

LC/MS Unknown Identifications Using MSMS Libraries

Part V: NIST Structure Searches

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- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties¹ EI GC-MS, LC-MS/MS, Chemical Ionization,³ Accurate Mass, Derivatization,^{4,5} MS library management, SciFinder⁶, ChempSpider⁶, Surfactant ID,⁷ NMR, GC-IR, organic synthesis, matrix ionization effects,⁸ etc.*



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

* https://en.wikipedia.org/wiki/Eastman_Chemical_Company



>50 Mass Specs Networked
Worldwide

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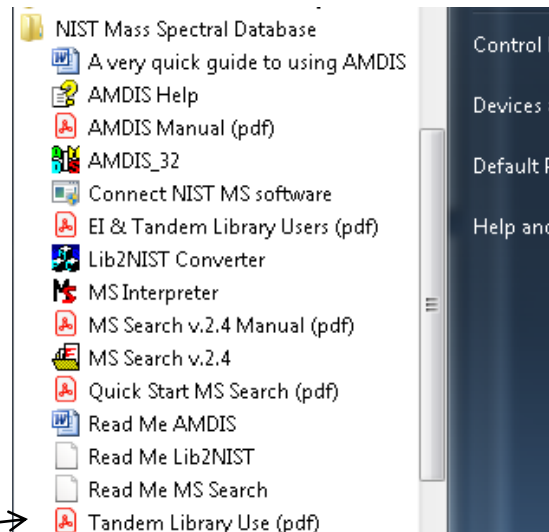
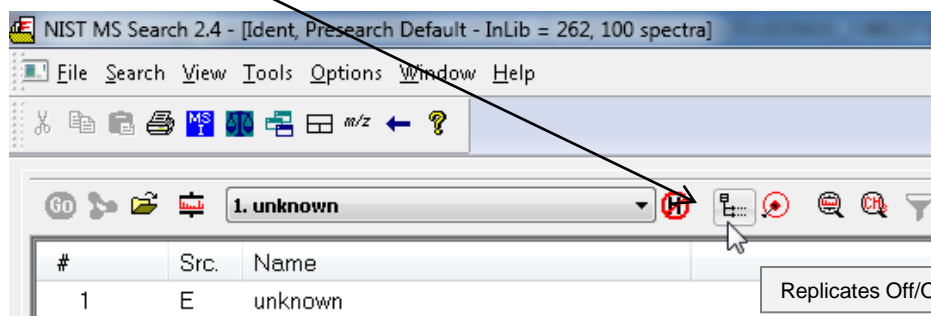
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LC/MS Unknown Identifications Using MSMS Libraries

- Part I: Overview of Software and User Customized Configurations
- Part II: NIST MSMS Search Software and Libraries
- Part III: More Detailed Discussion of MSMS Hybrid Search
- Part IV: Importing MSMS Spectra
- Part V: NIST Structure Searches
- Part VI: MS Interpreter Correlation of Substructure to MSMS Ions
- Part VII: Using and Creating Other MSMS Libraries
- Part VIII: Identification of Unknowns with "Spectraless" Libraries

Help Files for NIST Search

- "Hover" over Program Icon with mouse and function description displayed



- **Detailed** documentation for NIST Search⁸

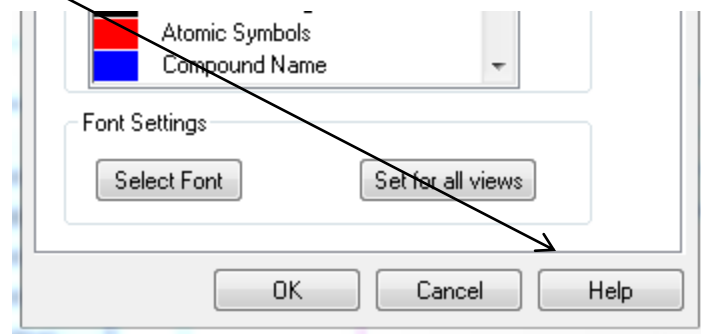
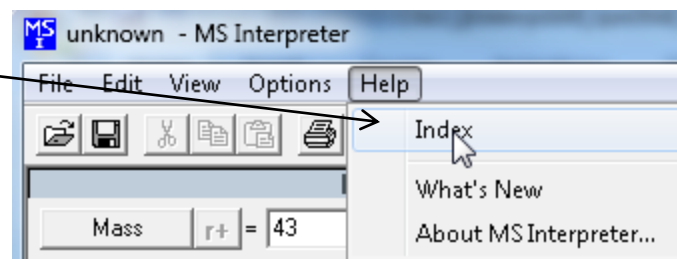
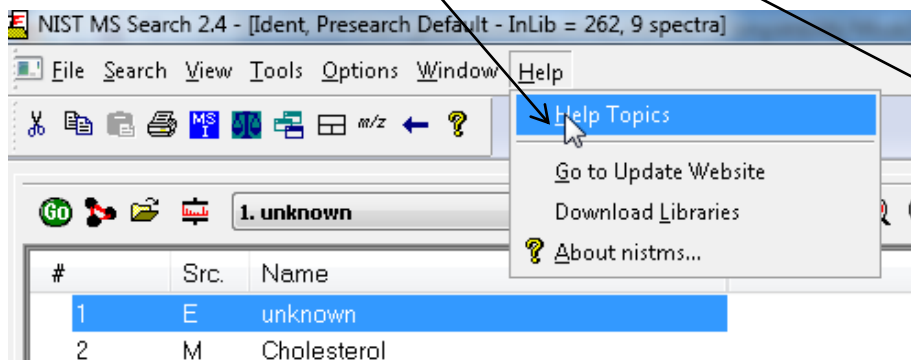
- MS Interpreter included in NIST manual⁸

- Windows Program Group

- NIST Tandem Search **Quick Start Guide**⁹

- "In program" assistance for both programs

- **Help button** at corner of all windows



NIST Software in General is “Windows Compliant”

- left click (LMB)** to select an item, **double LMB** on that item to perform operation
- right click (RMB)** in area or item to see operations that can be performed or to change properties of window
- LMB** on first item and last item to select group **while** holding **shift key**
- LMB** to select/deselect individual items **while** holding **Ctrl button**
- use up and down arrows **on keyboard** to step between entries
- some NIST windows such as librarian have no delete button to delete ions, **must** use delete key on keyboard!
- control a** (select all), **control x** (delete selected), **control c** (copy); **control v** (paste)
- control k** copies entries into windows in tab-separated text format, e.g., **paste** into Excel
- F1 MS Search help
- F9 send spectrum to MS Interpreter

Tip 3: **LMB** and **drag** to rearrange order of column headers

#	Lib.	Name	▼ Match	Prob. (%)	RI	B. Match	Syn	DBs
1	R	Undecane	955	44.8	1100	955	4	8
2	M	Undecane	945	44.8	1100	945	4	8
3	R	Undecane	944	44.8	1100	958	4	8
4	w1	Undecane	937	44.8	-	955	11	0
5	w1	Undecane	933	44.8	-	950	11	0
6	w1	Undecane	932	44.8	-	939	11	0

- LMB** on column of interest
- Can sort in lower value first or higher

Tip 1: When reviewing search results, use up and down arrows on keyboard to quickly step through results!



