Approaches for Identifying New Psychoactive Substances (NPS)

James Little
Mass Spec Interpretation Services

Current Trends in Seized Drug Analysis Symposium (2023)
Program Chairs: Dr. Ed Sisco and Dr. Simon Dunne
"Identification of NPS Using GC-MS & FTIR Data"
Jan. 25, 10:15 EST

cfsre The Center for Forensic Science Research & Education

Resources Found on Personal Website

- Todays workshop presentation
- Workshop handout with links to resources on my website
- ***Free*** tutorials and handouts for variety of analytical resources
- ***Much additional*** content ***not*** presented at Forensic@NIST2022 Workshop[1]

Experience in Unknown Identifications

- Retired Research Fellow, Eastman Chemical Company
- 42 Years Experience
- 6 Years Consulting at Mass Spec Interpretation Services
- Unknown Identification Using GC-MS, LC-MSMS, and ***Other*** Techniques

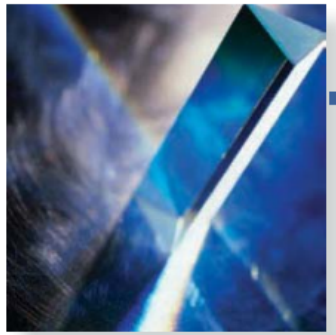
Eastman Chemical Company, Main Site, Kingsport, TN
50 Manufacturing Sites Worldwide, ~14,500 Employees

>50 Mass Specs Networked
<u>Worldwide</u>
Used for R&D

LCGC Article[2] Outlines Eastman's Approach for Unknown Identifications

In many cases, someone knows the identity of my unknown, I just need to find that information.. Thus the term "Known Unknowns.."

114 LCGC NORTH AMERICA VOLUME 31 NUMBER 2 FEBRUARY 2013 www.chromatographyonline.com



MS – THE PRACTICAL ART

Identifying "Known Unknowns" in Commercial Products by Mass Spectrometry

There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know.

Donald Rumsfeld

Former United States Secretary of Defense

"Tools in Toolkit" at Eastman to Identify "Known Unknowns"

- Sample history and known components in mixtures
- Commercial MSMS and EI libraries using NIST search
- Accurate mass analyses
- "Spectra-less databases" such as CAS Registry,[3] ChemSpider[4], etc.
- Chemical ionization (CI) GC-MS[5]
- Training courses for NIST EI and MSMS search software[6,7]
- Extensive EI and MSMS corporate user libraries ***shared and updated nightly***[8]
- NIST MS Interpreter fragmentation program[6,7]
- NMR[9] (H, C, P, F), 1D and 2D
- IR
- Synthesis of enriched samples by ***known chemistry*** in 100 mg quantities for confirmation by NMR and MS ***without purification***
- Deuterium exchange CI GC-MS for exchangeable protons[5]
- Deuterium exchange infusion MSMS for exchangeable protons
- Derivatizations for GC-MS[10,11]

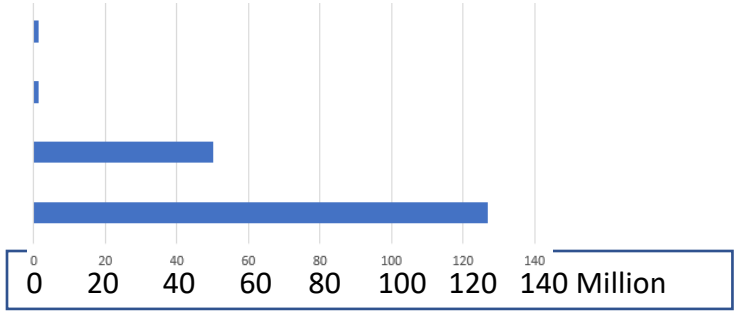
"Known Unknowns" Identified with "Spectra-less" Databases[3,4]

Which Contain *No* Searchable Spectra

SciFinder® A CAS SOLUTION
CAS Registry

ChemSpider
The free chemical database

~1.2M EI spectra in various libraries
~1.2M MSMS spectra in various libraries
~50M records in ChemSpider
~127 M records in CAS Registry, ~15K added each day



- ***Huge*** # of records and associated structures in ChemSpider and the CAS Registry are very useful for finding candidate structures for unknowns!
- ChemSpider free, fee for CAS Registry (SciFinder^n)
- Search CAS Registry by molecular formula and molecular weight
- Search ChemSpider by molecular formula and accurate mass data
- Searches refined by key words, no. of associated references, substructure, *etc.*
- Final candidate(s) reviewed using fragmentation, sample history, *etc.*

Complimentary NMR[9] Data Used with MS for Identifications

- ***When*** *utilized with* structures proposed by mass spec, NMR very useful for identifying unknowns even in mixtures
- Components confirmed in the mixture by NMR can be quantified *and then* used as standards for calibrating routine chromatographic techniques
- Primary standards are ***not needed*** for quantitation by NMR when an internal standard is added to the mixture

Low-Field Table Top NMR

Hi-Field NMR

- Hi field NMR's used at Eastman employ cryogens and are expensive to purchase and maintain
- New low field table-top systems use permanent magnets at a ***much lower*** associated cost
- Latter type are limited in sensitivity and resolution, but should be very useful for many applications

Novel NIST EI Hybrid Search*,[6]

Program Description:

- Hybrid search generates a similarity score matching fragments ***and*** neutral losses
- Greatly extends the scope of the library
- Mass difference must be confined to a single region of molecule and no significant alteration of fragmentation behavior
- *DeltaMass* is the molecular weight difference between query and library compound and reflects the modification of the molecule

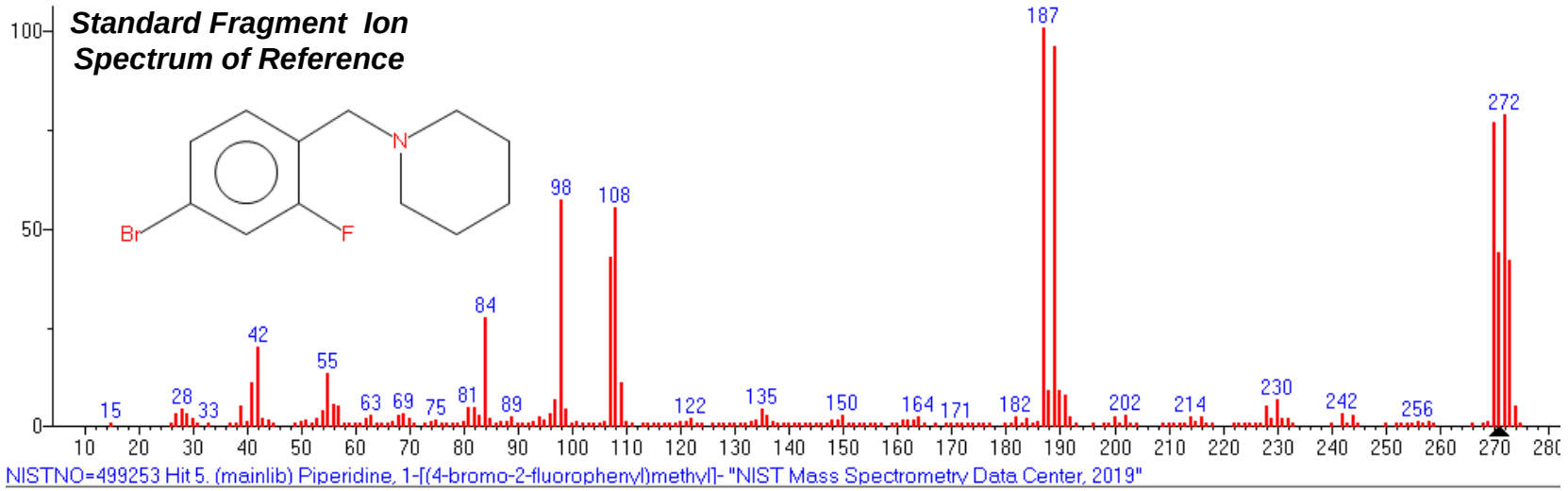
My Personal Experience:

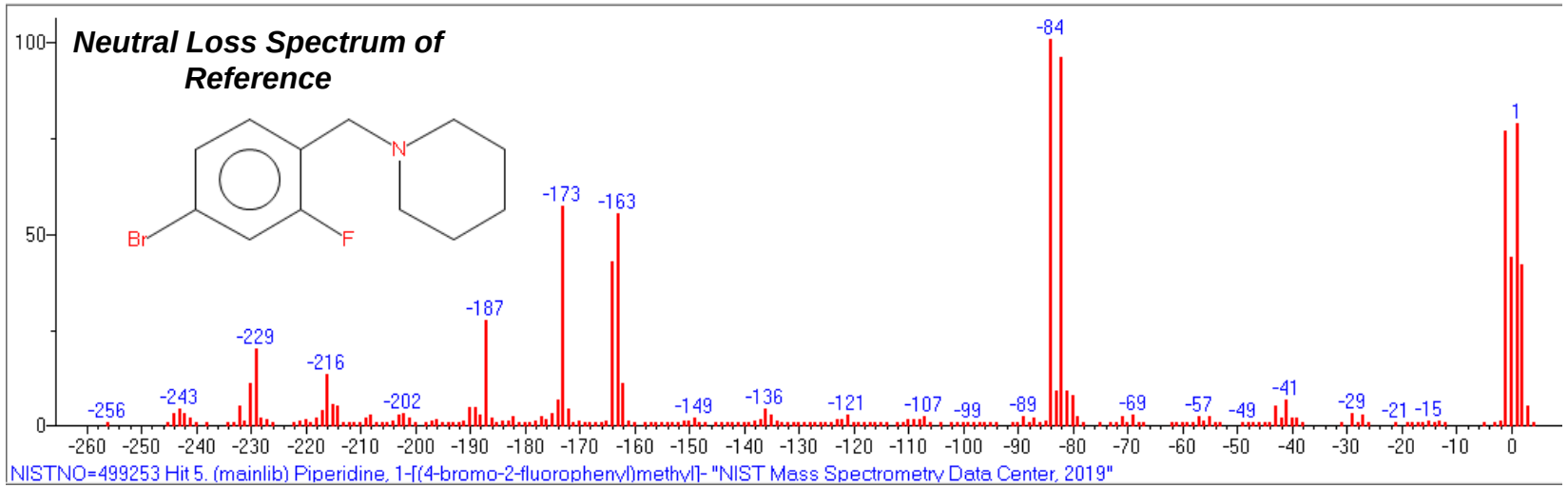
- Used for >40,000 searches in 3 years in evaluating new EI library spectra for NIST
- *Routinely amazed* by the types of similar compounds with high match factors
- Frequently yields useful results not noted in “simple” identity searches
- Very useful in identifying unknowns, finding similar model compounds, and supporting fragmentation mechanisms

*”Combining Fragment-Ion and Neutral-Loss Matching during Mass Spectral Library Searching: A New General Purpose Algorithm Applicable to Illicit Drug Identification,” A. Moorthy, W. Wallace, A. J. Kearsley, D. Tchekhovskoi, and S. Stein, *Analytical Chemistry* **2017** *89* (24), 13261-13268.

