LC/MS Unknown Identifications Using MSMS Libraries
Part VI: MS Interpreter Correlation of Substructure to MSMS Ions

12/27/20

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

- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties[1] EI GC-MS, LC-MS/MS, Chemical Ionization,[3] Accurate Mass, Derivatization,[4,5] MS library management, SciFinder[6], Chemspider[6], 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

>50 Mass Specs Networked
Worldwide

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

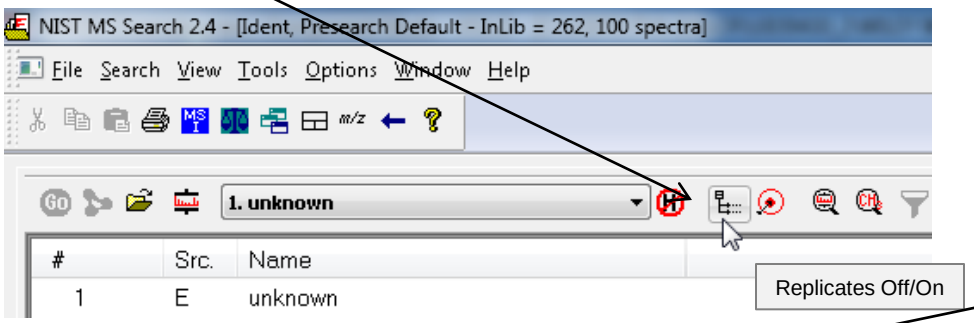
Table of Contents

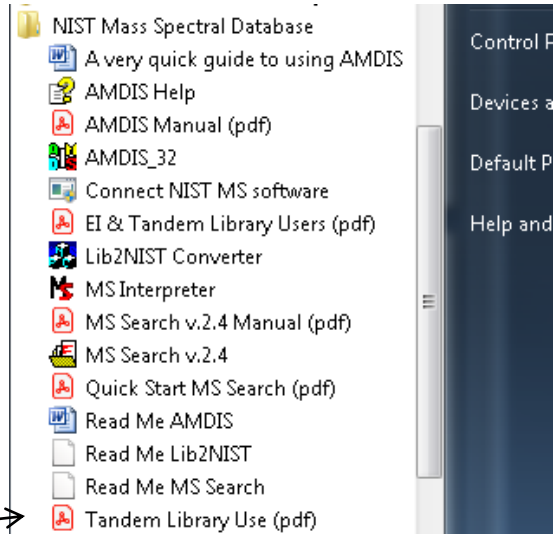
LC/MS Unknown Identifications Using MSMS Libraries

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

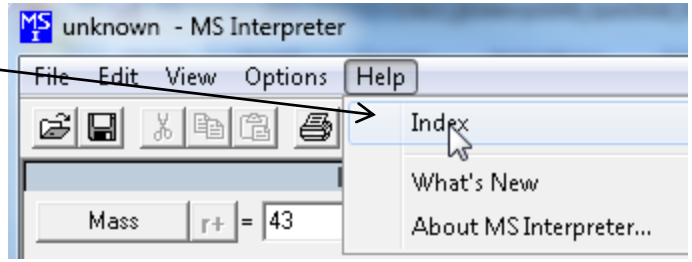
Help Files for NIST Search

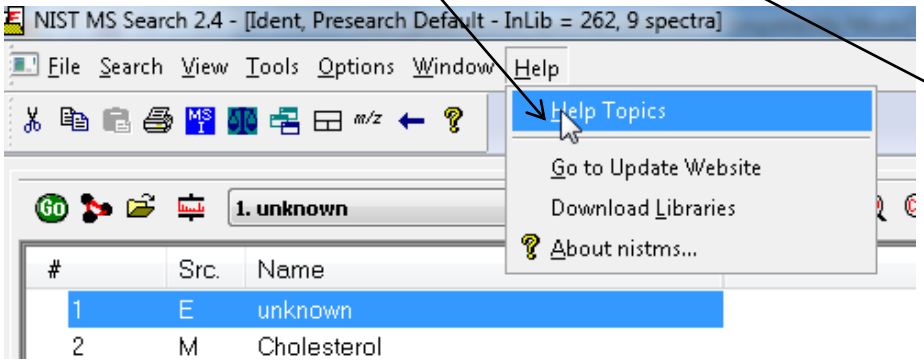
-"Hover" over Program Icon with mouse and function description displayed

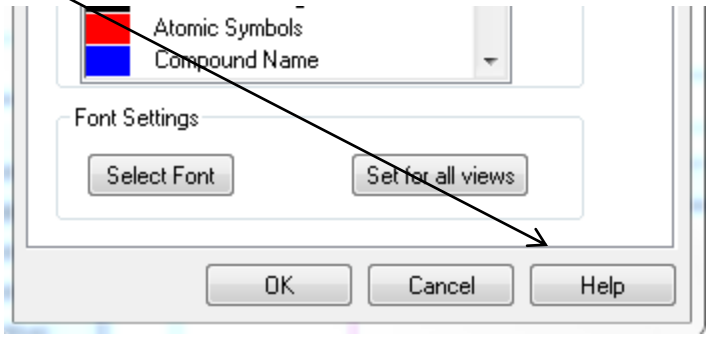




-***Detailed*** documentation for NIST Search[8]
-MS Interpreter included in NIST manual[8]
-Windows Program Group
-NIST Tandem Search ***Quick Start Guide***[9]
-"In program" assistance for both programs
-***Help button*** at corner of all windows



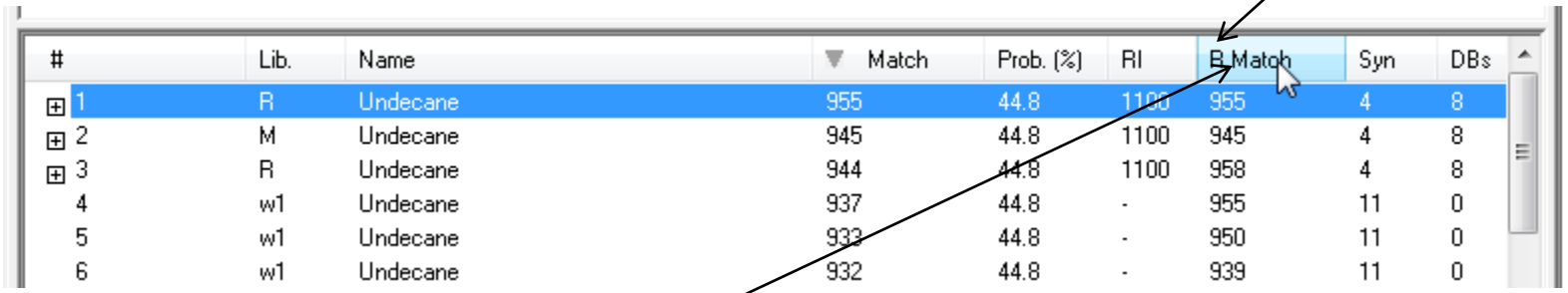




NIST Software in General is "Windows Compliant"

-***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!