Tip 2: When viewing structures in MS Interpreter, use left and right arrows on keyboard to quickly review results!



LC/MS Unknown Identifications Using MSMS Libraries NIST Structure Searches

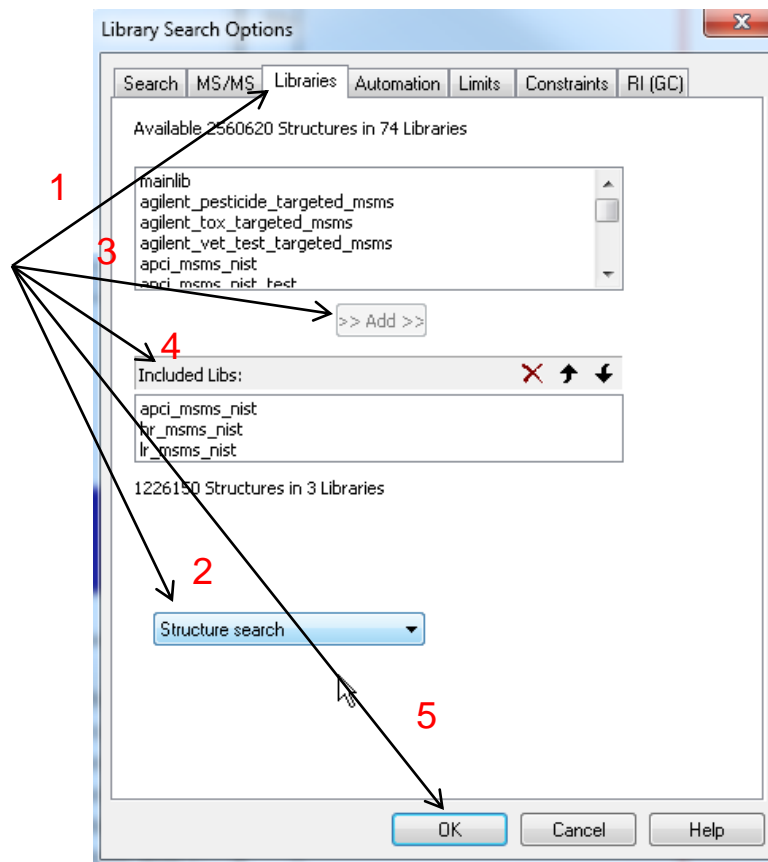
- **Many** Different Drawing Programs can be employed²
- Choose one that you **normally** use

Session Topics:

- Setting up **Library Search Options** for Structure Searches
- Importing structures from vendor programs²
- Searching for **similar** structures using **Structure Search**
- Searching for **exact** structure using **Library Search** with InChIKey
- Using “Compare” window to view series of spectra

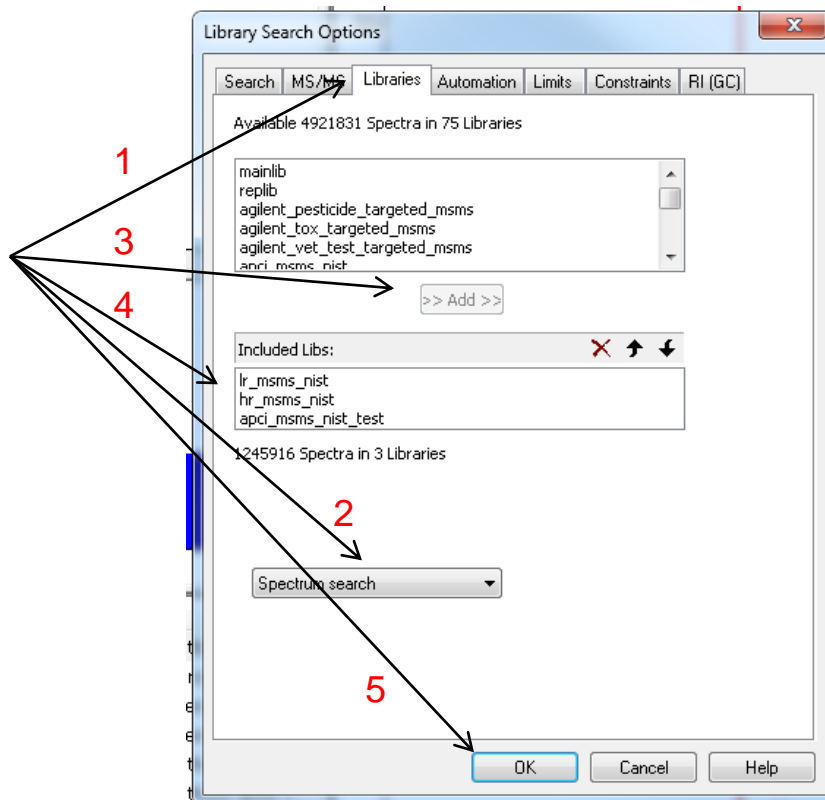
Critical Settings: Select the Libraries Used for Similar Structure Searches

- **Similar structure searches** are driven by libraries in **Structure Search**
- **LMB** on the **Libraries** tab and make sure **Structure Search** is selected
- Select the group of libraries to be searched by Similar Structure
- **>>Add>>** all libraries to be searched
- **Order** of libraries normally **only important** in Other Search Tab which return **maximum no.** of hits



