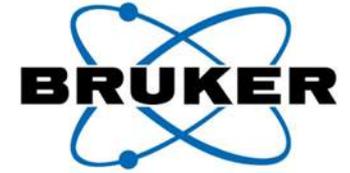


# Bruker Fusion-SV



Wenxin Xu, Ph.D.

Application

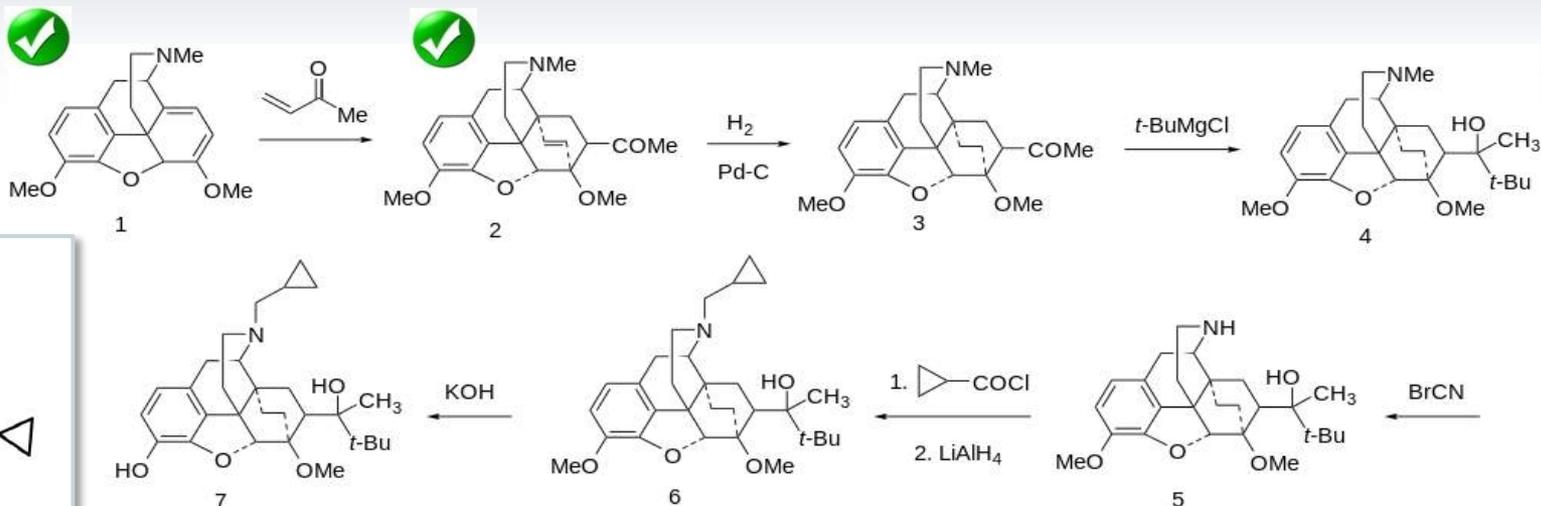
Sep, 2016

Would you like to learn more? Contact a customer service representative.

**Bruker**  
**FUSION-SV**

N  
HRAM-MS  
R

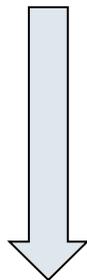
# Chemical Synthesis Control



Anything from:

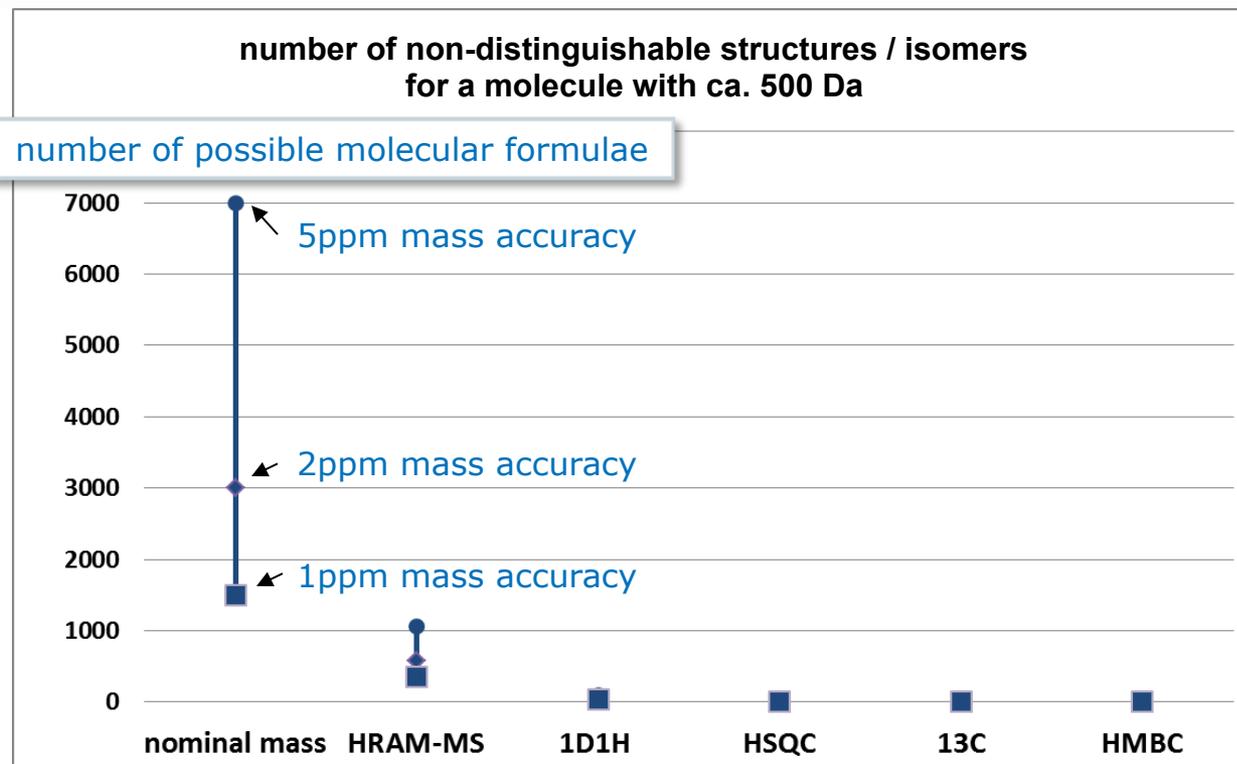
- ⇒ quick and dirty check of intermediates of synthetic process
- ⇒ Verification of final product
- ⇒ Several analytical technique used
- ⇒ depends on chemical question and required certainty

- They want to be sure, **not to file the wrong structure** as a drug candidate
- **Orthogonal data** is needed to minimize the chance for false positives
- MS can do things NMR can not (Cl/Br, S/O ...)
  - Nominal mass → leaves 1'000s of potential false positives
  - **HRAM-MS** → verifies the **molecular formula**
- **NMR** can do things MS can not
  - 100's of possible structures per molecular formula → potential false positives
  - 1D<sup>1</sup>H
  - HSQC
  - HMBC
  - <sup>13</sup>C