Hybrid Search *Combines Scores* of Standard Identity Search with Neutral Loss Search

- First searches the unknown standard spectrum against all library spectra and generates a ***standard identity*** match factor
- Then searches the unknown neutral loss spectrum against all library neutral loss spectra
- Generates a combined “hybrid” score



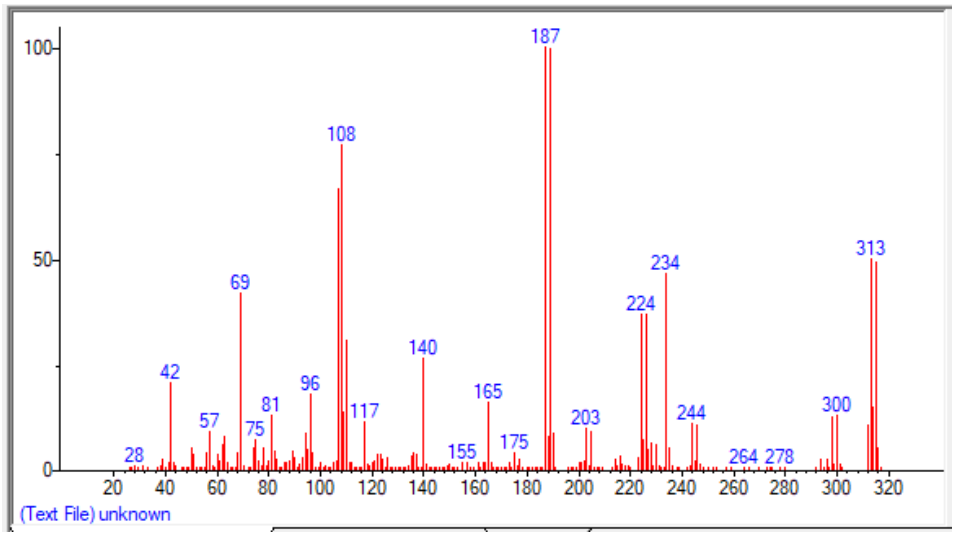


Hybrid Search Results for Unknown

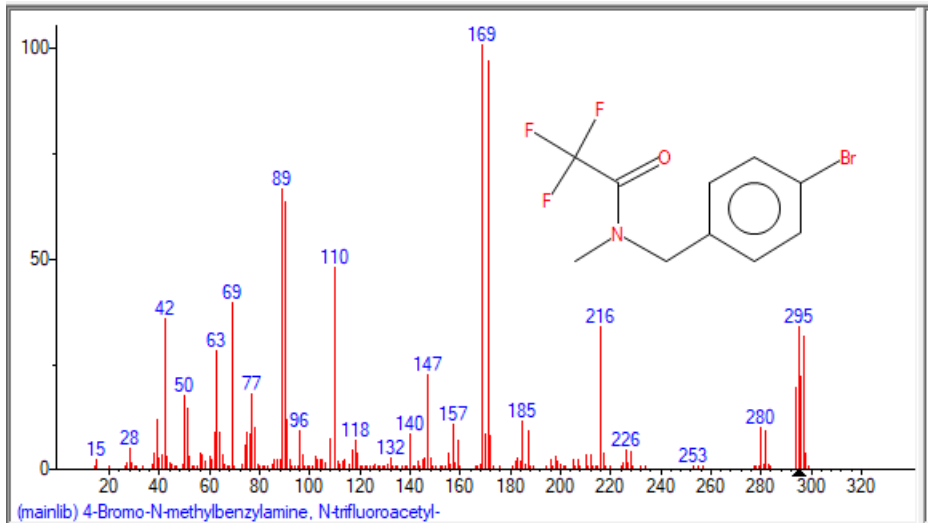
1) Best hybrid match factor is 908, next closest is 781
2) ***DeltaMass*** is 18, common for fluorine (mass 19) replacing hydrogen (mass 1) on ring, *i.e.* 19-1=18

#	Lib.	DeltaMass	Match	o.Match	Name
1	M	18	908	232	4-Bromo-N-methylbenzylamine, N-trifluoroacetyl-
2	M	70	781	559	N-[(5-Bromo-2-fluorophenyl)methyl]cyclopropanamine
3	M	91	766	437	1-Bromo-2-chloro-5-fluoro-4-methylbenzene
4	M	40	755	457	4-(4-Bromo-2-fluorobenzyl)morpholine

? Unknown

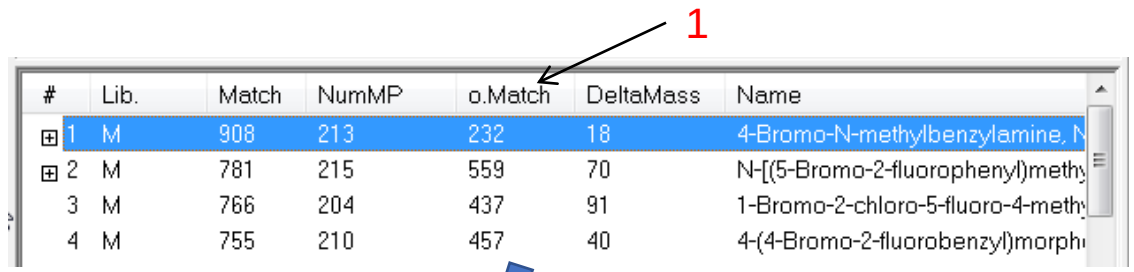


Hybrid Match 908

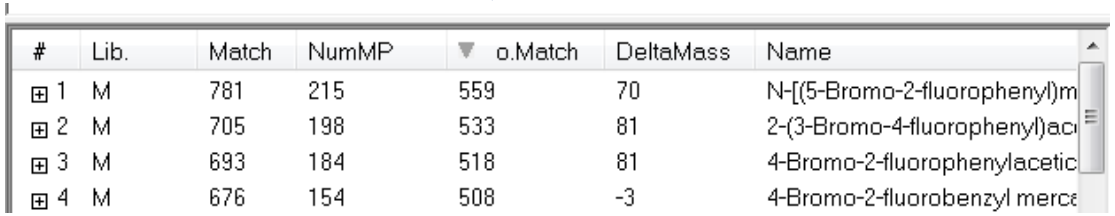


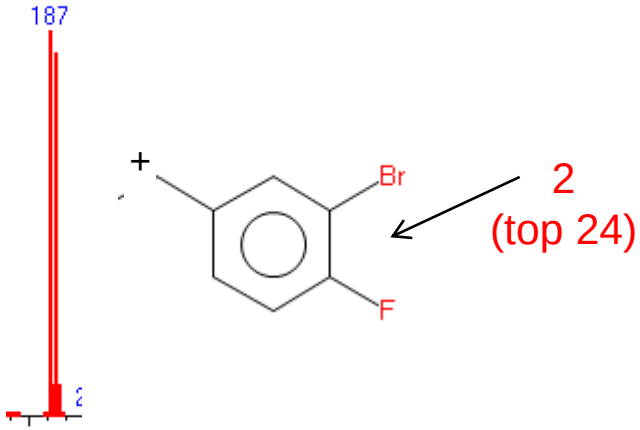
See Standard Identity Results *in Addition* to Hybrid

1. Resort “hybrid” search results by standard identity search match factor
2. Top 24 hits contain the sub substructure with F and Br on a benzyl group, *m/z* 187



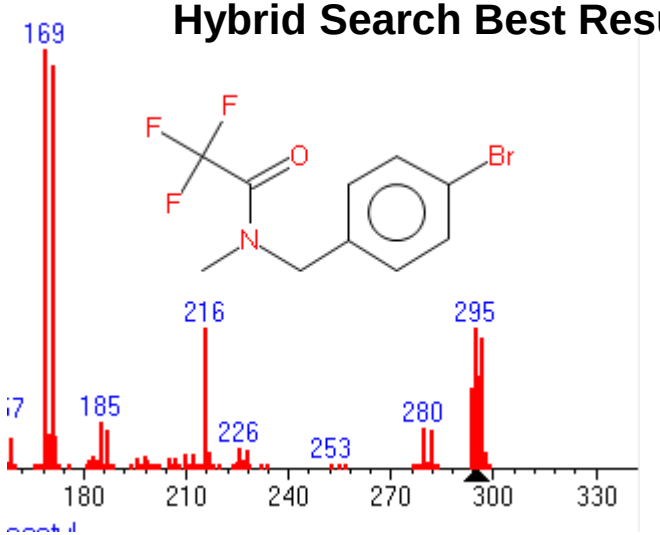
Resorted by o-match
EI Standard Identity Match Factor



