LC/MS Unknown Identifications Using MSMS Libraries
Part V: NIST Structure Searches
12/27/20

James Little
tvasailor@gmail.com
https://littlemsandsailing.com/
Kingsport, TN

- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties: EI GC-MS, LC-MS/MS, Chemical Ionization, Accurate Mass, Derivatization, MS library management, SciFinder, Chemspider, Surfactant ID, NMR, GC-IR, organic synthesis, matrix ionization effects, etc.*

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

>50 Mass Specs Networked
Worldwide

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

Table of Contents

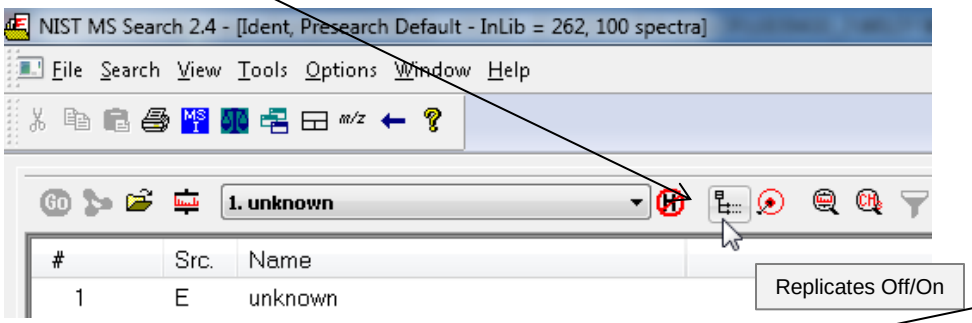
Topic	Slide #
Introduction of Presenter, James Little	1
Table of Contents	2
Series Topics	3
Help Files for NIST Search	4
NIST Software in General is “Windows Compliant”	5
Session Topics and Choice of Drawing Program	6
Critical Settings for Similar Structure Searches	7
Critical Settings for Exact Structure Searches	8
Critical Settings Turn Off Any Filtering Options Initially	9
Obtaining Structure from Drawing Program	10
Pasting Structure into NIST Search	11
Search for Similar Structures after Inserting Clipboard Structure	12
Search for Exact Structures	13
Tips for Displaying Similar and Exact Search Result with DeltaMass	14
Reverse Process: Sending Structures from NIST to Drawing Program	15
Using Compare Window to Display Multiple Spectra	16
Sending Structures Directly to Drawing Program	20
Live Demo YouTube Presentation	21
Presentation References (Internet Links)	22
Acknowledgements	23

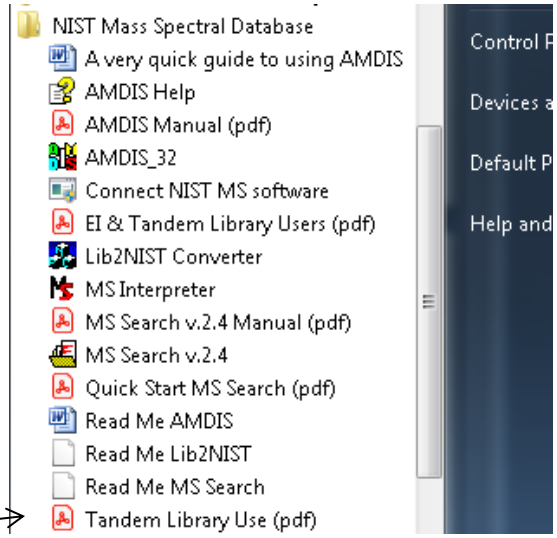
LC/MS Unknown Identifications Using MSMS Libraries

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
Part IX: Using Agilent MassHunter with NIST MSMS Search

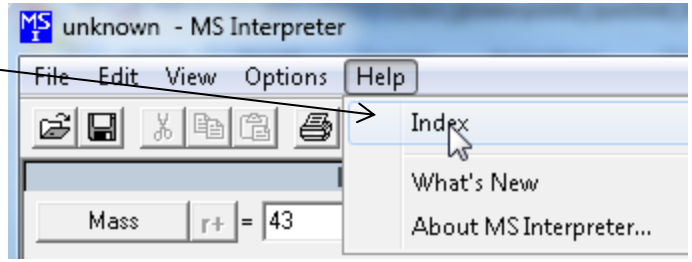
Help Files for NIST Search

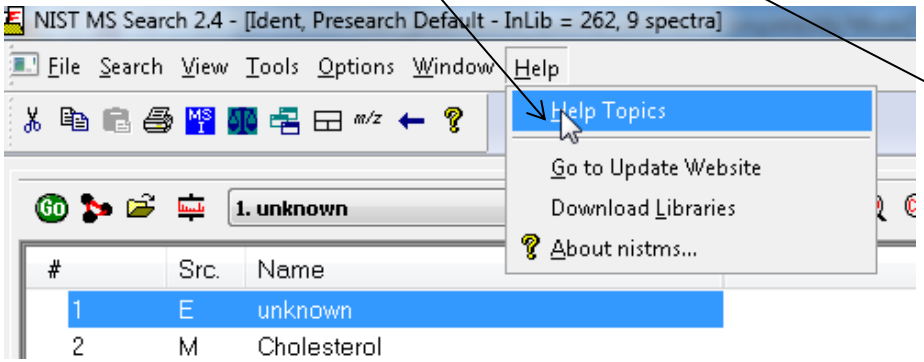
-"Hover" over Program Icon with mouse and function description displayed

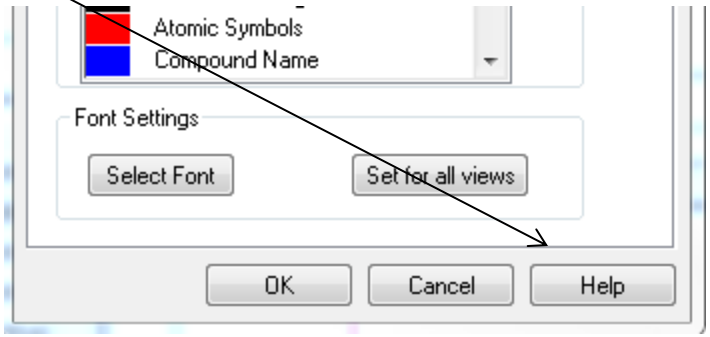




-***Detailed*** documentation for NIST Search[8]
-MS Interpreter included in NIST manual[8]
-Windows Program Group
-NIST Tandem Search ***Quick Start Guide***[9]
-"In program" assistance for both programs
-***Help button*** at corner of all windows



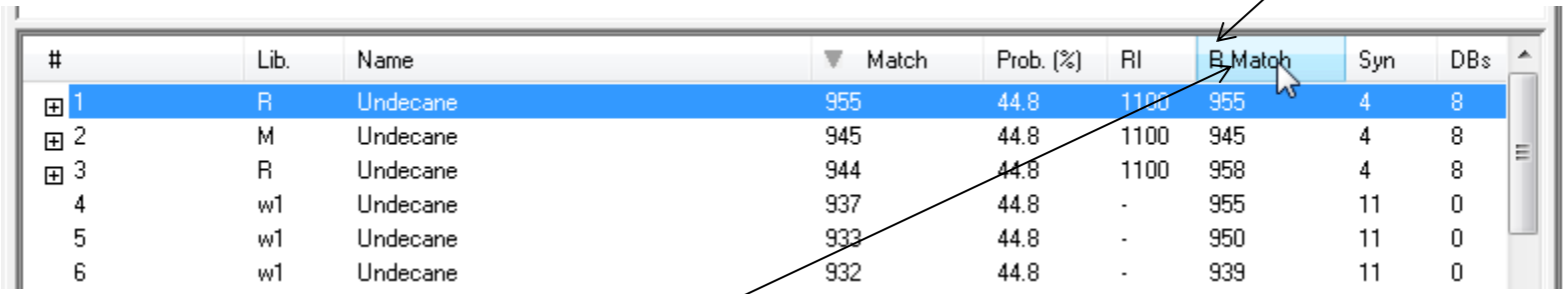




NIST Software in General is "Windows Compliant"