**Further reduction of  
potential wrong passes**

MW 467.298



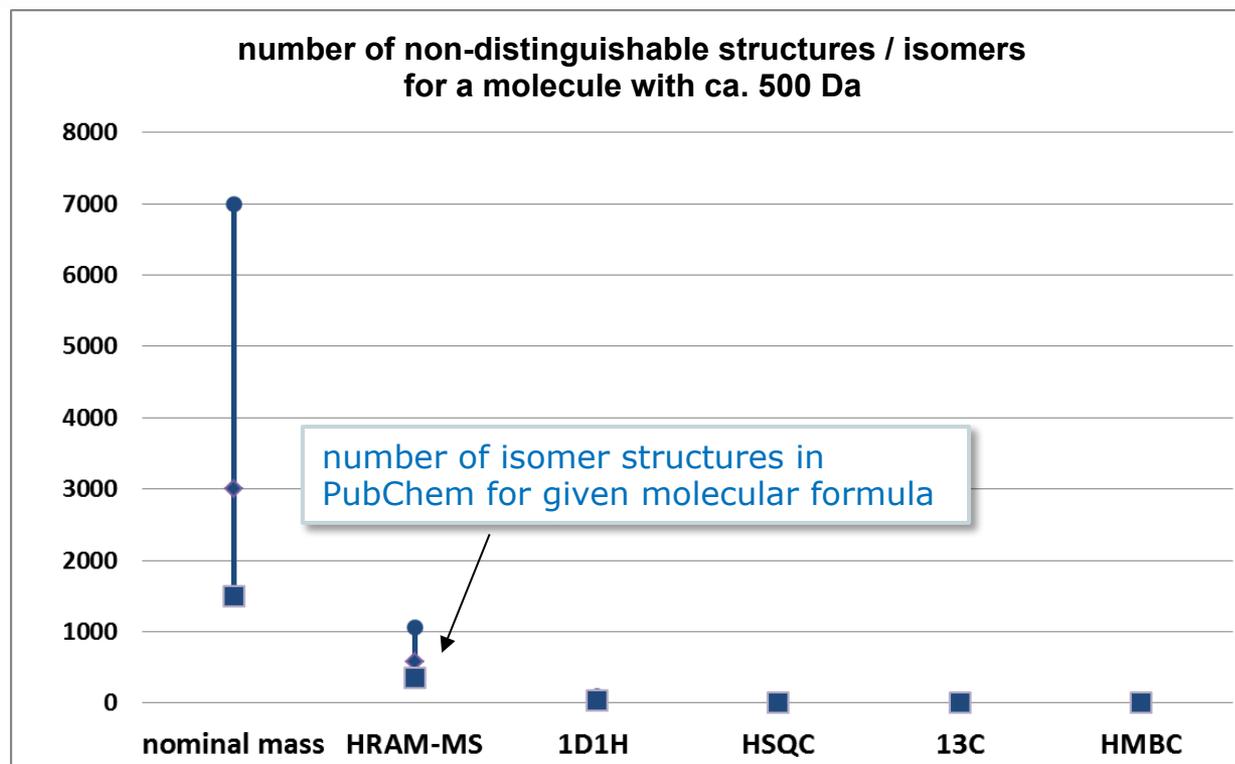
⇒ nominal mass only is not able to rule out 1000s of potentially false positives

# Structure Verification



MW 467.298

$C_{20}H_{22}NO_4$



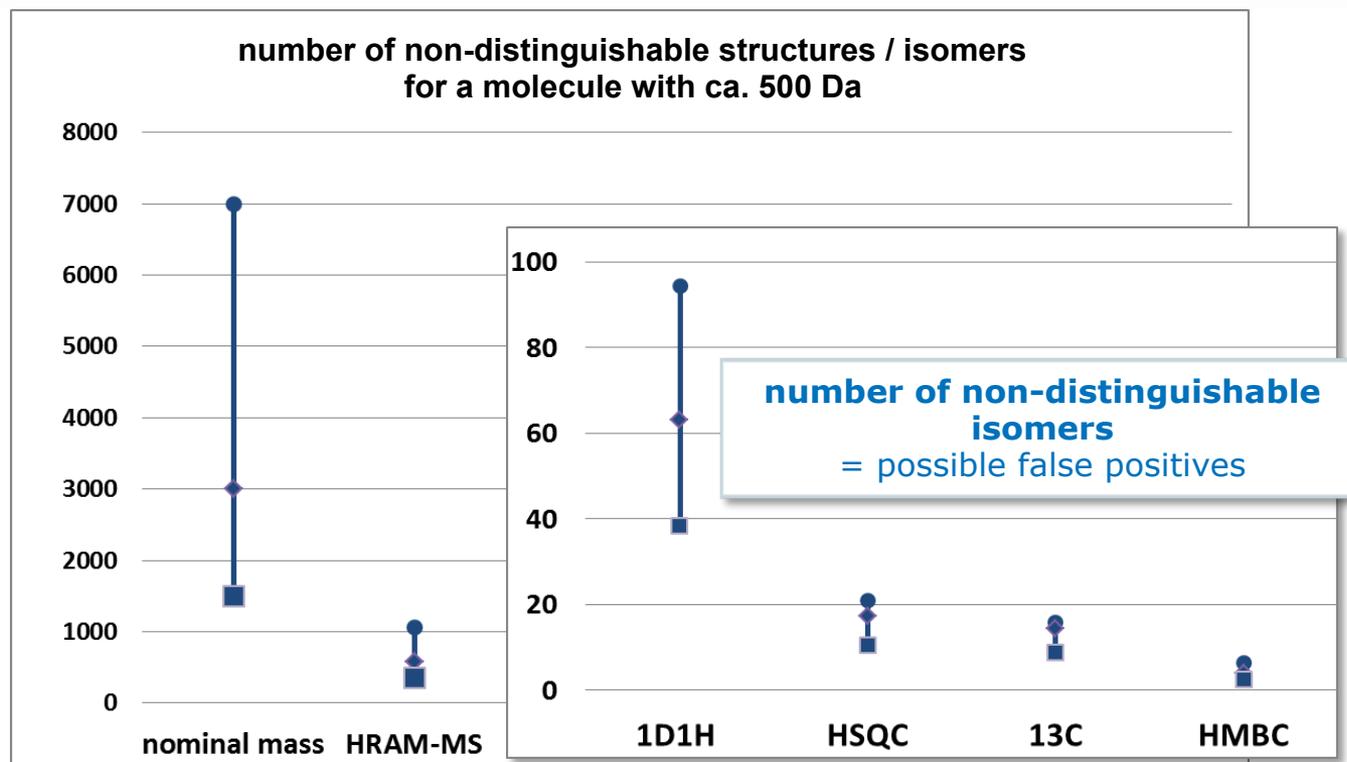
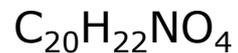
⇒ HRAM-MS verifies the molecular formula

⇒ still several 100 isomers that cannot be ruled out (potential false positives)

# Structure Verification



MW 467.298

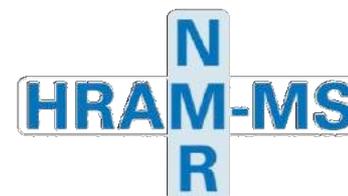


⇒  $^1H$  NMR still leaves many potential isomers that cannot be distinguished

⇒ clever combination of orthogonal information adds confidence



new, fully automatic analysis solution using HRAM-MS and NMR structure verification (SV)



Designed for Chemists

- structure centered
- workflow oriented
- Network embedded

# New: Bruker Fusion-SV 1.1



The screenshot displays the Bruker Fusion-SV 1.1 software interface. The main window shows a project navigator with a tree view of projects and compounds. A table lists data for various compounds, including MS, NMR\_1H, and NMR\_HSQC data. A 'Detailed Information' window is open, showing analysis results for AM-879-4201094. A '1H NMR Spectrum' window displays a spectrum plot. A 'Report' window shows a comment: 'Final compound successfully synthesized. Purity > 97%'. The interface includes a 'Generate Report' button and a 'Details' button.

Structure ID	Sample ID	MS	NMR	Report
Project_13				
Compound 2	Synthesis_2_A	✓	✓	Short 2015-11-02 10:42:13
MS	AN-329-42613559			
NMR_1H	AN-329-42613559			
NMR_HSQC	AN-329-42613559			
Compound 1	Synthesis_1_A	✓	✗	
Project_42				
Compound 4		-	✓	
Compound 3		-	-	
MS	AK-778-15446017			
NMR_1H	AK-778-15446017			
NMR_HSQC	AK-778-15446017			
Project_77				
Compound 99	Synthesis_Step_Final	✓	✓	Long 2015-11-02 10:42:47 Open
MS	AK-918-12349040			
NMR_13C	AK-918-12349040			
NMR_1H	AK-918-12349040			
NMR_HSQC	AK-918-12349040			

Include in Report	Status	✓	Evaluation	
<input checked="" type="checkbox"/> MS	Confirmed	✓	automatic	
<input checked="" type="checkbox"/> NMR_13C	Confirmed	✓	automatic	
<input checked="" type="checkbox"/> NMR_1H	Confirmed	✓	automatic	
<input checked="" type="checkbox"/> NMR_HMBC	Confirmed	✓	automatic	Only available for a
<input checked="" type="checkbox"/> NMR_HSQC	Confirmed	✓	automatic	

1H NMR Spectrum [ppm]

Final compound successfully synthesized.  
Purity > 97%

# Bruker Fusion-SV 1.1: Workbench



Bruker FUSION-SV - Admin - localhost

Projects | Verification Demo 2 Compound 4 | Verification Demo 2 Compound 3 | Project\_77 Compound 4

Project Navigator | Last Imported | Recently Reviewed

+ New | Import | Report | More

Structure ID	Sample ID	MS	NMR	Rep
Project_77				
Compound 4	Drug_2222_10_04_15	✓	✓	Generati
MS	AM-879_42010943_GC8_01_2029.d			
NMR_1H				
NMR_HSQC				
Project_78				
Compound 3				
MS				
NMR_1H				
NMR_HSQC	AK-778-15446017			
Structure Verification Demo 1				
Structure Verification Demo 2				
Verification Demo 1				