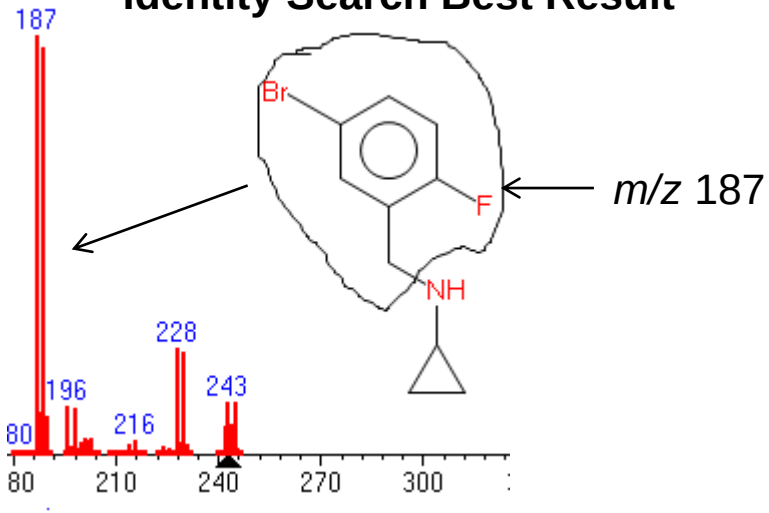


“Mentally” Merge Information of “Hybrid” and “Simple” Identity EI Search

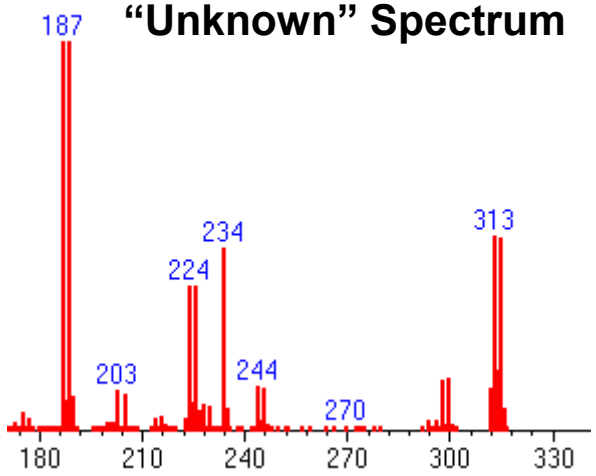
Hybrid Search Best Result



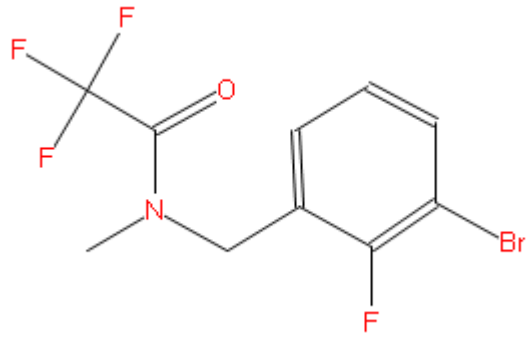
Identity Search Best Result



“Unknown” Spectrum



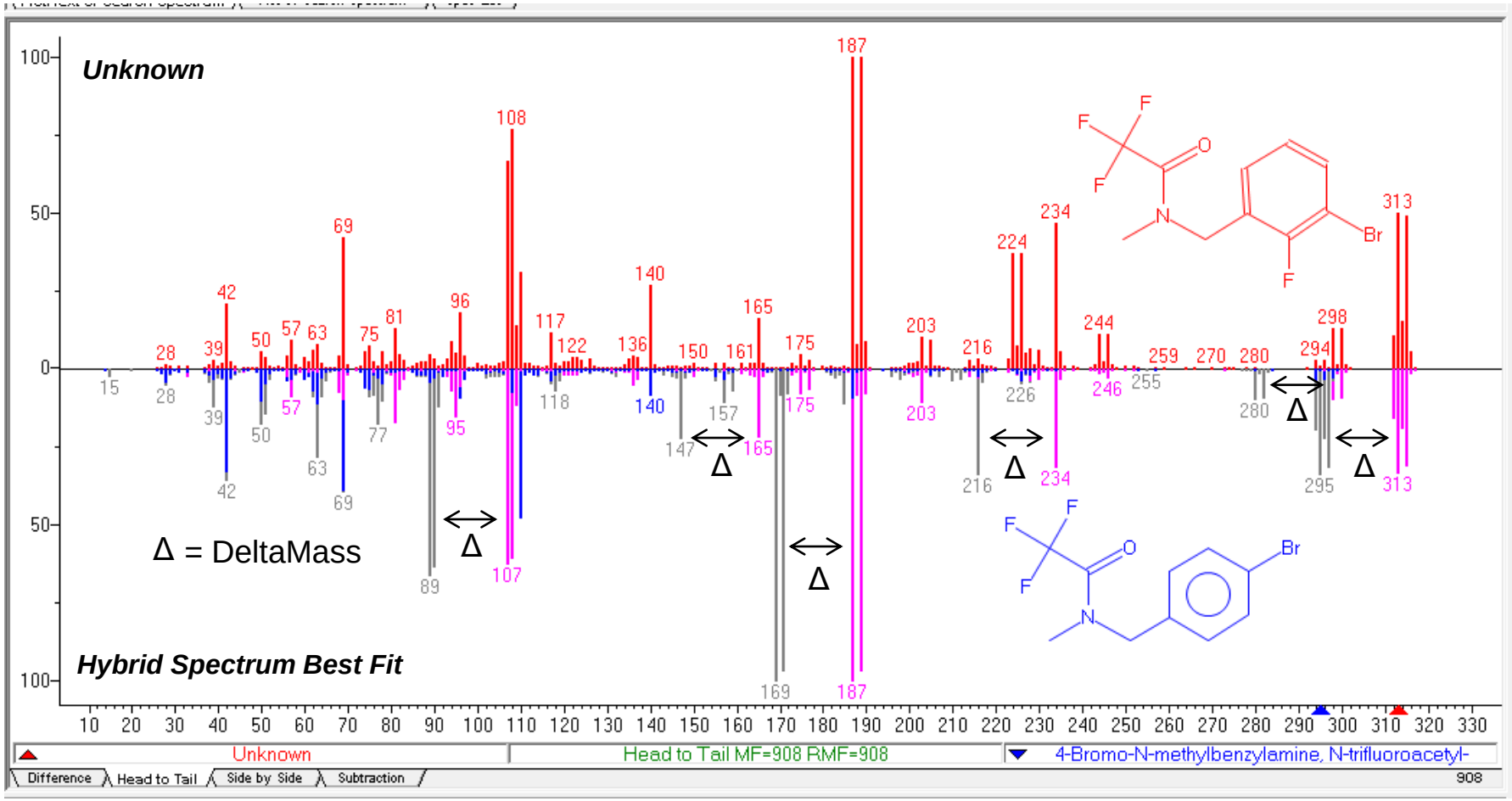
“Unknown Identity or Isomer”



Closer Look at Middle Display

Top is Unknown, Bottom is "Hybrid" Spectrum

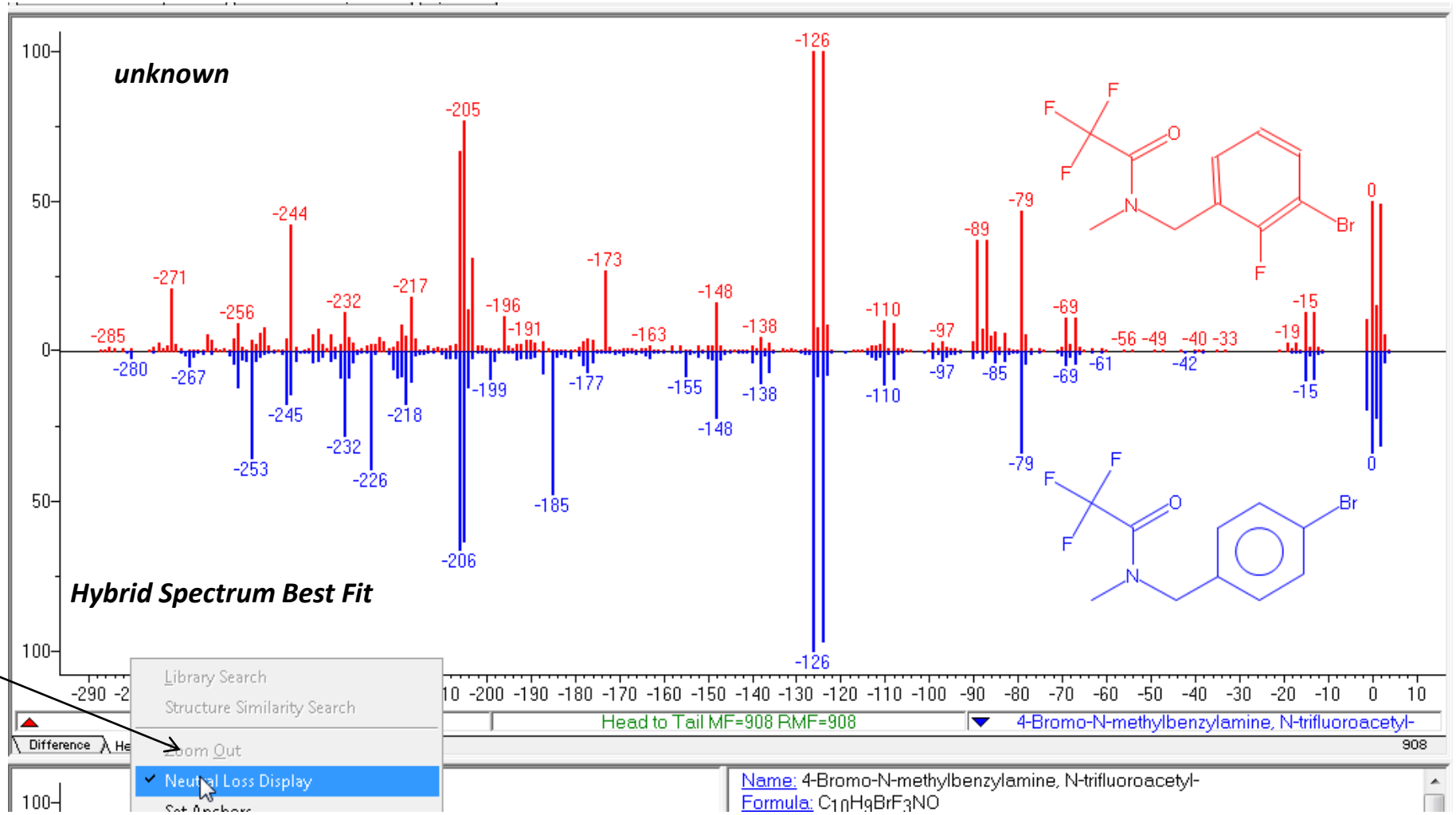
- In bottom spectrum, original ions in grey shifted by DeltaMass (Δ) 18 for user visual comparisons
- Can take a while to adjust to this view versus standard "Head to Tail" views



Alternate Comparison of Hybrid Spectrum: Neutral Loss Display

Top is Unknown, Bottom is "Hybrid" Spectrum

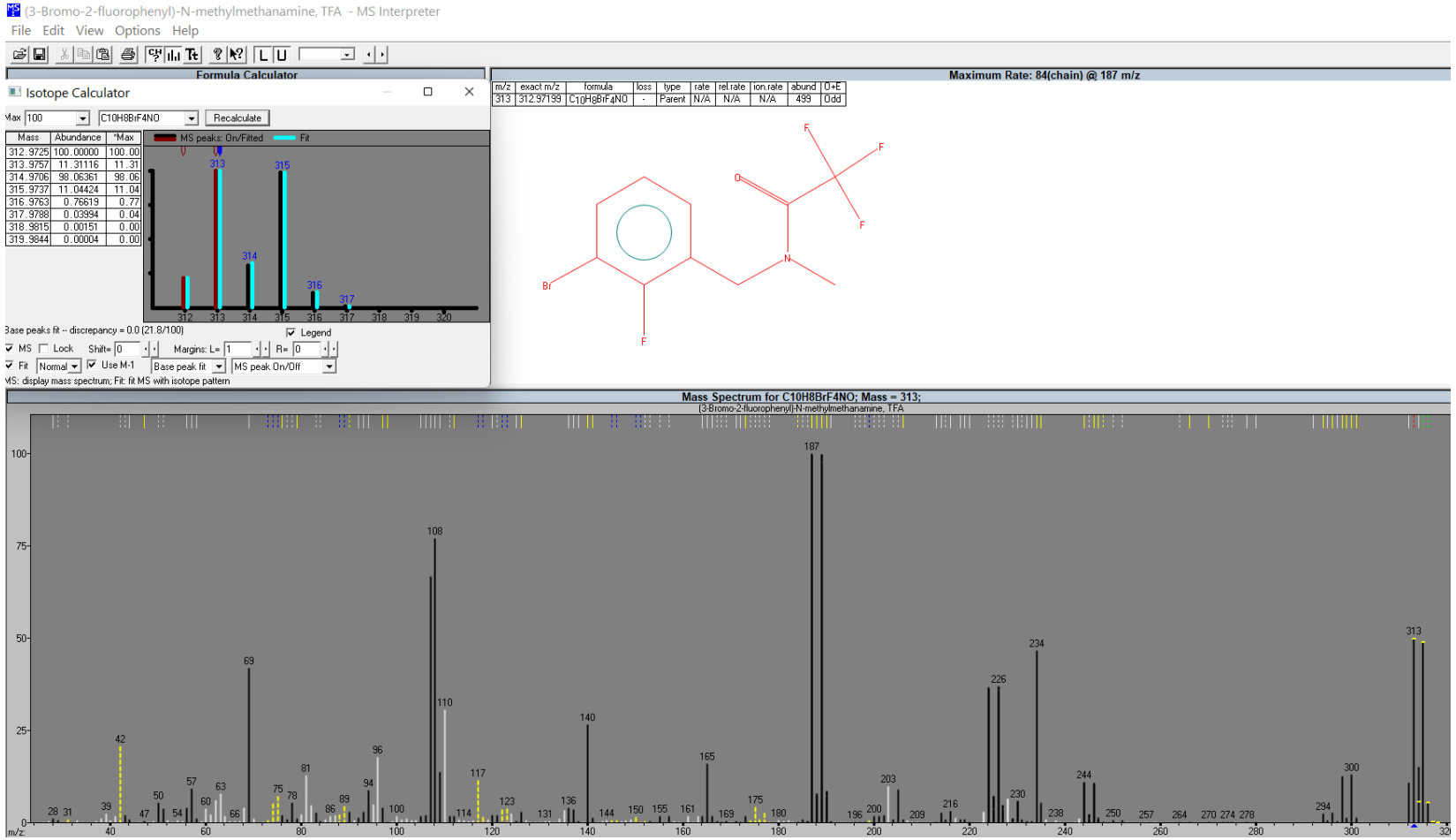
1. Can view alternatively as a neutral loss comparison
2. Shows whole spectrum of reference shifted by DeltaMass of 18
3. ***Easier*** and ***more efficient*** to ***look at "Hybrid" display*** with experienced eye!