-***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	R.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

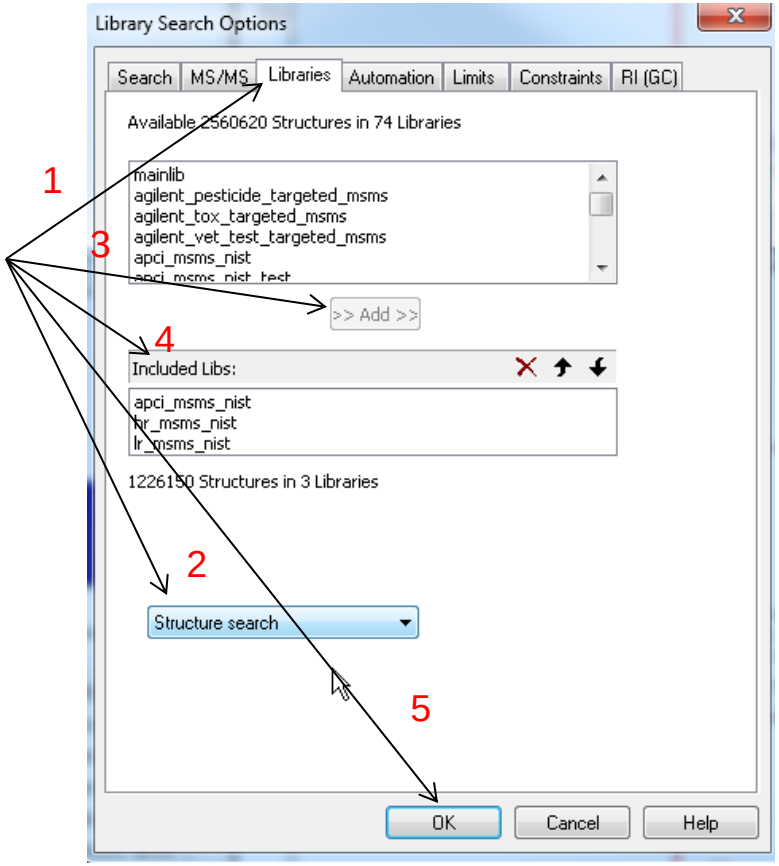
- ***Many*** Different Drawing Programs can be employed[2]
- Choose one that you ***normally*** use

Session Topics:

-Setting up ***Library Search Options*** for Structure Searches
-Importing structures from vendor programs[2]
-Searching for ***similar*** structures using ***Structure Search***
-Searching for ***exact*** structure using ***Library Search*** with InChIKey
-Using “Compare” window to view series of spectra

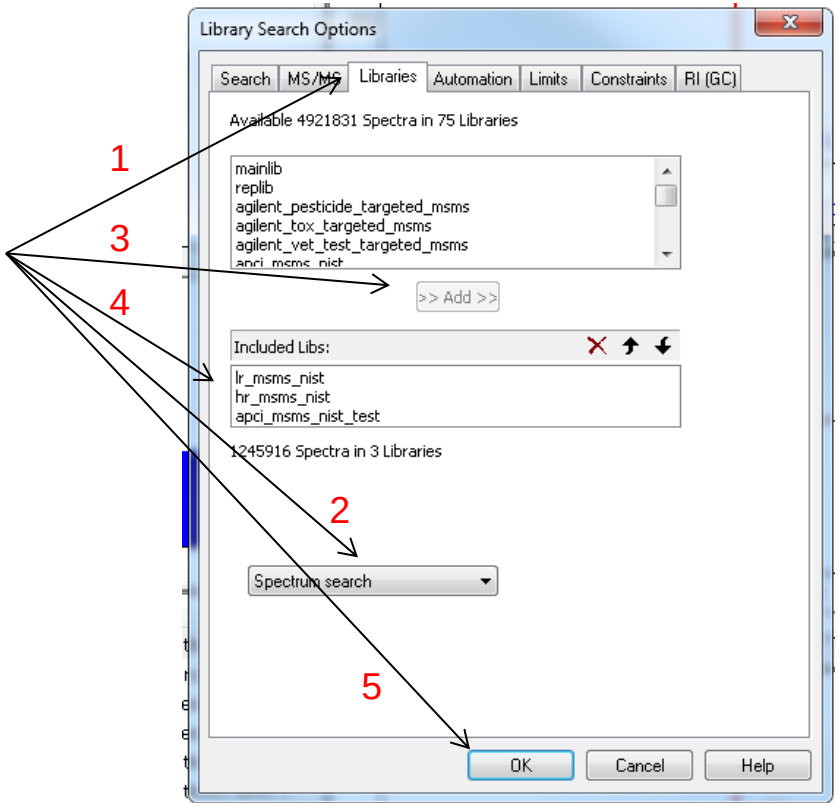
Critical Settings: Select the Libraries Used for *Similar Structure* Searches

- ***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



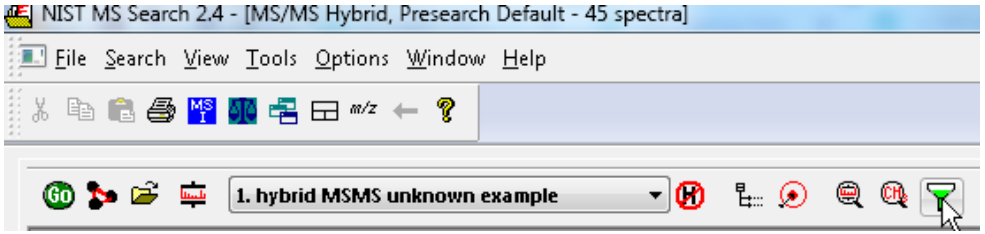
Critical Settings: Select the Libraries Used for *Exact Structure* Searches

- ***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



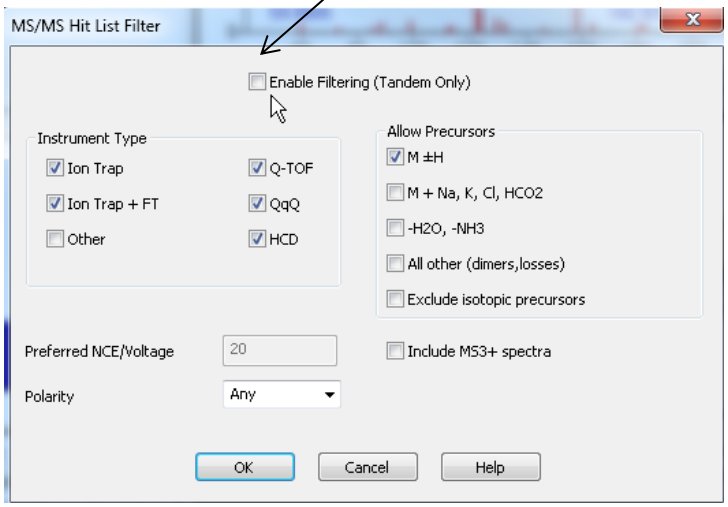
Critical Settings: Turn Off Any Filtering Options Initially

