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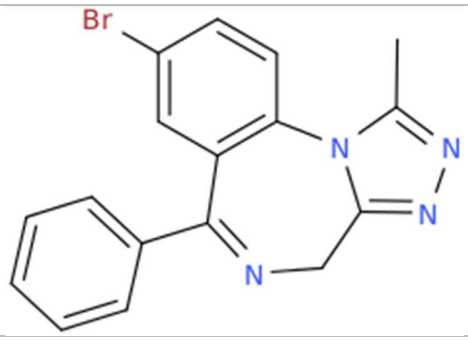
ANALYTICAL REPORT

Bromazolam (C17H13BrN4)

12-bromo-3-methyl-9-phenyl-2,4,5,8-tetraazatricyclo[8.4.0.0²,⁶]tetradeca-1(14),3,5,8,10,12-hexaene

Remark – other NPS detected: none

Sample ID:	1868-17
Sample description:	powder
Sample type:	test purchase /ISF projekt (NFL-SI)
Date of entry (DD/MM/YYYY) into NFL database:	06/12/2017
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	12-bromo-3-methyl-9-phenyl-2,4,5,8-tetraazatricyclo[8.4.0.0²,⁶]tetradeca-1(14),3,5,8,10,12-hexaene
Other names	8-bromo-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
Formula (per base form)	C17H13BrN4
M_w (g/mol)	353,22
Salt form/anions detected	base
StdInChIKey (per base form)	KCEIOBKDDQAYCM-UHFFFAOYSA-N
Other NPS detected	none
Additional info (purity..)	pure by GC-MS, HPLC-TOF

[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 6.1 min, then heating at 50 ^{0}C/min up to 325 ^{0}C and finally 9.1 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 solid 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