Part VI: MS Interpreter[9-12] Correlation of Substructure to MSMS Ions

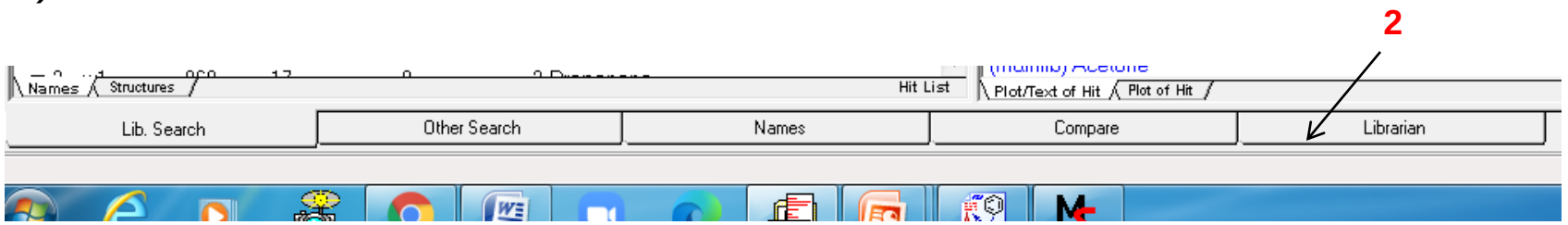
Session Topics:

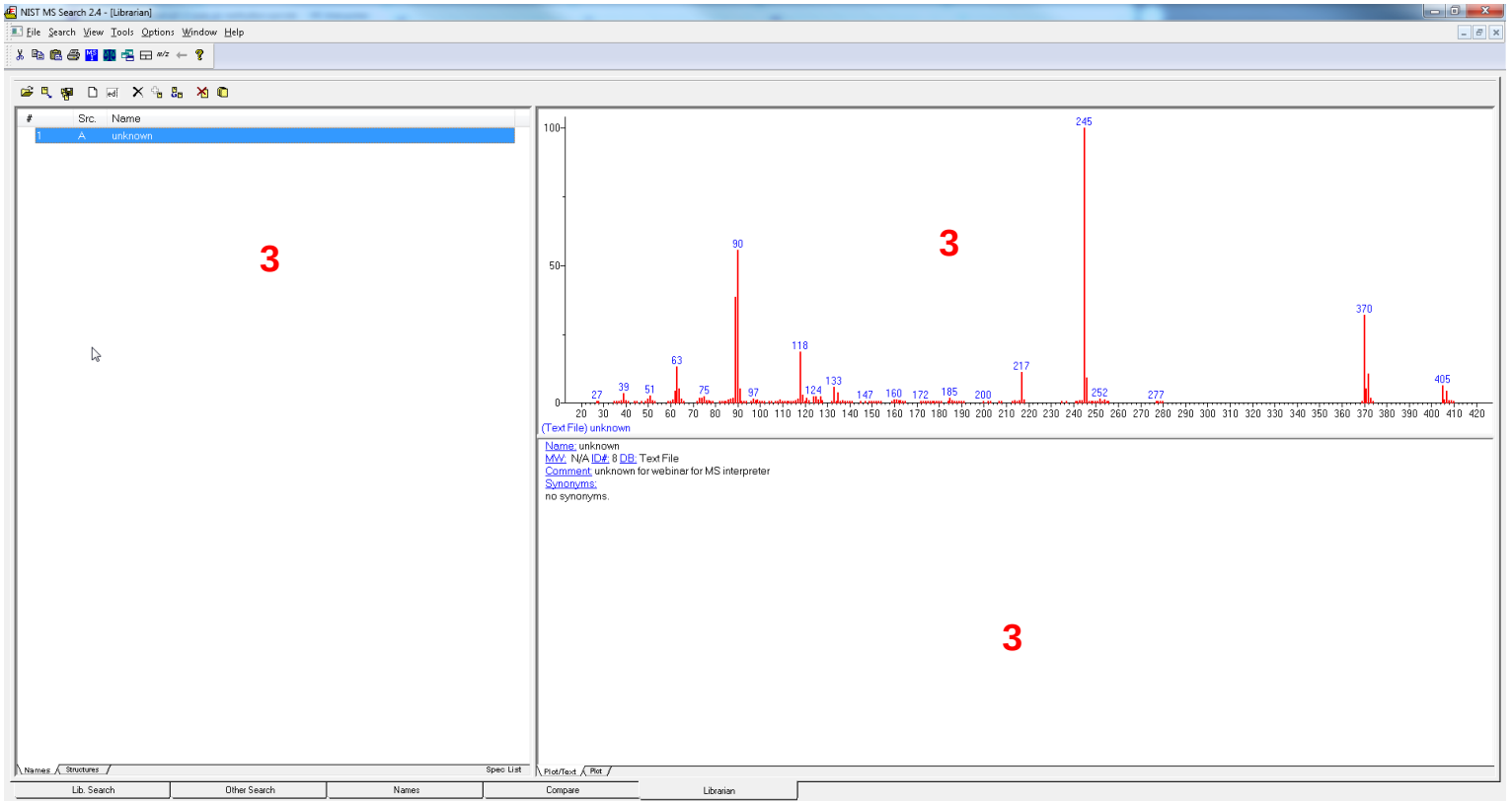
-Setting up ***Library Search Options*** MS Interpreter
-Associating structure with spectrum
-Processing data

MS Interpreter for Correlating Structure to Spectrum:

Adding a Structure to a Spectrum

1) ***Import*** the mass spectrum from instrument data system
2) ***Open Librarian tab*** at bottom of MS Search Window
3) ***View*** in ***Librarian*** tab



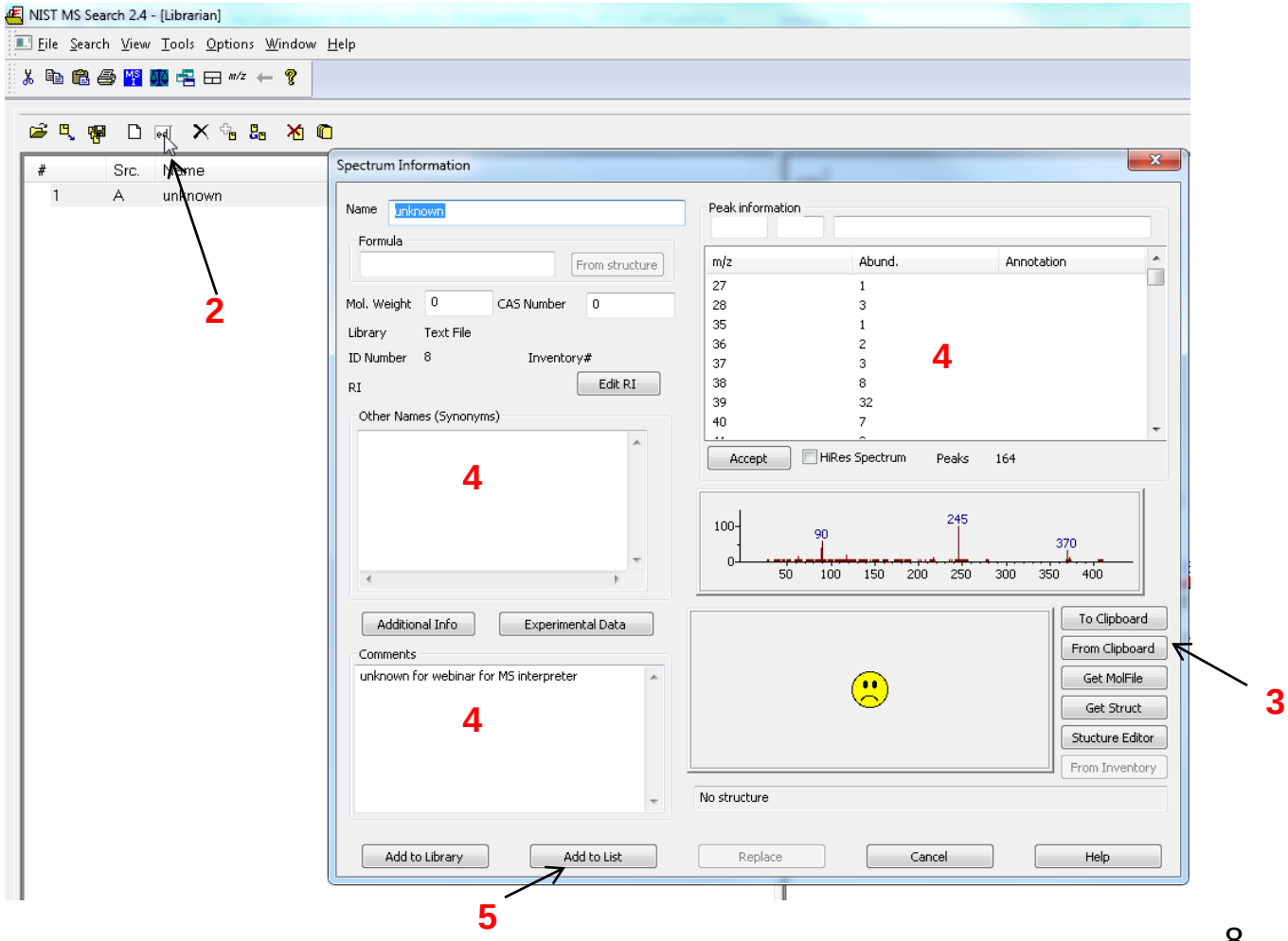


MS Interpreter for Correlating Structure to Spectrum:

Adding a Structure to a Spectrum

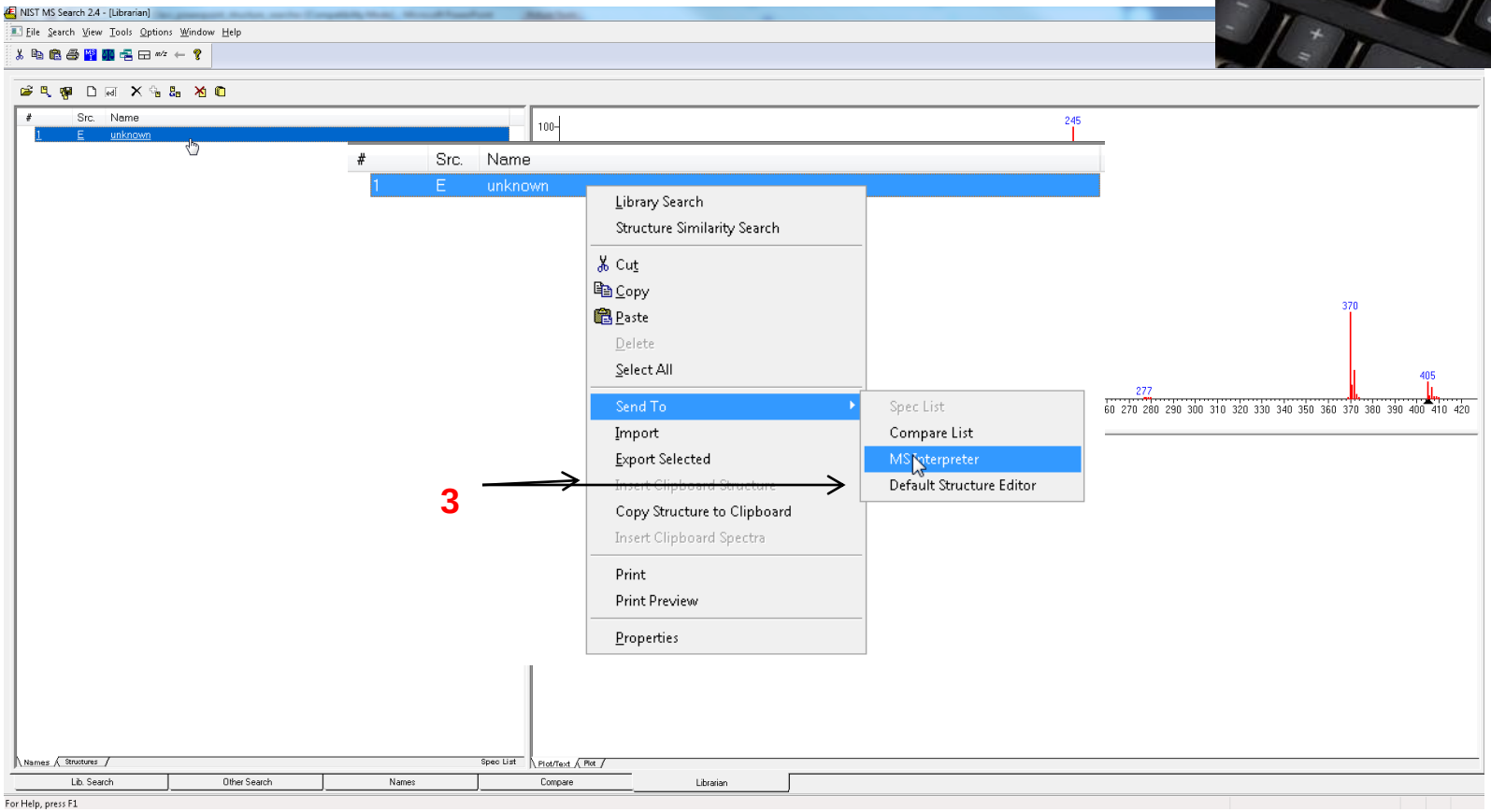
1) ***Draw*** the proposed structure with drawing program and ***copy*** into clipboard
2) ***Open Librarian tab*** and select ***"ed "*** icon with **left mouse click**
3) ***LMB*** on **From Clipboard** to associate structure with spectrum
4) ***Modify*** name fields, synonyms, comments fields, or even ***edit*** spectrum
5) ***Add to List***

Tip: This is basically the approach used to ***create a user library*** and/or user library ***entry***. This will be discussed in detail in Presentation VII in this series. ***Instead*** of "Add to List", the "Add to library" button will be selected. Also, one can "Replace" a current spectrum in user library after correcting it.