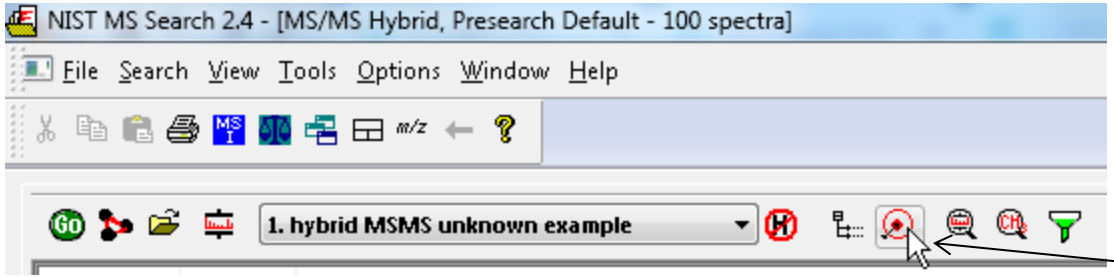
- ***Initially*** turn off any filtering options
- Make sure (***1***) "Enable Filtering" is ***not*** checked
- Make sure (***2***) "Best Matching Only" is ***not pressed***
- Turn on ***after*** search if desired to limit
- These can be changed ***without*** performing search again



1

not checked initially



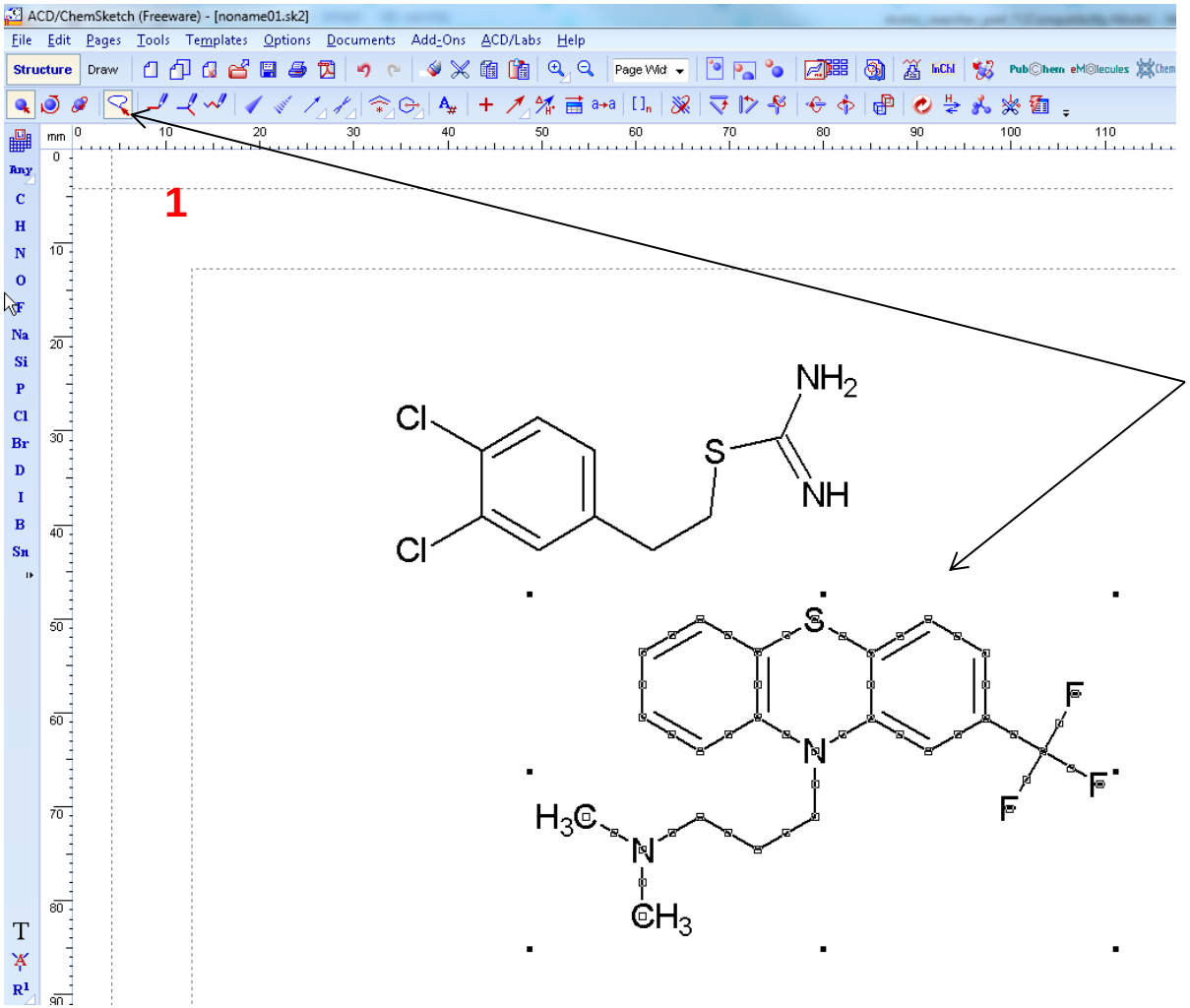


2

not depressed initially

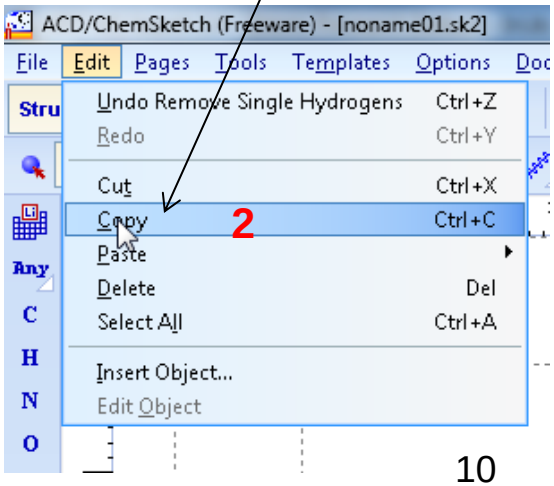
NIST Structure Search

Obtaining Structure from ChemSketch



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

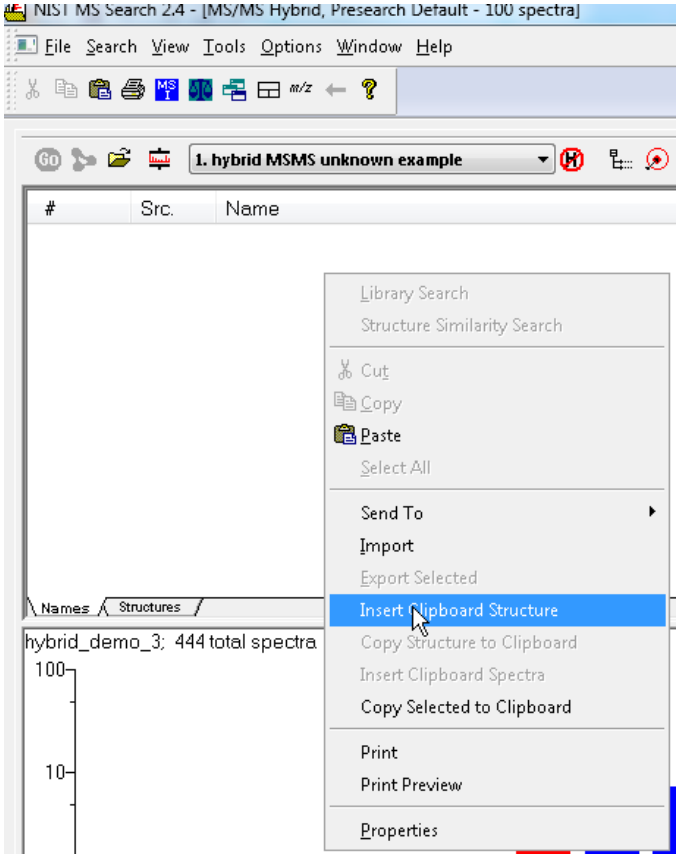