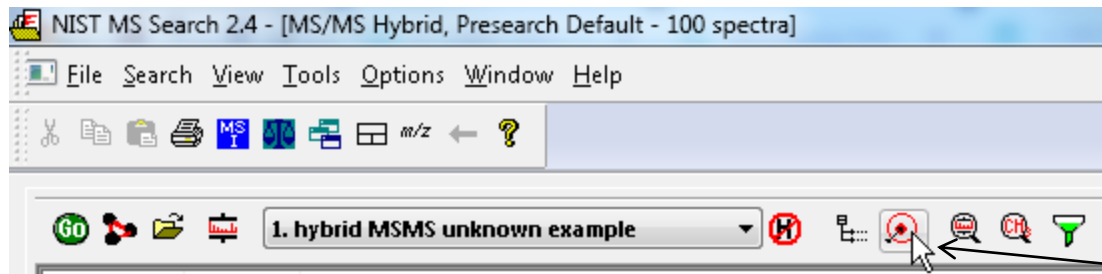
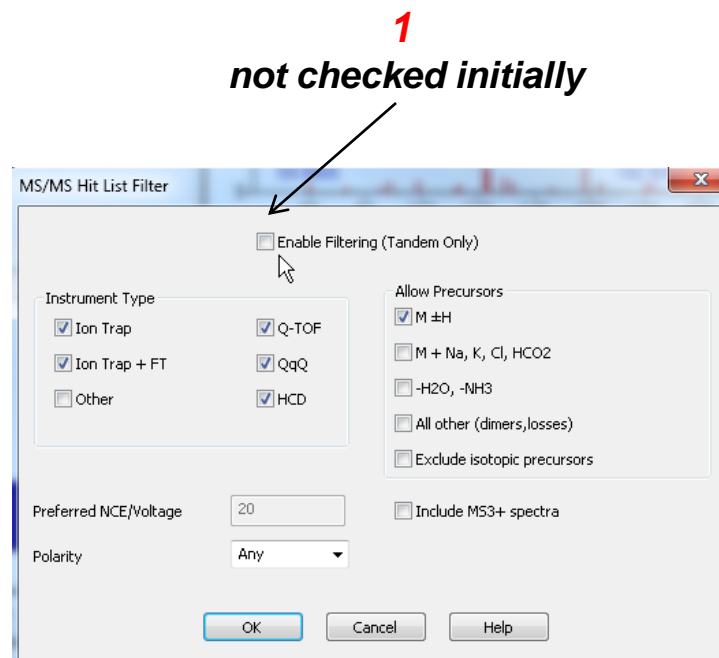
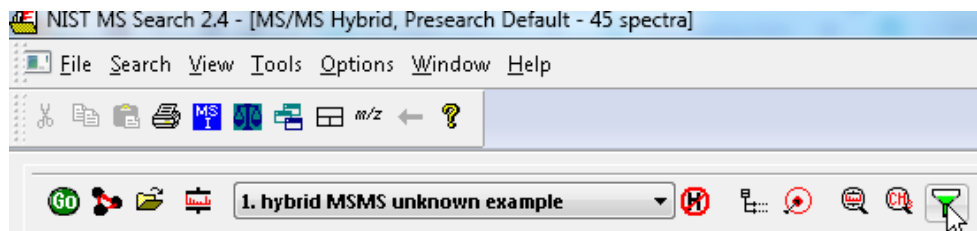
Critical Settings: Select the Libraries Used for *Exact Structure* Searches

- **Exact structure searches** are driven by libraries in **Spectrum Search**
- Somewhat surprising, but because InChIKey not actual structure used
- **LMB** on the **Libraries** tab and make sure **Spectrum Search** is selected
- Select the group of libraries to be searched by Exact Structure
- **>>Add>>** all libraries to be searched
- **Order** of libraries normally **only important** in Other Search Tab which return **maximum no.** of hits



Critical Settings: Turn Off Any Filtering Options Initially

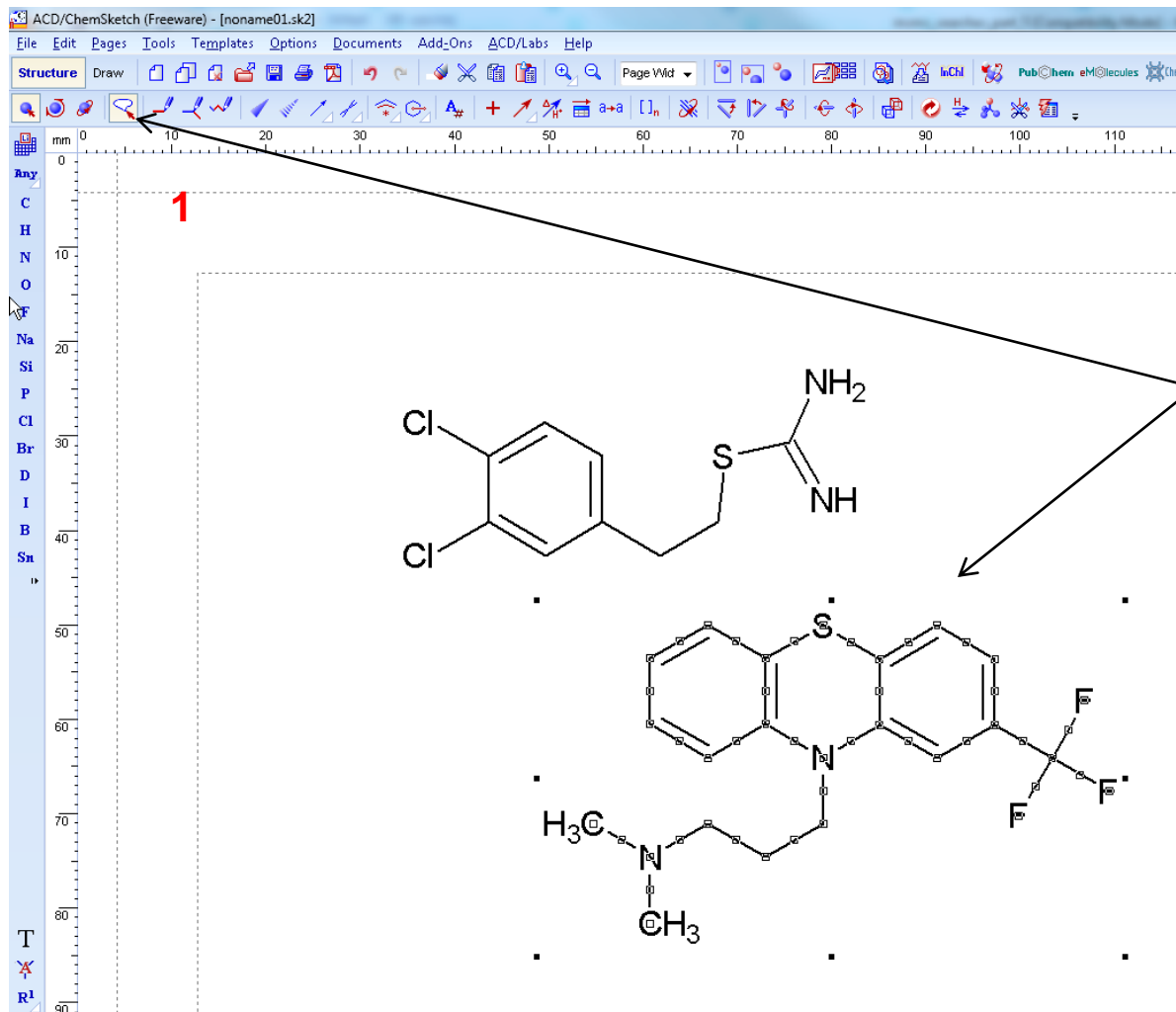
- **Initially** turn off any filtering options
- Make sure (1) “Enable Filtering” is **not** checked
- Make sure (2) “Best Matching Only” is **not pressed**
- Turn on **after** search if desired to limit
- These can be changed **without** performing search again