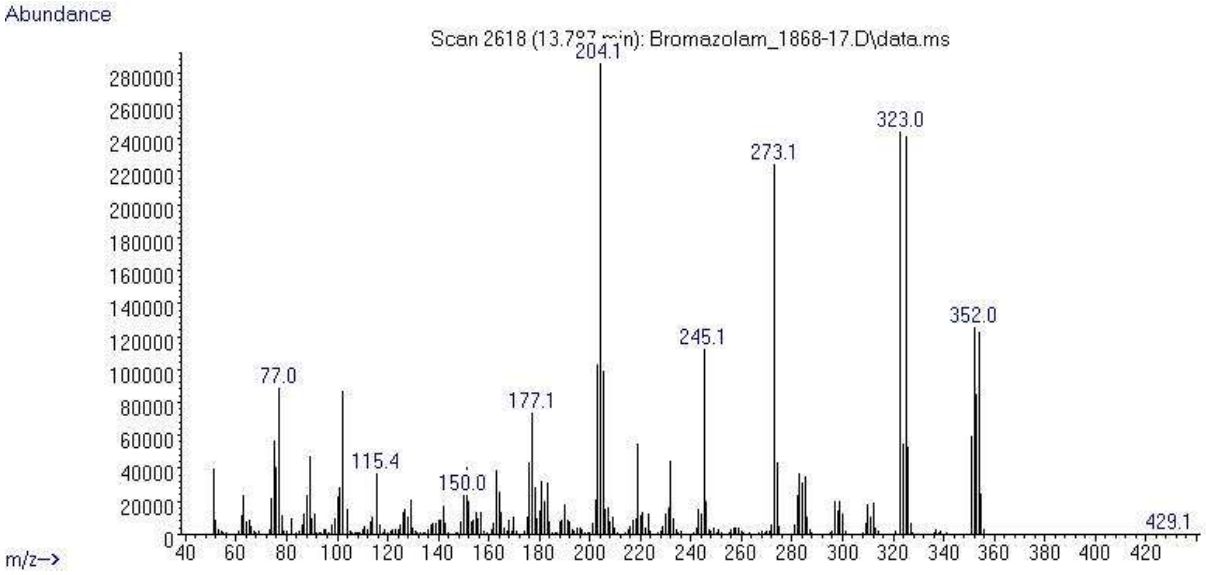
Supporting information

Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	/

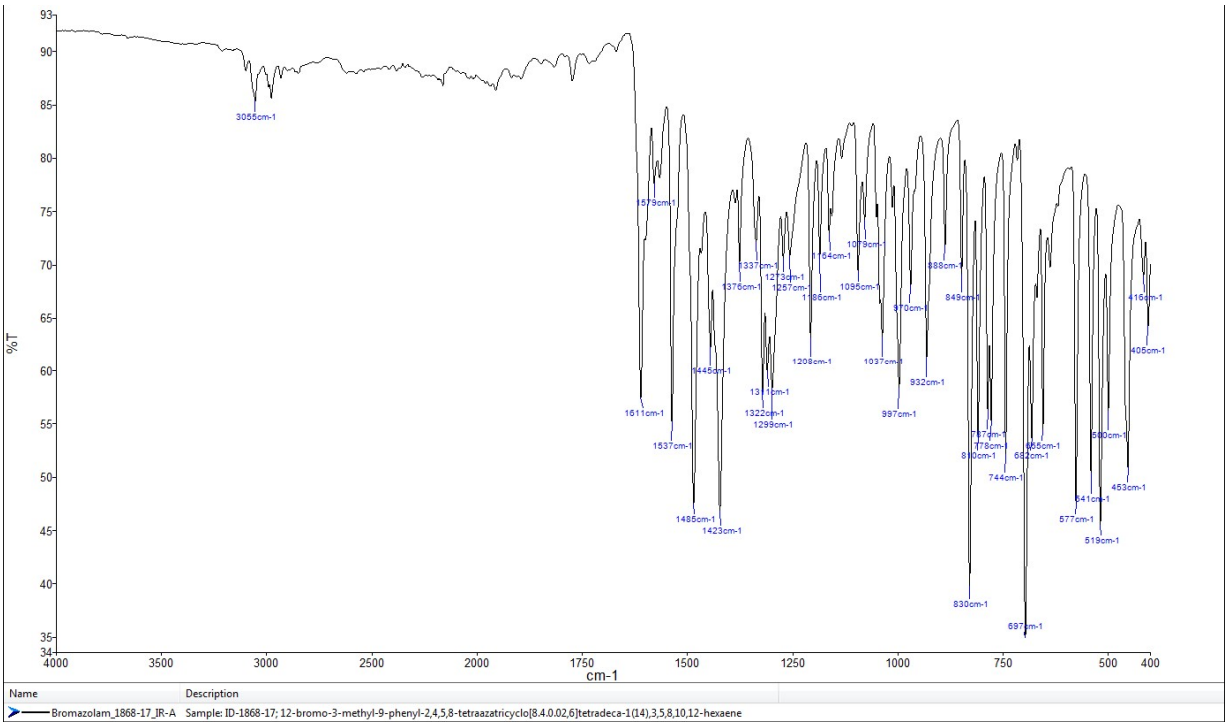
Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 13,79 BP(1): 204; BP(2): 323,BP(3) :325,
HPLC-TOF	+	Exact mass (theoretical): 352,0324; measured value Δppm:-0,76; formula:C17H13BrN4
FTIR-ATR	+	direct measurement (sample as received)
FTIR (solid phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	+	
validation		
other		