2) Then copy into clipboard

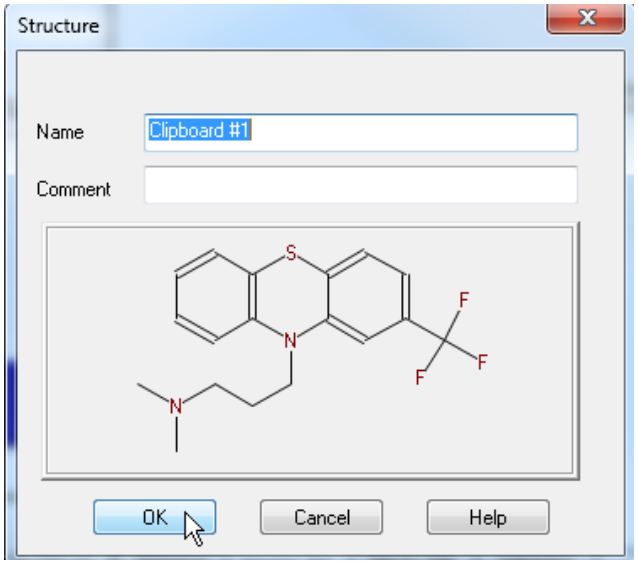


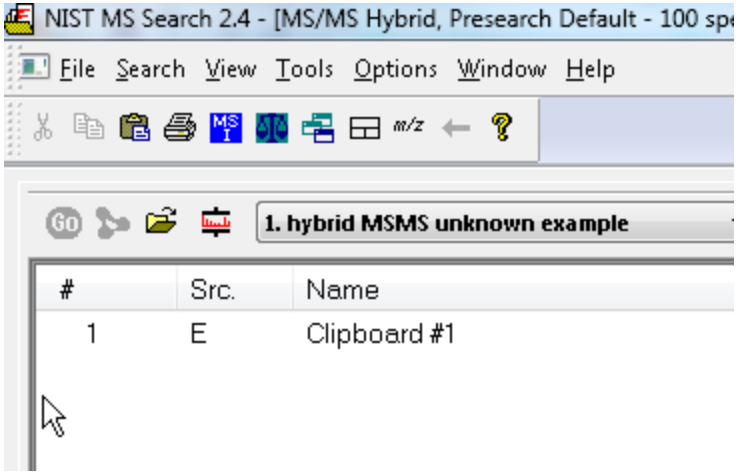
NIST Structure Search

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”

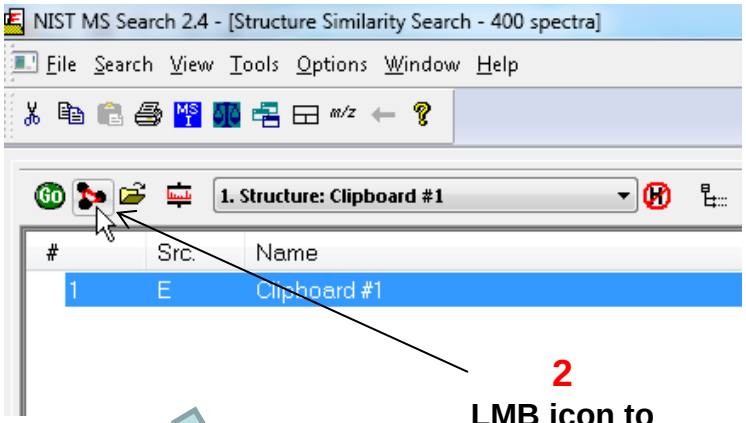






Search for Similar Structures after “Insert Clipboard Structure”

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

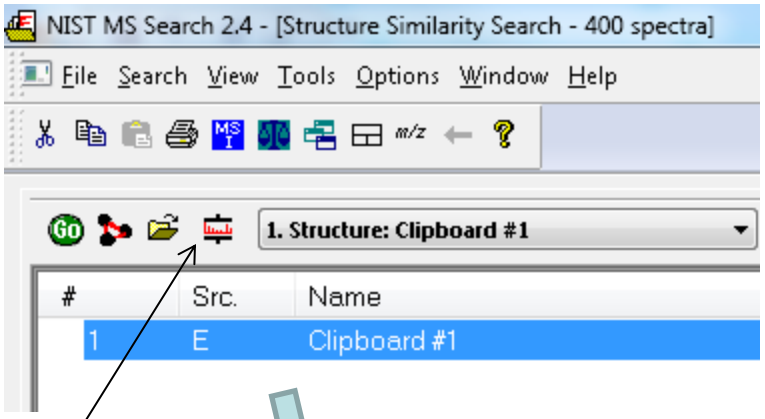
2
LMB icon to perform search

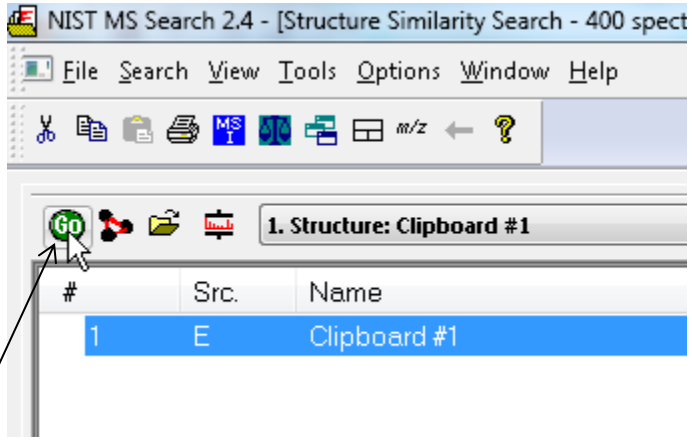
3
Results of search

#	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 MS^3 or MS^2+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



