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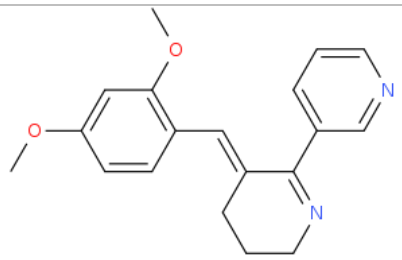
ANALYTICAL REPORT[1]

GTS-21 (C19H20N2O2)

(3E)-3-[(2,4-dimethoxyphenyl)methylidene]-3,4,5,6-tetrahydro-2,3'-bipyridine

Remark – other NPS detected: none

Sample ID:	1539-16
Sample description:	powder - yellow-intensive
Sample type:	test purchase /RESPONSE -purchasing
Date of sample receipt (M/D/Y):	2/16/2016
Date of entry (M/D/Y) into NFL database:	4/7/2016
Report updates (if any) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure[2] (base form)	
Systematic name	(3E)-3-[(2,4-dimethoxyphenyl)methylidene]-3,4,5,6-tetrahydro-2,3'-bipyridine
Other names	3-(2,4-Dimethoxybenzylidene)anabaseine;
Formula (per base form)	C19H20N2O2
M_w (g/mol)	308,38
Salt form/anions detected	hydrochloride
StdInChIKey	RPYWXZCFYPVCNQ-RVDMUPIBSA-N
Compound Class	Piperidines & pyrrolidines
Other NPS detected	none
Add.info (purity..)	pure by GC-MS, HPLC-TOF (small additional peak of the same mass)

[1] This report has been produced with the financial support of the Prevention of and fight against crime Programme of the European Union (grant agreement number JUST/2013/ISEC/DRUGS/AG/6413). The contents of this report are the sole responsibility of the National Forensic Laboratory and can in no way be taken to reflect the views of the European Commission.

[2] 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 6.1 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

