

CycloBranch

Tutorials

Outline

- Tutorial 1: Does a peptide have a cycle ?
- Tutorial 2: How to determine a tag ?
- Tutorial 3: How to determine a complete sequence ?
- Tutorial 4: How to determine a branched sequence ?
- Tutorial 5: How to determine terminal modifications ?
- Tutorial 6: How to compare a theoretical spectrum with a peaklist ?
- Tutorial 7: How to search in a database of MS/MS spectra ?
- Tutorial 8: How to search MS spectrum against a database of compounds ?
- Tutorial 9: Draw Peptide Tool
- Tutorial 10: Identification of Branch-Cyclic Peptide with a Long-sized Branch
- Tutorial 11: Identification of Peptide Family using Similarity Search
- Tutorial 12: Identification of Siderophores in MS/MS data
- Tutorial 13: Identification of Siderophores in MALDI-MS and LC-MS data
- Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

Tutorial 1: Does a peptide have a cycle ?

- Are y -ions observed in linear mode? **No.**
- Are multiple overlapping series of b -ions observed in cyclic mode? **Yes.**
- Are scrambled ions observed in cyclic mode? **Yes.**

→ The peptide likely contains a cycle.

Example 1a

Settings...

Peaklist

Peptide Type: **Linear**

Peaklist File: PeakLists/cyclosporin_recalibrated.txt

Precursor m/z Ratio: 1202.848645

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db33_v2.txt

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 2

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☒

Enable Similarity Search: ☐

Application

Mode: De Novo Search Engine

Sequence Database File:

Maximum Number of Threads: 6

Score Type: Number of b-ions + dehydrated b-ions

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- C
- C*
- Cx
- C*x
- X
- X*
- Xx
- X*x
- Y**
- Y*
- Yx
- Y*x

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence: [NMe-Bmt]-[Abu]-[NMe-Gly]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Example 1a

CycloBranch											
File Search Tools View Help											
Filter Clear											
	* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	N-terminal Modification	C-terminal Modification	Matched Peaks	Sum of Relative Intensities	B	Y
1	1	[His]-[C14:0-Me(13)-OH(3)]-[Ala]-[Val]-[N...	C62H111N11O12	1201.841368	10			9	196.958944	8	0
2	2	[C14:0-Me(13)-OH(3)]-[His]-[Leu]-[NMe-...	C62H111N11O12	1201.841368	10			9	142.435576	8	0
3	3	[His]-[C14:0-Me(13)-OH(3)]-[Ala]-[Val]-[N...	C62H111N11O12	1201.841368	10			9	185.871909	8	0
4	4	[C14:0-Me(13)-OH(3)]-[His]-[Ala]-[Val]-[N...	C62H111N11O12	1201.841368	10			9	196.958944	8	0
5	5	[His]-[C14:0-Me(13)-OH(3)]-[Ala]-[Val]-[V...	C62H111N11O12	1201.841368	10			9	88.066762	8	0
6	6	[C14:0-Me(13)-OH(3)]-[His]-[Leu]-[Gly]-[...	C62H111N11O12	1201.841368	10			9	171.804917	8	0
7	7	[C14:0-Me(13)-OH(3)]-[His]-[Ala]-[Val]-[N...	C62H111N11O12	1201.841368	10			9	185.871909	8	0
8	8	[His]-[C14:0-Me(13)-OH(3)]-[Leu]-[Gly]-[...	C62H111N11O12	1201.841368	10			9	160.717882	8	0
9	9	[C14:0-Me(13)-OH(3)]-[His]-[Ala]-[Val]-[V...	C62H111N11O12	1201.841368	10			9	88.066762	8	0
10	10	[His]-[C14:0-Me(13)-OH(3)]-[Leu]-[Gly]-[...	C62H111N11O12	1201.841368	10			9	74.079100	8	0
11	11	[C14:0-Me(13)-OH(3)]-[His]-[Ala]-[Val]-[V...	C62H111N11O12	1201.841368	10			9	99.153797	8	0
12	12	[His]-[C14:0-Me(13)-OH(3)]-[Leu]-[NMe-...	C62H111N11O12	1201.841368	10			9	54.589761	8	0
13	13	[His]-[C14:0-Me(13)-OH(3)]-[Ala]-[Val]-[V...	C62H111N11O12	1201.841368	10			9	99.153797	8	0

Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 1
Number of edges: 239

Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 92

Comparing theoretical spectra of candidates with the peak list... ok

CycloBranch successfully finished at 09:26:59 (time elapsed: 0 hrs, 0 min, 0 sec).