Create New Project

Project name: Project\_79

Project description: DrugDevelopment No\_123

Share project with other users

OK Cancel

# Bruker Fusion-SV 1.1: Workbench

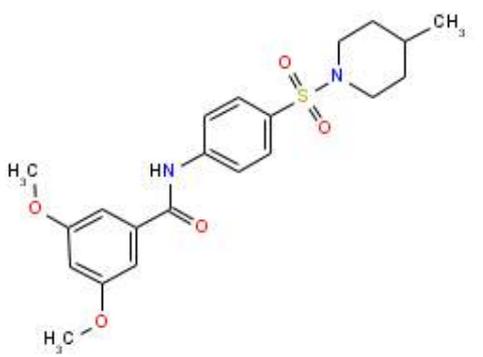


New Verification

Project Name:   Sample ID:

Structure ID:

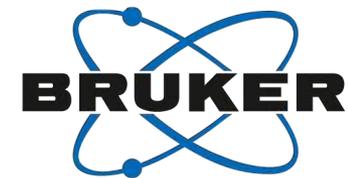
- ▲  AJ-292-4169549 [6]
  - AJ-292-4169549
  - AJ-292-4169549\_001
  - AJ-292-4169549\_002
  - AJ-292-4169549\_003
  - AJ-292-4169549\_004
  - AJ-292-4169549\_005
- ▶  AK-778-1544601 [2]
- ▶  AM-879-4201094 [1]
- ▶  AN-329-4261355 [1]
- ▶  Structure verification demo data [4]



The chemical structure shown is a sulfonamide derivative. It consists of a central benzene ring substituted with a methoxy group (-OCH<sub>3</sub>) at the 3-position and a methyl group (-CH<sub>3</sub>) at the 4-position. This ring is connected via an amide bond (-NH-C(=O)-) to another benzene ring, which is further substituted with a sulfonamide group (-SO<sub>2</sub>-N(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>) at the 1-position.

.mol or .sdf

# Structure Editor



Structure Editor

The image displays a chemical structure editor window. The central canvas shows a complex organic molecule. It features a benzimidazole ring system. One of the nitrogen atoms in the imidazole ring is substituted with a p-chlorophenyl group. The 2-position of the benzimidazole ring is substituted with a 2-(4-methylphenyl)acetate side chain. The interface includes a top toolbar with various editing tools like selection, zoom, and drawing. On the left and right sides, there are vertical toolbars for selecting and drawing different ring systems and atoms. At the bottom, there is a status bar with the text "Select or move atoms", "new structure", and the molecular formula "C24 H20 N O3 Cl" with a molecular weight of "MW: 405.874". Below the status bar is a row of element symbols: C, H, N, O, S, F, P, I, Cl, Br, X, A, R. In the bottom right corner, there is a small icon for "Au" and a grid pattern.

Chemical structure shown: CC1=CC=C(C=C1)C(=O)CC2=C(C3=CC=CC=C3N2)C4=CC=C(C=C4)Cl

Structure Editor

Select or move atoms      new structure      C<sub>24</sub> H<sub>20</sub> N O<sub>3</sub> Cl      MW: 405.874

C H N O S F P I Cl Br X A R

# Import MS & NMR data

Structure ID	Sample ID	MS	NMR	Report
Structure Verification Demo 1				
Structure Verification Demo 2				
project01				
▶ AJ-292-4169549_001	AJ-292-4169549	✓	✓	<a href="#">Long 2016-08-23 16:18:20</a>
project02				
▶ AK-778-1544601	AK-778-1544601	✓	✗	<a href="#">Long 2016-08-23 16:17:06</a>
project03				
▶ AM-879-4201094	AM-879-4201094	✓	✓	<a href="#">Long 2016-08-23 16:37:54</a>
project04				
▶ AN-329-4261355	AN-329-4261355	✓	✓	<a href="#">Long 2016-08-23 16:57:48</a>
project05				
AJ-292-4169549_003	AJ-292-4169549_003			<input type="button" value="Import"/>

# Verify



Structure ID	Sample ID	MS	NMR	Report
Structure Verification Demo 1				
Structure Verification Demo 2				
project01				
project02				
project03				
project04				
project05				
AJ-292-4169549_005	AJ-292-4169549_005			
MS	AJ-292_41695498_RA8_01_2120.d			
NMR_13C	AJ-292-41695498 6 1			
NMR_1H	AJ-292-41695498 1 1			
NMR_HMBC	AJ-292-41695498 5 1			
NMR_HSQC	AJ-292-41695498 3 1			

# Verification review



The screenshot displays the Bruker EUSION SV software interface. The top navigation bar includes 'Projects' and 'Project\_13 Compound 2' (highlighted in red). The main window is divided into several panels:

- MS Calibration Info:** Shows 'Base Calibration' and 'Calibration graph' tabs. The 'Calibration graph' tab displays a table of calibration data:

Reference m/z	Resulting m/z	Intensity	Error [ppm]	Error [mDa]
158.96407	158.96407	38840	0.01	0.00
226.95149	226.95148	173722	-0.06	-0.01
294.93892	294.93896	13358	0.13	0.04
362.92634	362.92628	66512	-0.16	-0.06
430.91377	430.91381	98171	0.10	0.04
498.90119	498.90117	63970	-0.03	-0.01
566.88861	566.88861	64156	-0.01	-0.00
634.87604	634.87604	55119	0.01	0.00
702.86346	702.86346	40384	-0.00	-0.00

The right panel shows a mass spectrum with peaks labeled at m/z 400.914, 412.912, 413.875, 414.917, and 415.981. A blue callout box states: "accurate mass and isotopic pattern information".

The bottom-left panel shows the chemical structure of a brominated compound with the formula  $C_{18}H_{13}Br_2N_2O_3$  and a monoisotopic mass of 467.0684 Da.

The bottom-right panel shows the  $^1H$  NMR spectrum with peaks at 1.1 ppm (3H) and 2.9 ppm (3H). A blue callout box states: "assignment of NMR data".

The 'Manual Spectra Alignment' dialog box is open, showing the following information:

- Currently marked positions in spectra are:
- 1D: 2.200 ppm [Use]
- HSQC: 2.148 ppm [Use]
- Spectra reference frequency (SR) will be changed to align positions to:
- Align to: 2.200 ppm
- Buttons: OK, Cancel

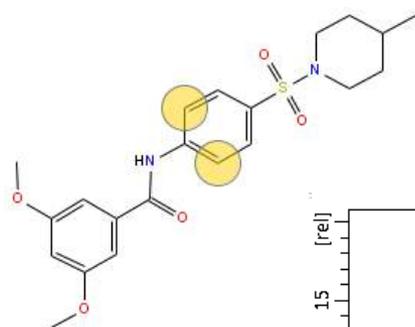
# New: Bruker Fusion-SV 1.1



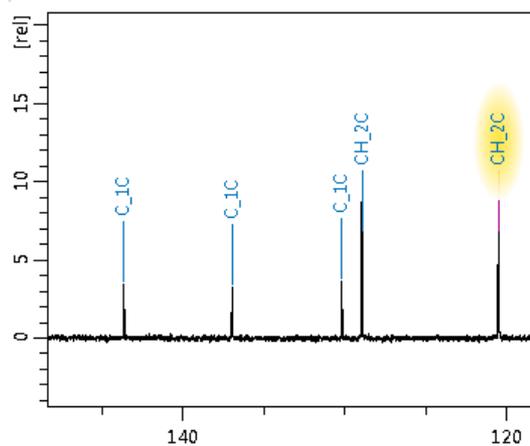
Automatic analysis of combined **1D<sup>1</sup>H**, **HSQC**, **HMBC** and **1D<sup>13</sup>C** data for

⇒ Automatic processing of NUS data (HSQC, HMBC)

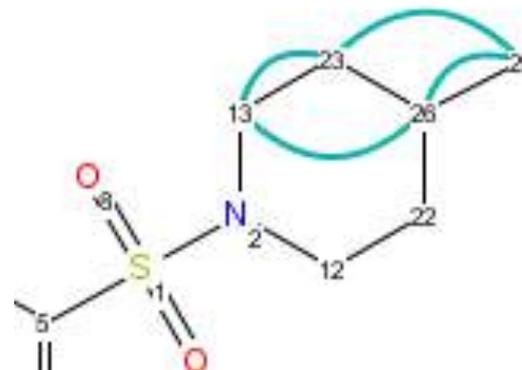
⇒ Spectra interpretation easily understandable



**<sup>13</sup>C assignment**



**HMBC correlations**



# Generate Report



101: Conf

Posi
176.3
165.3
135.1
132.1
130.1
128.1
126.3
125.1
53.4
50.6
47.7
41.1
39.9
37.7
18.2

19

20

Br

Bru

Bru

Bru

Printed: November 2, 2015 10:42:42 AM GMT+01:00

15

### Assignments as Patent String

Journal Type: **Journal of the American Chemical Society (JACS)**

Detail Level: **High**  Ascending  Descending

<sup>1</sup>H NMR (400 MHz, DMSO): d, ppm 12.72 (1H, s), 11.58 (1H, s), 7.90 (2H, d, J = 8.7 Hz), 7.80 - 7.71 (3H, m), 7.27 (2H, dd, J = 7.2, 7.2 Hz), 7.22 - 7.14 (3H, m), 3.02 - 2.95 (2H, m), 2.88 - 2.76 (1H, m), 2.69 (2H, t, J = 7.4 Hz), 1.11 (6H, d, J = 6.7 Hz).