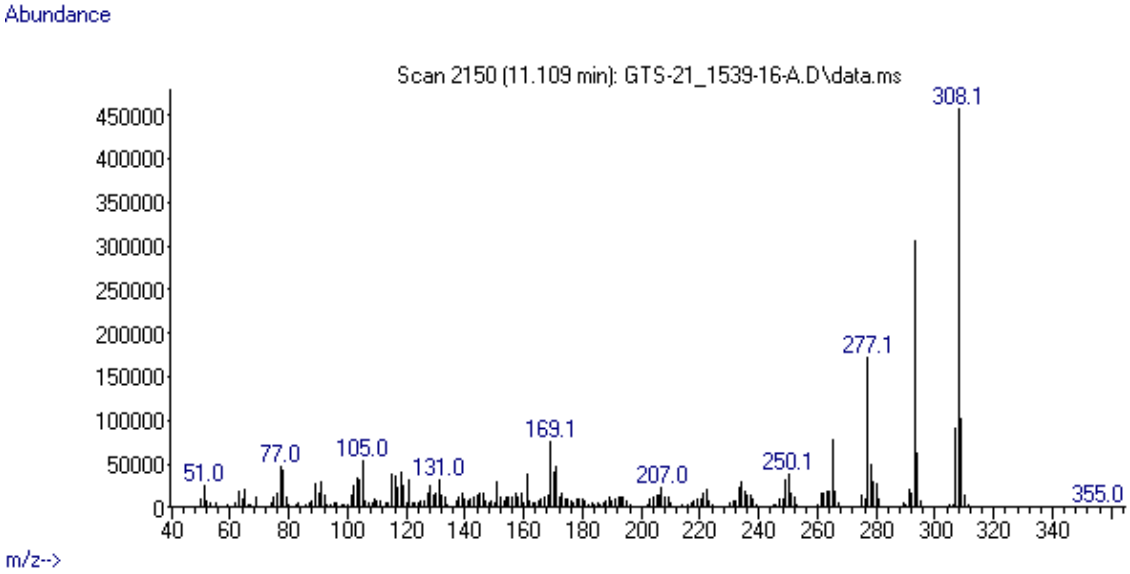
Supporting information

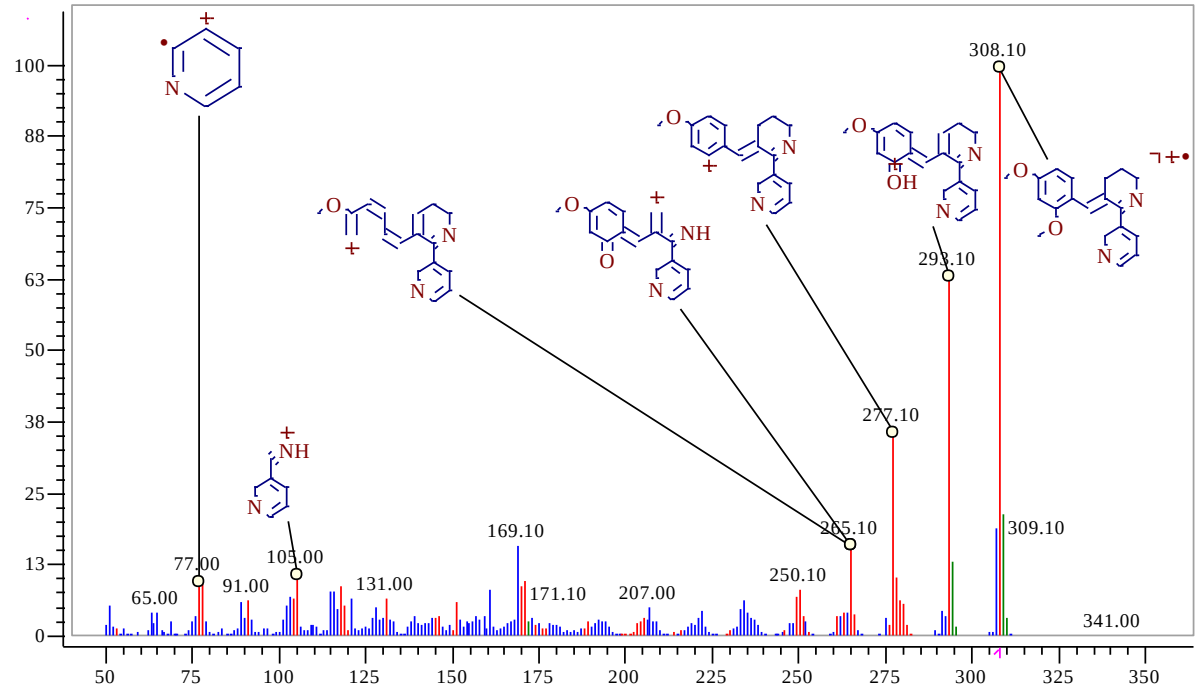
Solubility in	result/remark
CH_2Cl_2	partially
MeOH	soluble
H_2O	not tested

Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 11,03 BP(1): 308; BP(2): 293,BP(3) :277,
HPLC-TOF	+	Exact mass (theoretical): 308,1525; measured value Δppm:-0,57; formula:C19H20N2O2
FTIR-ATR	+	direct measurement (sample as received)
FTIR (condensed phase) always as base form	+	
IC (anions)	+	
NMR (in FKKT)	+	
validation		
other		