ANALYTICAL RESULTS

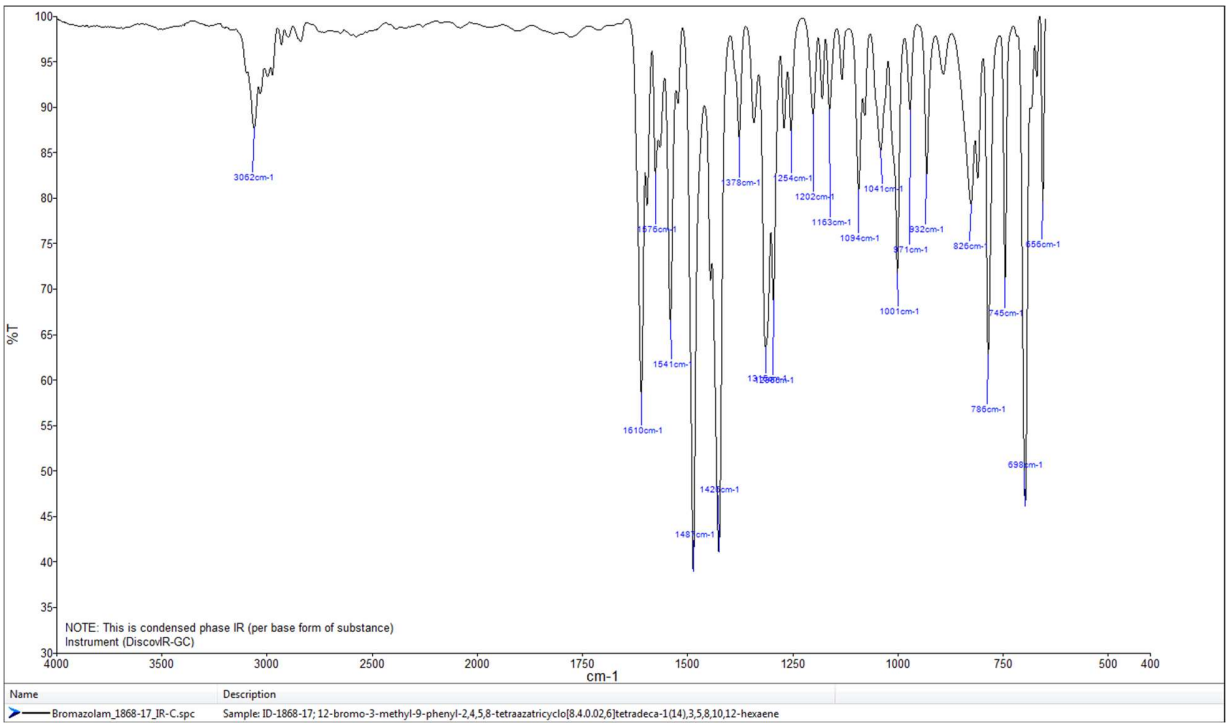
MS (EI)



FTIR-ATR - direct measurement (sample as received)



IR (solid phase – after chromatographic separation)



TOF REPORT

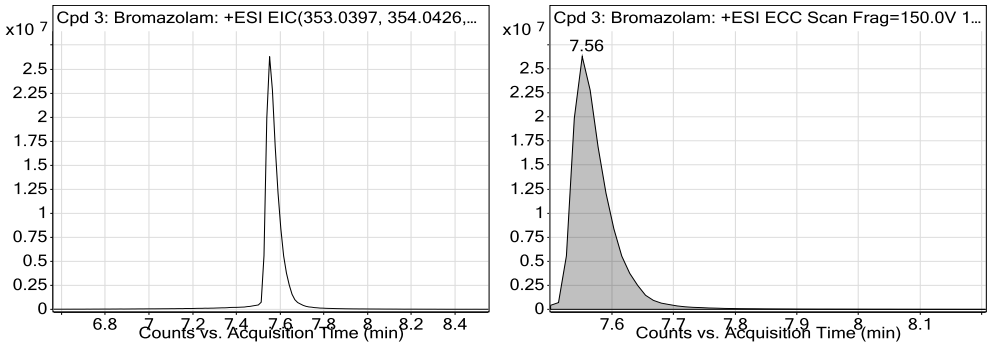
Data File	1868-17.d	**Sample Name**	1868-17
Sample Type	Sample	**Position**	P2-A7
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-19_07_2017-XDB-C18-ESI-final.m	**Acquired Time**	10/20/2017 9:51:21 AM
IRM Calibration Status	Success	**DA Method**	Drugs_NFL.m
Comment	MeOH		