<sup>13</sup>C NMR (101 MHz, DMSO): d, ppm 179.8, 179.8, 141.7, 139.1, 137.9, 129.1, 128.8, 127.6, 126.7, 124.7, 44.6, 40.0, 35.7, 34.9, 19.3.

Copy OK

# MS & NMR Analysis Settings



**MS Analysis Settings**

Method: instrument01 [New] [Delete]  
instrument01

Calibration

Mass lists: Na Acetate (pos), Na Formate (pos) [Edit...]  
Mass tolerance [Da]: 0.01

Chromatogram

BPC num peaks: 5  
EIC num peaks: 3

Chromatogram Peak Finder

Sensitivity [%]: 95.0  
Intensity threshold mode: Absolute  
Intensity threshold: 1000.0  
Area threshold mode: Off  
Area threshold: 10.0

Verification

Adducts: [M+H]<sup>+</sup>, [M+Na]<sup>+</sup>, [M+K]<sup>+</sup>, [M+NH4]<sup>+</sup>  
Mass tolerance [Da]: 0.005  
Isotopic fidelity: Medium  
Saturation intensity: 1.00000e+08  
Low abundance warning[%]: 0.0

[Apply] [OK] [Cancel]

**NMR Analysis Settings**

project01 [New] [Delete]  
project01

Expert Settings

Quantification reference directory [Browse]

Known impurities [Edit]

Suppression frequency: unsuppressed [Edit]

Eretic signal:  @ -1.0 ppm

13C decoupling

[Apply] [OK] [Cancel]

# NMR Analysis Settings



NMR Analysis Settings - Define Impurities

Known Impurities

TMS     TSP     H2O     MeOH  
 DMSO\_D5     DMSO\_H6     Dimethylsulfon     EtOH  
 iPrOH     C6H6     Acetone     CH3CN  
 CHD2CN     Ethylacetate     DMF     NH4  
 CYCLOHEXANE     ACETIC\_ACID

Custom Impurities

Active	Identifier	Shift [ppm]	Multiplicity	Coupling	Protons	Carbons	
							<b>New</b>
							Edit
							Delete
							Cancel

NMR Analysis Settings - Edit Impurity

Identifier default

Protons

Enable

Shift [ppm] from  to

Multiplicity DOUPLET

Coupling [Hz]

Number of Protons ?

Carbons

Enable

Shift[ppm] from  to

Number of Carbons 1

OK Cancel

# NMR Analysis Settings



NMR Analysis Settings

project01 New Delete

project01

Expert Settings

Quantification reference directory  Browse

Known impurities  Edit

Suppression frequency  Edit

Eretic signal  @  ppm

13C decoupling

Apply

OK Cancel

NMR Analysis Settings - Edit Suppression Frequencies

Suppression Frequencies Location

Unsuppressed

Suppression Frequency File

NMR Parameter O1P

Custom

Edit Suppression Frequencies

Filename for suppression frequencies

Number of suppression frequencies

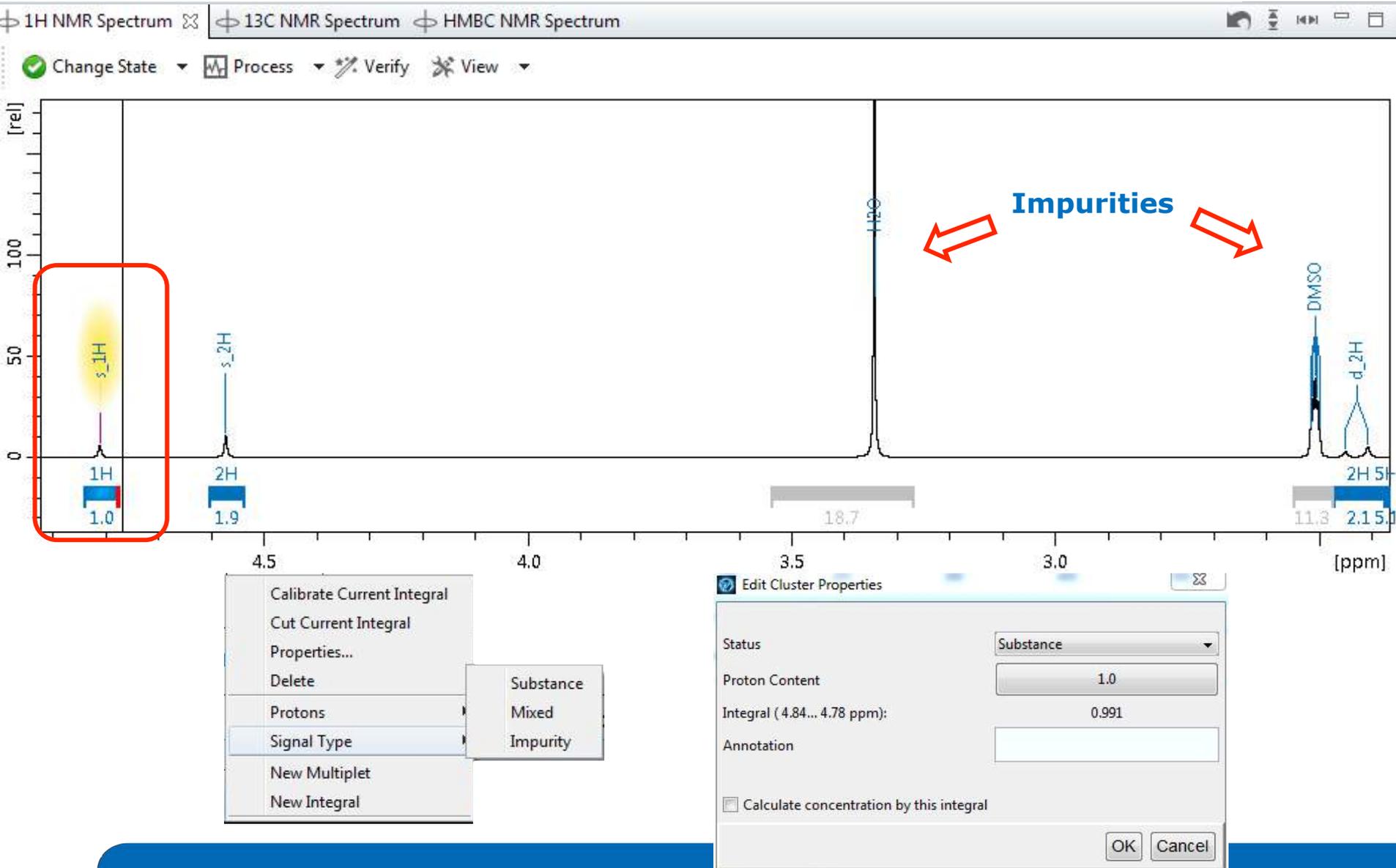
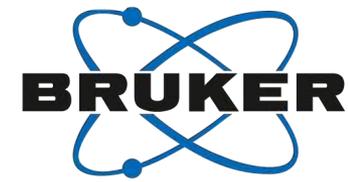
Suppression frequency [ppm]

Remove region around solvent [Hz]