ANALYTICAL RESULTS

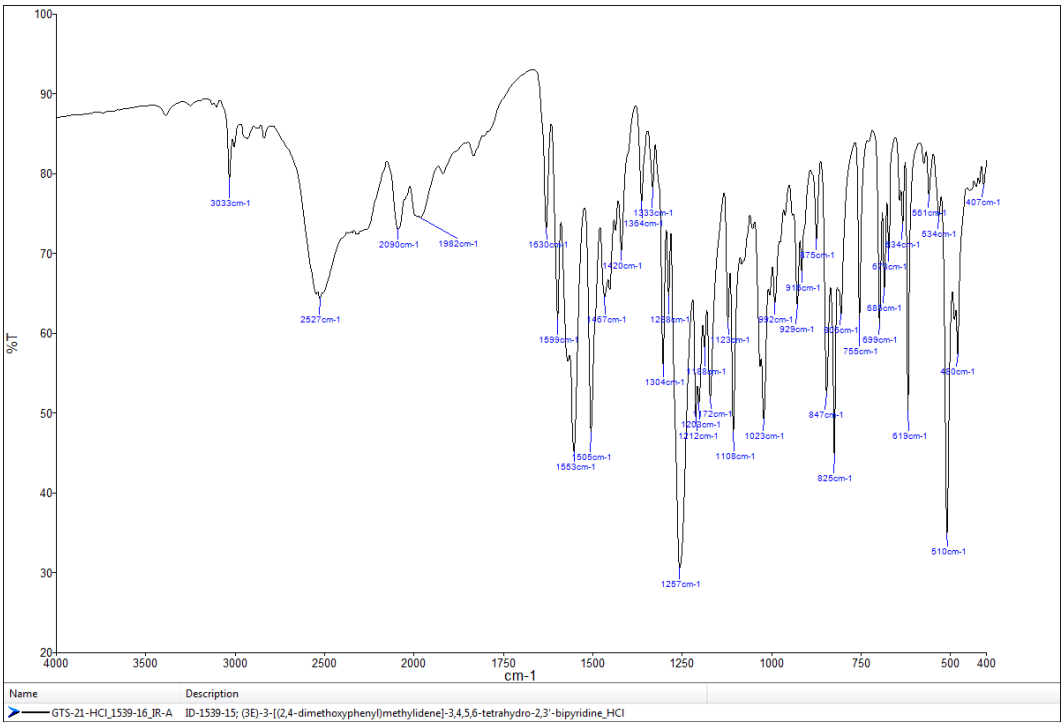
MS (EI) spectrum



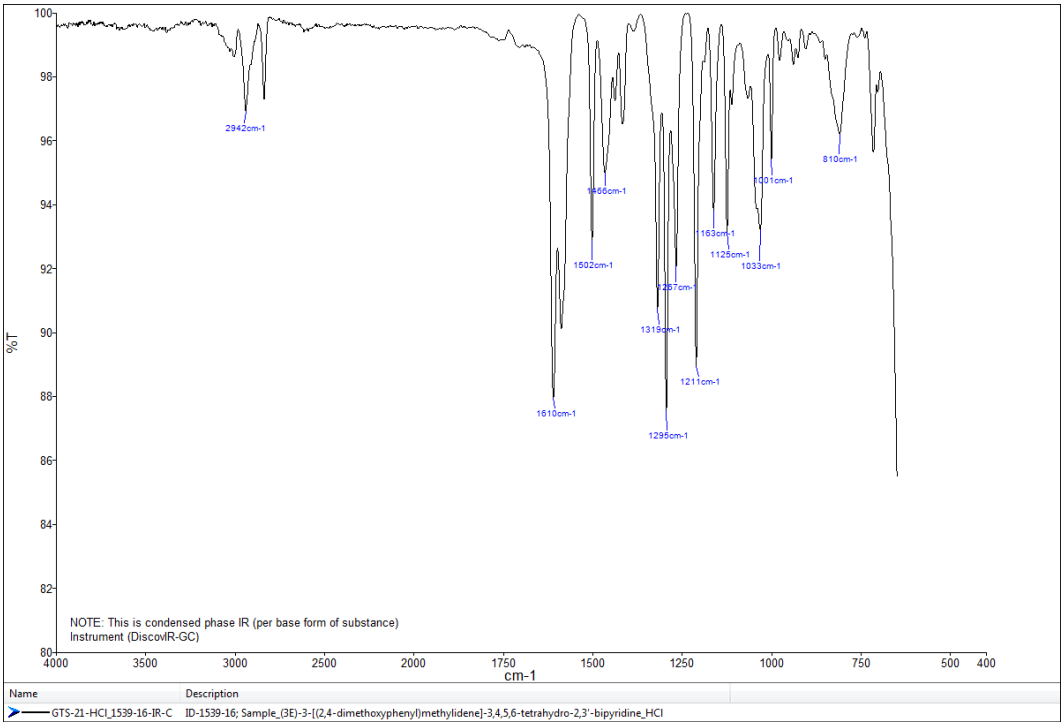


Mass spectrum interpretation by Thermo Scientific Mass Frontier (7.0) software

FTIR-ATR - direct measurement (sample as received)