2
not depressed initially

NIST Structure Search

Obtaining Structure from ChemSketch



1) Use "lasso" tool to select structure to import into NIST program

2) Then copy into clipboard

ACD/ChemSketch (Freeware) - [noname01.sk2]

File	Edit	Pages	Tools	Templates	Options	Doc
Stru	Undo Remove Single Hydrogens	Ctrl+Z				
	Redo	Ctrl+Y				
	Cut	Ctrl+X				
	Copy	Ctrl+C				
	Paste					
	Delete	Del				
	Select All	Ctrl+A				
	Insert Object...					
	Edit Object					

10

NIST Structure Search

Pasting Structure into NIST Search

- **RMB** to select “Insert Clipboard Structure”
- Enter name if you desire, select OK
- Structure is added to “Spec List Box”

The screenshot shows the NIST MS Search 2.4 interface. At the top, the title bar reads "NIST MS Search 2.4 - [MS/MS Hybrid, Presearch Default - 100 spectra]". Below the menu bar (File, Search, View, Tools, Options, Window, Help), there is a toolbar with icons for file operations and a search box. A dropdown menu is open over a table with columns "#", "Src.", and "Name". The table contains one entry: "hybrid_demo_3; 444 total spectra". The context menu includes options like "Library Search", "Structure Similarity Search", "Cut", "Copy", "Paste", "Select All", "Send To", "Import", "Export Selected", "Insert Clipboard Structure" (highlighted), "Copy Structure to Clipboard", "Insert Clipboard Spectra", "Copy Selected to Clipboard", "Print", "Print Preview", and "Properties".

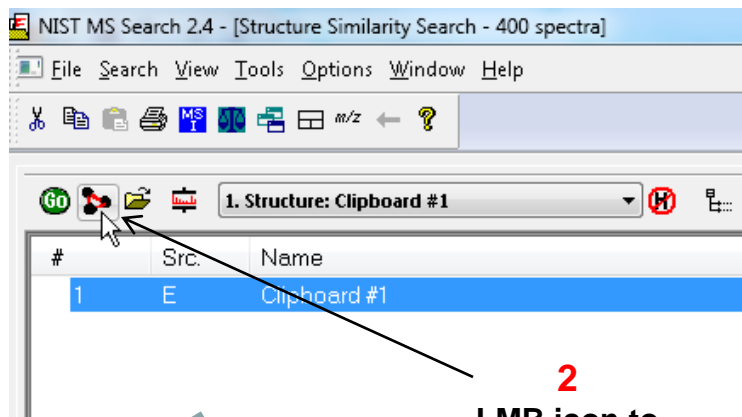
The "Structure" dialog box is shown, with a title bar "Structure" and a close button. It contains a "Name" field with the text "Clipboard #1", a "Comment" field, and a large area displaying a chemical structure of a benzothiazine derivative with a dimethylamino group and a trifluoromethyl group. At the bottom, there are "OK", "Cancel", and "Help" buttons. A mouse cursor is pointing at the "OK" button.

The screenshot shows the NIST MS Search 2.4 interface after the structure has been added. The table now has two entries:

#	Src.	Name
1	E	Clipboard #1

Search for Similar Structures after “Insert Clipboard Structure”

- 1) **LMB** on structure search **icon** to search **after** highlighting entry
- 2) **LMB** structure search icon
- 3) Results reported in lower left window **sorted** by similarity (1000 good fit)



1
LMB to highlight
desired entry

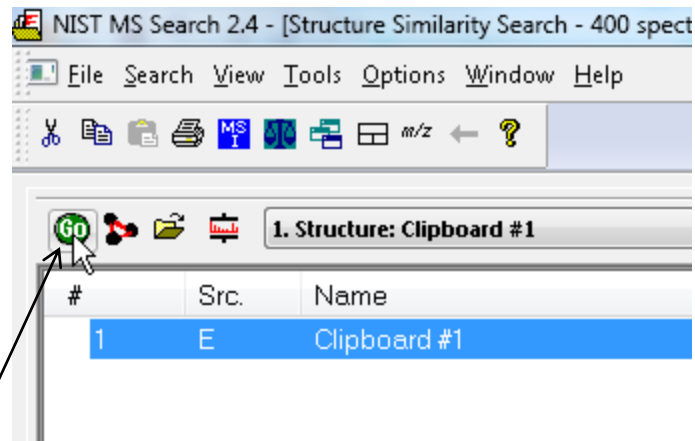
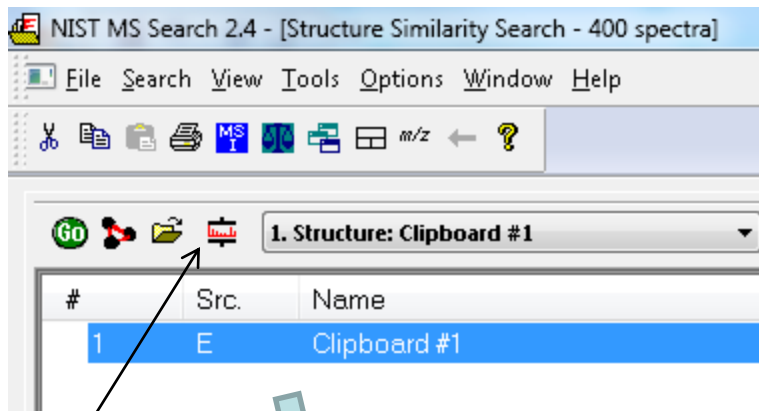
2
LMB icon to
perform search