MS Interpreter for Correlating Structure to Spectrum:
Sending to MS Interpreter

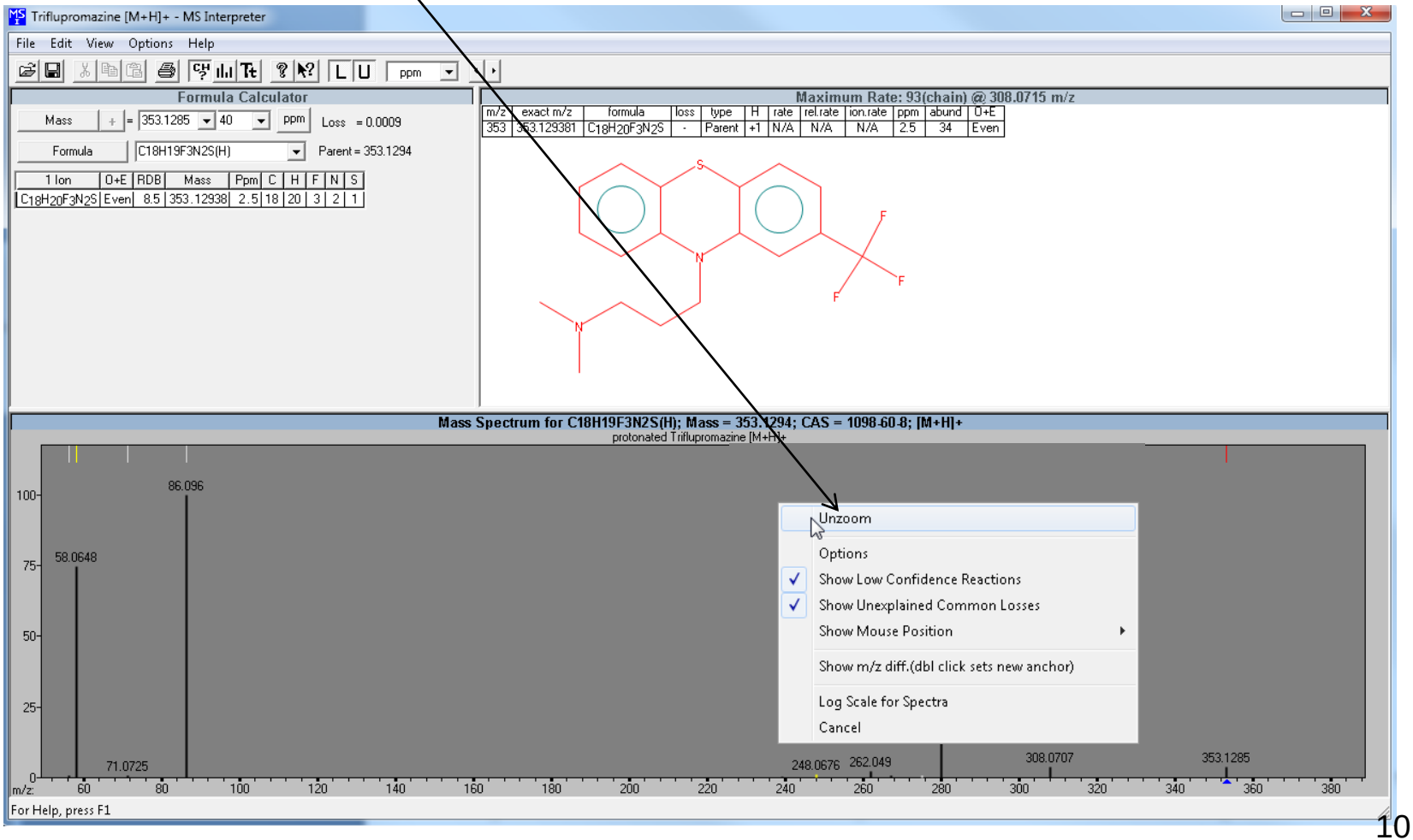
1) Entry is now ready to send to MS interpreter
2) Program has automatically calculated nominal molecular weight, exact mass, molecular formula, and InChIKey
3) ***Right mouse*** click on selected entry, then LMB on Send to/MS Interpreter
4) An ***alternative*** to step 3, is to ***send*** the entry to MS interpreter ***using F9*** key



MS Interpreter for Correlating Structure to Spectrum:

Processing Data in MS Interpreter

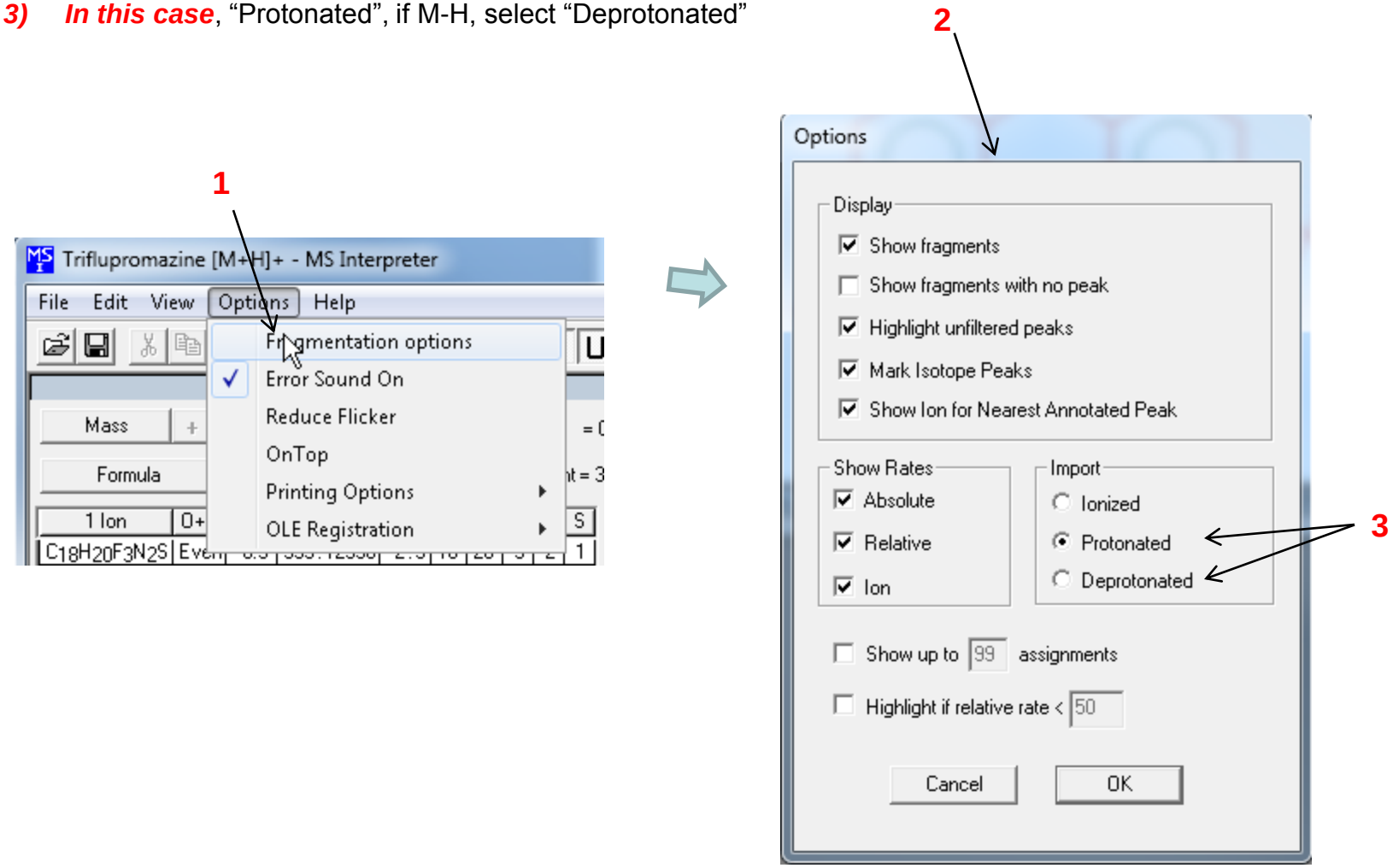
-All ions in black, yellow, and red correlated to structure automatically
-Ions in white are not assigned
-Spectrum can be expanded like any spectrum in NIST programs by ***LMB*** and ***dragging*** to form box
-***RMB*** and then ***LMB Unzoom*** to return to original size



MS Interpreter for Correlating Structure to Spectrum:

Suggested Default Settings for MS/MS (Tandem)