IR (condensed phase – after chromatographic separation)



TOF REPORT

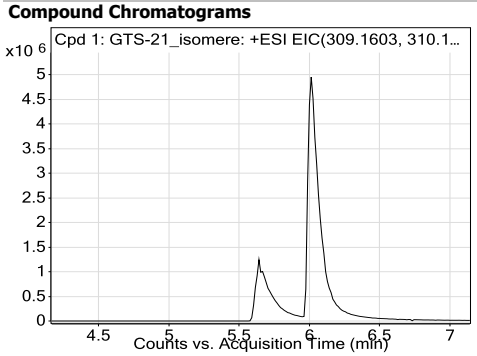
Data File	GTS-21_1539-16_TOF.d	**Sample Name**	ID_1539-16
Sample Type	Sample	**Position**	P1-D7
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-1512015-XDB-C18-ESI-poz-pod.m	**Acquired Time**	2/19/2016 11:28:32 AM
IRM Calibration Status	Success	**DA Method**	Drugs_NFL.m
Comment	extract in MeOH		

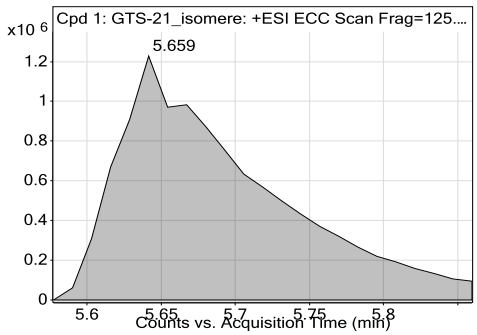
Compound Table

Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 1: GTS-21_isomere	GTS-21_isomere	C19 H20 N2 O2	5.659	308.1533
Cpd 2: GTS-21	GTS-21	C19 H20 N2 O2	6.022	308.1527

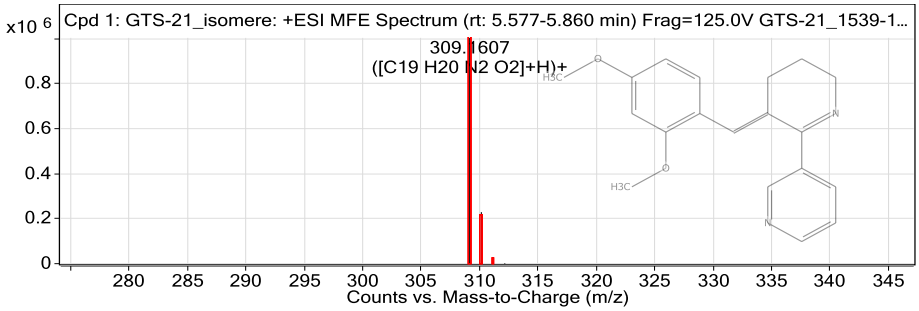
Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
GTS-21_isomere	309.1607	5.659	308.1533	5.66	C19 H20 N2 O2	308.1525	-2.51