1

Powerful NIST MS Interpreter[6,7] Program Correlates Ions to Structures

- Ions in “color and black” explained by program
- Ions in white not explained
- Isotope ratios
- Logical fragments
- Mechanisms and relative fragmentation rates
- Detailed descriptions in my ***free*** courses



*Hybrid Search **Needs** Nominal MW of Species to Work Properly!*

- Many EI spectra do not have molecular ions (~20% in NIST library)
- ***User*** must decide by:
  - Letting program determine automatically
  - User proposing from logical losses at higher *m/z* in spectrum
  - Chemical Ionization
  - Use value proposed by two different NIST algorithms within software

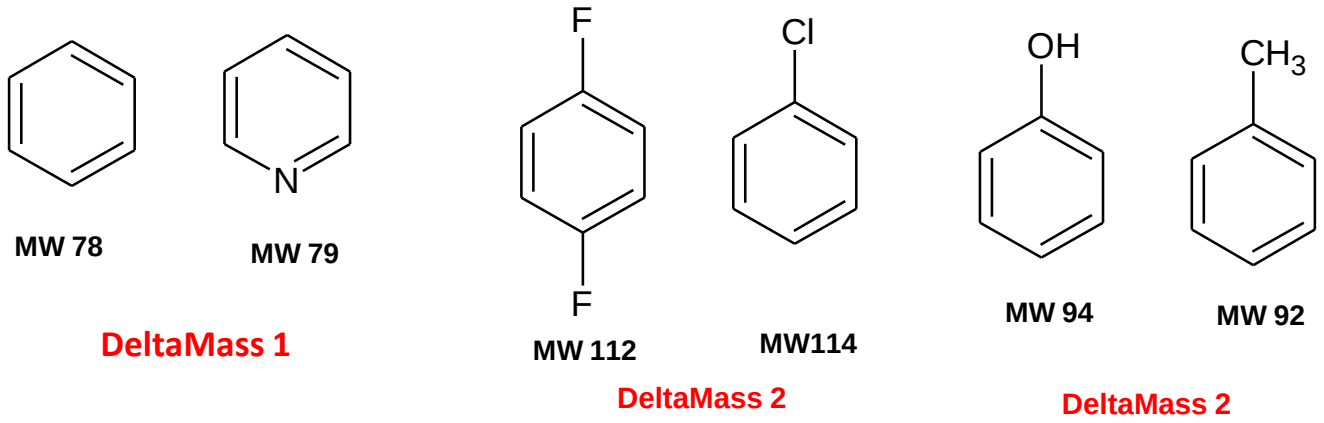
DeltaMass Table[12]

- Useful Hybrid DeltaMass values noted in my spectral evaluations for NIST
- ~600 values in Excel spreadsheet
- Value note can be + /–, depending on species present/absent

DeltaMass Nominal	Group/Element (1)	Group/Element (2)
1	CH3SO2 group	NH2SO2 group
1	methyl on armoatic ring	amine on aromatic ring
1	CH2NH2 group	CH2CH3 group
1	nitrogen in heterocyclic aromatic ring	phenyl aromatic ring
1	amine on aromatic ring	phenol ring
1	N in six membered heterocylic aromatic	aromatic ring no nitrogen incorporated
1	insertion of nitrogen in place of carbon in 5-membered heterocyclic ring	no insertion
1	TBDMS derivative attached to two NH groups	TDDMS derivative inserted on O and one in group on aromatic ring
1	CH2NH2 onaromatic ring	CH2OH on aromatic ring
1	acid on aromatic ring	amide on aromatic ring
1	acid and amide on aromatic ring	two acids
1	methyl and methoxy on aromatic ring	dimethylamine on aromatic ring
1	methoxy on aromatic ring	N-methyl group on aromatic ring
1	methyl on pyrimidine ring	methyl on pyrimidine ring
2	MS2 of a M+2 isotope ion in compound such as Br, Cl	MS2 of a M+2 isotope ion in compound such as Br, Cl
2	two fluorines on aromatic ring	one chlorine on aromatic ring
2	cyclopentyl ring	furan ring
2	alkene	alkyne group
2	Sulfonyl fluoride	Sulfonic acid
2	OH on aromatic ring with nitrogen in ring	NH2 on aromatic ring with no nitrogen in ring
2	aromatic aldehyde	methoxy ether of phenol
2	3-methylbutylamide group	piperidine amide group

Associating Some *Simple* Structures with DeltaMass Values

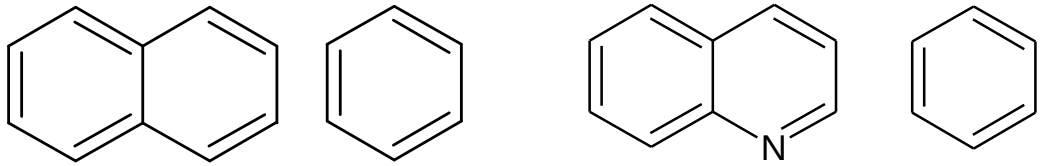
- Some simple ***small*** MW compounds to illustrate types of substructural information
- ***Note:*** Odd values of DeltaMass contain one nitrogen change in structure, thus ***"Nitrogen Rule"***
- Isotope ratios and/or accurate mass helpful with redundancies
- Of course, these substructures can be a part of ***larger*** molecules



MW 78 **MW 79** **DeltaMass 1**

MW 112 **MW114** **DeltaMass 2**

MW 94 **MW 92** **DeltaMass 2**



MW 128 **MW 78** **DeltaMass 50**

MW 129 **MW 78** **DeltaMass 51**

DeltaMass Table[12]

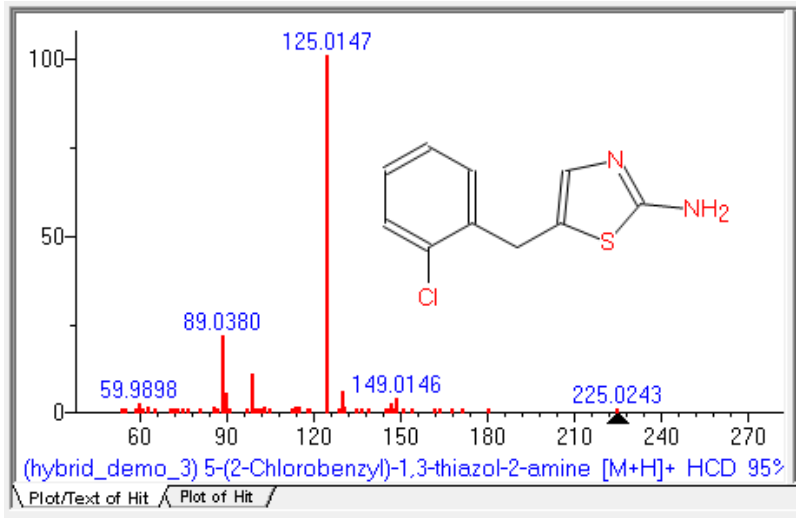
- Values in Excel DeltaMass Table only shown in nominal mass
- E.g. below see entries for DeltaMass = 34 nominal
- Manually added accurate DeltaMass column in example below

			Manually Calculated
34	pyridinyl group	dimethylamino group	33.9843
34	Sulfonamide group on aromatic	nitro on aromatic	34.9877
34	chlorine on aromatic ring	phenyl ring	33.9611
34	CF3 on aromatic	chlorine replacing	34.0263

versus observed = 33.9610

Hybrid Search *Also* Used for LC-MSMS Unknowns[7]

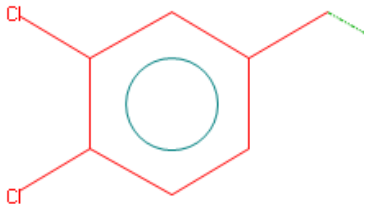
"Hybrid" Best Result
Accurate Mass DeltaMass, +Cl-H

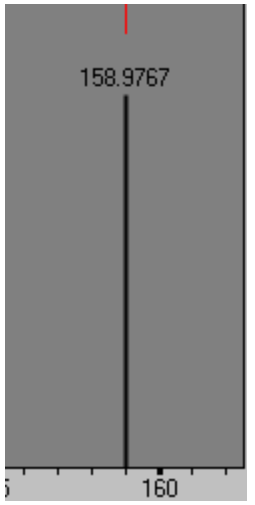


Top 20 **"Identity Searches" show 2 Cl's on benzyl ring**

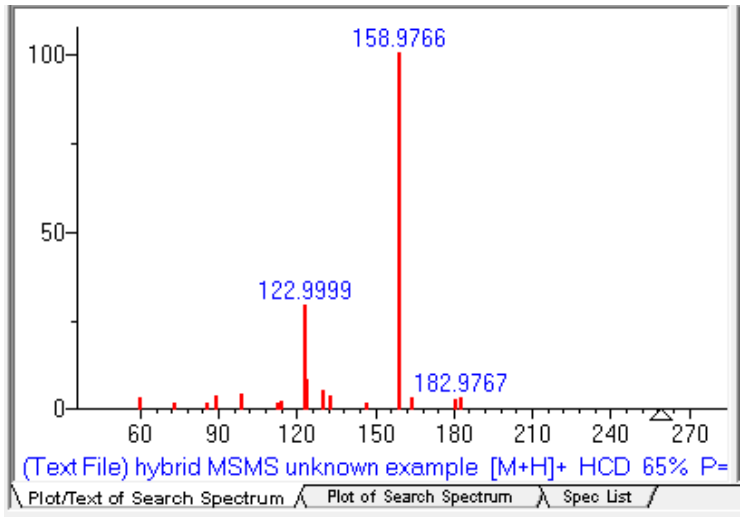
Maximum Rate: 65(chain) @ 158.9763 m/z

m/z	exact m/z	formula	loss	type	rate	rel.rate	ion.
159	158.976282	$C_7H_5Cl_2$	CH_4N_2S	H-Displacement	65	78	5

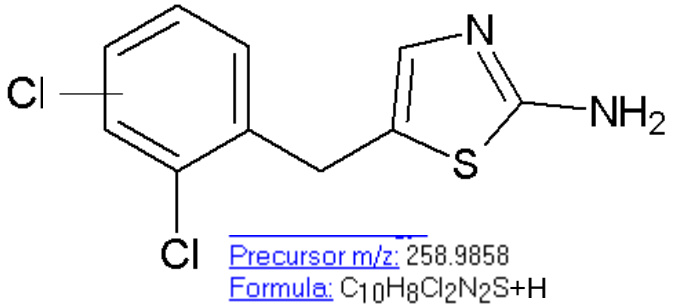




"Unknown" Spectrum



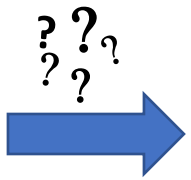
"Proposed Structure for Unknown"



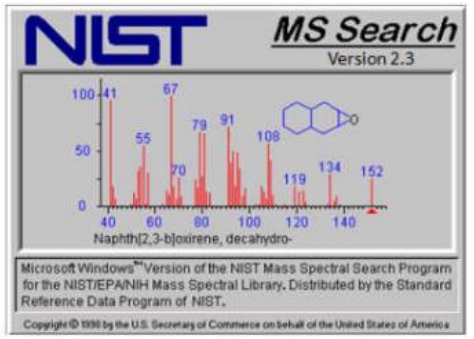
“Real-World Example” of the Identification of Fentanyl-Related Compound[13]