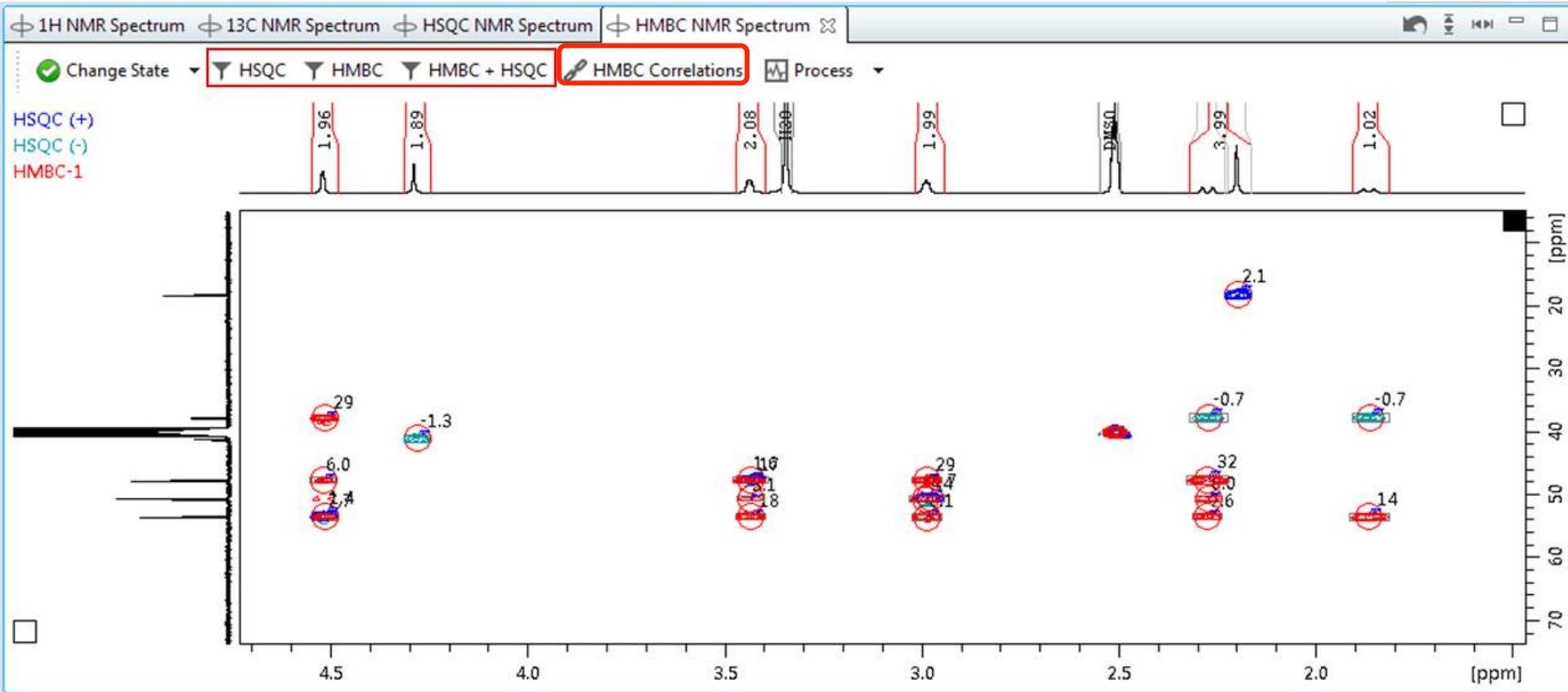
Outer region around solvent [Hz]

OK Cancel

# Modifying results from an automated analysis



# HMBC spectrum



# HMBC Correlation

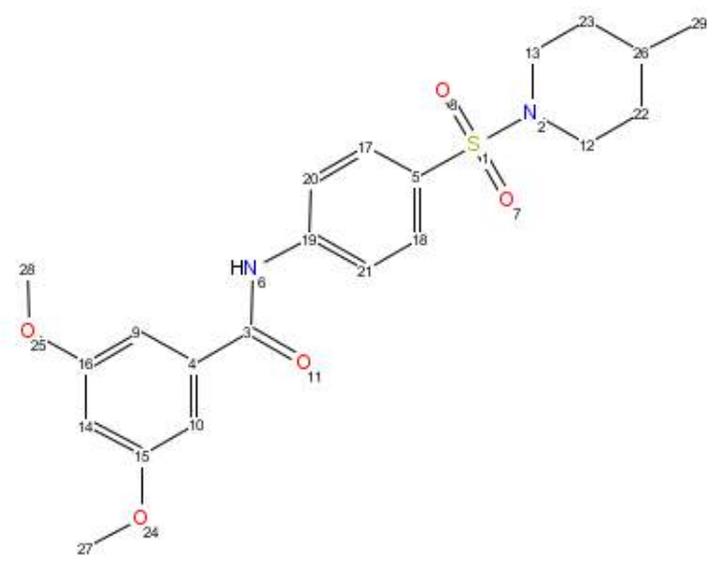
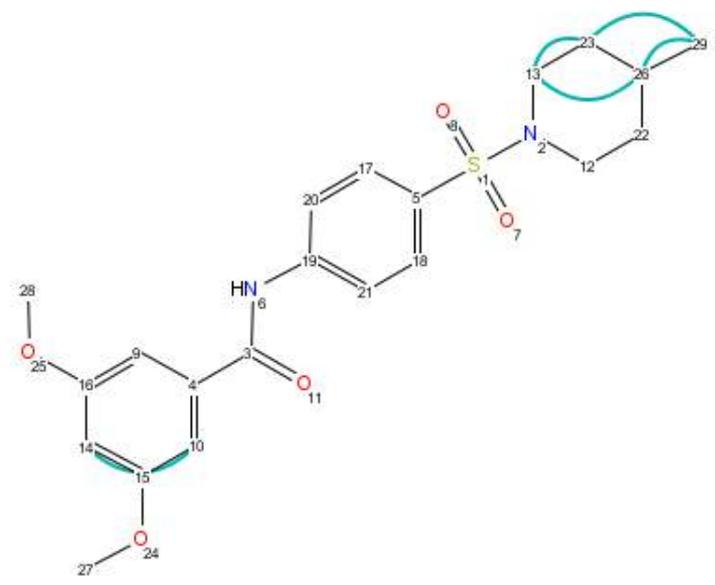


HMBC Correlations



Confirmed correlations:

Violated correlations:



OK



# Substructure search



Bruker FUSION-SV - Admin hbqsswtest-0

Projects Structure...on Demo 2 Compound 4

Analyte Group Navigator View Analyte Editor View Analyte SubStructure Search

Clear filter

PO4500270762 [79]  
Structure verification demo data [4]  
Compound 1  
Compound 2  
Compound 3  
Compound 4

Compound:  Modified Structure  
Formula:  C7H7NO  
Monoisotopic Mass:  121.05276  
Process:  Start

**Results Overview Found 14 Hits**

Nr.	Name	Formula	Mass
1	Compound 3	C24H20ClNO3	405.1130
2	Compound 1	C21H26N2O5S	418.1560
3	PO4500270762_006	C22H30N2O3	370.2256
4	PO4500270762_029	C16H14N2O2	266.1055
5	PO4500270762_004	C25H29NO4	407.2097
6	PO4500270762_021	C21H19NO2	317.1416
7	PO4500270762_065	C14H11N3O3	269.0800
8	PO4500270762_078	C13H11NO4	245.0688
9	PO4500270762_074	C15H16N2O3	272.1161
10	Compound 4	C32H35ClN2O4	546.2290
11	PO4500270762_036	C23H26N2O4	394.1893
12	PO4500270762_014	C26H18N2O3	406.1317
13	PO4500270762_057	C15H14N2O2S	286.0776
14	PO4500270762_069	C15H15NO4	273.1001

Edit Structure Import Structure Clear All

# Substructure search



Brucker Fusion-SV - Admin - hbqsswtest-0

Projects Structure...on Demo 2 Compound 4

Analyte Group Navigator View Analyte Editor View Analyte SubStructure Search

Clear filter

- PO4500270762 [79]
- Structure verification demo data [4]
  - Compound 1
  - Compound 2
  - Compound 3
  - Compound 4

Compound:  Modified Structure

Formula:  C7H7NO

Monoisotopic Mass:  121.05276

Process:  Start

**Results Overview Found 14 Hits**

Nr.	Name	Formula	Mass
1	Compound 3	C24H20ClNO3	405.1130
2	Compound 1	C21H26N2O5S	418.1560
3	PO4500270762_006	C22H30N2O3	370.2256
4	PO4500270762_029	C16H14N2O2	266.1055
5	PO4500270762_004	C25H29NO4	407.2097
6	PO4500270762_021	C21H19NO2	317.1416
7	PO4500270762_065	C14H11N3O3	269.0800
8	PO4500270762_078	C13H11NO4	245.0688
9	PO4500270762_074	C15H16N2O3	272.1161
10	Compound 4	C32H35ClN2O4	546.2290
11	PO4500270762_036	C23H26N2O4	394.1893
12	PO4500270762_014	C26H18N2O3	406.1317
13	PO4500270762_057	C15H14N2O2S	286.0776
14	PO4500270762_069	C15H15NO4	273.1001