Compound Chromatograms

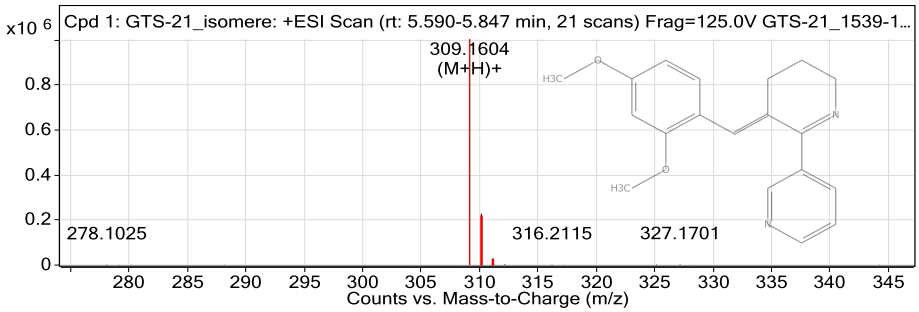




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



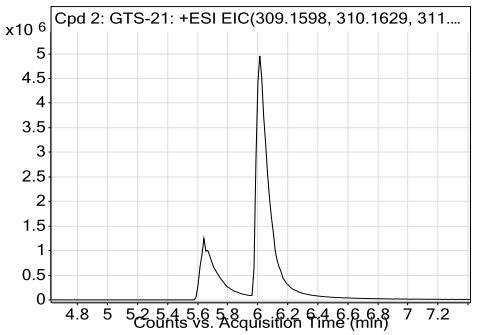
MS Spectrum Peak List

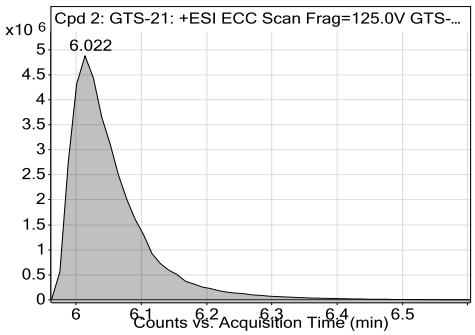
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
309.1607	1	1002911.44	C19 H20 N2 O2	(M+H)+
310.1633	1	228815.69	C19 H20 N2 O2	(M+H)+
311.1657	1	29317.02	C19 H20 N2 O2	(M+H)+
312.1691	1	2985.66	C19 H20 N2 O2	(M+H)+

Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
GTS-21	309.1598	6.022	308.1527	6.02	C19 H20 N2 O2	308.1525	-0.57