3
Results of search

#	Lib.	DotProd	DeltaMass	Prec. Type	Energy	Name
1	hr	1000	0	[M+H] ⁺	30%	Triflupromazine [M+H] ⁺ HCD 30% P=3..
2	hr	1000	0	[M+H] ⁺	130%	Triflupromazine [M+H] ⁺ HCD 130% P=..
3	hr	1000	0	[M+H] ⁺	40%	Triflupromazine [M+H] ⁺ HCD 40% P=3..
4	hr	1000	0	[M+H] ⁺	45%	Triflupromazine [M+H] ⁺ HCD 45% P=3..
5	hr	1000	0	[M+H] ⁺	25V	Triflupromazine [M+H] ⁺ QTOF 25V P=...
6	hr	1000	0	[M+H] ⁺	35%	Triflupromazine [M+H] ⁺ HCD 35% P=3..
7	hr	1000	0	[M+H] ⁺	160%	Triflupromazine [M+H] ⁺ HCD 160% P=..
8	hr	1000	0	[M+H] ⁺	4V	Triflupromazine [M+H] ⁺ QTOF 4V P=3..
9	hr	1000	0	[M+H] ⁺	27V	Triflupromazine [M+H] ⁺ QTOF 27V P=...

Search for Exact Structures

- 1) **LMB** on **Library Search Options**
- 2) MS/MS Hybrid show DeltaMass, if not zero, indicates MS³ or MS²+2 isotope was origin of the same structure
- 3) **Select** Search tab and select **InChIKey**, then **OK**
- 4) **Click** on **Library Search not Structure Search** icon
- 5) Process results using tips used for similarity search



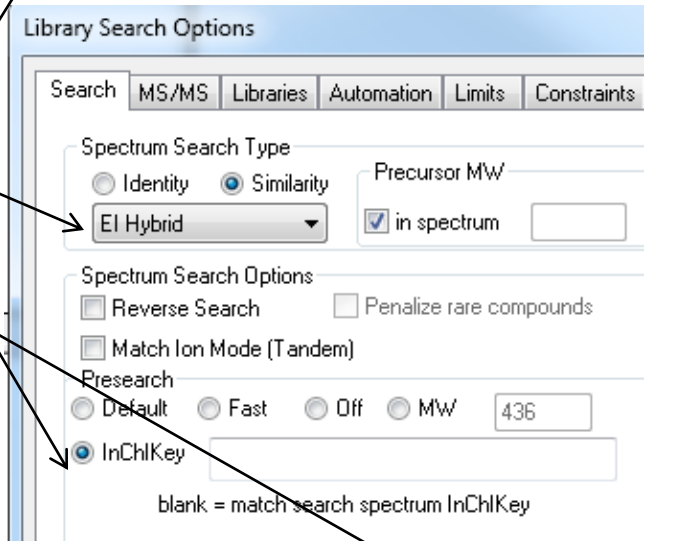
3

“Go” icon, *not* “structure icon”

1

2

3



#	Lib.	Match	Prob. (%)	RI	Name
1	M	0	20.0	-	Acetone-D6
2	M	0	20.0	-	Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone

4

Tips for Displaying Structure Search Results

- 1) **RMB** on display box and select properties
- 2) add **delta mass** to table for either Exact or Similar search
- 3) **run** similarity search again and then **sort** by clicking on DeltaMass header
- 4) **Scroll down** until group with DeltaMass=0 found

Distance RI Name

1000	267	Formaldehyde
1000	-	Carbon monoxide
1000	401	Acetaldehyde
1000	487	Acetone
1000	-	Acetone
1000	598	2-Butanone
1000	688	3-Pentanone
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde

Library Search Properties

Hit Text Info

Spec List Text Info

Hits List Spec List Plot of Hit

Structure View Options

Structure Size in %: 50

Structures Only

Items to Display

Distance Str

DeltaMass Str

R.Match EI

Match no RI EI

Short Library Name

Tip 1: When reviewing search results, use up and down arrows on keyboard to quickly step through results!

#	Lib.	DotProd	Distance	RI	DeltaMass	Name
25	w1	1000	1000	408	14	Acetaldehyde
26	w1	1000	1000	408	14	Acetaldehyde
27	M	1000	1000	487	0	Acetone
28	w1	1000	1000	455	0	Acetone
29	w1	1000	1000	455	0	2-Propanone
30	w1	1000	1000	455	0	2-Propanone
31	w1	1000	1000	455	0	2-Propanone
32	w1	1000	1000	455	0	2-Propanone
33	w1	1000	1000	455	0	2-Propanone
34	w1	1000	1000	455	0	2-Propanone

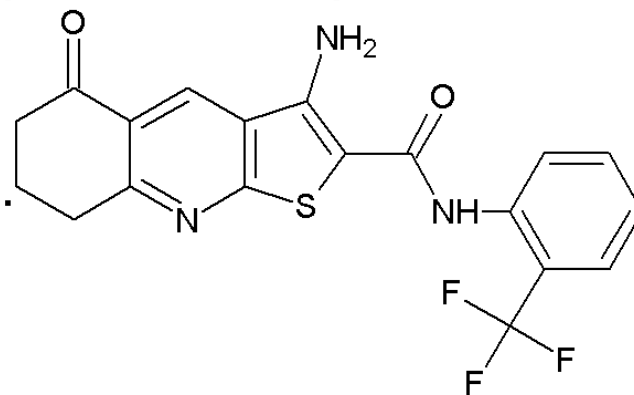
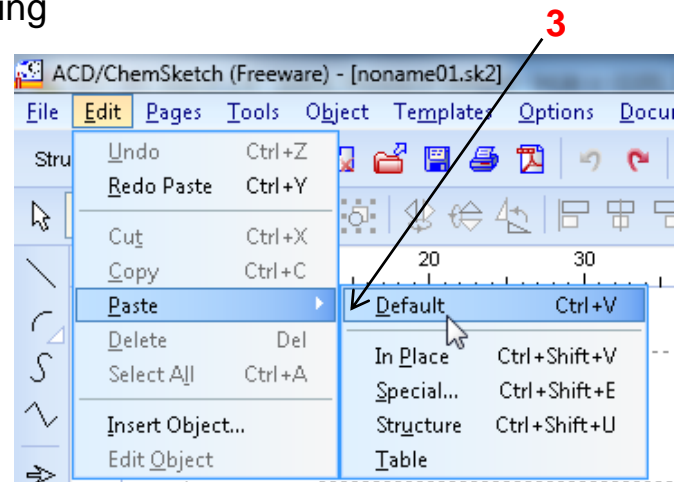
Reverse Process: Sending Structure from MS Search to Drawing Program

