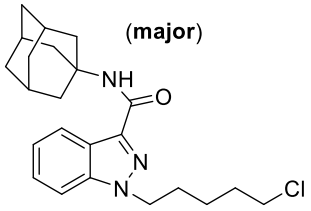
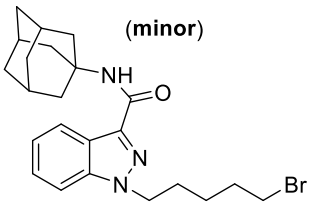
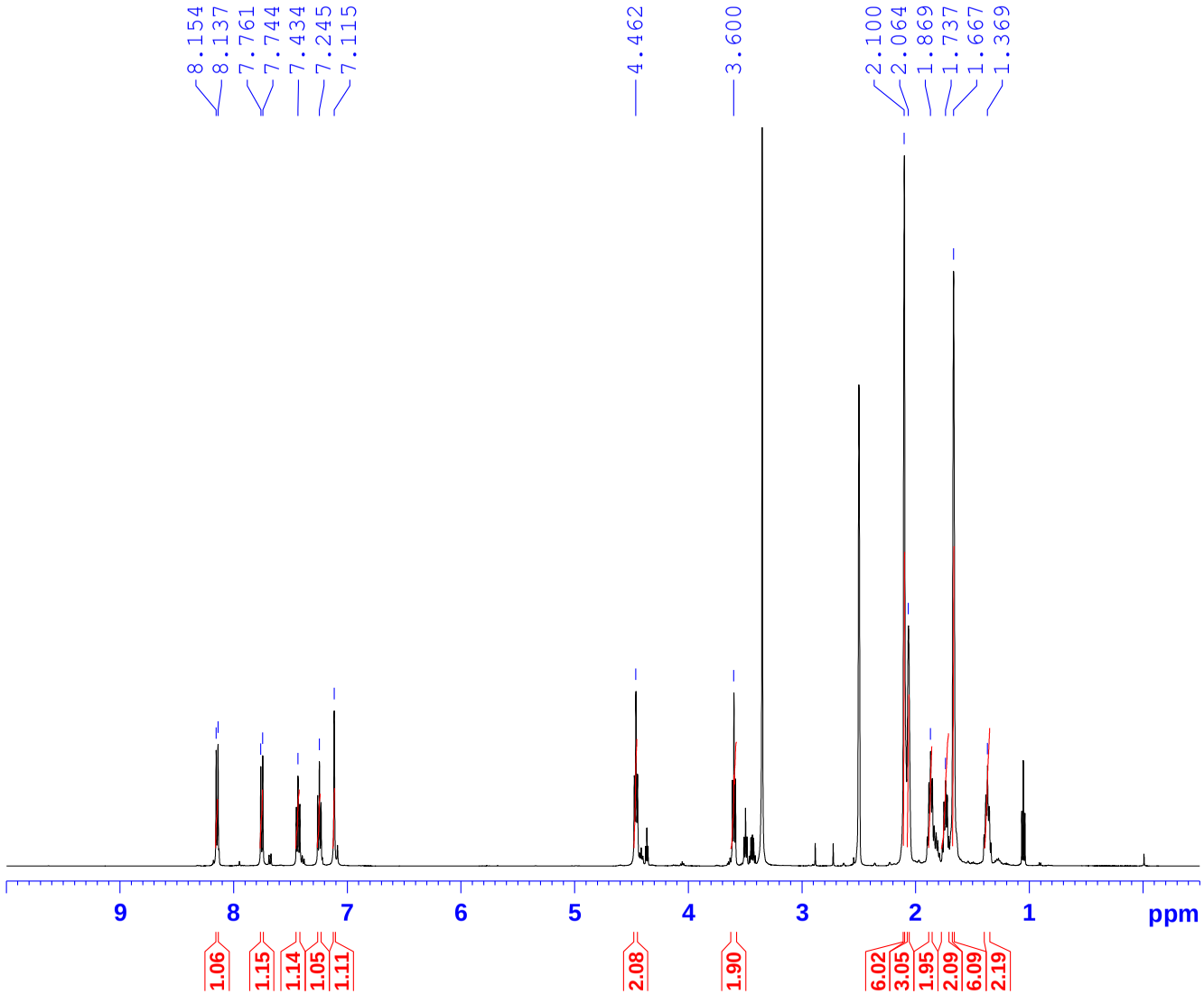
University
of Ljubljana