Edit Structure Import Structure Clear All

Mapping sub structure is high lighted

# Substructure search



Analyte Editor View | Analyte SubStructure Search

Compound:  Modified Structure

Formula:  C6H6

Monoisotopic Mass:  78.04695

Process:

**Results Overview** Found 176 Hits

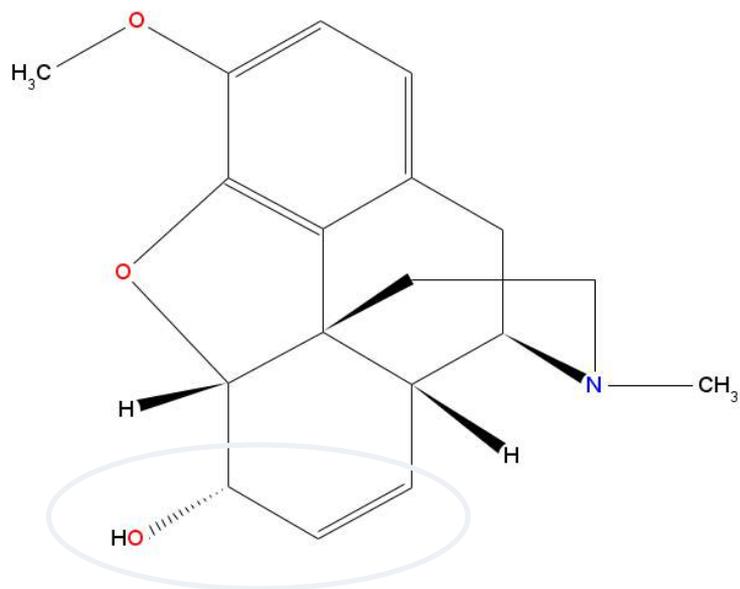
Name	Formula	Mass
New Structure 2 jhgjhg	C17H15N...	281.1052
New Structure	C17H14Cl...	315.0662
Compound 4	C32H35Cl...	546.2285
Compound 3	C24H20Cl...	405.1132
Compound 2	C19H23N...	405.1181
Compound 1	C21H26N...	418.1562
AO-476-40672079_001	C26H24Cl...	477.1278
AO-476-40672079_001	C26H24Cl...	477.1278
AO-299-15047109_001	C29H31N...	513.2198
AO-299-15047109	C29H31N...	513.2198
AO-081-41887499_001	C26H26Br...	527.0943
AO-081-41887499	C26H26Br...	527.0943
AO-081-40770617_001	C21H19Cl...	415.0854
AO-081-40770617	C21H19Cl...	415.0854
AN-919-15231075_001	C25H26Cl...	435.1714
AN-919-15231075	C25H26Cl...	435.1714
AN-919-15231074_001	C25H26Cl...	435.1714
AN-919-15231074	C25H26Cl...	435.1714
AN-919-15231054_001	C26H24Cl...	477.1278
AN-919-15231054	C26H24Cl...	477.1278
AN-329-42613590_001	C19H21N...	403.1024

Edit Structure | Import Structure | Clear All

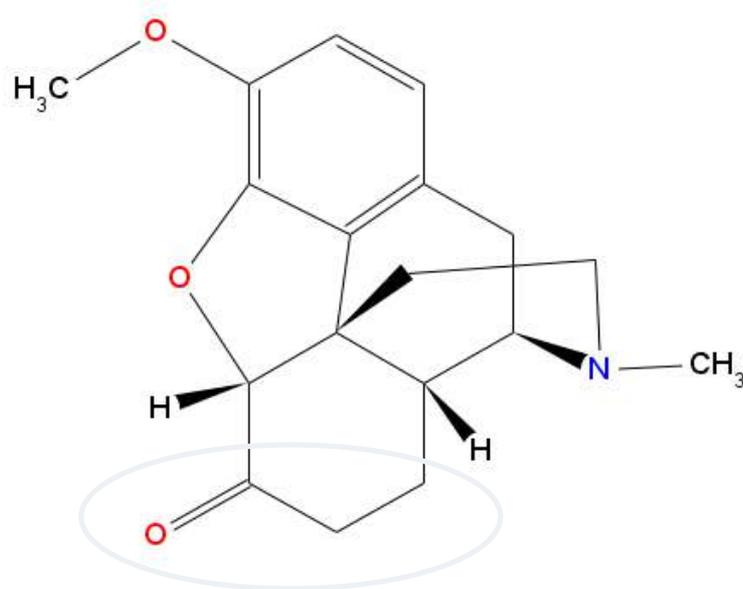
The chemical structure shows a complex heterocyclic molecule. It features a central benzene ring highlighted in yellow, which is part of a larger fused ring system. The structure includes a thiophene ring, a pyridine ring, and a piperidine ring. There are several nitrogen atoms (NH) and a sulfur atom (S) in the structure. A chlorine atom (Cl) is attached to a phenyl ring, and a methyl group (CH3) is attached to the piperidine ring. The overall structure is a complex polycyclic system.

## Example

# Codeine and Hydrocodone



Codeine  
 $C_{18}H_{21}NO_3$   
 299.365



Hydrocodone  
 $C_{18}H_{21}NO_3$   
 299.365

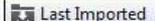
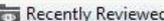
# Example

## Codeine and Hydrocodone

Bruker FUSION-SV

Projects

Codeine codeine  Codeine codeine 

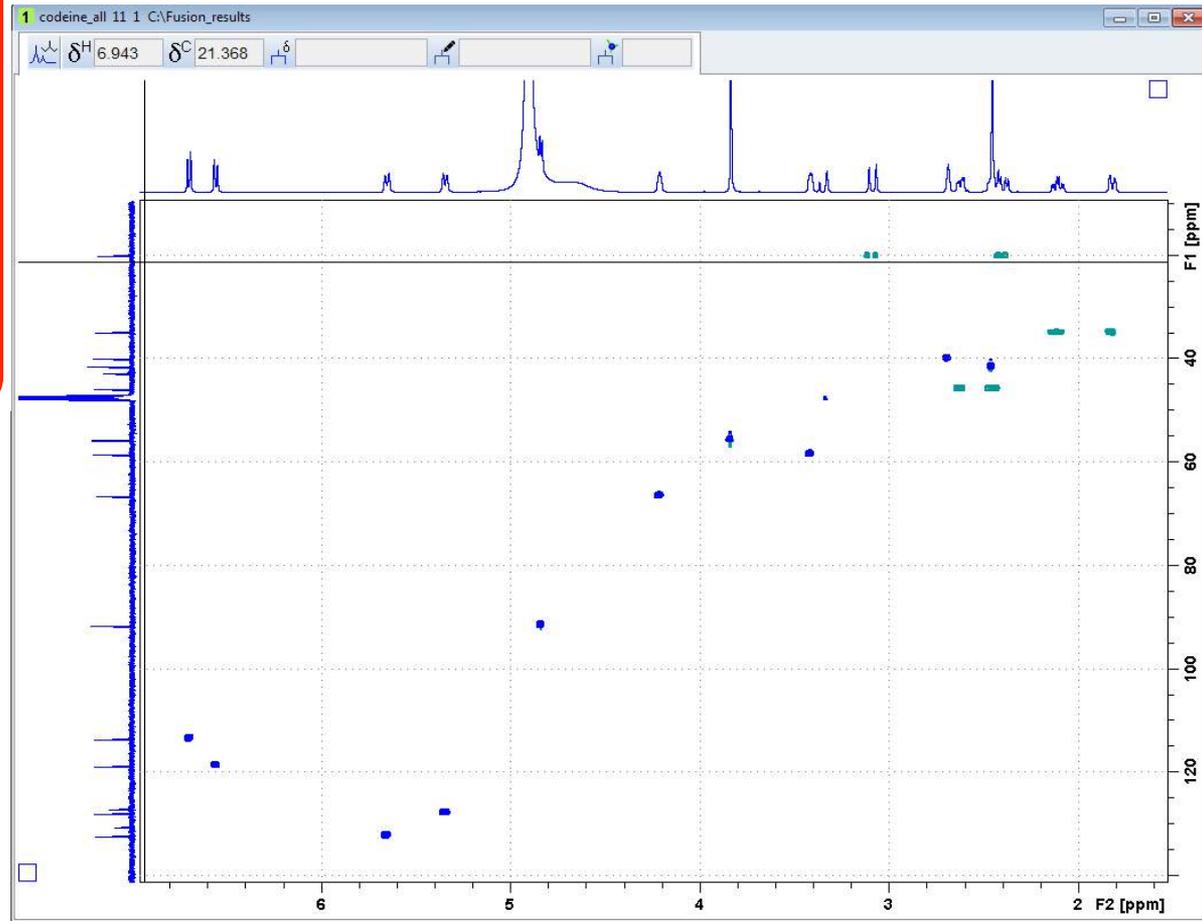
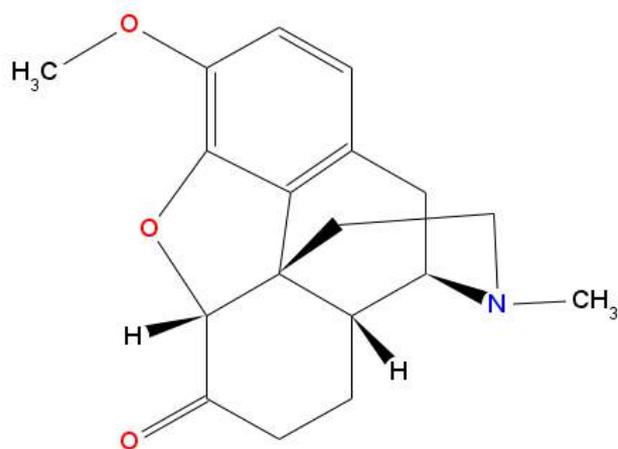
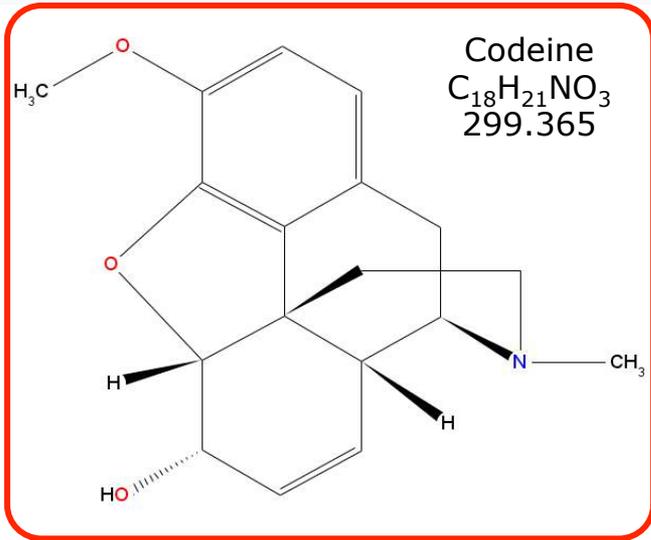
Project Navigator  