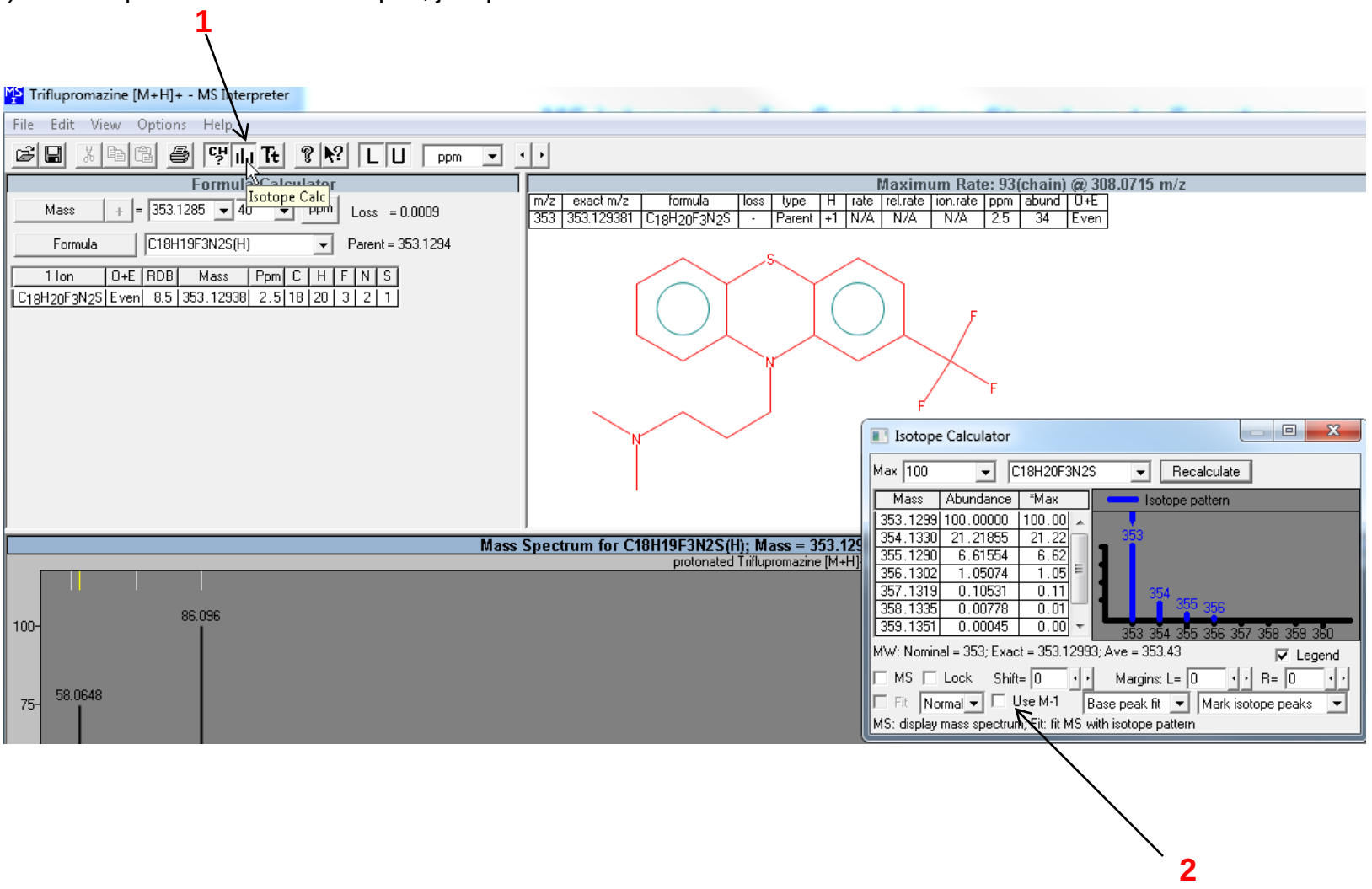
1) Select **Options** with **LMB** click then **Fragmentation options**
2) Setup options as noted in window
3) ***In this case***, “Protonated”, if M-H, select “Deprotonated”



MS Interpreter for Correlating Structure to Spectrum:

Suggested Default Settings for MS/MS (Tandem)

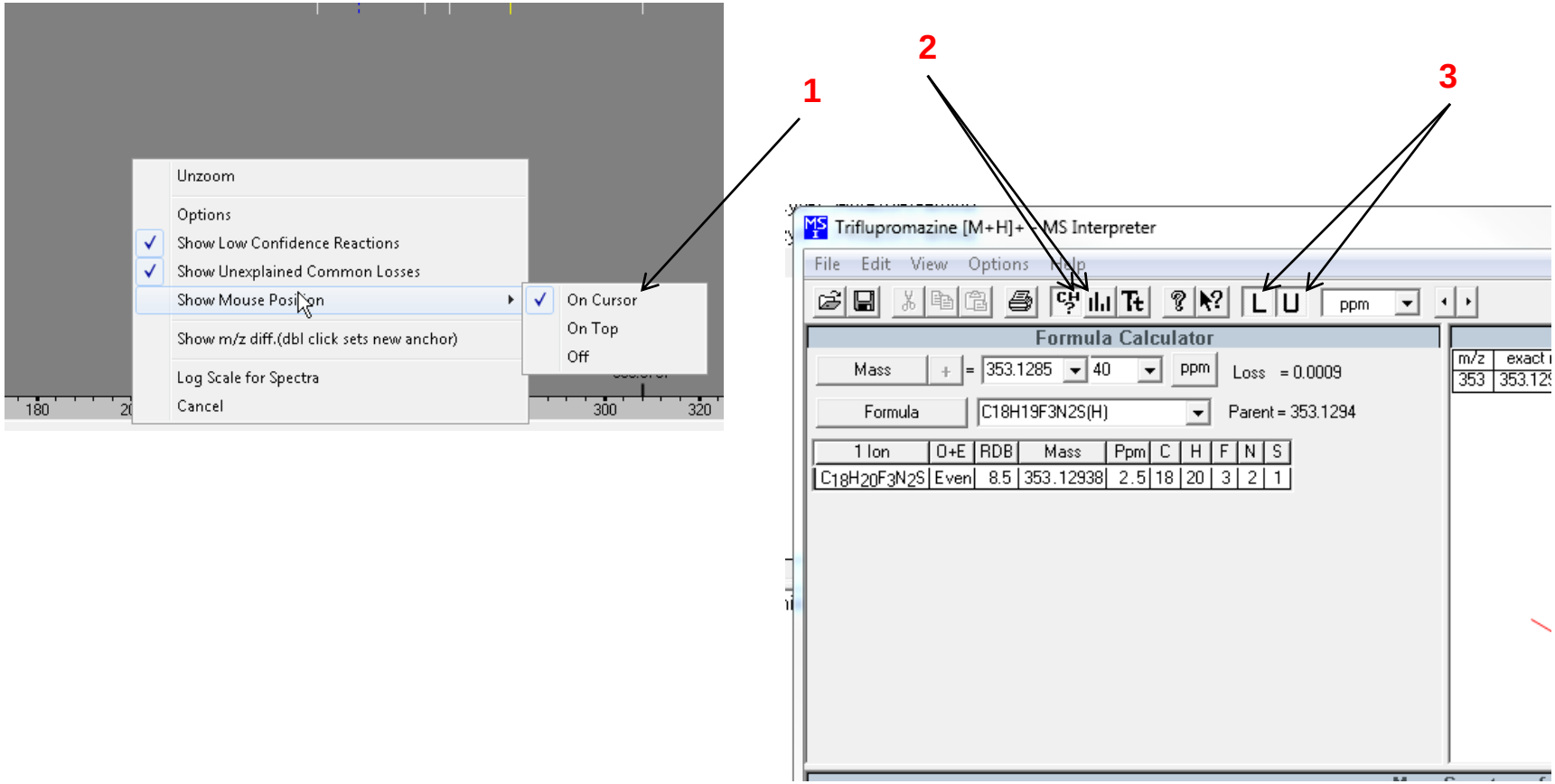
1) Select ***Isotopes Calc*** icon ***if your*** spectrum has isotopes
2) Select the Defaults as appropriate
3) This spectrum has ***no*** isotopes, just precursor



MS Interpreter for Correlating Structure to Spectrum:

Suggested Default Settings for MS/MS (Tandem)