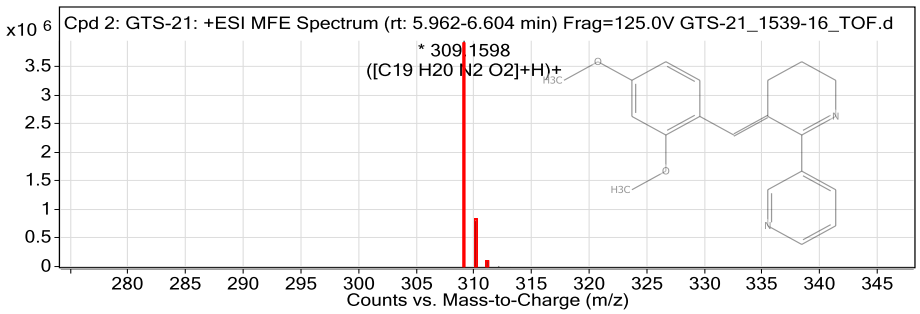
Compound Chromatograms

TOF REPORT

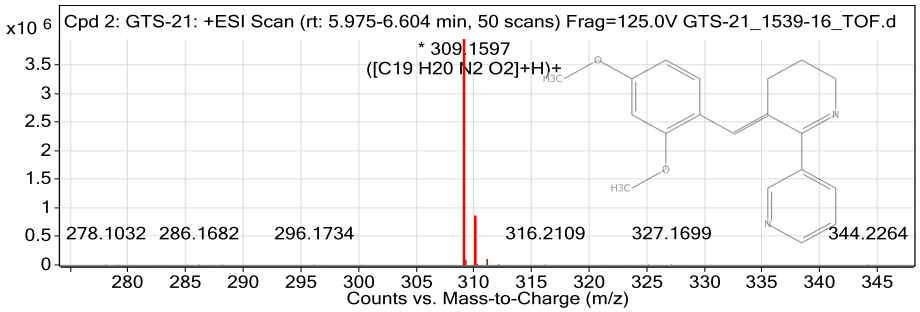




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

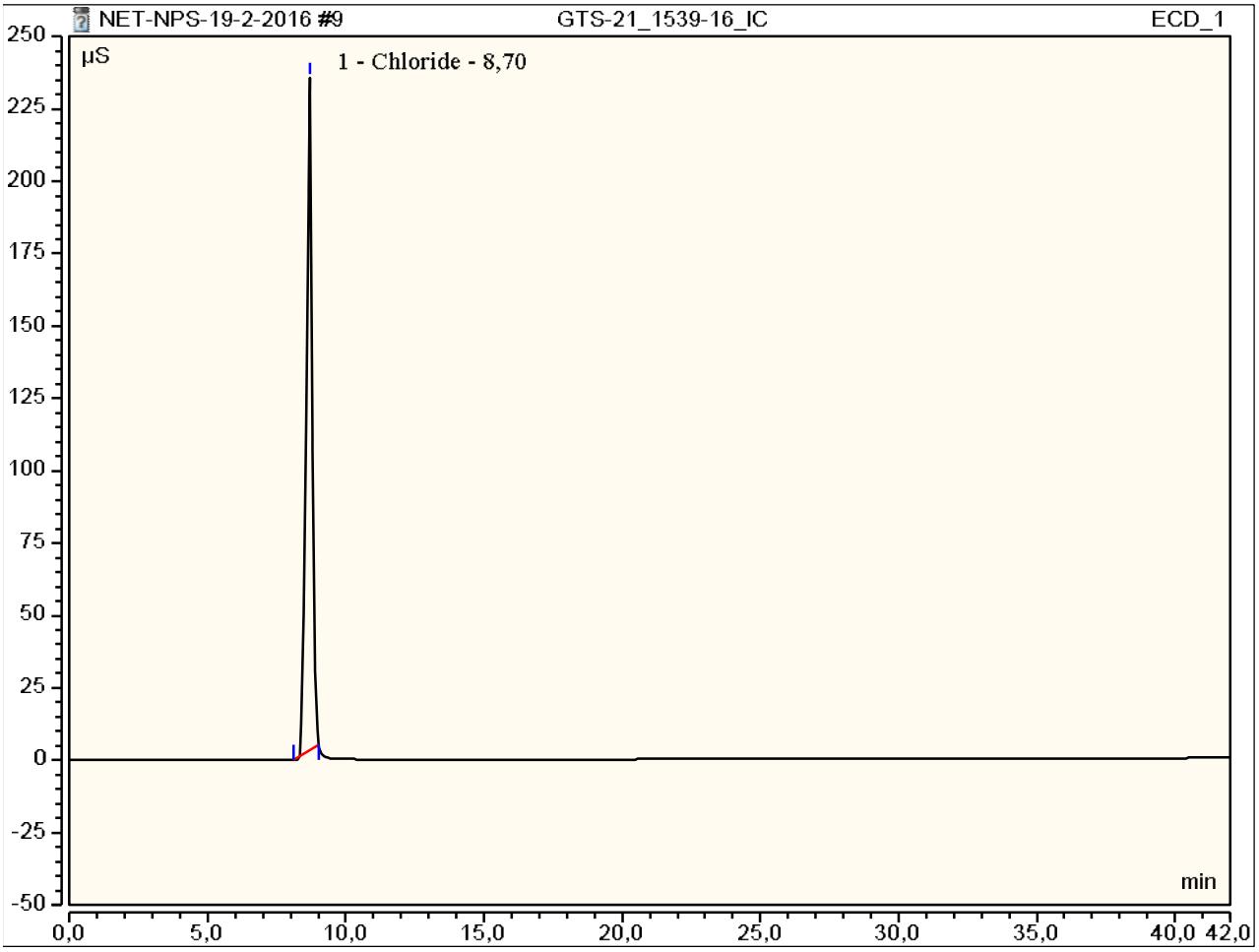
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
309.1598	1	3949073.5	C19 H20 N2 O2	(M+H)+
310.1638	1	794772.73	C19 H20 N2 O2	(M+H)+
311.166	1	102593.96	C19 H20 N2 O2	(M+H)+
312.1684	1	10011.94	C19 H20 N2 O2	(M+H)+
313.1727	1	297.69	C19 H20 N2 O2	(M+H)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**GTS-21_1539-16_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**22-feb-2016 / 16:45**	*Run Time:*	**42,00**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount mg/L
1,00	8,70	**Chloride**	BMB	58,53	232,25	n.a.
		TOTAL:		58,53	232,25	0,00