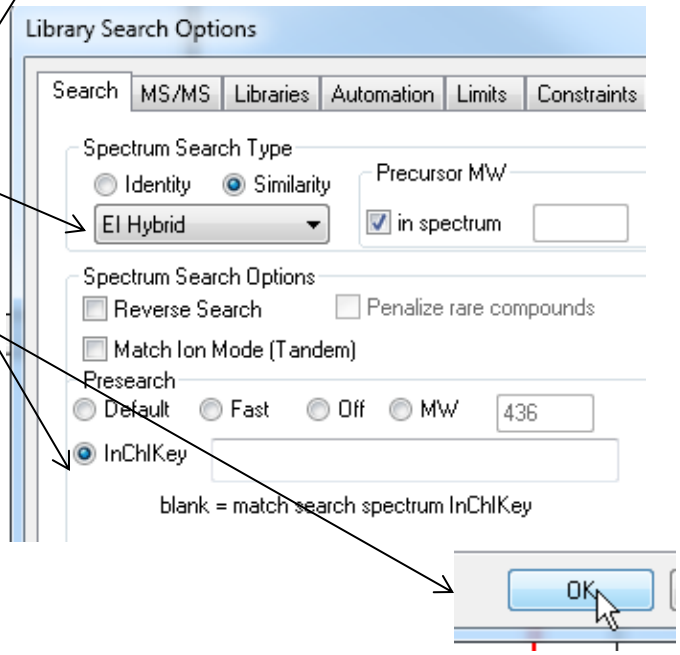


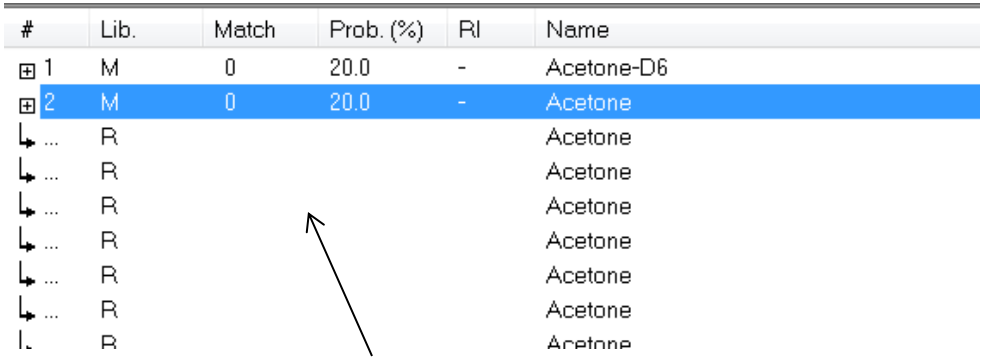
3
"Go" icon, ***not*** "structure icon"

1

2

3

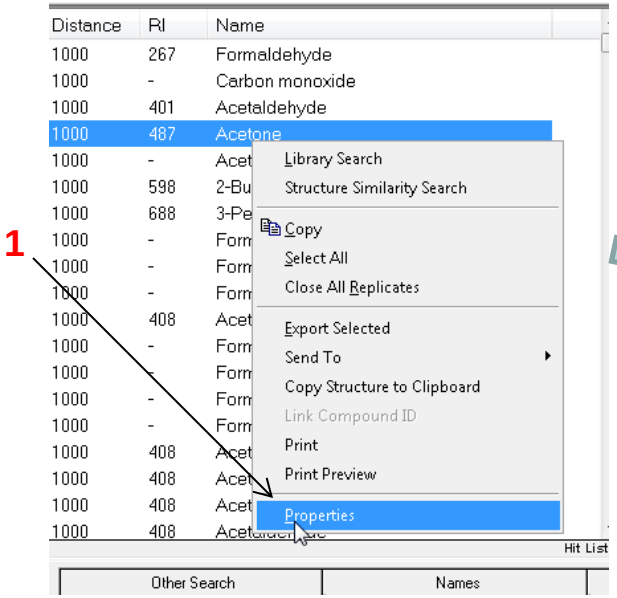




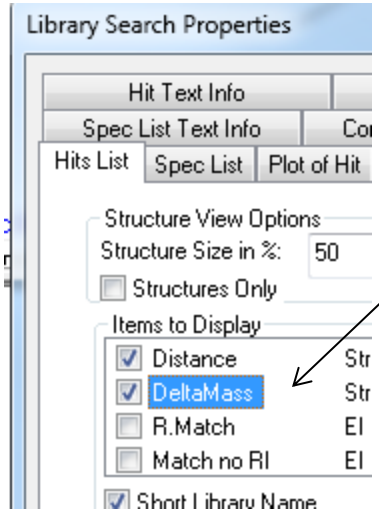
4

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 seach
3) ***run*** similarity search again and then ***sort*** by clicking on DeltaMass header
4) ***Scroll down*** until group with DeltaMass=0 found