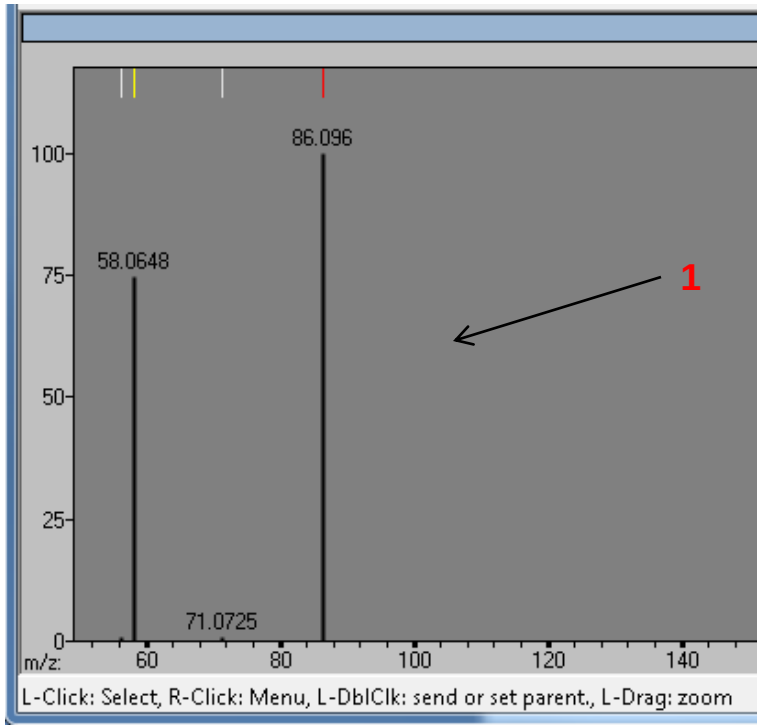
1) ***RMB*** in spectrum window, then ***LMB*** to select ***Show Mouse Position/On Cursor***
2) ***Formula Isotope button depressed*** to display window, also Formaula Isotope Calc if appropriate
3) ***Low confidence mechanism*** and ***unknown mechanism*** buttons ***depressed*** to show additional fragmentation

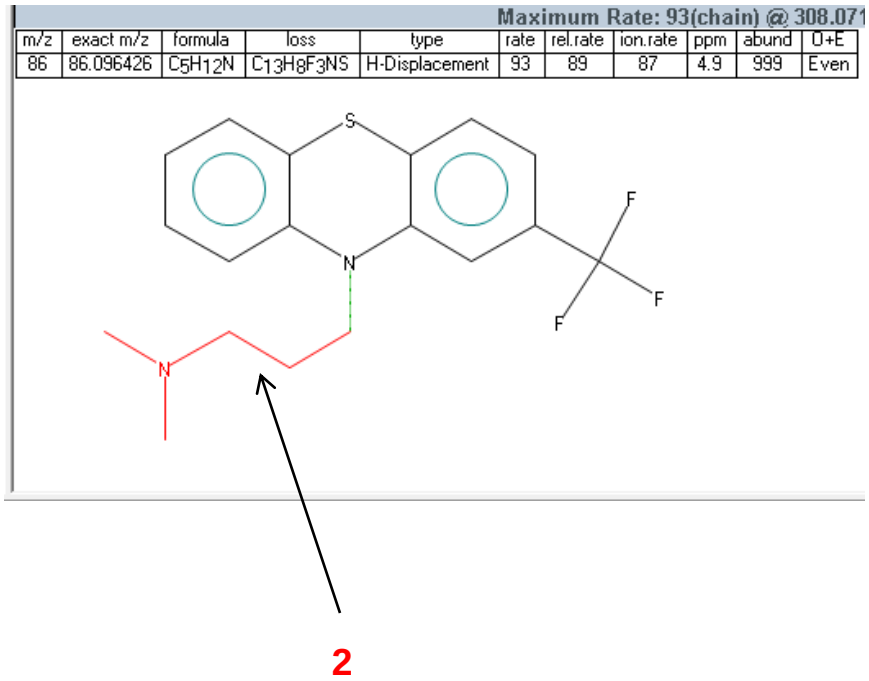


MS Interpreter for Correlating Structure to Spectrum:

Displaying the Proposed Structures

1) ***LMB*** on the ion of interest and the
2) Structure in ***highlighted in red*** in top window





MS Interpreter for Correlating Structure to Spectrum:

*Stepping Through **More** Than One Proposed Structure*

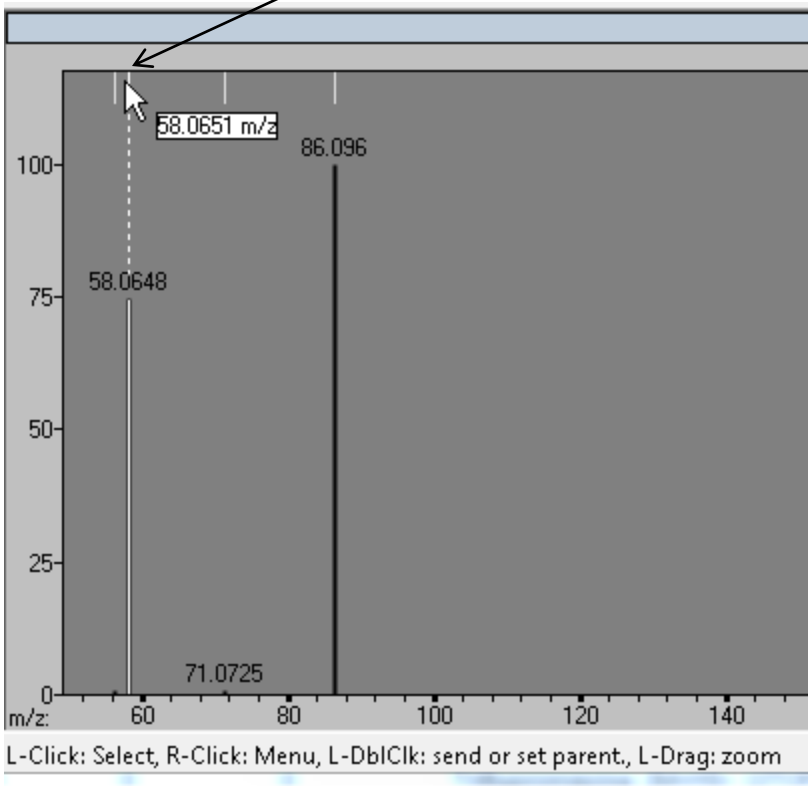
1) ***LMB*** on the ***tick mark*** for ion of interest , the display will toggle through ***all 4*** in this example

2) If ***more than one*** is present, the box will indicate the number, in this case ***¼***

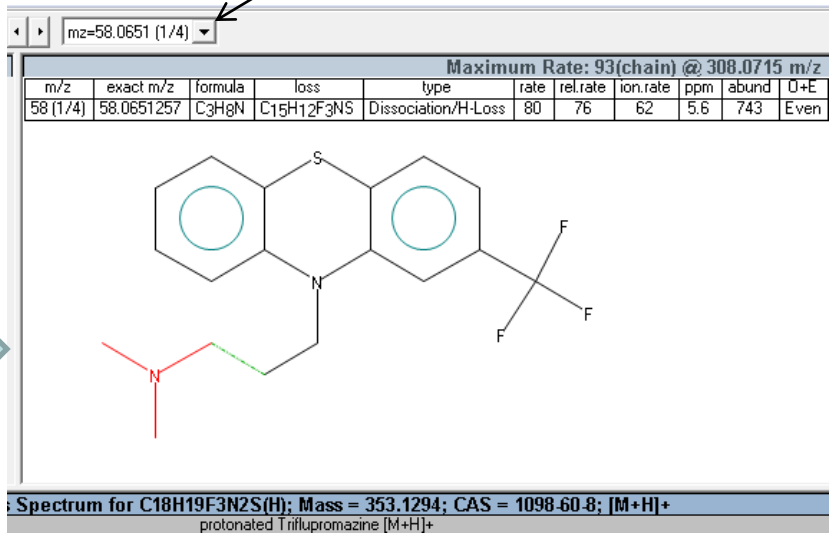
3) Also, you can select one of the tick marks with LMB then use your ***left*** and ***right*** arrows ***on keyboard*** to step through the assigned ions in the mass spectrum