Compound Table

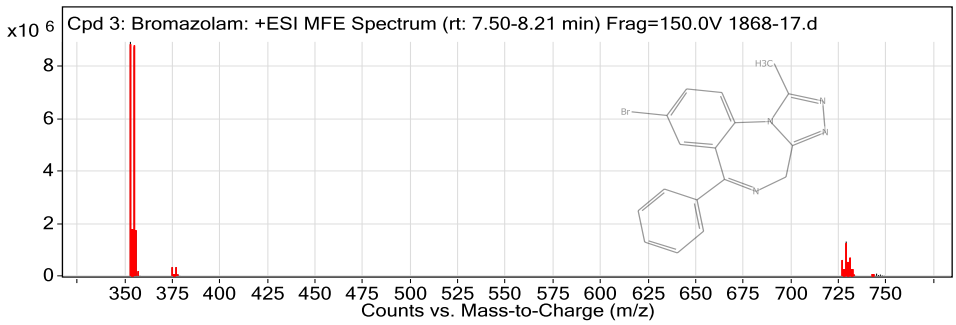
Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 3: Bromazolam	Bromazolam	C17 H13 Br N4	7.56	352.0326

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
Bromazolam	353.0399	7.56	352.0326	7.56	C17 H13 Br N4	352.0324	-0.76