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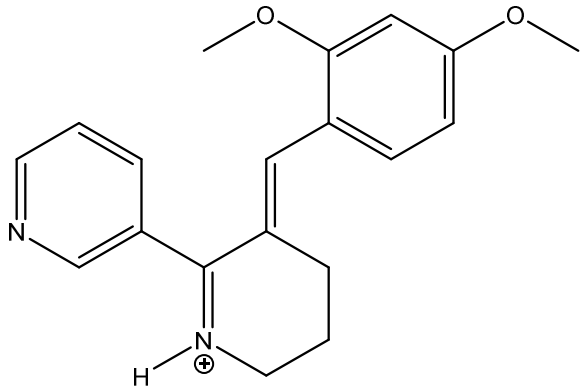
Co-funded by the Prevention of and Fight against Crime Programme of the European Union

REPORT

Sample ID:	**1539-16**
Our notebook code:	P-1539-16
NMR sample preparation:	15 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:	
Chemical name:	(E)-3-(2,4-dimethoxybenzylidene)-3,4,5,6-tetrahydro-[2,3'-bipyridin]-1-ium cation
Comments:	- Structure elucidation based on 1D and 2D NMR spectra - Sample is pure according to NMR.
Supporting information:	Copies of ^{1}H and ^{13}C NMR spectra
Author:	Prof. Dr. Janez Košmrlj, Doc. Dr. Krištof Kranjc
Date of report:	April 4, 2016

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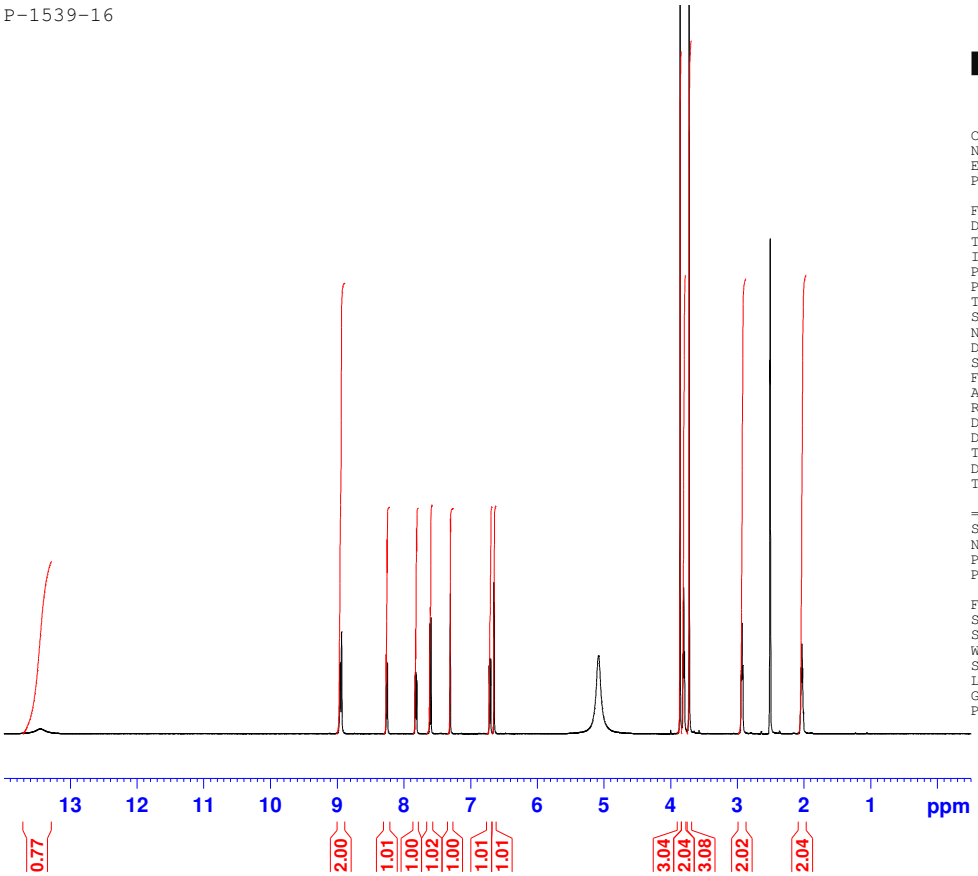
P-1539-16