1. Any structure in MS Search Program can be sent to drawing program
2. **LMB** on entry, then **RMB** to "**Copy Structure to Clipboard**"
3. Then paste from Windows Clipboard into Drawing Program
4. Useful for modifying an existing structure for searching

#	Lib.	Match	Prob. (%)	Name
1	w1	593	53.1	1-iodo-4-isocyanatobenzene
2	M	568	16.2	3-Hydroxy-4-iodobenzonitrile
3	w1	530	3.88	3-Amino-5-keto-NH2-(trifluoromethyl)phe...
4	M	522		Library Search pyrimidine
5	w1	521		Structure Similarity Search ylthio)-4-(4-hydro...
6	M	512		benzoate
7	w1	512		ro-5-methyl-N[3...
8	M	511		idole
9	M	511		ro-5-methyl-N[3...
1..	M	511		nitrile
1..	M	508		c)pyridine
1..	M	504		benzoate
1..	M	497		
1..	R	490		
1..	M	484		-dicyanophenyl)-
1..	w1	484		-1-yl)phthalonitrile
1	M	480		l)-1,3-benzoxaz...

Lib. Search Other Search Name

2



Using “Compare” Window to Display Multiple Spectra

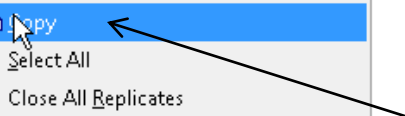
- 1) **Select** three spectra, e.g., from your exact search of acetone using control **left mouse click** while **holding control key** on keyboard
- 2) **RMB** somewhere within **blue highlighted area** and select **copy with left mouse** button

#	Lib.	Match	R.Match	o.Match	o.R.Match	DeltaMass	Name
1	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 3V P=3...
2	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 4V P=3...
3	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 5V P=3...
4	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 7V P=3...
5	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 10V P=...



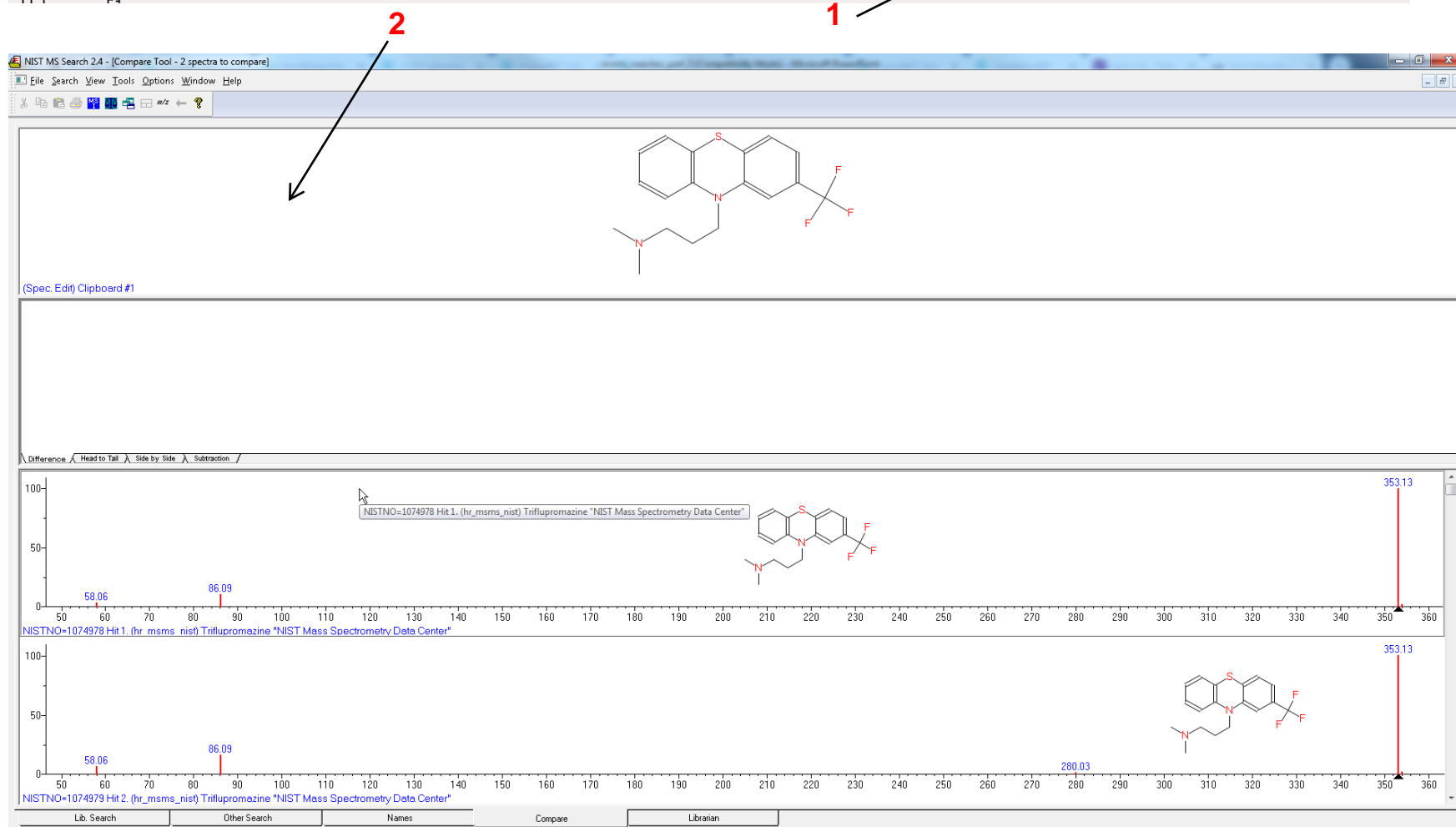
#	Lib.	Match	R.Match	o.Match	o.R.Match	DeltaMass	Name
1	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 3V P=3...
2	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 4V P=3...
3	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 5V P=3...
4	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 7V P=3...
5	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 10V P=...
6	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 15V P=...
7	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 15V P=...
8	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 20V P=...
9	hr	0	0	0	0	0	Triflupromazine [M+H] ⁺ QTOF 20V P=...