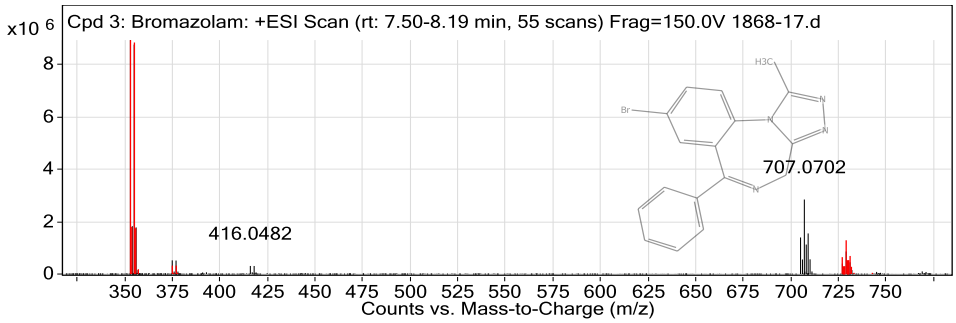
Compound Chromatograms



MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Charge	Abund	Formula	Ion/Isotope
353.0399	1	8929483	C17 H13 Br N4	(M+H)+
354.0429	1	1766012.09	C17 H13 Br N4	(M+H)+
355.038	1	8725159	C17 H13 Br N4	(M+H)+
356.0409	1	1716990.12	C17 H13 Br N4	(M+H)+
375.0219	1	336314.38	C17 H13 Br N4	(M+Na)+
377.0201	1	331927.76	C17 H13 Br N4	(M+Na)+
727.0546	1	607187.46	C17 H13 Br N4	(2M+Na)+
729.0526	1	1308699.38	C17 H13 Br N4	(2M+Na)+
730.0555	1	480759.89	C17 H13 Br N4	(2M+Na)+
731.0516	1	680828.89	C17 H13 Br N4	(2M+Na)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**1868-17**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**20-okt-2017 / 13:04**	*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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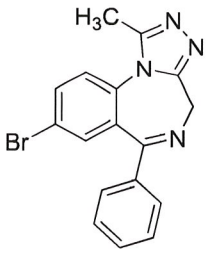
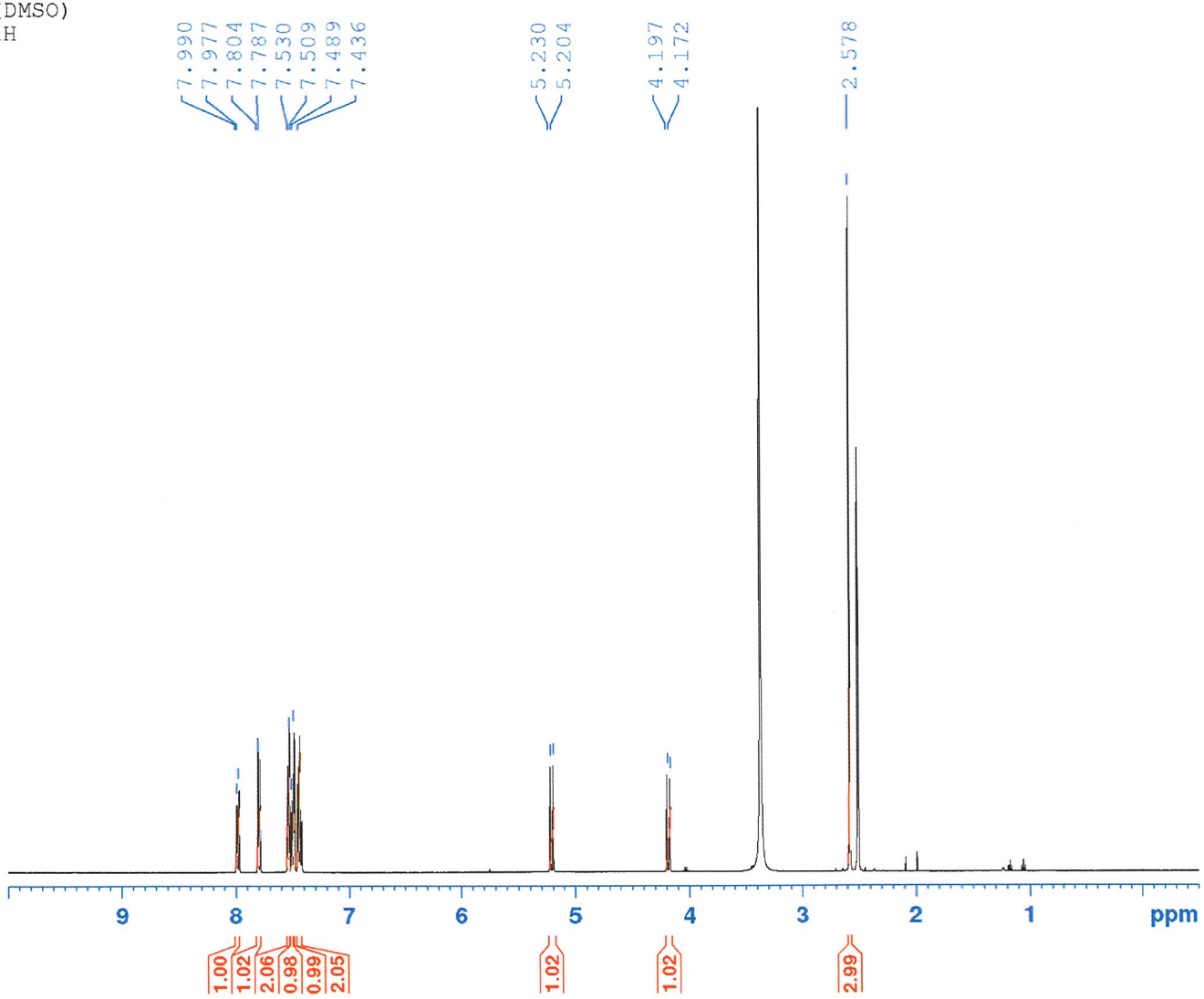
R E P O R T

Contract No.	C1714-17-460078 (Republic of Slovenia, Ministry of the Interior, POLICE)
Sample ID:	**1868-17**
Received date:	January 11, 2018
Our notebook code:	NFL-1868-17
NMR sample preparation:	19.9 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: $C_{17}H_{13}BrN_4$ Exact Mass: 352,0324 Molecular Weight: 353,2230
Chemical name:	8-bromo-1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4] diazepine
Comments:	- Structure elucidation based on 1D and 2D NMR spectra and HRMS. - >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:	January 26, 2018