1

click on tick marks



2



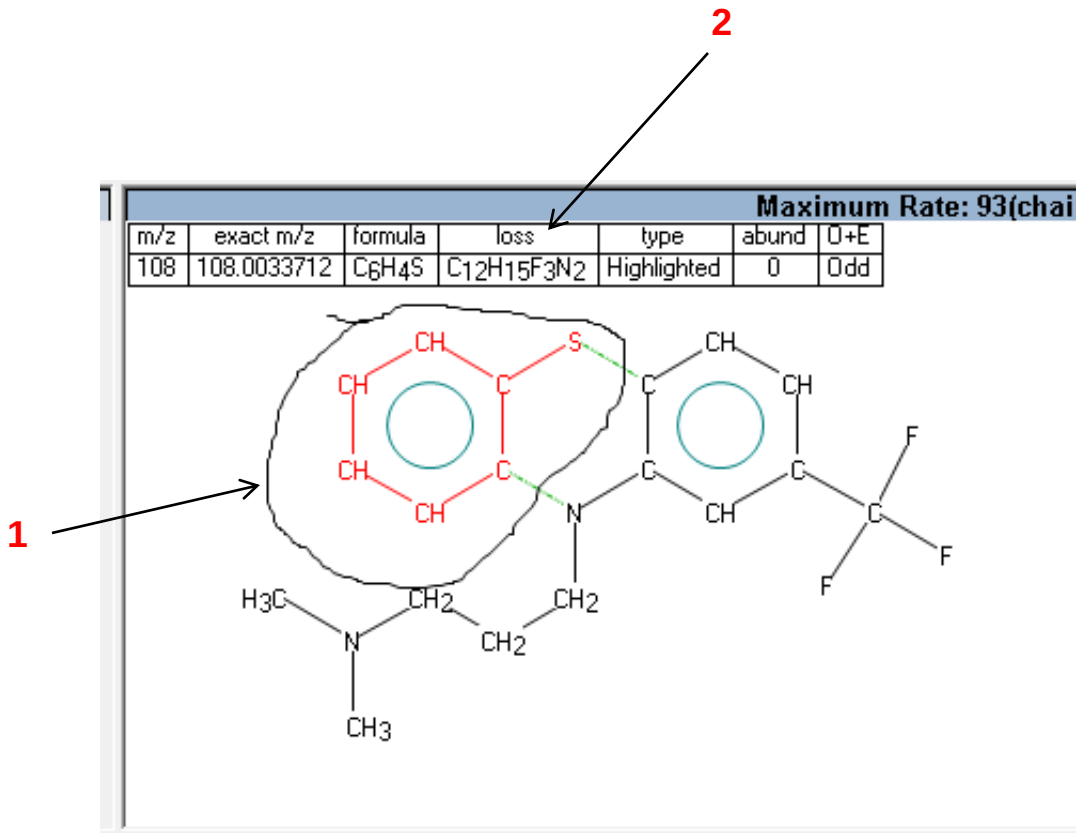
3

MS Interpreter for Correlating Structure to Spectrum:

Other Miscellaneous Tips: Lassoing Ions Manually

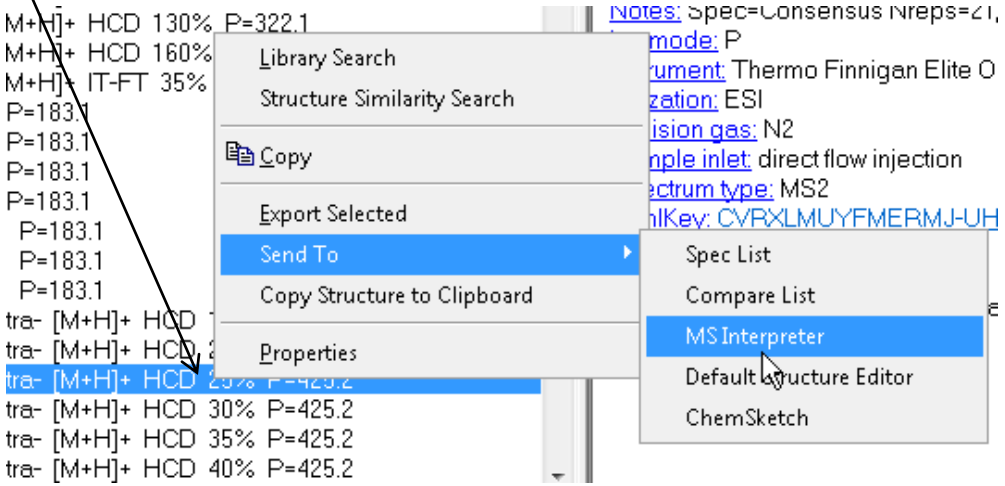
1) ***Lasso*** your own substructure by ***LMB*** and ***circling,*** shows type as ***highlighted***

2) The formula , exact mass, and loss will be shown in the “Formula Calculator” window



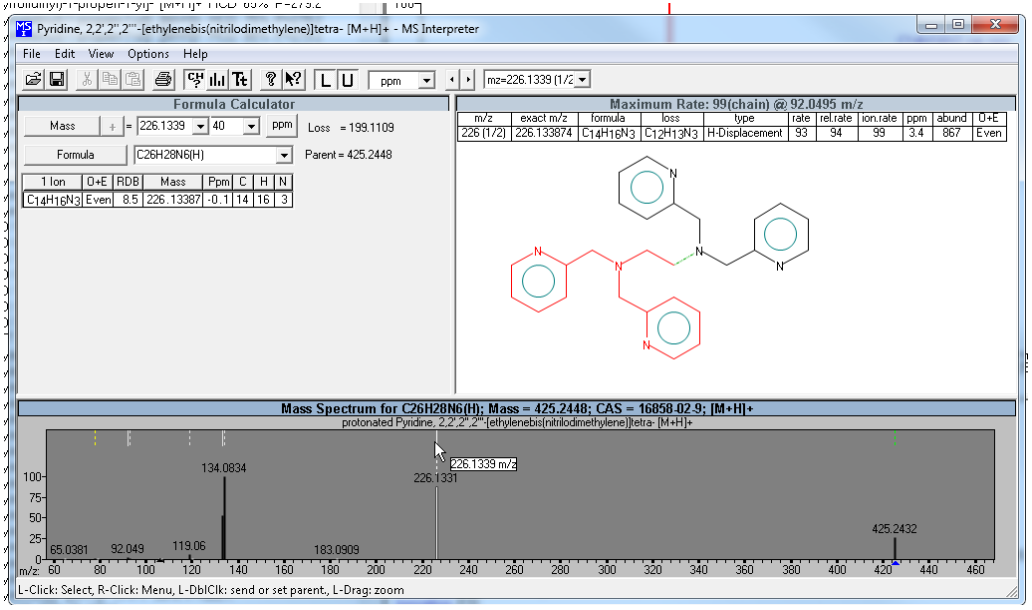
Any Structure and *Associated Spectrum* in NIST Program Can be *Sent* to *MS Interpreter*

1



1. ***Either LMB*** select then ***RMB*** **Send To/MS Interpreter**
2. ***Or*** select ***F9*** on keyboard