Example 1b

Settings...

Peaklist

Peptide Type: **Cyclic**

Peaklist File: PeakLists/cyclosporin_recalibrated.txt **Select**

Precursor m/z Ratio: 1202.848645

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db33_v2.txt **Select**

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 2

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: **Select**

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☒

Enable Similarity Search: ☐

Application

Mode: De Novo Search Engine

Sequence Database File: **Select**

Maximum Number of Threads: 6

Score Type: Number of b-ions + dehydrated b-ions

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Select All **Clear All** **Reset**

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence: [NMe-Bmt]-[Abu]-[NMe-Gly]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NM] **Edit**

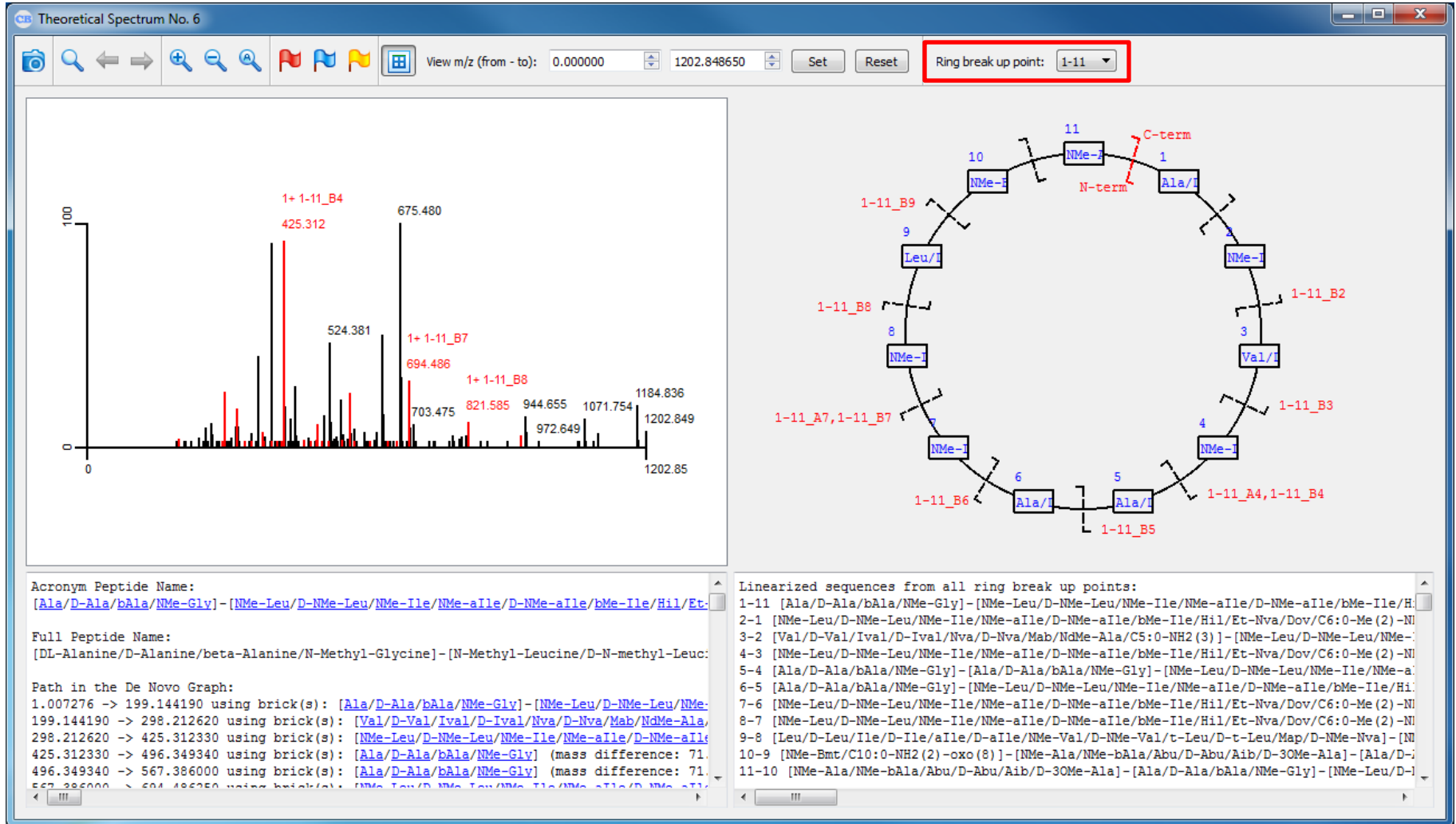
N-terminal Modification:

C-terminal Modification:

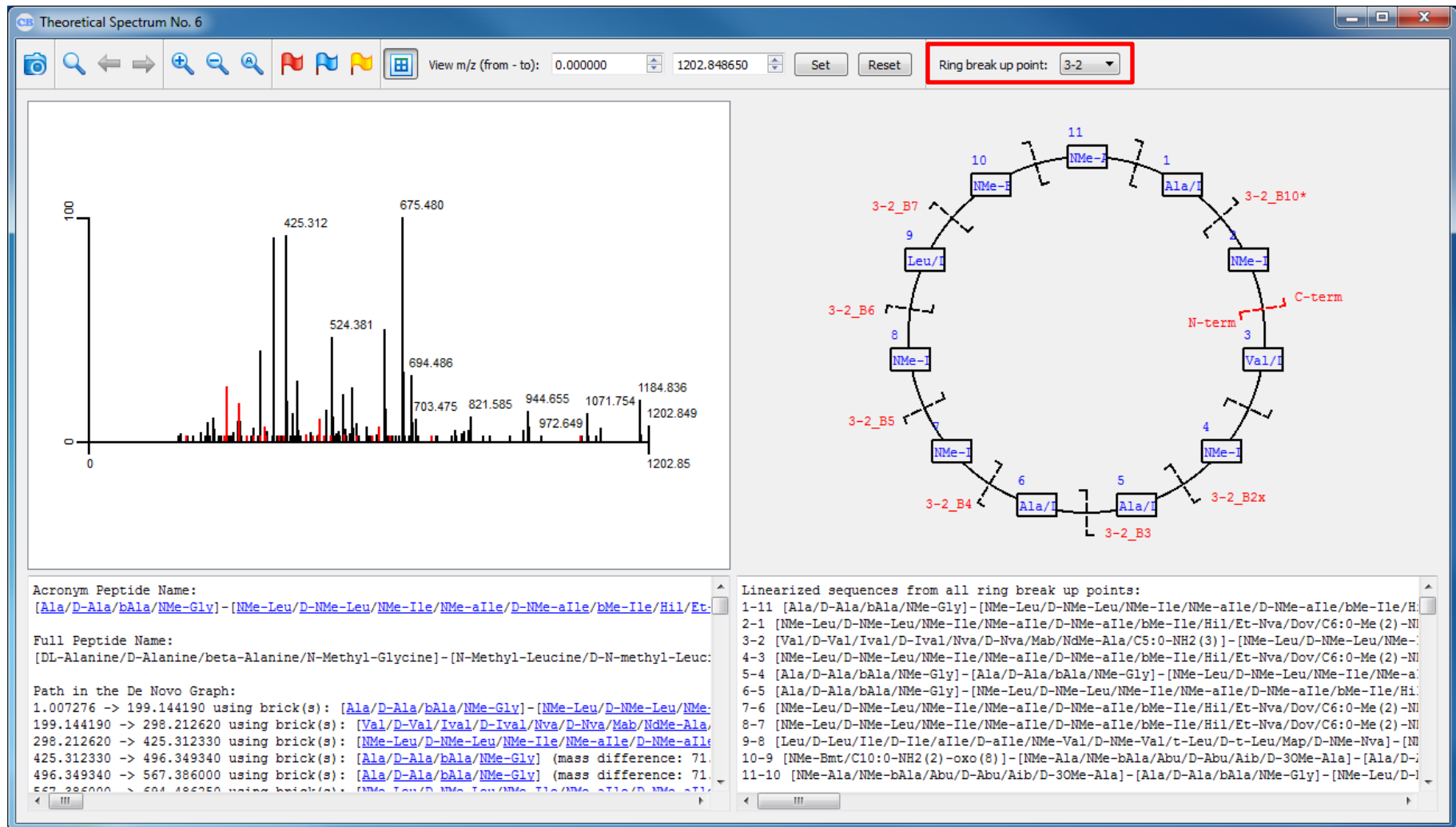
Branch Modification:

OK **Cancel** **Apply** **Load** **Save 'tutorial1b.ini'** **Save As...**

Example 1b



Example 1b



Example 1b

CycloBranch

File Search Tools View Help

Filter Clear

	* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Matched Bricks	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	A	A*	Ax	A*x	B	B*	Bx	B*x	Scrambled Peaks
1	1	[NMe-Leu]-[NMe-Leu]-[Gly]-[Leu]-[Val]-[...]	C62H111N11O12	1201.841368	11	119	90	52.325581	858.370316	7	1	0	0	38	22	4	0	17
2	2	[Ala]-[NMe-Leu]-[Val]-[Val]-[Leu]-[Gly]-[N...]	C62H111N11O12	1201.841368	11	119	90	52.325581	858.370316	7	1	0	0	38	22	4	0	17
3	3	[NMe-Leu]-[NMe-Leu]-[Gly]-[NMe-Leu]-[...]	C62H111N11O12	1201.841368	11	119	90	52.325581	858.370316	7	1	0	0	37	21	4	0	19
4	* 4	[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-...]	C62H111N11O12	1201.841368	11	124	90	52.325581	858.370316	8	1	0	0	37	21	4	0	18
5	5	[Ala]-[NMe-Leu]-[Val]-[NMe-Ala]-[NMe-L-...]	C62H111N11O12	1201.841368	11	119	90	52.325581	858.370316	7	1	0	0	37	21	4	0	19
6	* 6	[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[...]	C62H111N11O12	1201.841368	11	124	90	52.325581	858.370316	8	1	0	0	37	21	4	0	18
7	7	[NMe-Leu]-[NMe-Leu]-[Ala]-[Val]-[NMe-...]	C62H111N11O12	1201.841368	11	118	90	52.325581	858.370316	7	0	0	0	35	22	4	0	21
8	8	[Ala]-[NMe-Leu]-[Leu]-[NMe-Leu]-[Leu]-[...]	C62H111N11O12	1201.841368	11	136	90	52.325581	858.370316	3	1	1	0	36	21	3	0	24
9	9	[NMe-Leu]-[NMe-Leu]-[Ala]-[Val]-[Val]-[V...]	C62H111N11O12	1201.841368	11	120	90	52.325581	858.370316	8	0	0	0	36	21	4	0	20
10	10	[NMe-Leu]-[NMe-Leu]-[Gly]-[Val]-[Leu]-[...]	C62H111N11O12	1201.841368	11	114	90	52.325581	858.370316	8	1	0	0	36	21	5	0	18
11	11	[NMe-Leu]-[NMe-Leu]-[Ala]-[Leu]-[NMe-...]	C62H111N11O12	1201.841368	11	121	90	52.325581	858.370316	7	0	0	0	37	20	4	0	21
12	12	[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[NMe-...]	C62H111N11O12	1201.841368	11	120	90	52.325581	858.370316	7	1	0	0	36	21	4	0	20
13	13	[NMe-Leu]-[NMe-Leu]-[Gly]-[NMe-Ala]-[...]	C62H111N11O12	1201.841368	11	120	90	52.325581	858.370316	7	1	0	0	36	21	4	0	20
14	14	[Leu]-[NMe-Ala]-[NMe-Leu]-[Ala]-[Val]-[...]	C62H111N11O12	1201.841368	11	121	90	52.325581	858.370316	3	0	1	0	35	22	4	0	24

Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 0
Number of edges: 186

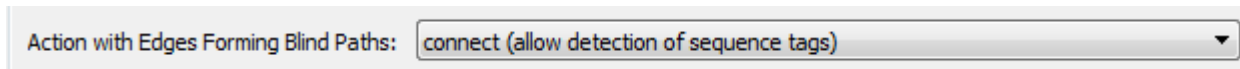
Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 18096

Comparing theoretical spectra of candidates with the peak list...
.....10002000300040005000600070008000900010000
.....1100012000130001400015000160001700018000 ok

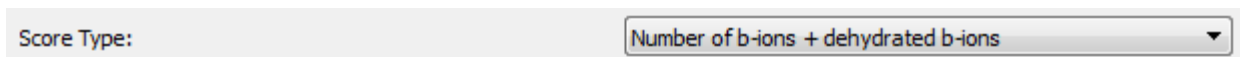
CycloBranch successfully finished at 09:44:04 (time elapsed: 0 hrs, 2 min, 12 sec).