O. David Sparkman, University of the Pacific
James Little, Mass Spec Interpretation Services

White Powder



NIST Hybrid Search

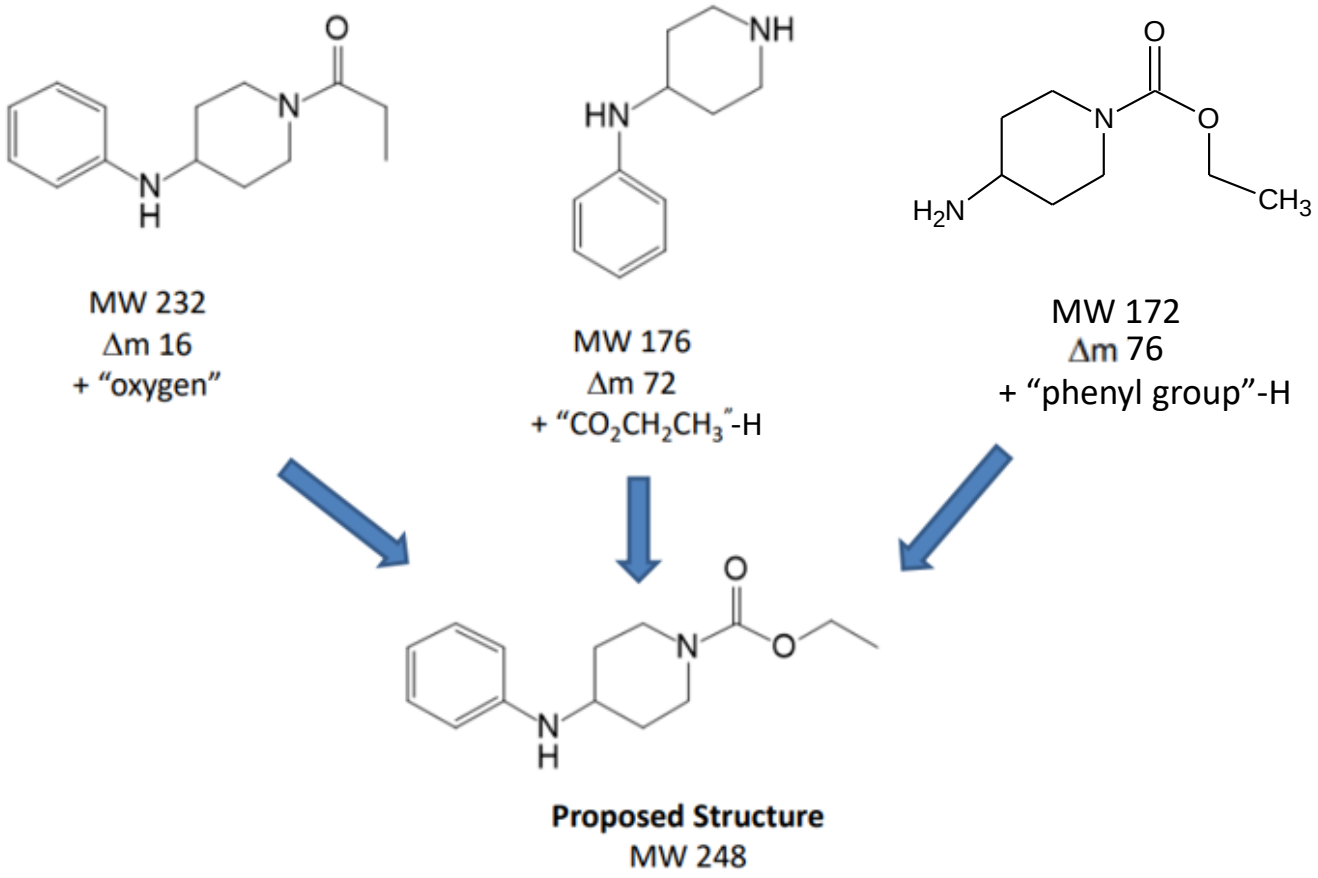


Wiley KnowItAll IR Search

ChemSpider
The free chemical database

Proposed Identification Using Hybrid Search Data

- Delta Masses from Top Hits and Correlation to Proposed Structure



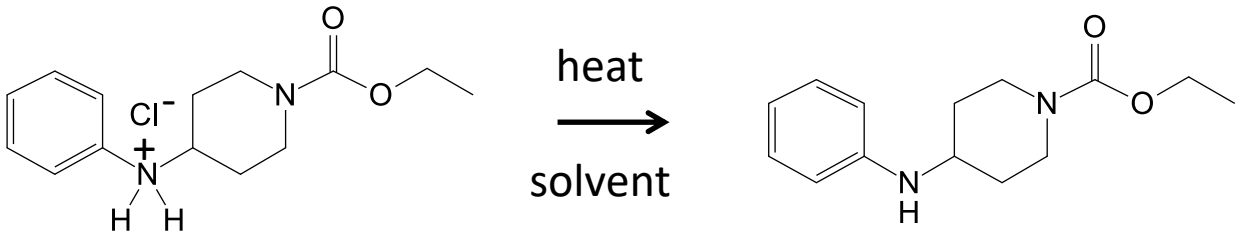
Additional Data Used in Identification

- IR Search in Wiley KnowItAll[16] to confirm ethyl carbamate group
- IR and mass spectral data matched that obtained from CLIC member laboratory upon request from the original investigator

JCLIC.ORG

CLANDESTINE LABORATORY INVESTIGATING CHEMISTS ASSOCIATION

Final Conclusions:



IR
HCl Salt

EI GC-MS
Free Base

Wiley KnowItAll Software for Unknown Identifications

- Vendor neutral data processing solution
- Processes IR, Raman, MS, NMR, and UV data
- Supports over 170 vendor data formats
- Adaptive MS search similar to NIST hybrid

Spectra:
~264K IR
~25K Raman
~1.25M EI
~894K NMR
~30K UV

Wiley KnowItAll

Drugs Often Found as Hydrochloride Salts

spectroscopyonline.com/view/organic-nitrogen-compounds-v-amine-salts

Spectroscopy

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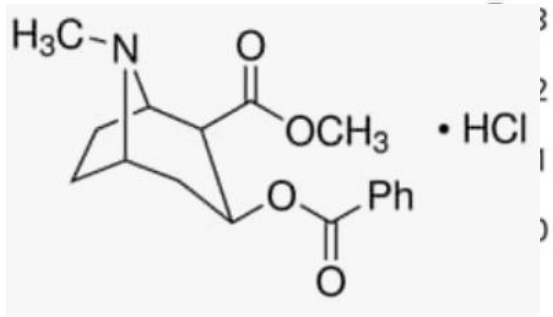
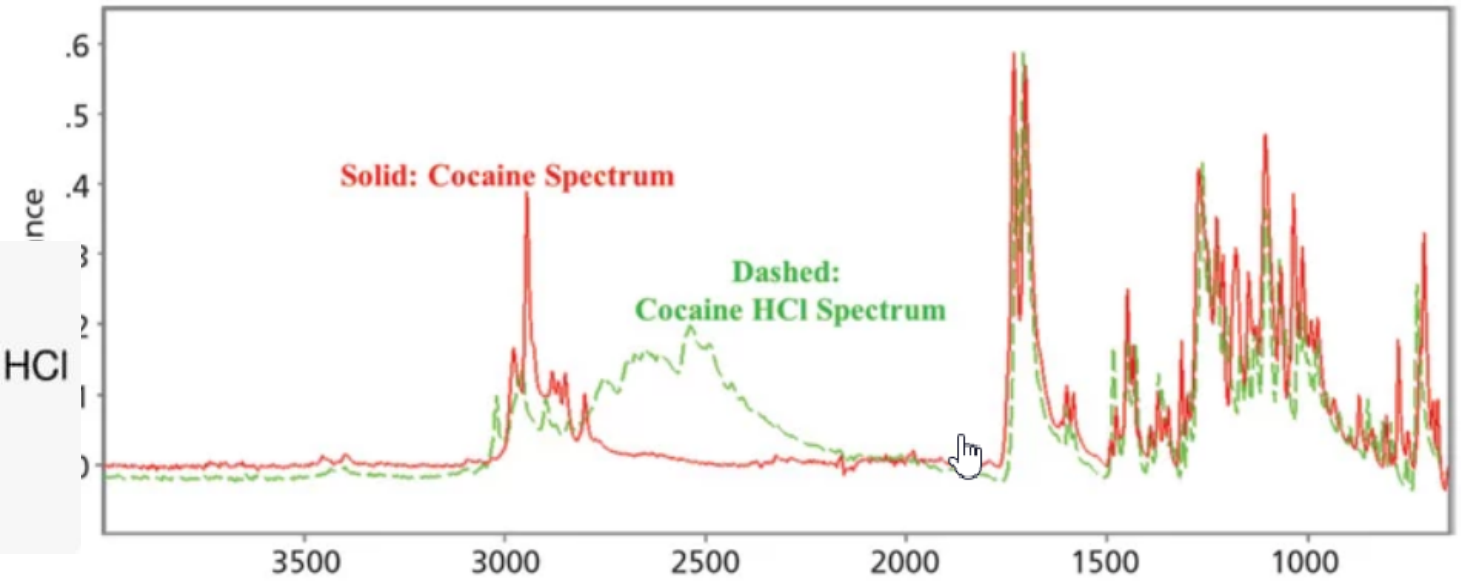
AN MJH life sciences BRAND

compound is rendered water soluble, and thus more bioavailable.

Being able to distinguish amines from amine salts even has legal implications. Cocaine is found in two forms, the hydrochloride amine salt and the amine or "free base" with the street name *crack cocaine* (7). In the United States, possession of these two illicit substances carries different penalties, as the crack version is considered more dangerous. One of the major uses of Fourier transform infrared spectroscopy (FT-IR) in forensic labs is distinguishing cocaine hydrochloride from cocaine. Fortunately, this is easy as can be seen in Figure 2, which shows an overlay of the IR spectra of cocaine base and cocaine HCl.



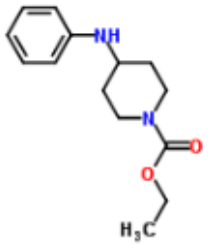
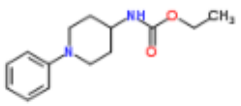


Alternate Approach Using ChemSpider and the Hybrid Search Results

- See ***separate videos*** on using ChemSpider and SciFinder^n with Hybrid Results
- Found one additional structure by ChemSpider, might be hard to differentiate by EI mass spec or IR data

Found 2 results

Search term: **Structure Search - Similarity AND MF = 'C_{14}H_{20}N_{2}O_{2}'**

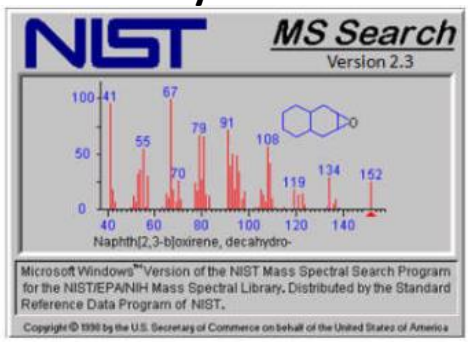
ID	Structure	Molecular Formula	Molecular Weight	# of Data Sources	# of References	# of PubMed	# of RSC
28677384		$C_{14}H_{20}N_2O_2$	248.3208	16	19	0	0
32606266		$C_{14}H_{20}N_2O_2$	248.3208	3	4	0	0

“Real-World Example” of the Identification of PCP-Related Compound[14]