Faculty *of Chemistry*
and Chemical Technology

R E P O R T

Contract No.	C1714-19-460155 (Republic of Slovenia, Ministry of the Interior, POLICE)
Sample ID:	**2190-20**
Received date:	September 10, 2020
Our notebook code:	NFL-2190-20
NMR sample preparation:	22.8 mg dissolved in 0.7 mL DMSO-d_6
NMR experiments:	^{1}H, ^{13}C, ^{1}H–^{1}H *gs*-COSY, ^{1}H–^{13}C *gs*-HSQC, ^{1}H–^{13}C *gs*-HMBC, ^{1}H–^{15}N *gs*-HMBC
Proposed structure with formula, exact mass, molecular weight:	 Chemical Formula: $C_{23}H_{30}ClN_3O$ Exact Mass: 399,2077 Molecular Weight: 399,9630  Chemical Formula: $C_{23}H_{30}BrN_3O$ Exact Mass: 443,1572 Molecular Weight: 444,4170
Chemical name:	*N*-((3*s*,5*s*,7*s*)-adamantan-1-yl)-1-(5-chloropentyl)-1*H*-indazole-3-carboxamide (**major**), *N*-((3*s*,5*s*,7*s*)-adamantan-1-yl)-1-(5-bromopentyl)-1*H*-indazole-3-carboxamide (**minor**)
Comments:	- Structure elucidation based on 1D and 2D NMR spectra and HRMS. - The sample consists of herein identified **major** and **minor** compounds in molar ratio of 1 : 0.15, based on ^{1}H NMR spectrum. - Resonances for the **major** compound are integrated and/or peak picked in the attached ^{1}H and ^{13}C NMR spectra. - >97% 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:	September 15, 2020

NFL-2190-20
1H

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EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200925
Time              18.24
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                   32
DS                    2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG                 80.6
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           500.1300044 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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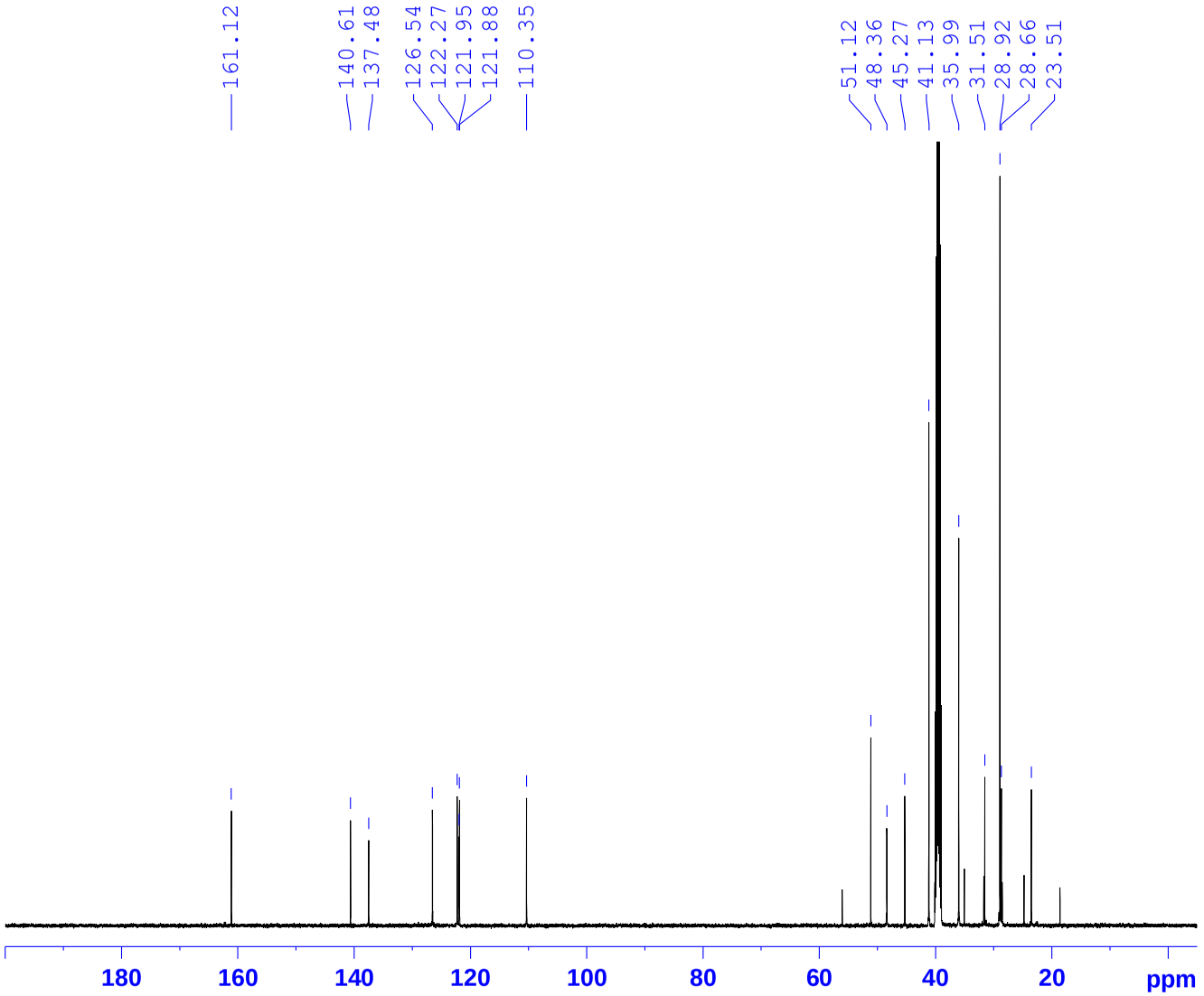
NFL-2190-20
13C

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EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
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Time               15.27
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  3072
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           1.00000000 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           125.7578452 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```