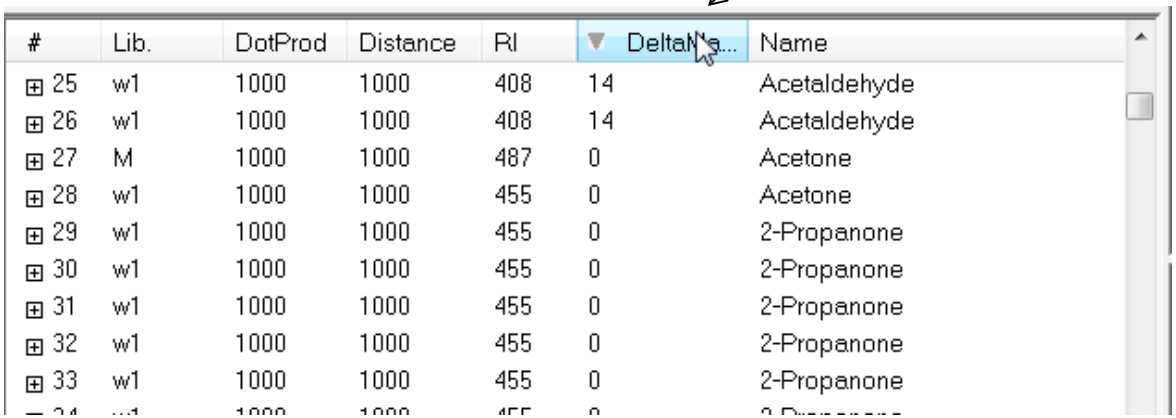


1



2

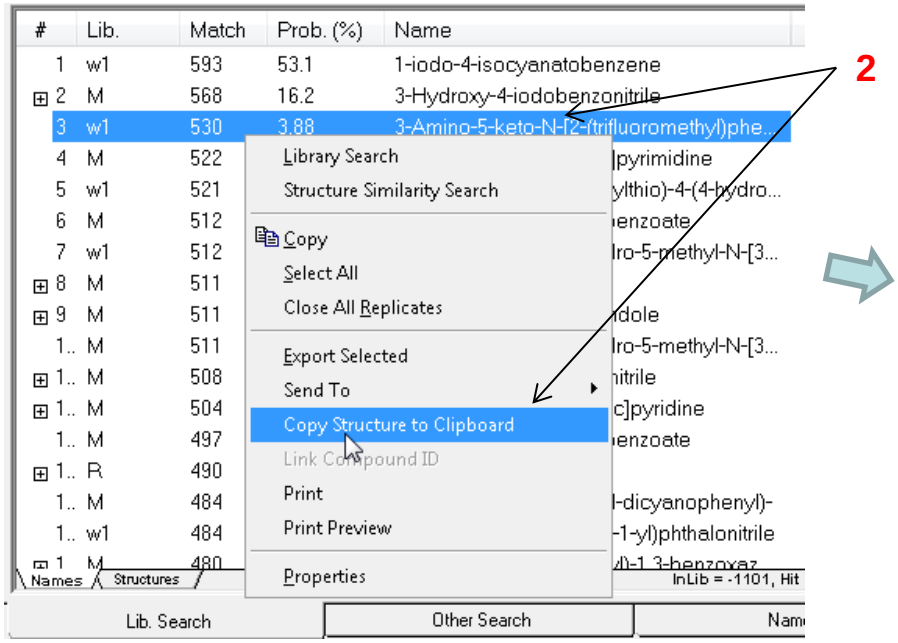
3

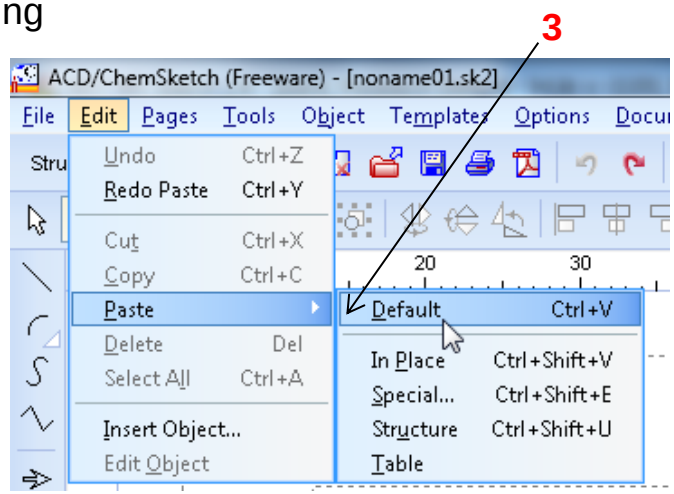


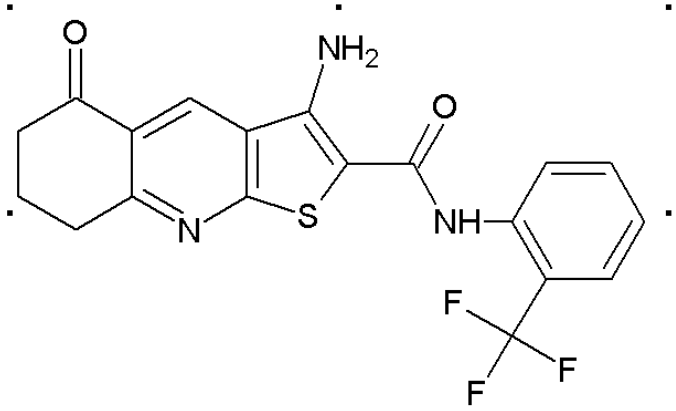
Tip 1: When reviewing search results, use up and down arrows on keyboard to quickly step through results!