- Library Search
- Structure Similarity Search
- Copy**
- Select All
- Close All Replicates
- Export Selected
- Send To

2

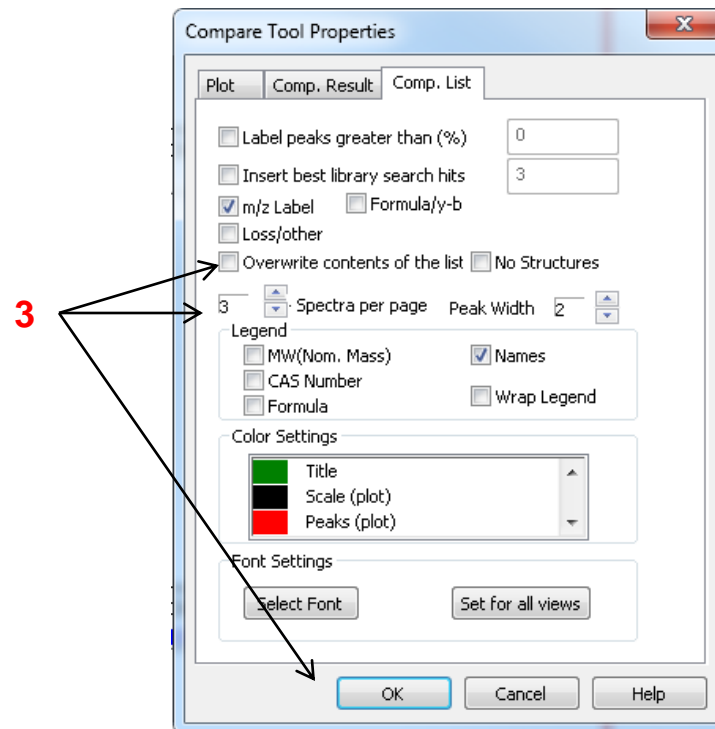
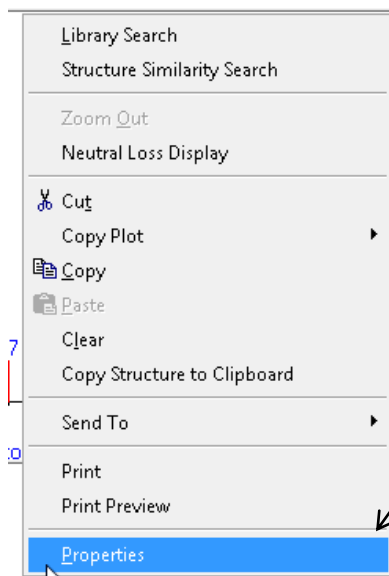
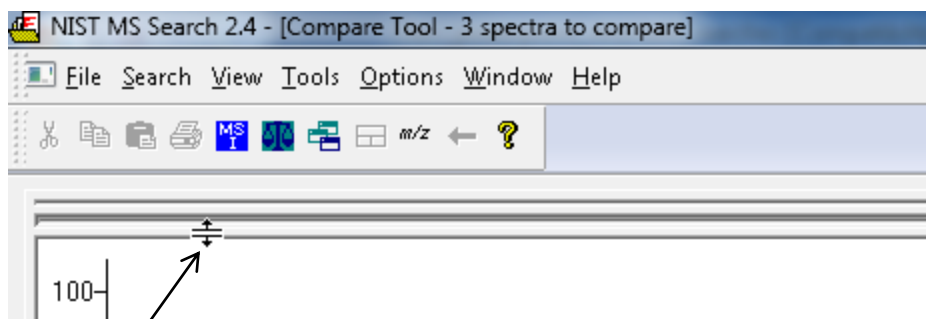
Using “Compare” Window to Display Multiple Spectra

- 1) Select **Compare** tab at bottom of program
- 2) Normally setup to transfer the 3 best library hits for comparison as shown below



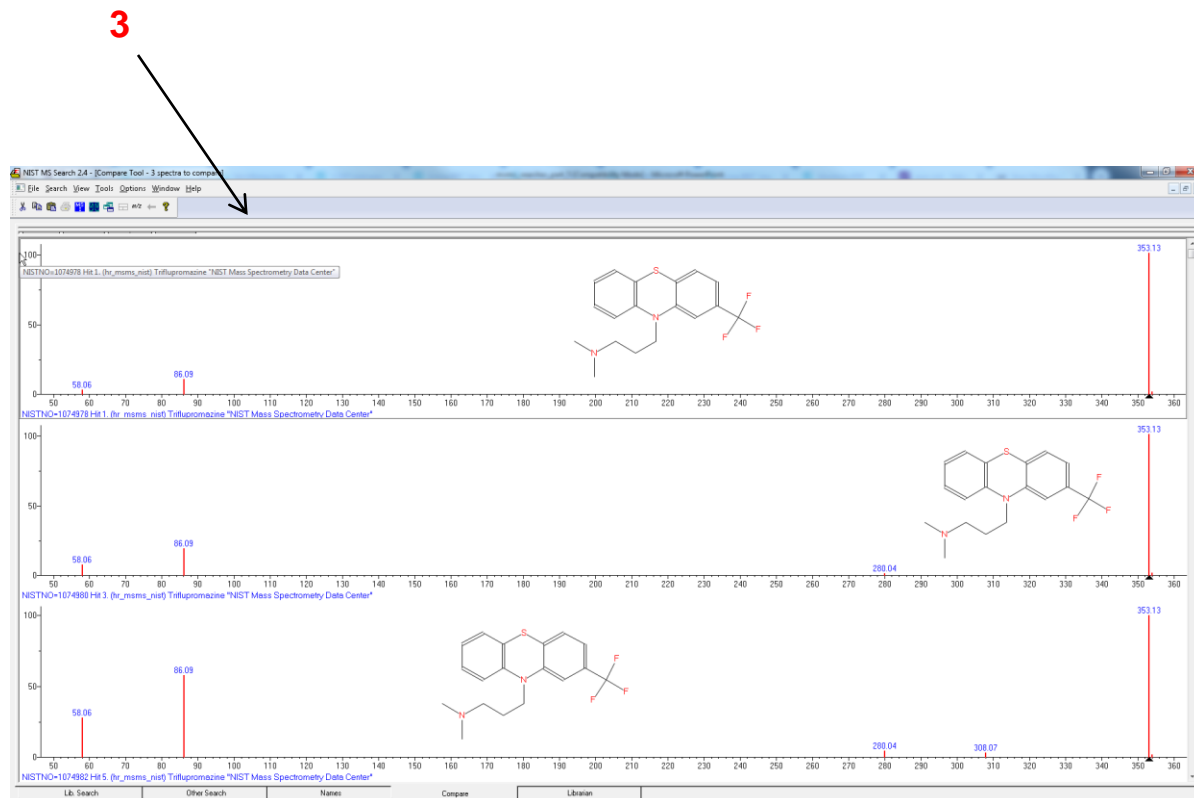
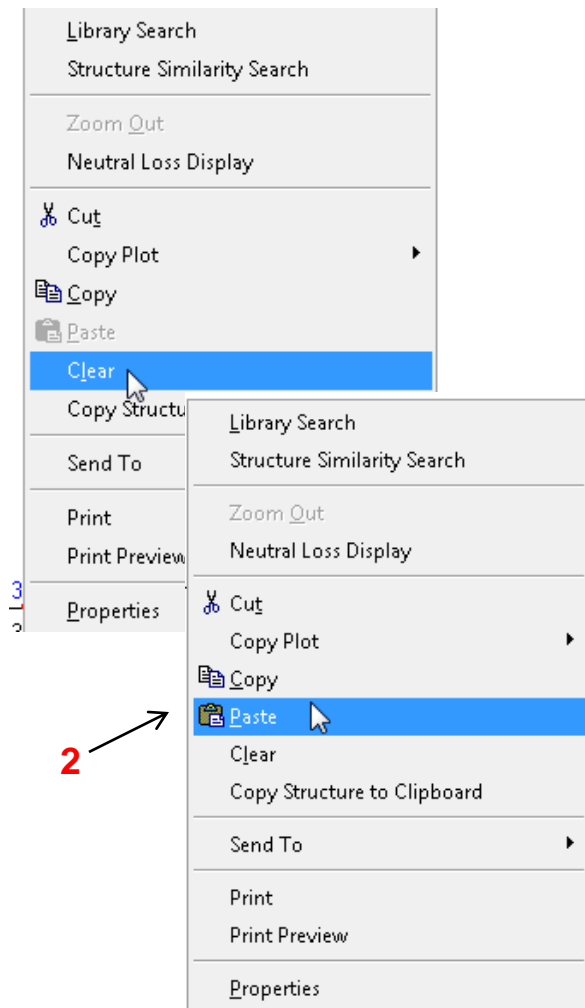
Using “Compare” Window to Display Multiple Spectra