Tutorial 2: How to determine a tag ?

- Detection of sequence tags must be enabled.



- Proper scoring function must be chosen.
(e.g., number of matched peaks,
sum of relative intensities of matched peaks,
number of b-ions or number of b-ions +
dehydrated b-ions)

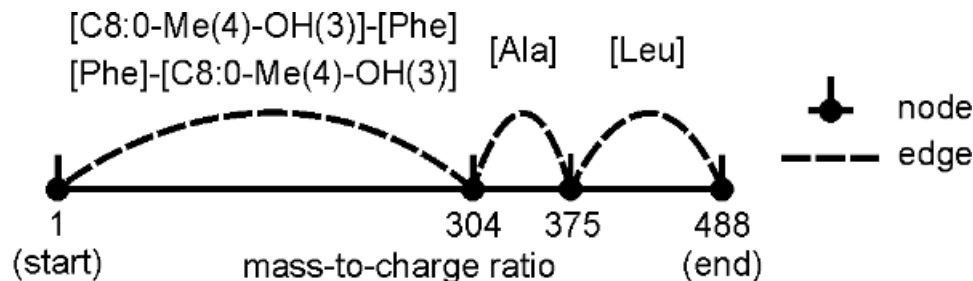


Tutorial 2: How to determine a tag ?

- A threshold of minimum relative intensity of peaks must be set high. **3 – 5 %**

Minimum Threshold of Relative Intensity: 5.000

- Maximum number of combined building blocks must be small. **1/1/1**



Database of Building Blocks

Building Blocks Database File:

Maximum Number of Combined Blocks (start):

Maximum Number of Combined Blocks (middle):

Maximum Number of Combined Blocks (end):

Example 2

Settings...

Peaklist

Peptide Type: Cyclic

Peaklist File: PeakLists/cyclosporin_recalibrated.txt

Precursor m/z Ratio: 1202.848645

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 5.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db33_v2.txt

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Action with Blind Paths in De Novo Graph: connect (allow detection of sequence tags)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: De Novo Search Engine

Sequence Database File:

Maximum Number of Threads: 1

Score Type: Number of b-ions + dehydrated b-ions

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

A
A*
Ax
A*x
B
B*
Bx
B*x
C
C*
Cx
C*x

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence: [NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Example 2

- Correct tag of Cyclosporin A “[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]” was reported as a top hit.

The screenshot displays the CycloBranch software interface. At the top is a menu bar with 'File', 'Search', 'Tools', 'View', and 'Help'. Below the menu is a toolbar with various icons for file operations, search, and analysis. The main window contains a table with the following columns: Result ID, Peptide Sequence, Summary Formula, Monoisotopic Mass, Number of Bricks, Matched Bricks, Matched Peaks, Ratio of Matched Peaks [%], Sum of Relative Intensities, and a series of columns labeled A, A*, Ax, A*x, B, B*, Bx, B*x. The table lists 9 results, with the first result (ID 1) highlighted in yellow. Below the table, there is a status bar with text indicating the progress of the analysis: 'Removing edges forming paths which can be substituted by longer paths... ok', 'Edges removed: 0', 'Number of edges: 162', 'Calculating the number of paths forming peptide sequence candidates... ok', 'Number of paths found: 1037', 'Comparing theoretical spectra of candidates with the peak list... ..1000 ok', and 'CycloBranch successfully finished at 10:19:55 (time elapsed: 0 hrs, 0 min, 1 sec)'.

	Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Matched Bricks	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	A	A*	Ax	A*x	B	B*	Bx	B*x
1	1	[297.205344]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[268.179100]	C33H60N6O6 (partial)	636.457434	8	34	24	48.000000	634.883076	1	0	0	0	17	5	0	0
2	2	[254.199524]-[Leu]-[Gly]-[Ala]-[NMe-Leu]-[Ala]-[NMe-Leu]-[Leu]-[268.179100]	C34H61N7O7 (partial)	679.463247	9	26	23	46.000000	525.473821	2	0	0	0	15	5	0	0
3	3	[254.199524]-[Val]-[Ala]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[268.179100]	C34H61N7O7 (partial)	679.463247	9	26	21	42.000000	512.961394	0	0	0	0	15	5	0	0
4	4	[297.205344]-[NMe-Leu]-[Ala]-[NMe-Leu]-[Ala]-[NMe-Leu]-[Leu]-[268.179100]	C33H60N6O6 (partial)	636.457434	8	30	22	44.000000	568.554551	2	0	0	0	15	4	0	0
5	5	[254.199524]-[Ala]-[Val]-[Ala]-[NMe-Leu]-[Ala]-[NMe-Leu]-[Leu]-[268.179100]	C34H61N7O7 (partial)	679.463247	9	24	22	44.000000	504.385295	2	0	0	0	15	4	0	0
6	6	[367.283554]-[Gly]-[Ala]-[NMe-Leu]-[Ala]-[NMe-Leu]-[Leu]-[268.179100]	C28H50N6O6 (partial)	566.379183	8	24	22	44.000000	516.584853	2	0	0	0	14	5	0	0
7	7	[254.199524]-[Ala]-[Val]-[Val]-[Val]-[Ala]-[NMe-Leu]-[Leu]-[268.179100]	C34H61N7O7 (partial)	679.463247	9	23	22	44.000000	538.681951	2	0	0	0	15	4	0	0
8	8	[254.199524]-[Leu]-[Gly]-[Val]-[Val]-[Ala]-[NMe-Leu]-[Leu]-[268.179100]	C34H61N7O7 (partial)	679.463247	9	25	22	44.000000	564.834090	2	0	0	0	14	5	0	0
9	9	[254.199524]-[Leu]-[Gly]-[Val]-[Leu]-[Gly]-[NMe-Leu]-[Leu]-[268.179100]	C34H61N7O7 (partial)	679.463247	9	27	21	42.000000	602.734418	1	0	0	0	14	5	0	0

Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 0
Number of edges: 162

Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 1037

Comparing theoretical spectra of candidates with the peak list...
.....1000 ok

CycloBranch successfully finished at 10:19:55 (time elapsed: 0 hrs, 0 min, 1 sec).

Tutorial 3: How to determine a complete sequence ?

- Mass-to-charge error tolerances must be small.
1 – 2 ppm

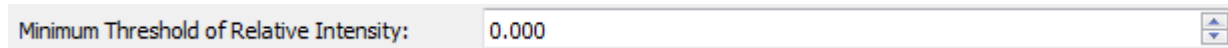
Precursor m/z Error Tolerance:	1.000 ppm
Fragment m/z Error Tolerance:	1.000 ppm

- Detection of sequence tags should be disabled.

Action with Edges Forming Blind Paths:	remove (speed up the search)
--	---

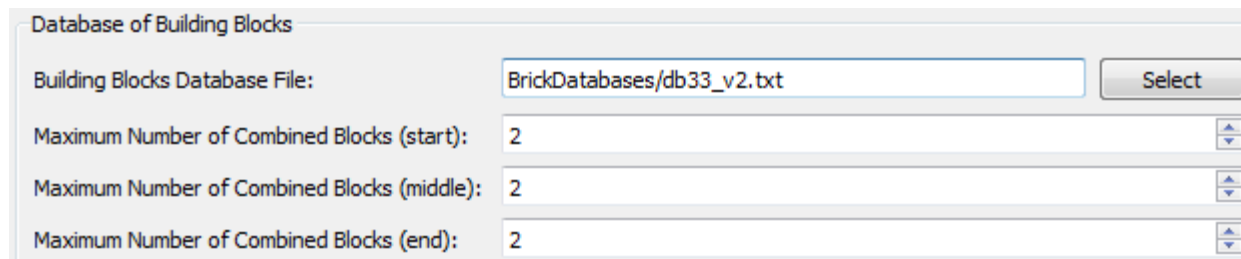
Tutorial 3: How to determine a complete sequence ?

- Threshold of minimum relative intensity should be small.



Minimum Threshold of Relative Intensity: 0.000

- Maximum numbers of combined building blocks must be increased.
 - proper values must be determined empirically from the gaps between peaks



Database of Building Blocks

Building Blocks Database File: BrickDatabases/db33_v2.txt Select

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 2

Maximum Number of Combined Blocks (end): 2

Tutorial 3: How to determine a complete sequence ?