NFL-1868-17
(DMSO)
1H

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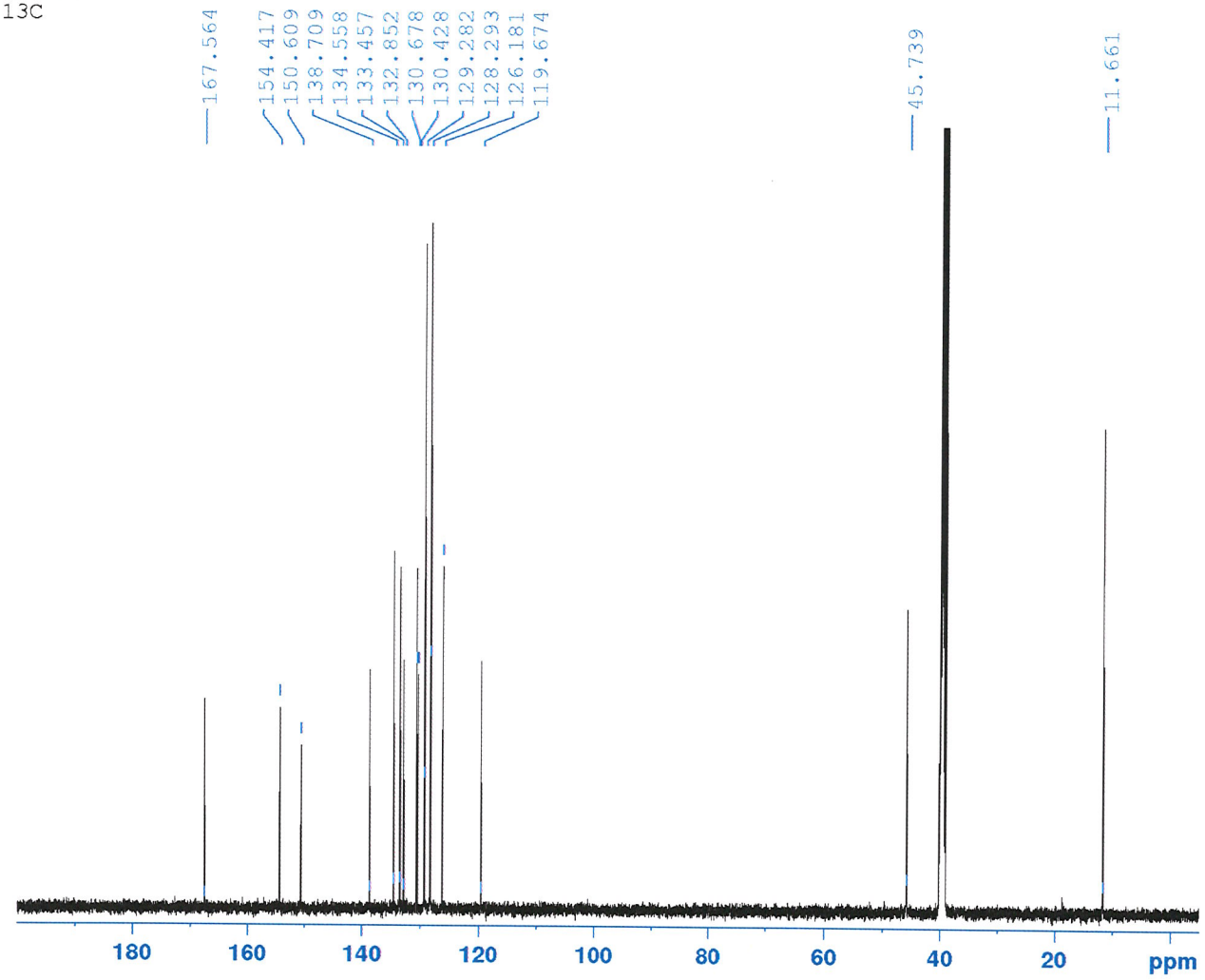
Current Data Parameters
NAME          NFL-1868-17
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20180113
Time               10.57
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                  90.5
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          500.1300015 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

NFL-1868-17
(DMSO)
13C



Current Data Parameters
NAME NFL-1868-17
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180113
Time 12.57
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2048
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 125.7578440 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40