- 1) **Compress top two** windows by **selecting bar** with left mouse click and **scrolling closed** with mouse
- 2) Select prosperities window in bottom windows and then select **Properties** with **LMB**
- 3) Setup options as shown below (**turn off** “Overwrite contents”)



Using “Compare” Window to Display Multiple Spectra

- 1) **Go to Compare window** by selecting **tab at bottom** of Search Display Window
- 2) **RMB** in window and then **LMB** on **Clear** to delete spectra present
- 3) **RMB spectra** selected in slide 15 **should appear** in window for comparison



Sending Structures Directly to Drawing Program

NOTE: Tried this, but didn't always transfer, the "Default Structure Editor" location associated with Mol files by Windows worked better in the pull down menu than the one I assigned to "ChemSketch"!

If you want to send the structure to more than one program, you must create a text file named autoimp.str and place it in the same folder as the NISTMS search program. Make sure you display files with extension and the file is truly named autoimp.str and NOT autoimp.str.txt! The file must contain the following general type command line:

```
"Name" "Fully extended file name" "%1"
```

In my particular case, I created the following autoimp.str file:

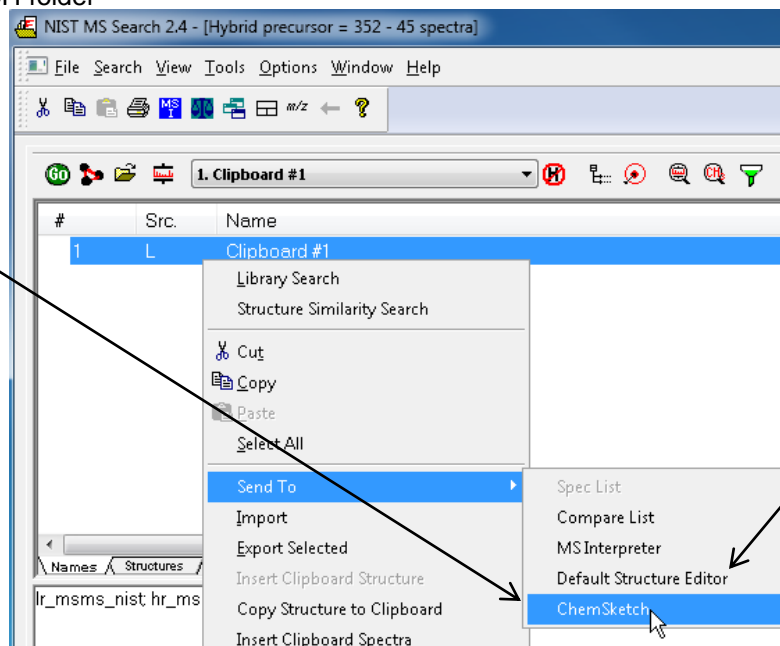
```
"ChemSketch" "C:\ACDFREE8\CHEMSK.EXE" "%1"
```

I actually found this file already present on my drive in my ChemSketch folder. I am **not sure** who created it and placed it there?

I made a copy and placed it in my NIST20/MSSEARCH folder

Now can use "Send To" "ChemSketch".

Now select entry of interest with **LMB** and Then **RMB** and select to send. ChemSketch Program will **open automatically** and the structure will be transferred.



NOTE: This worked better!

***Live Demo* on YouTube**
LC/MS Unknown Identifications Using MSMS Libraries
Part V: NIST Structure Searches

Presentation References (*Internet Links*)

1. [James Little Mass Spectral Resource Website](#)
2. [NIST Search Software Detailed Manual](#)
3. [Chemical Ionization for MW Determination](#)
4. [Trimethylsilyl Derivatives for GC-MS](#)
5. [Methyl Ester Derivatives for GC-MS](#)
6. [SciFinder/ChemSpider and Accurate Mass LC-MS Data for Unknown ID's](#)
7. [Surfactant Identification](#)
8. [Lipid Matrix Ionization Effects in LC-MS](#)

Acknowledgements

- Stephen Stein (NIST)
- David Sparkman (NIST Contractor)