2

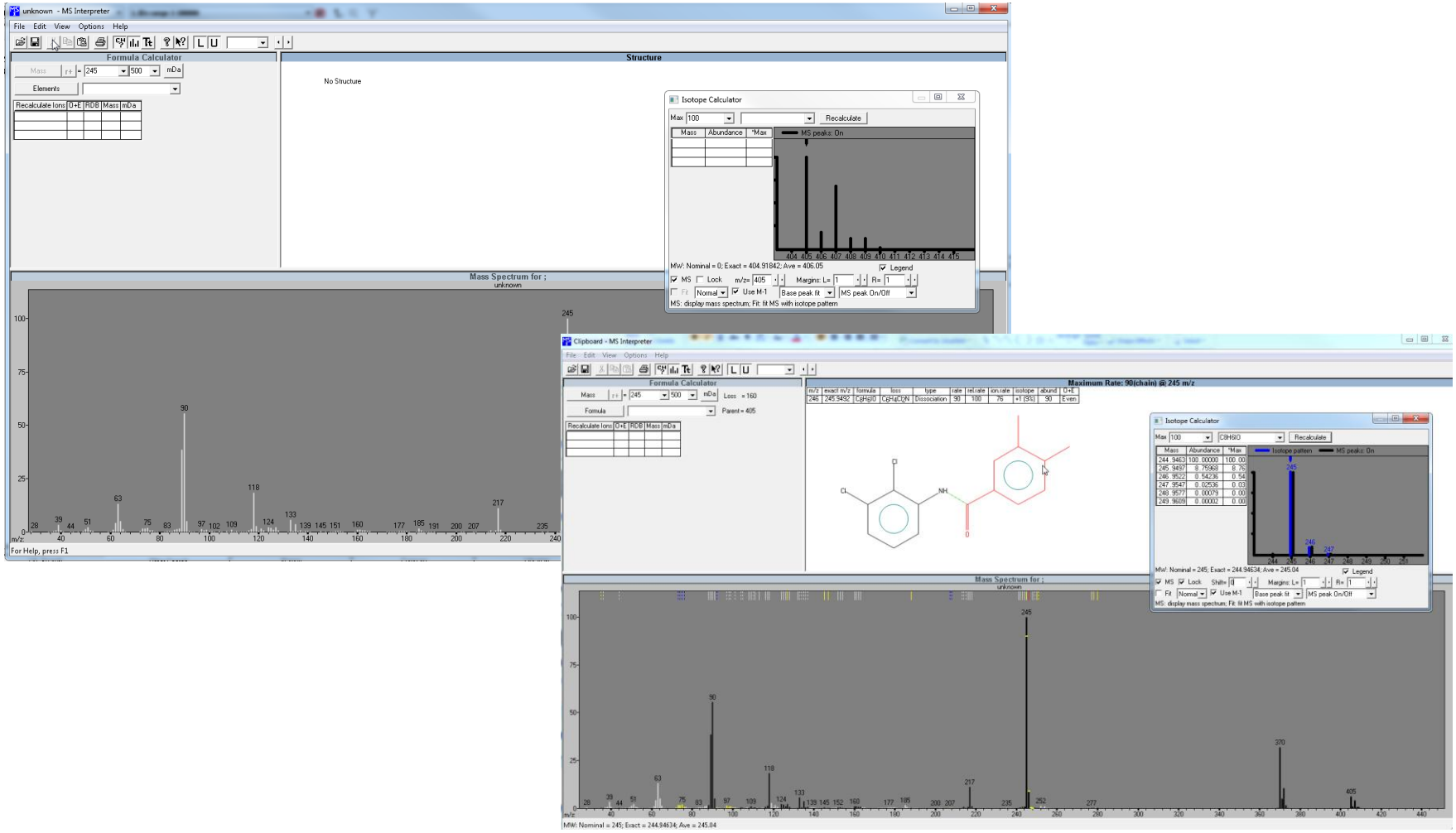


MS Interpreter for Correlating Structure to Spectrum:

Other Miscellaneous Tips: Direct Structure Paste

1) ***Copy structure*** into clipboard ***from drawing program***, then ***directly paste*** into MS interpreter with control V on keyboard or ***pull-down menu/paste***

2) The structure ***cannot be saved*** with spectrum upon exiting MS Interpreter

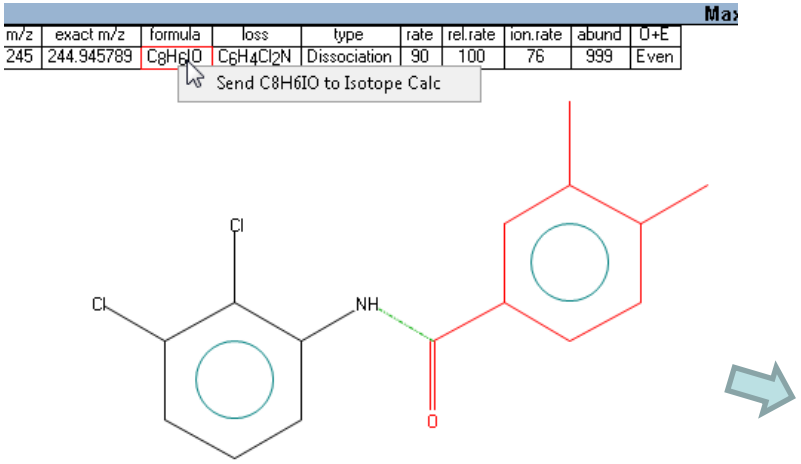


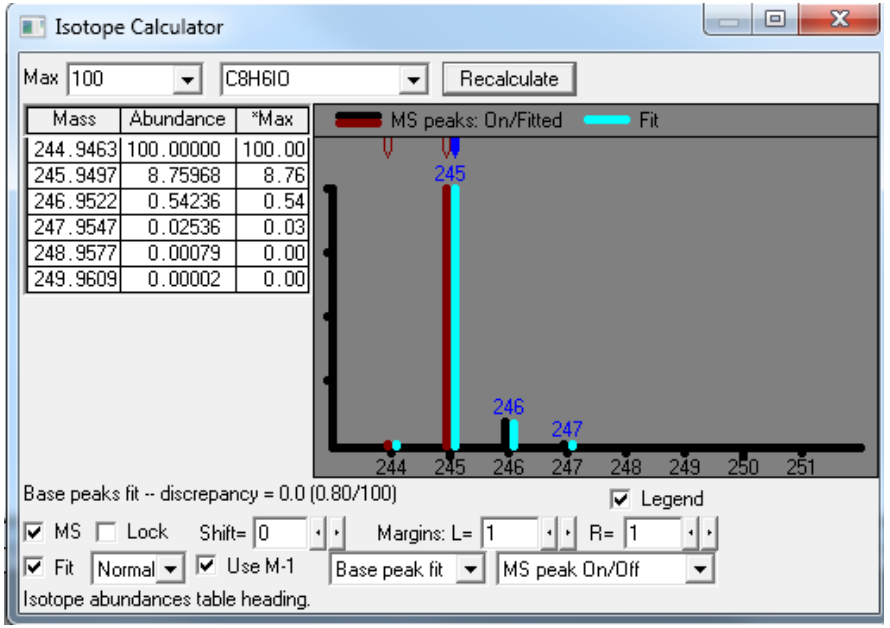
MS Interpreter for Correlating Structure to Spectrum:

If your MS/MS (Tandem) Spectrum is acquired with isotopes

1) ***RMB*** on the formula for the ion, then ***LMB*** on ***Send "x" to Isotope Calc***

2) Theoretical isotope ratio ***compared*** to observed





Live Demo on YouTube
LC/MS Unknown Identifications Using MSMS Libraries
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*
9. *New Developments in the Modeling of Ion Fragmentation by MS Interpreter Software*
10. *Enhancements to NIST MS Interpreter for Modeling High Mass Accuracy Tandem Mass Spectra*
11. *NIST Tandem Quick Start Guide*
12. *NIST Flyer: New Hybrid Search and MS Interpreter for EI and Tandem Spectra*

Acknowledgements

- Stephen Stein (NIST)
- Alexey Mayorov (NIST)
- Yuri Mirokhin (NIST)
- Dmitrii Tchekhovskoi (NIST)
- David Sparkman (NIST consultant)