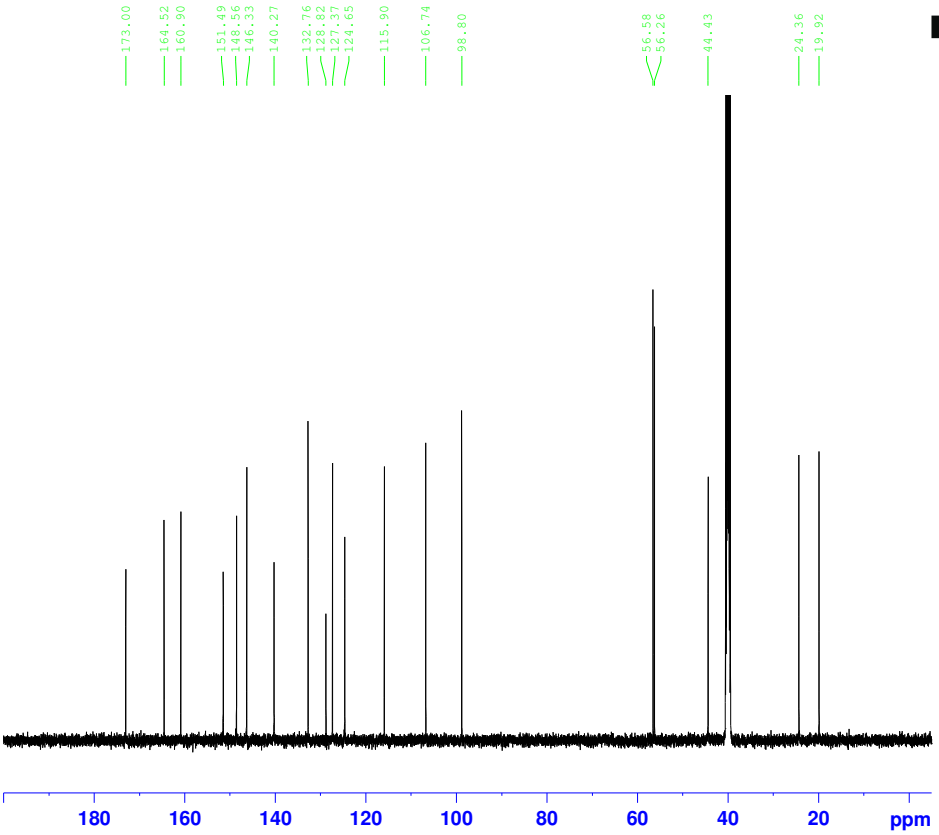
Current Data Parameters
NAME P-1539-16
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160324
Time 2.47
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 3.2768500 sec
RG 90.5
DW 50.000 usec
DE 6.50 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 8.90 usec
PLW1 26.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

P-1539-16



Current Data Parameters
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EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160324
Time 4.45
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 300.0 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 125.7703637 MHz
NUC1 13C
P1 9.00 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 0.32179001 W
PLW13 0.16186000 W

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