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

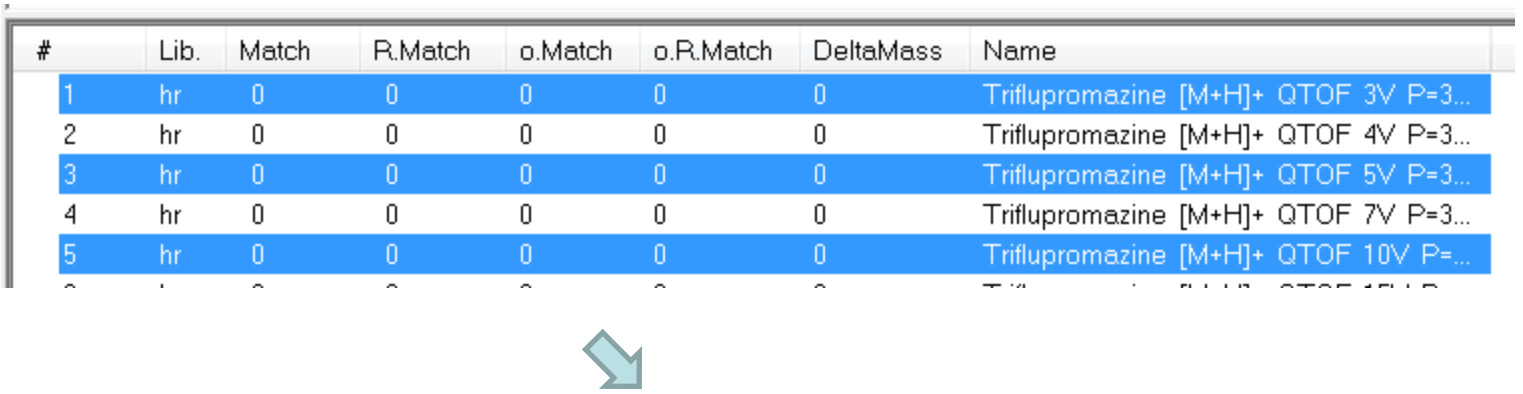




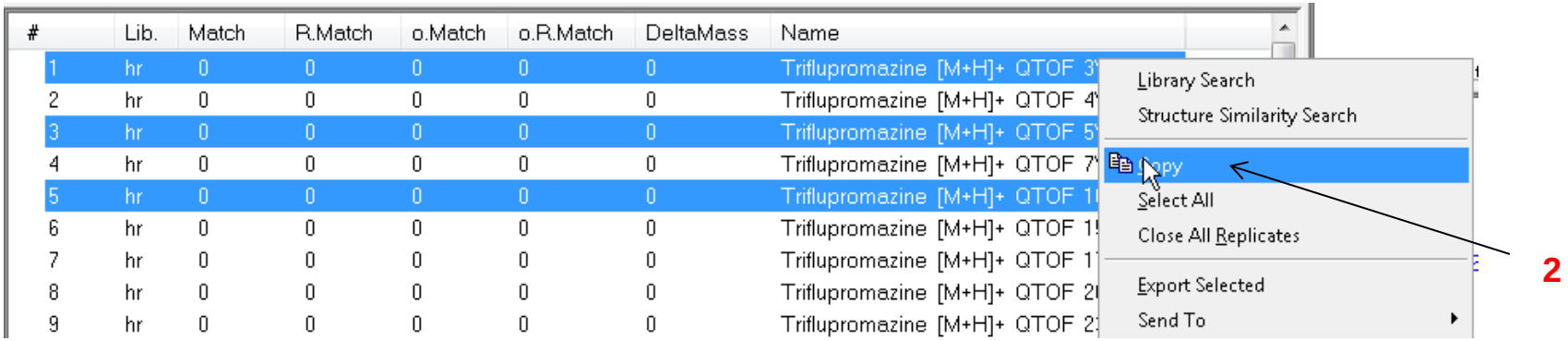


Using *"Compare"* Window to Display Multiple Spectra

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

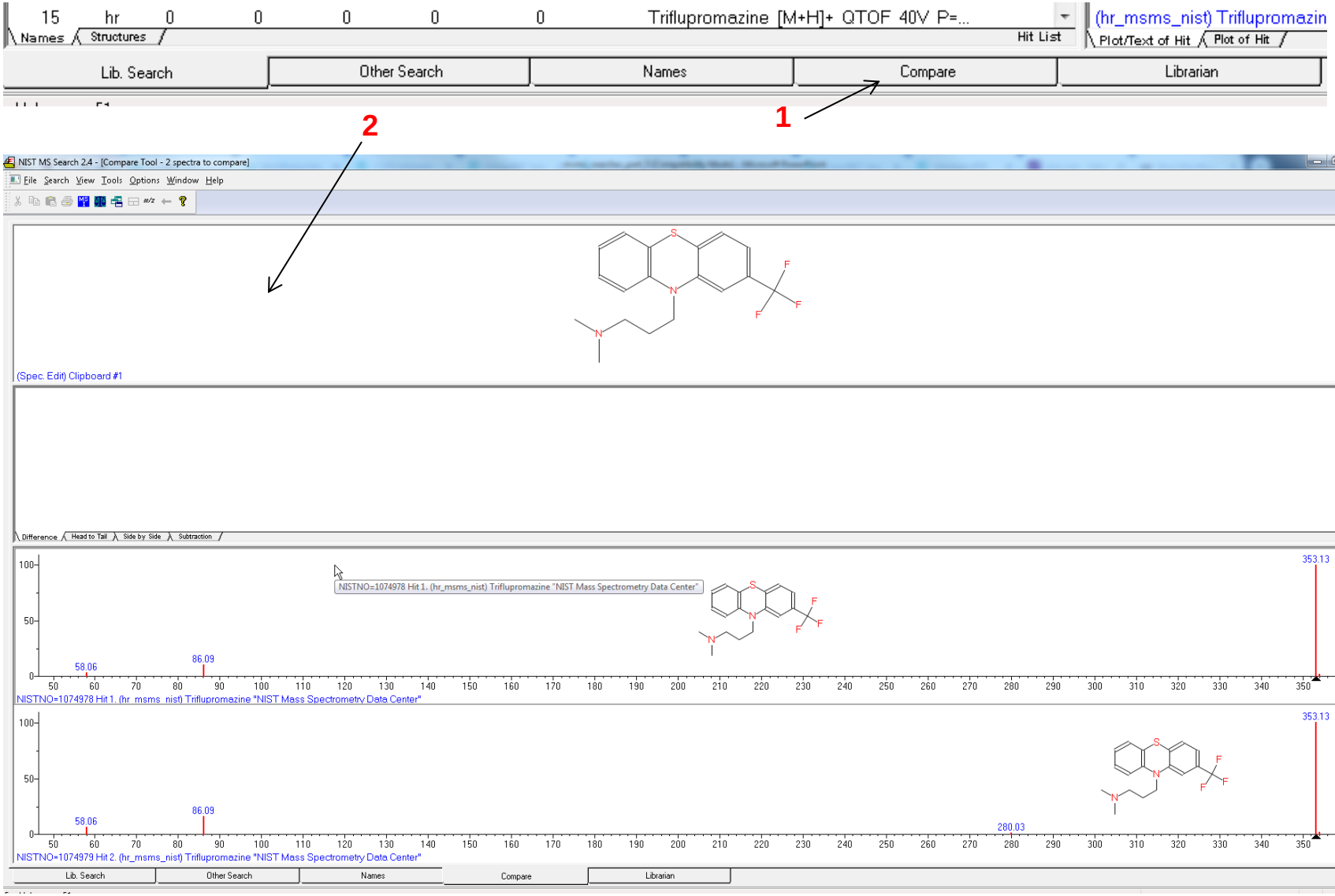


#	Lib.	Match	R.Match	o.Match	o.R.Match	DeltaMass	Name
1	hr	0	0	0	0	0	Triflupromazine [M+H]+ QTOF 3V P=3...
2	hr	0	0	0	0	0	Triflupromazine [M+H]+ QTOF 4V P=3...
3	hr	0	0	0	0	0	Triflupromazine [M+H]+ QTOF 5V P=3...
4	hr	0	0	0	0	0	Triflupromazine [M+H]+ QTOF 7V P=3...
5	hr	0	0	0	0	0	Triflupromazine [M+H]+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



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

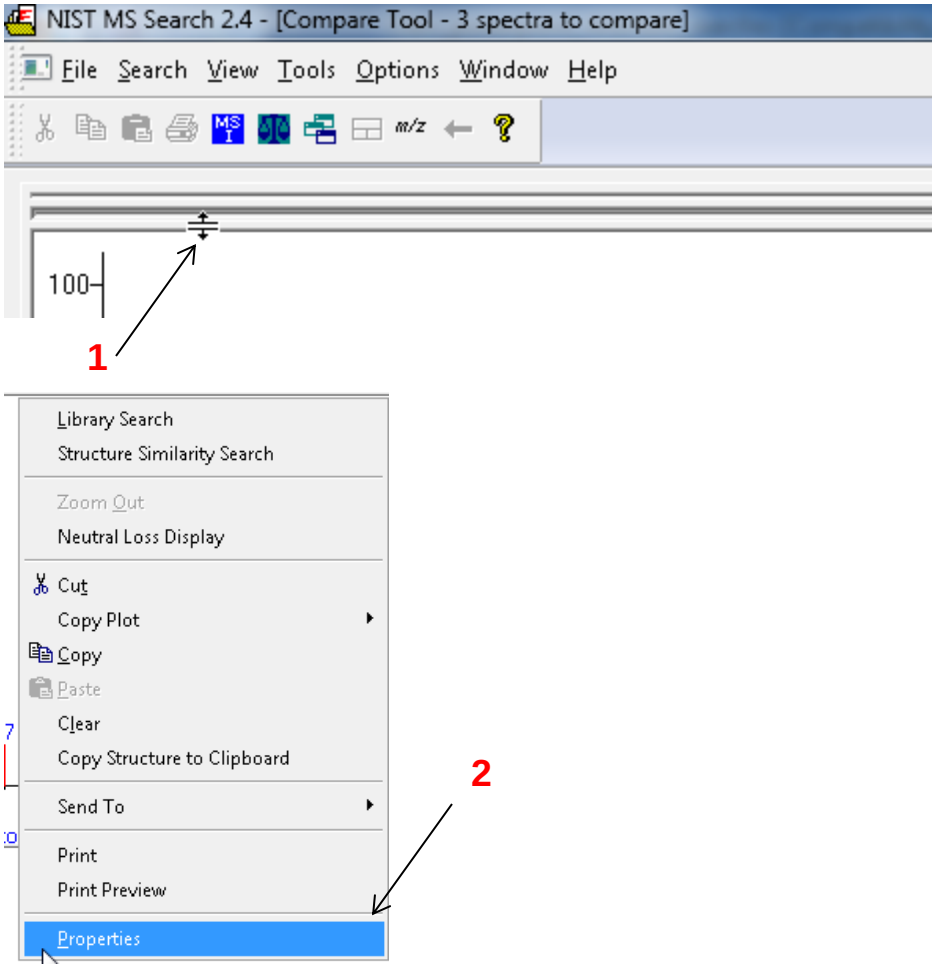


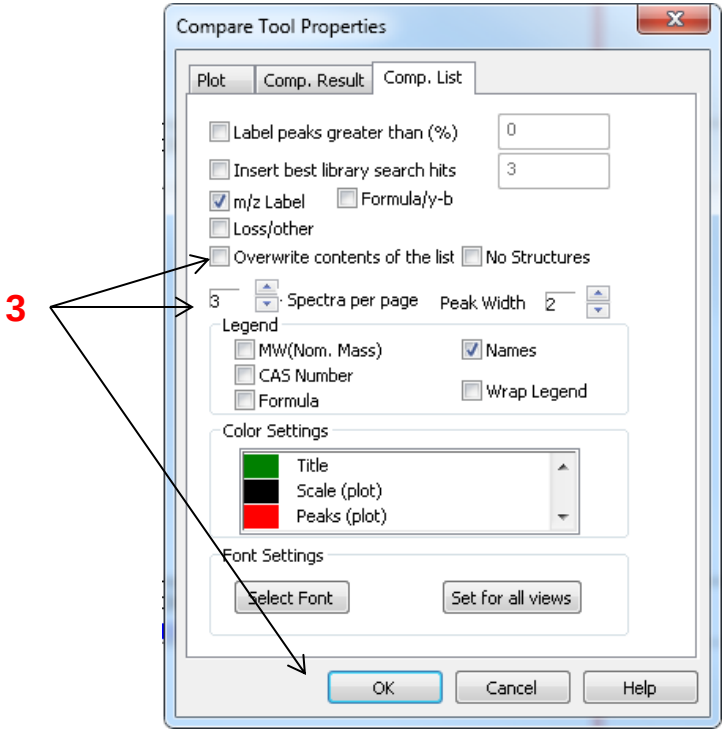
Using *"Compare"* Window to Display Multiple Spectra