O. David Sparkman, University of the Pacific
James Little, Mass Spec Interpretation Services

White Powder

NIST Hybrid Search

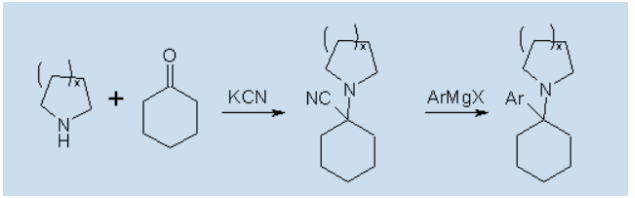


“Spectra-less” Databases

SciFinder® A CAS SOLUTION

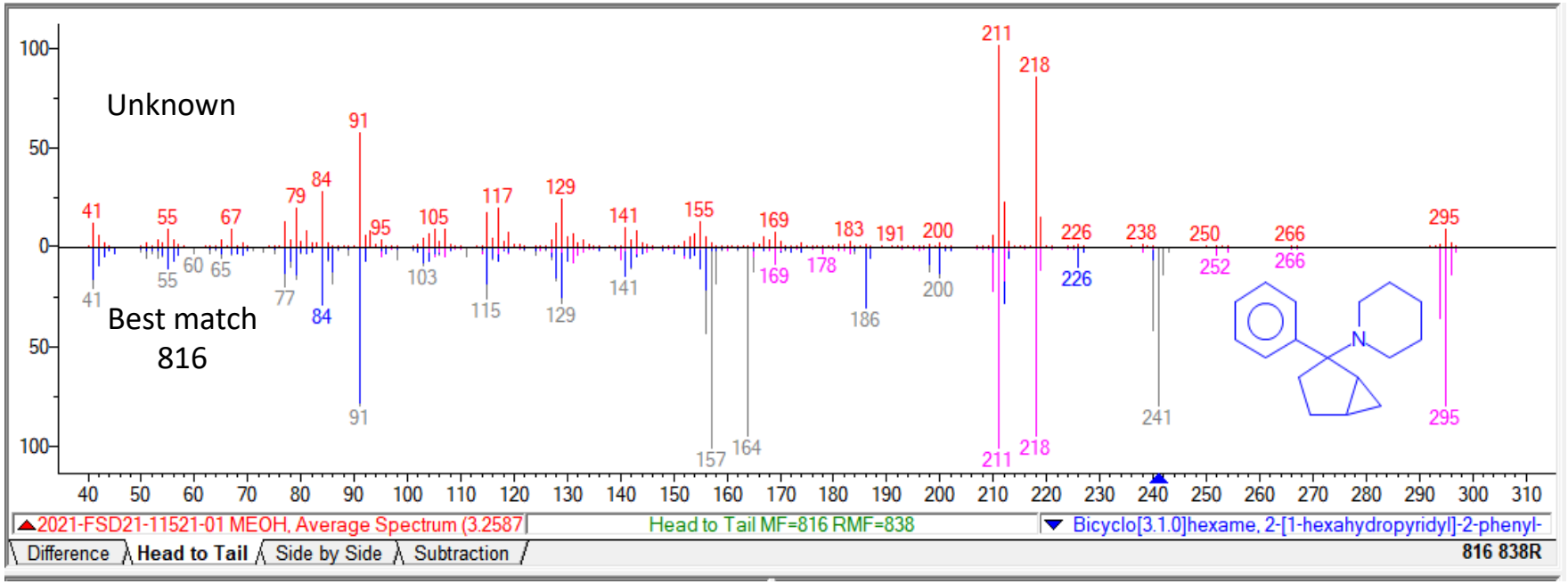
ChemSpider The free chemical database

Chemistry



Hybrid Search Results for EI GC/MS

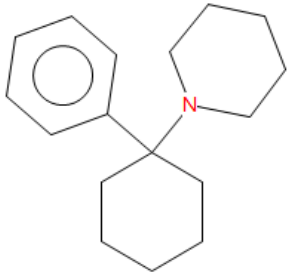
- Hybrid search yields one promising hit
- Match is >800
- DeltaMass = 54



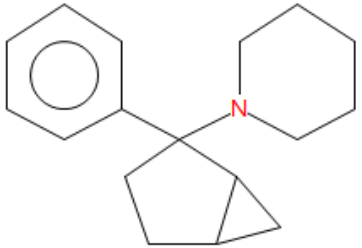
#	Lib.	DeltaM...	Match	R.Match	o.Match	NumMP	o.NumMP	Syn	DBs	Name
1	M	54	816	838	348	142	93	1	0	Bicyclo[3.1.0]hexame, 2-[1
2	M	77	756	761	337	161	100	3	0	1,3-Cyclohexanedione, 5-(
3	M	53	731	733	345	166	125	4	1	Methyl 4-hydroxy-3,5-dinitr
4	M	54	727	729	364	169	134	0	0	N-Allyl-N-methyl-1-phenylh
5	M	55	703	706	563	172	137	1	0	1-Ethoxy-7-phenylvinylider
⊞ 6	M	69	699	750	587	149	97	2	0	Indan, 1-benzylidenehexah

Summary of MS Results

- Hybrid search indicates PCP-related compound
- Δmass = 54 from the hybrid search *not* associated with values noted in my Delta mass table
- Accurate mass data for the unknown indicated a molecular formula of $C_{21}H_{29}N$ via DART MS analyses (E. Sisco, NIST)
- Best match has a molecular formula of $C_{17}H_{23}N$
- Indicates addition of C_4H_6
- Plus, additional double bond equivalent (DBE)



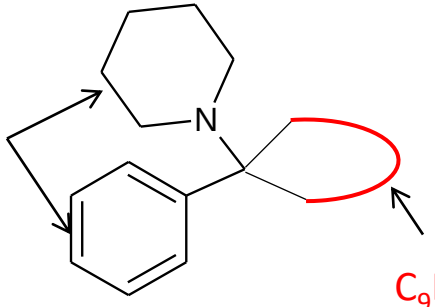
"Angel Dust"
Phenylcyclohexyl piperidine
PCP
Phencyclidine



Best Match
$C_{17}H_{23}N$
MW 241
7 DBE's

$+C_4H_6$

$C_{12}H_{15}N$
5 DBE's



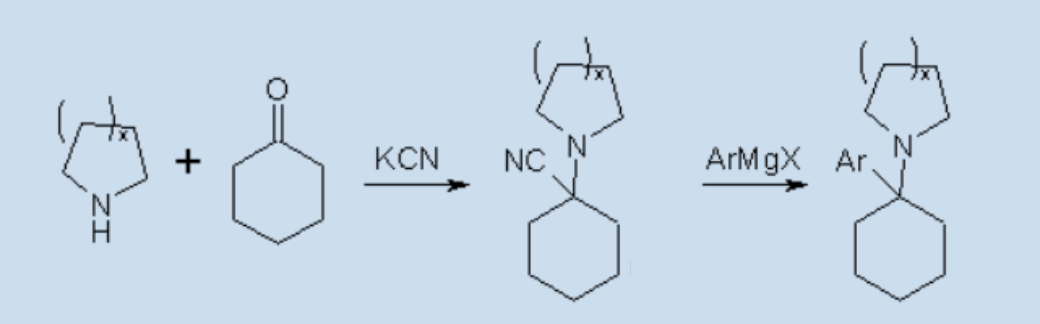
C_9H_{14}
3 DBE's

Total Structure
$C_{21}H_{29}N$
MW 295
8 DBE's

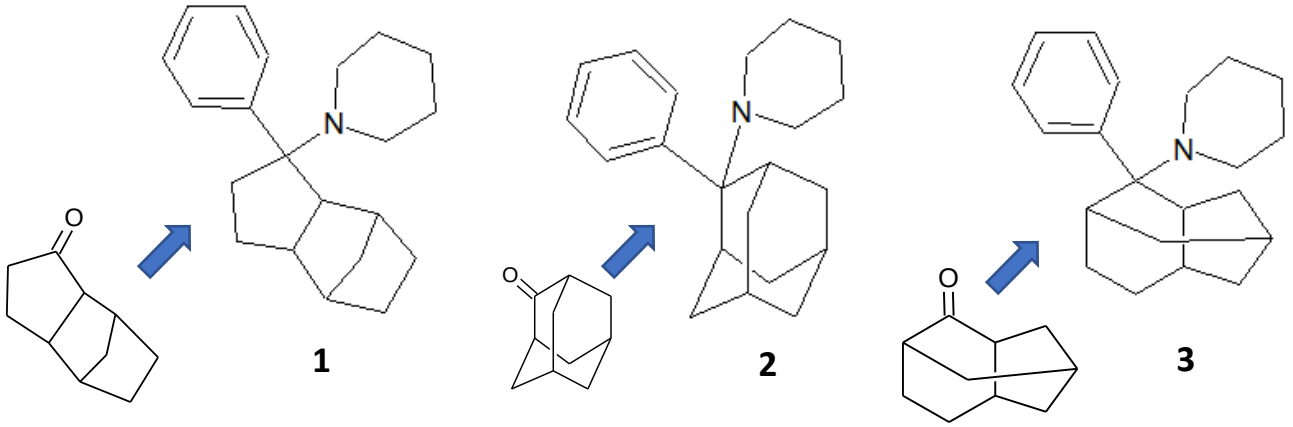
Structures Proposed from Chemistry

- Considered chemistry * to propose 3 structures
- PCP related species *could* be made with the reaction below from 3 commonly available ketones

Proposed Chemistry



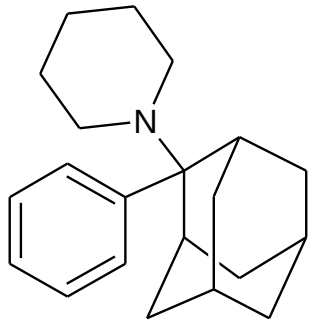
3 Proposed Structures from Chemistry and Easily Obtained Ketones



***Illicit Synthesis of Phencyclidine (PCP) and Several of Its Analogs**
by A.T. Shulgin and D.E. Mac Lean, *Clin. Toxicol. 9(4), 553-560 (1976)*
https://www.designer-drug.com/pte/12.162.180.114/dcd/chemistry/pcp.shulgin.html

Conclusions Leading to Identification of Unknown

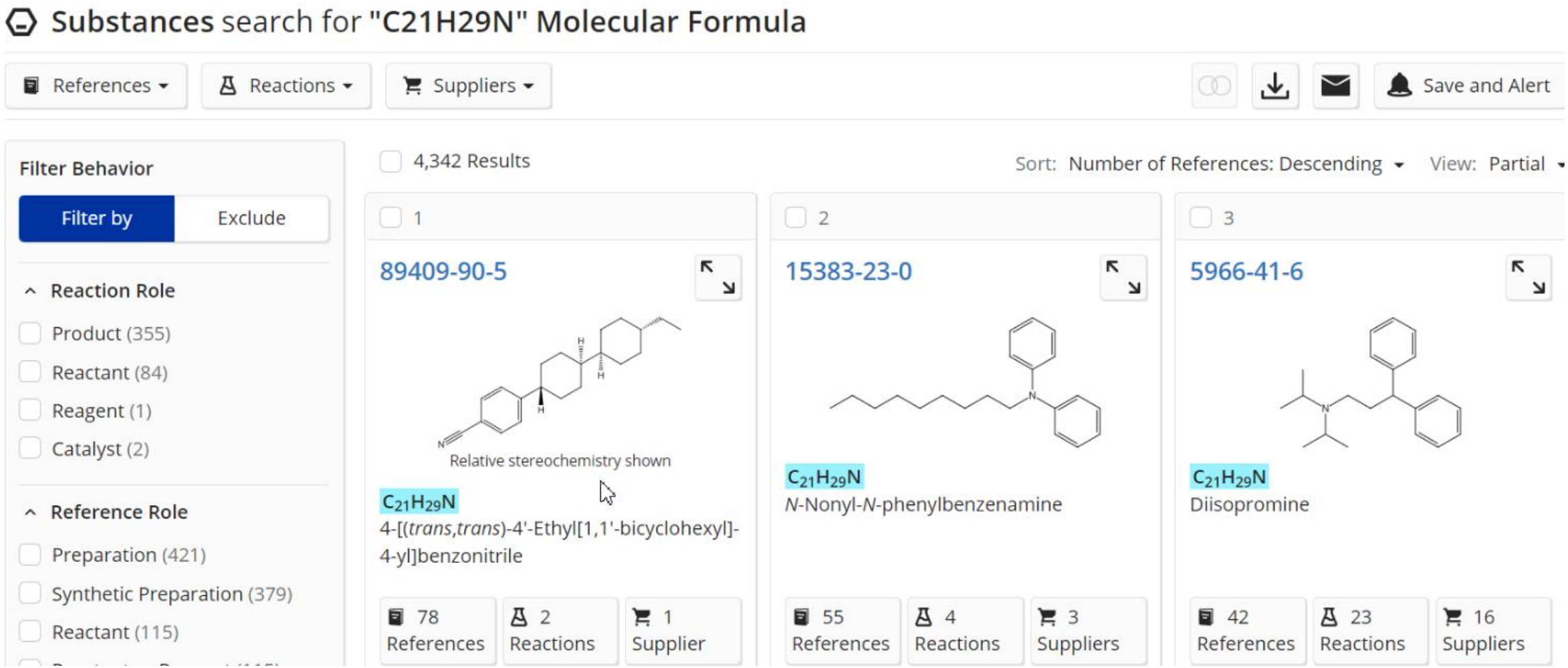
- Hybrid search was critical in suggesting PCP-related substructure
- DeltaMass was not easily associated to a definitive fragment in my DeltaMass table
- Molecular formula from Accurate mass DART critical step (E. Sisco, NIST)
- Initially, molecular formula and DBE's plus chemistry to propose 3 structures
- Structure confirmed by detailed 2D proton/carbon NMR as structure below (A. Urbas, NIST)
- Later, "Spectra-less" approach with ChemSpider and SciFinder^{n} demonstrated