+ New Import Report More

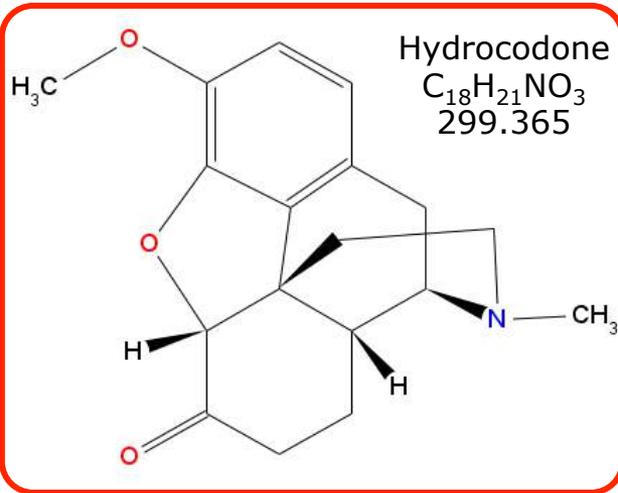
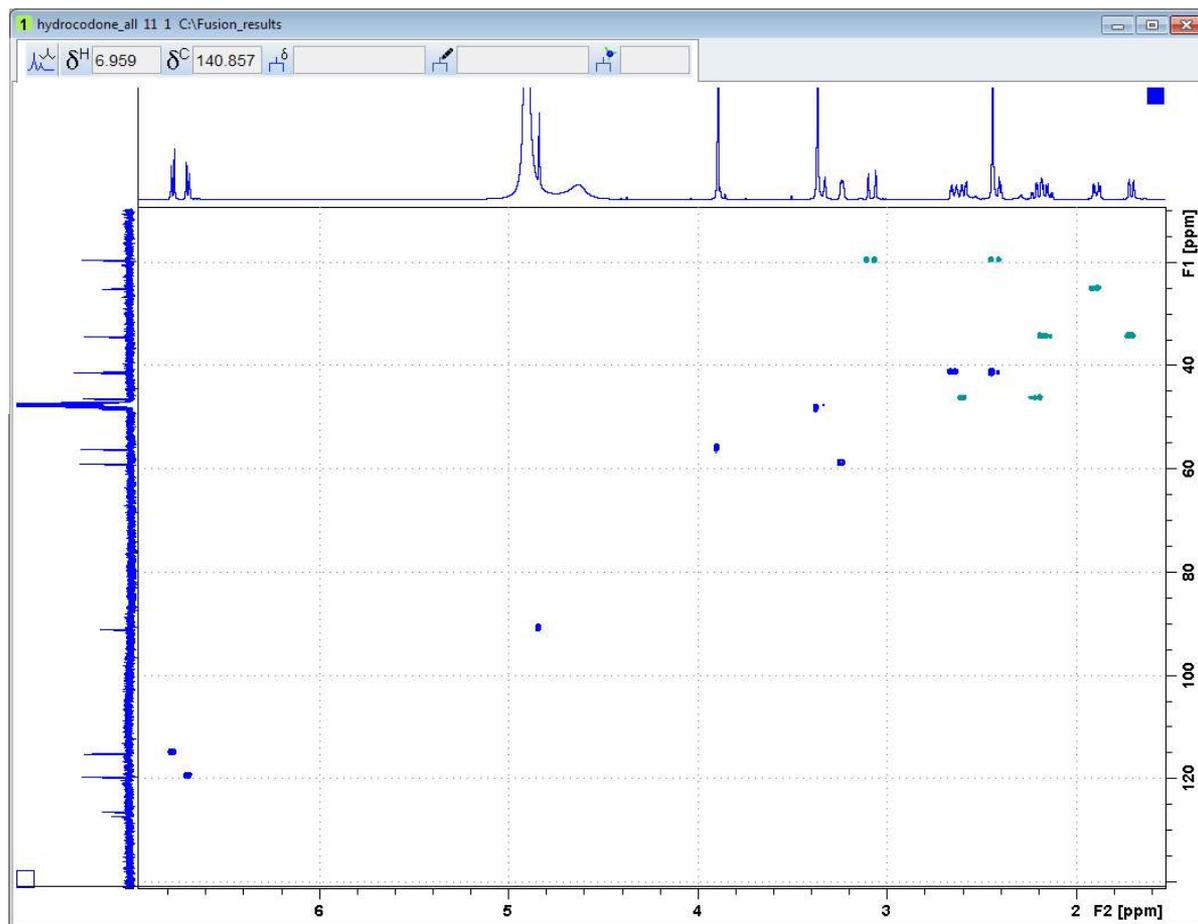
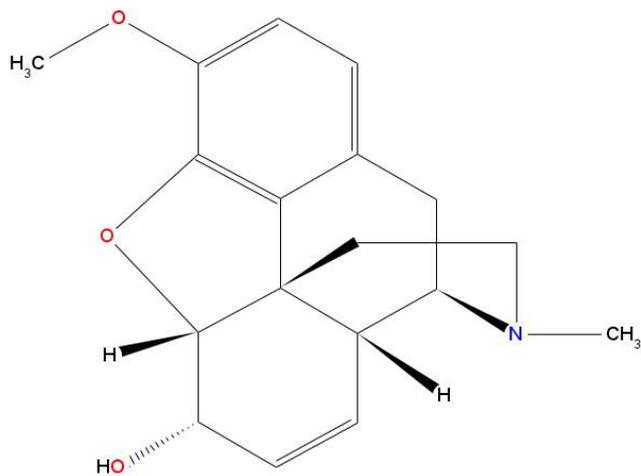
Structure ID	Sample ID	MS	NMR	Report
codeine	codeine			
MS	Codeine_1ppm_GE4_01_675.d			
NMR_13C	codeine_1H_13C 13 1			
NMR_1H	codeine_1H_13C 10 1			
<b>Codeine_TN</b>				
hydrocodone	hydrocodone			
hydrocodone	hydrocodone			
MS	Codeine_1ppm_GE4_01_675.d			
NMR_13C	codeine_1H_13C 13 1			
NMR_1H	codeine_1H_13C 10 1			

- with the wrong structure FUSION reports NOT OK from NMR

...But from the data we can tell!