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***

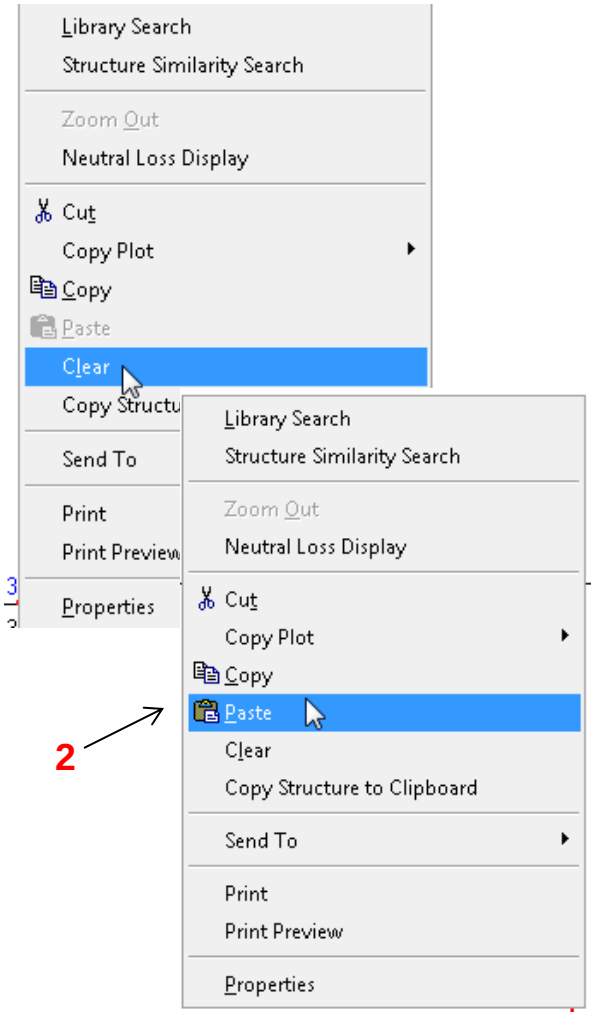
3) Setup options as shown below (***turn off*** "Overwrite contents")

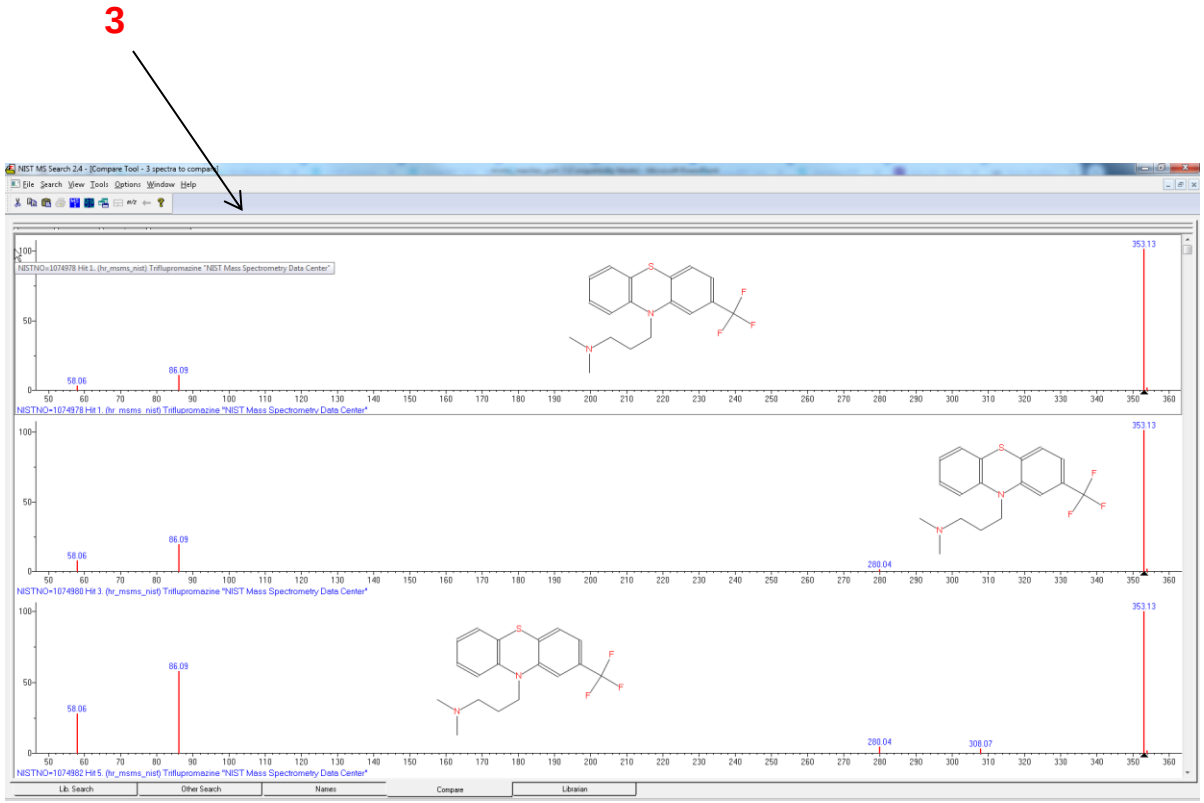




Using *"Compare"* Window to Display Multiple Spectra

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:

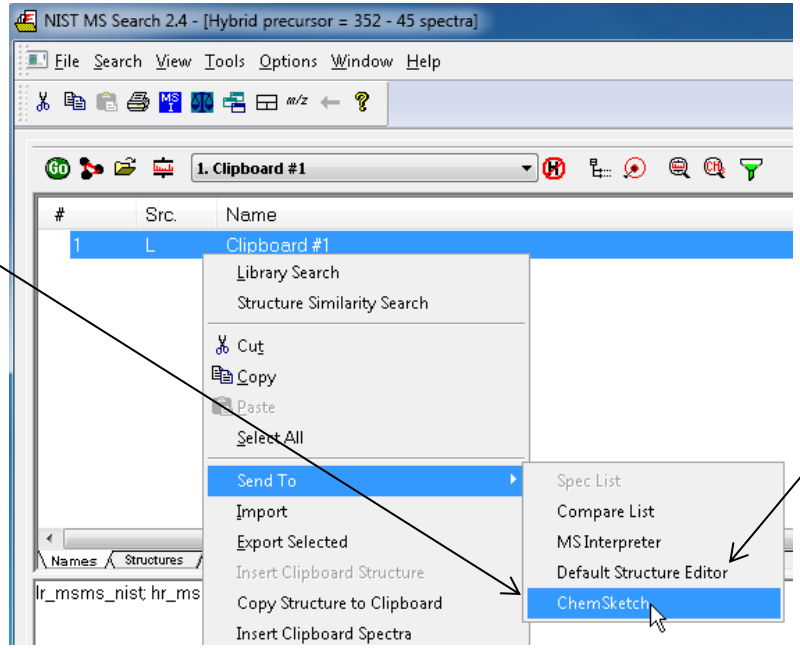
"ChemSketch" "C:\ACDFREE8\CHEMSK.EXE" "%1"

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

Acknowledgements

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