Identity of PCP-Related Unknown
CAS No. 72241-00-7

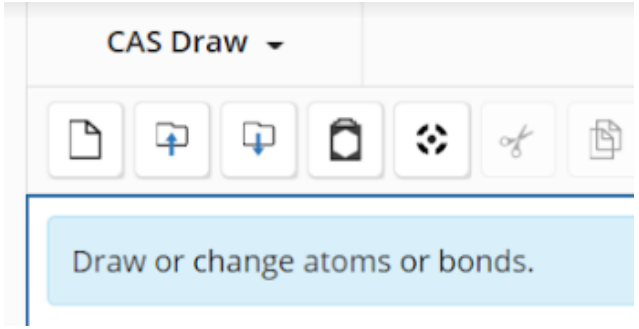
Alternate Approach Using SciFinder^n and the Hybrid Search Results

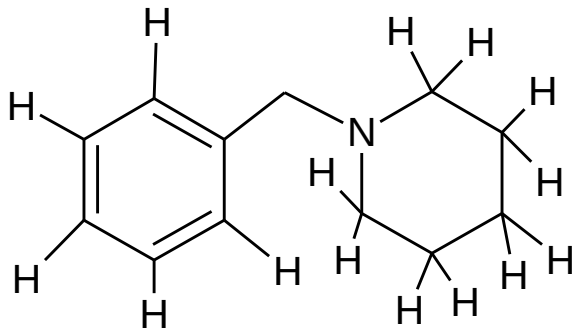
- SciFinder^n using molecular formula search, ***see separate video for details***
- Results of SciFinder^n search for $C_{21}H_{29}N$ sorted by #'s of associated references
- *4,342 Too many* to be useful

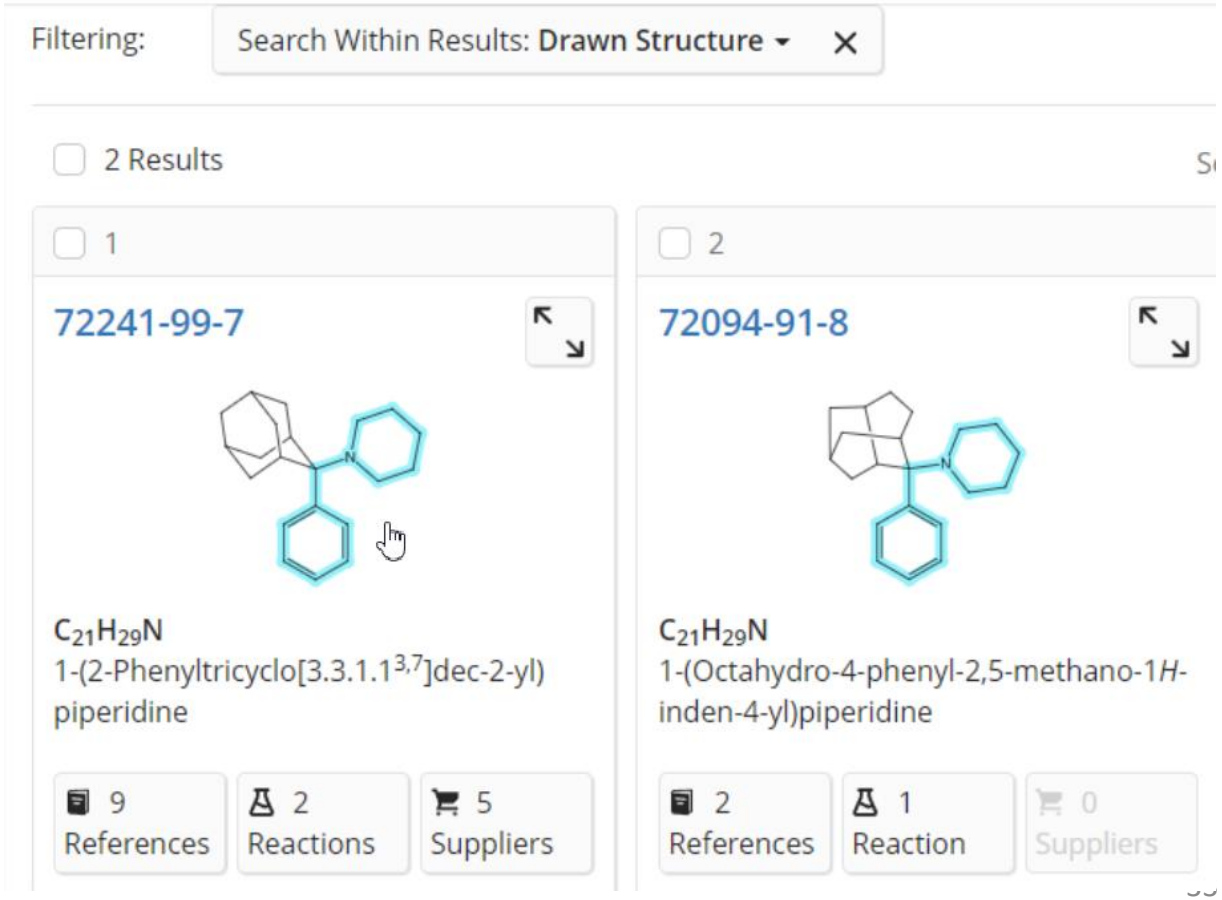


Refined Search by Number of Groups Attached to Piperidine Ring or Benzene in SciFinder^n (Adam Howard, Eastman)

- Approach *not* included in initial "known unknown" reference, ***see separate video for details***
- Allow no substitution on both rings by including hydrogens and a molecular formula of $C_{21}H_{29}N$
- Only 2 the of the 4,342 results had consistent structures
- Result #1 had 9 references and 4 suppliers, Result #2 had 2 references and 0 suppliers
- Found 28 when using molecular weight ***instead*** of molecular formula



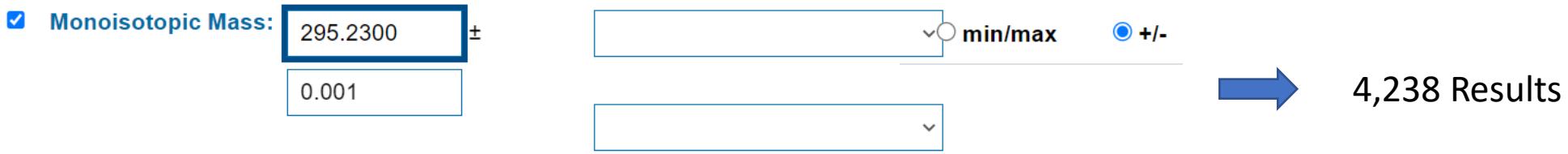




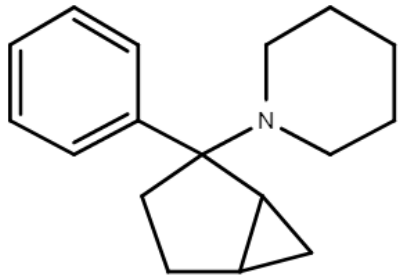
Alternate Approach Using ChemSpider Using Monoisotopic Mass and Similarity Structure Search

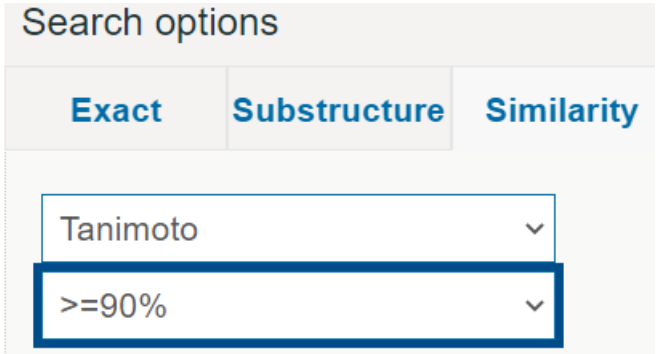
***Not* Included in Original ASMS Article, *See Website* for Details**

Just Monoisotopic Mass Search with Window



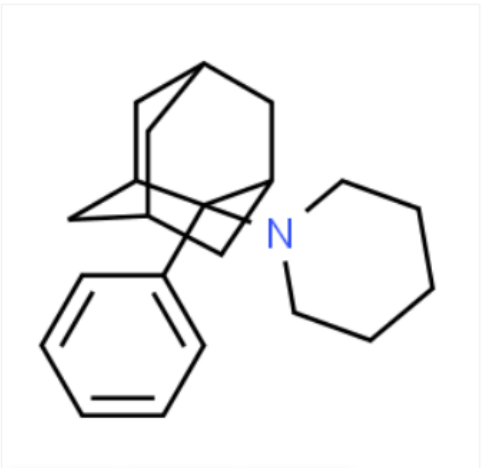
Both Monoisotopic Mass Search +/- Mass Error ***and*** Tanimoto (***Similarity***) Search





Found 1 result

Search term: **Structure Search - Similarity AND MM >= 295.229 AND MM <= 295.231 AND abs(Monoisotopic_Mass - 295.23) as mass_defect AND SingleComponent AND NonIsotopic**