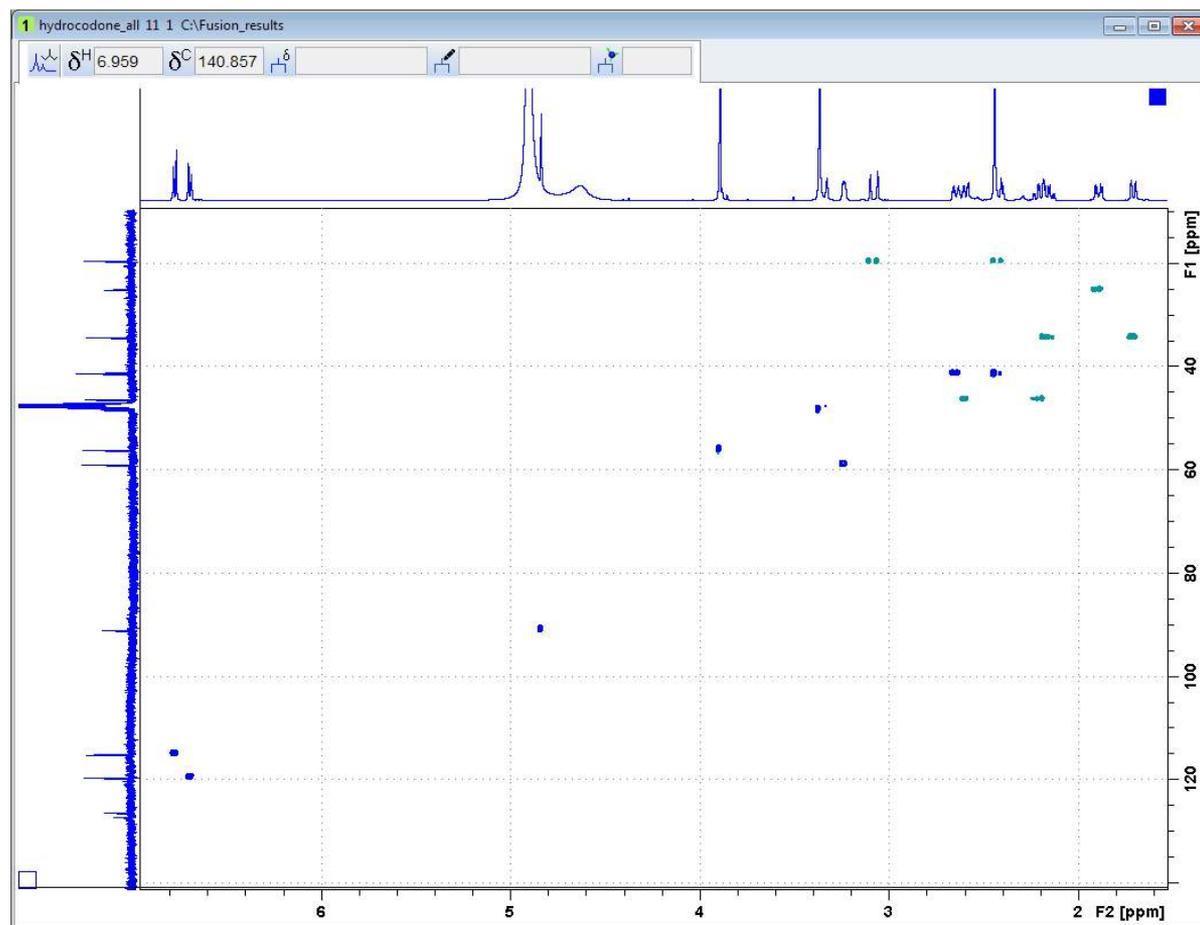
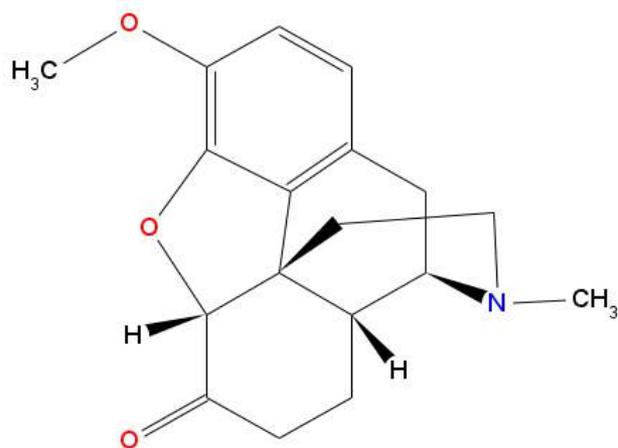


...But from the data we can tell!



# ...But from the data we can tell!

- HSQC of hydrocodone tells us something has happened, and this isn't hydrocodone anymore!



# Let's Demo Fusion!

- CMC-assist and FUSION are a black box yes or no
- Be prepared when going through the data to see some incorrect assignments

## Bruker Fusion-SV License Request

Last Name*	<input type="text"/>	Comments, questions, send license to (if different from above), etc.:	<input type="text"/>
First Name*	<input type="text"/>	Type of license request*	
Title	<input type="text"/>	<input checked="" type="radio"/> New License	
Company/Institute*	<input type="text"/>	<input type="radio"/> Demo License	
Street	<input type="text"/>	Please don't forget the order number if you want to upgrade or transfer a license	
City*	<input type="text"/>	Order number	<input type="text"/>
State (USA only)	<input type="text"/>	Add. order numbers	<input type="text"/>
ZIP/Postal Code	<input type="text"/>		
Country	China <input type="button" value="v"/>		
Email*	<input type="text"/>		<input type="button" value="Submit"/>

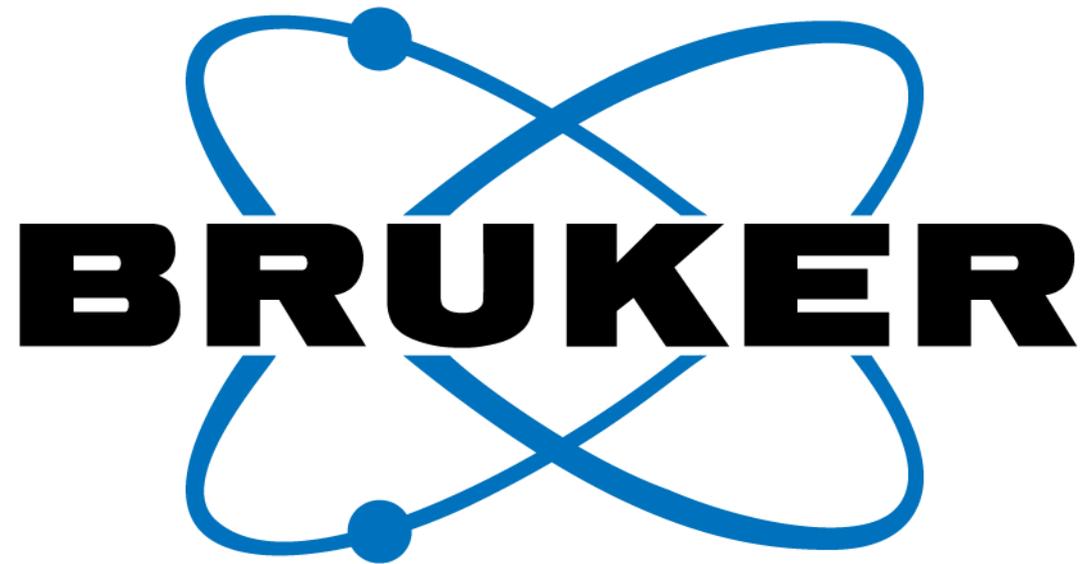
[https://www.bruker.com/nmr\\_license\\_requests.html](https://www.bruker.com/nmr_license_requests.html)

# Bruker Fusion-SV: Unique Solution for SV Synthesis Control



full integration HRAM-MS and NMR data (orthogonal techniques)

- significantly increased accuracy and throughput of Structure Verification
  - easily useable – also for non-experts
  - fully automated data analysis
  - proprietary NMR auto-analysis algorithms based on human logic emulation
  - unique *Smart Formula*<sup>™</sup> for mass accuracy and True Isotopic Pattern (TIP)
- ⇒ Bruker FUSION-SV coalesces NMR data with HRAM-MS data and yields a combined result



[www.bruker.com](http://www.bruker.com)

## Q & A

是否有问题?

请您在 **Q&A 面板中**提交您的问题

我们做的如何?

当您退出webinar的时候, 请填写您在此次webinar的**评价**, 我们非常感谢您的反馈。

**Thank you!**



[Wenxin.xu@bruker.com](mailto:Wenxin.xu@bruker.com)  
[Lu.shan@bruker.com](mailto:Lu.shan@bruker.com)