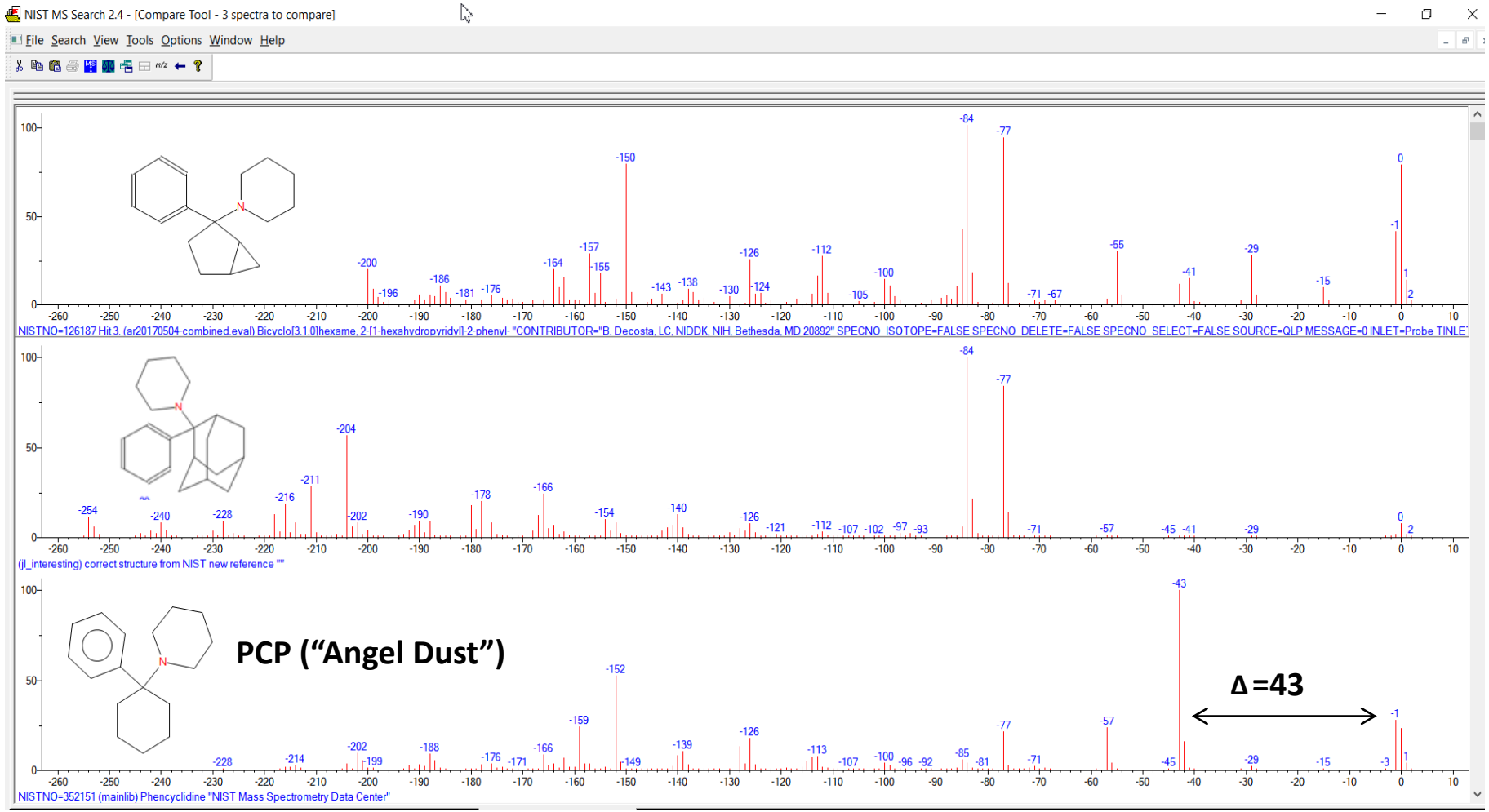


1-(2-Phenyladamantan-2-yl)piperidine

Molecular Formula	$C_{21}H_{29}N$
Average mass	295.462 Da
Monoisotopic mass	295.230011 Da
ChemSpider ID	58052

Addendum: Differences in EI Fragmentation of PCP-Related Compounds

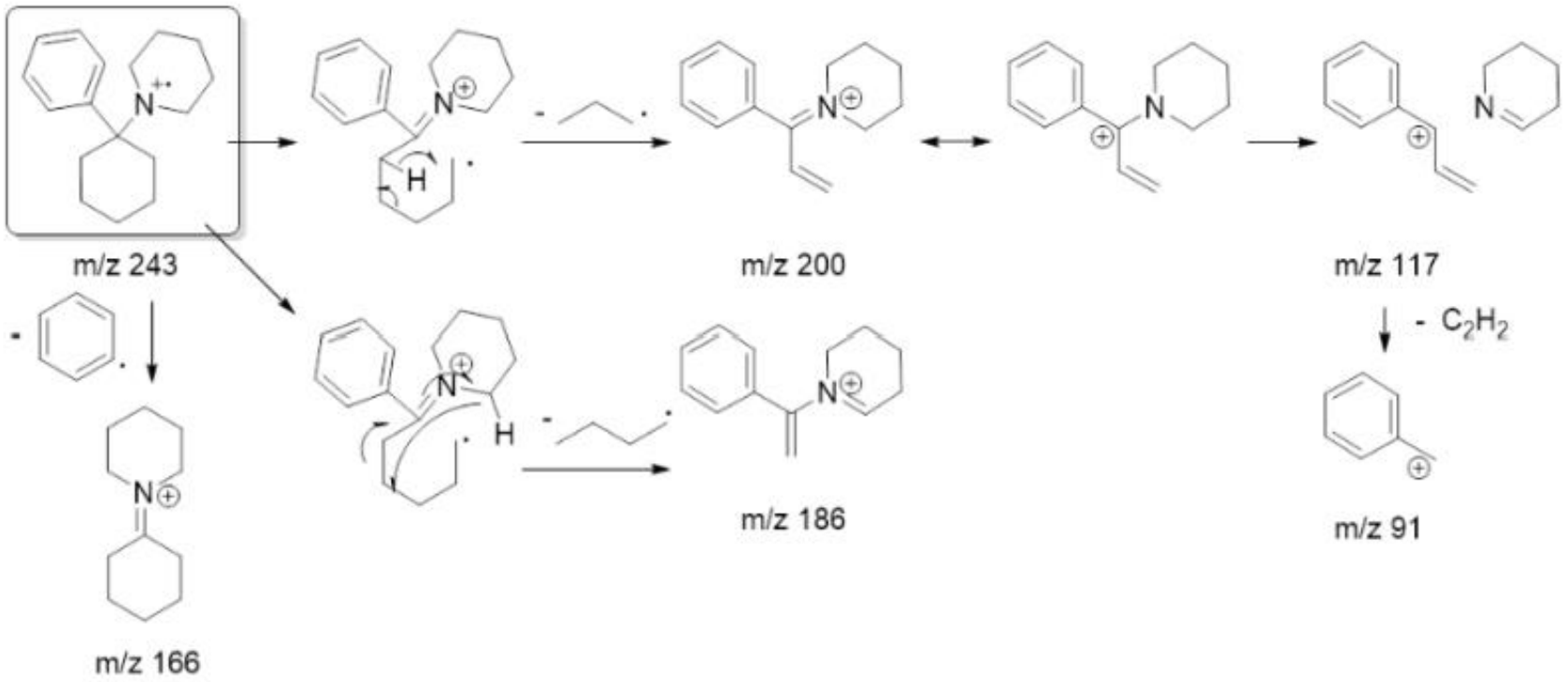
- Major differences between neutral loss spectra of the three compounds below
- Thought the parent compound, PCP ("Angel Dust") would be more similar to other two
- The presence of fused cyclohexyl ring drives loss of 43, C_3H_7 shown in neutral loss spectra below
- Same mechanism for loss of 43 ***not*** accessible by other two compounds
- If best hit with MW of 241 not present, hybrid search ***would have failed*** to yield useful information



Explanation of 43 Loss in PCP EI Mass Spectrum

(Also Detailed Mechanism for Identified Unknown)[15]

Martin Garraffo, NIST



Conclusions

- Hybrid search is a very valuable addition to the identification process
- Extends the utility of all ***commercial*** and ***user*** EI and MSMS libraries
- One of the “many tools in my toolkit” to identify unknowns including:
  - “Spectra-less” databases
  - IR data
  - NMR data
  - Accurate mass data
  - Chemistry
- Free detailed training for EI hybrid searches and other techniques found on my website

In many cases, someone knows the identity of my unknown, I just need to find that information..

Hyperlinks to References in Presentation

1. Seized Illegal Drugs Forensic at Forensic@NIST2022 Workshop
2. LCGC Article Eastman's Approach for Unknown Identification
3. Using CAS Registry/SciFindern for Unknown Identifications
4. Using ChemSpider for Unknown Identifications
5. GC-MS Chemical Ionization for Molecular Weight Determinations
6. Tutorial for EI NIST Search Software
7. Tutorial for MSMS NIST Search Software
8. Creating and Sharing User EI and MSMS Libraries with Automatic Updates
9. NMR Tips
10. Trimethylsilyl Derivatizations and Associated Artifacts for GC-MS Analyses
11. Easy Method for Formation of Methyl Esters of Carboxylic Acids with Trimethylsilyldiazomethane
12. Delta Mass Table for Unknown Identifications
13. Identification of "Real World" Fentanyl Related Compound Using Wiley KnowItAll Adaptive ("Hybrid") and IR Searches
14. Identification of "Real World" PCP-Related Compound Using NIST Hybrid Search
15. EI Fragmentation Mechanisms for PCP-Related Compounds
16. Wiley KnowItAll Tutorial for Both Mass Spec and IR Software

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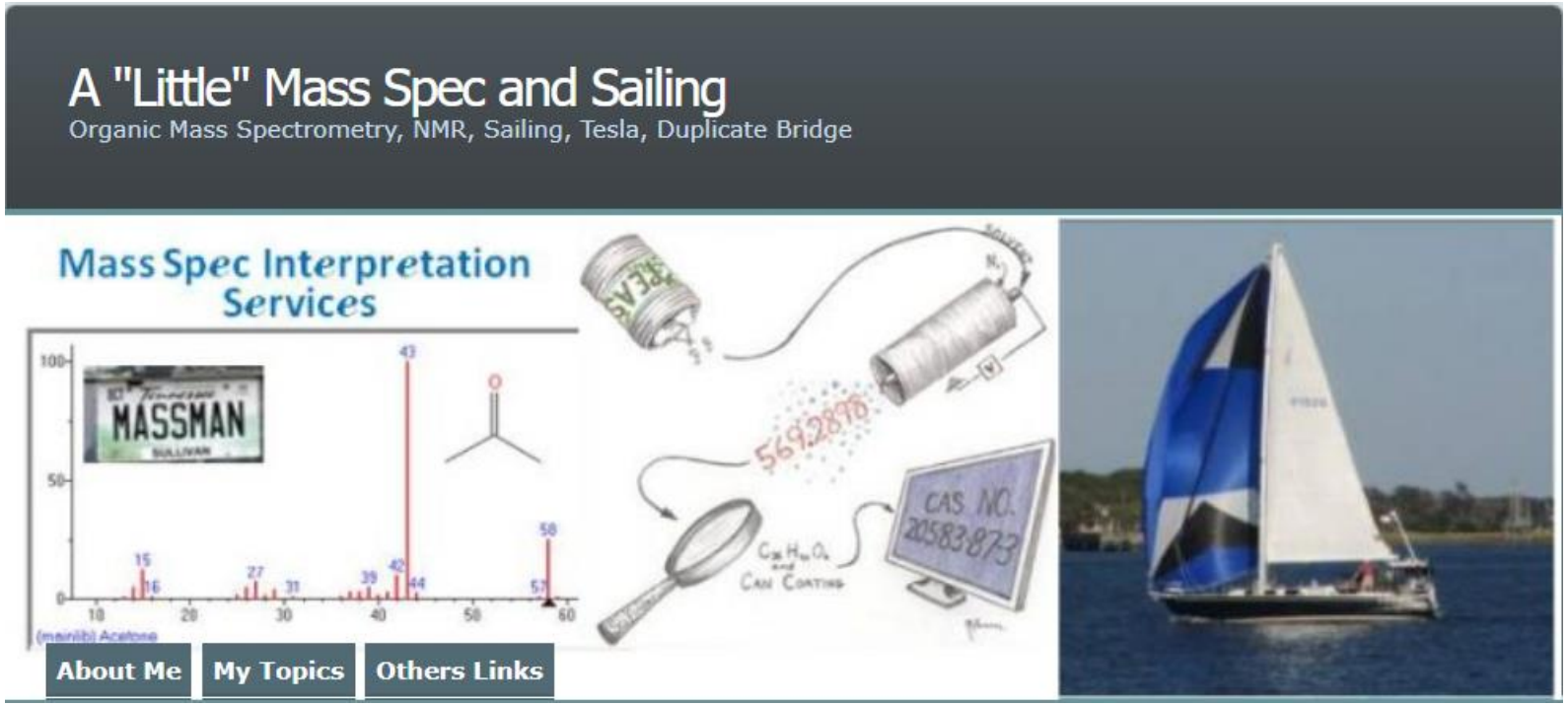
- Aaron Urbas, NIST
- Gary Mallard, NIST Contractor
- Sandy Markey, NIST Contractor
- Curt Cleven, Eastman
- Adam Howard, Eastman
- Laura Adduci GSK, formerly Eastman
- Stewart Millen, Eastman
- Antony Williams formerly ChemSpider, RSC (now EPA)
- Stacey Edwards, ETSU School of Pharmacy
- Michelle D’Souza, Wiley
- Karl Nedweed, Wiley
- Ty Abshera, Wiley
- Bobby Booth, Wiley
- Graeme Whitley, Wiley

Tour of Website



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- Helium Conservation