- Sequence tag(s) should be used.
 - sequences without the tag are skipped

Peptide Sequence Tag:

[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]

- sequences without the tag are processed and sequences with the tag are yellow

Searched Peptide Sequence

Sequence:

[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]

Edit

CycloBranch														
File Search Tools View Help														
Filter Clear														
* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Matched Bricks	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	A	A*	Ax	A*x	B	B*
1	[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[NMe-Ala]-[NMe-Bmt]-[Leu]	C62H111N11O12	1201.841368	11	124	72	41.860465	804.697520	8	1	0	0	37	21
2	[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[NMe-Bmt]-[NMe-Ala]	C62H111N11O12	1201.841368	11	124	72	41.860465	804.697520	8	1	0	0	37	21
3	[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[Gly]-[NMe-Leu]-[Leu]-[NMe-Bmt]-[NMe-Ala]	C62H111N11O12	1201.841368	11	117	66	38.372093	786.487847	6	0	1	0	35	22
4	[NMe-Ala]-[Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[NMe-Bmt]-[NMe-Ala]	C62H111N11O12	1201.841368	11	113	72	41.860465	813.979630	9	1	1	0	36	20
5	[Leu]-[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Leu]-[Val]-[NMe-Ala]-[NMe-Ala]-[NMe-Bmt]	C62H111N11O12	1201.841368	11	121	71	41.279070	804.926232	8	1	1	0	35	21
6	[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Leu]-[Val]-[NMe-Ala]-[NMe-Ala]-[NMe-Bmt]-[Leu]	C62H111N11O12	1201.841368	11	121	71	41.279070	804.926232	8	1	1	0	35	21
7	[Leu]-[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Leu]-[Val]-[NMe-Ala]-[NMe-Ala]-[NMe-Bmt]-[Leu]	C62H111N11O12	1201.841368	11	126	67	38.953488	812.921414	3	1	1	0	37	19

Tutorial 3: How to determine a complete sequence ?

- Examples of sequence tags:
 - [Val] **one-block tag**
 - [Val]-[Leu] **two-block tag, etc.**
 - ([Val]-[Leu]-*){2} **[Val]-[Leu]-[Val]-[Leu]**
 - ([Val]|[Leu]) **[Val] or [Leu]**
 - [Pro]-[Ile]-[Ile]\([Orn]-[N-Ac-Ile]\)[Phe]
branching at [Orn], branch is formed from [N-Ac-Ile]
 - ECMAScript syntax is supported (except “[” and “]”)
<http://www.cplusplus.com/reference/regex/ECMAScript/>

Example 3

Settings...

Peaklist

Peptide Type:

Peaklist File:

Precursor m/z Ratio:

Precursor Ion Adduct:

Charge:

Precursor m/z Error Tolerance:

Fragment m/z Error Tolerance:

Fragment m/z Error Tolerance for Deisotoping:

Minimum Threshold of Relative Intensity:

Minimum m/z Ratio:

Database of Building Blocks

Building Blocks Database File:

Maximum Number of Combined Blocks (start):

Maximum Number of Combined Blocks (middle):

Maximum Number of Combined Blocks (end):

Maximum Cumulative Mass of Blocks:

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Action with Blind Paths in De Novo Graph:

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☒

Enable Similarity Search: ☐

Application

Mode:

Sequence Database File:

Maximum Number of Threads:

Score Type:

Maximum Number of Candidate Peptides Reported:

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence:

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Example 3

- Correct sequence of Cyclosporin A “[NMe-Bmt]-[Abu]-[NMe-Gly]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]” was reported as a top hit.

CycloBranch

File Search Tools View Help

Filter Clear

* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Matched Bricks	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	A	A*	Ax	A*x	B	B*	Bx	B*x
1 * 1	[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[NMe-Ala]-[NMe-Bmt]-[Leu]	C62H111N11O12	1201.841368	11	124	72	41.860465	804.697520	8	1	0	0	37	21	4	0
2 * 2	[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[NMe-Bmt]-[NMe-Ala]	C62H111N11O12	1201.841368	11	124	72	41.860465	804.697520	8	1	0	0	37	21	4	0
3	[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[Gly]-[NMe-Leu]-[Leu]-[NMe-Bmt]-[NMe-Ala]	C62H111N11O12	1201.841368	11	117	66	38.372093	786.487847	6	0	1	0	35	22	1	0
4	[NMe-Ala]-[Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[Leu]-[NMe-Bmt]-[NMe-Ala]	C62H111N11O12	1201.841368	11	113	72	41.860465	813.979630	9	1	1	0	36	20	4	0
5	[Leu]-[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Leu]-[Val]-[NMe-Ala]-[NMe-Ala]-[NMe-Bmt]	C62H111N11O12	1201.841368	11	121	71	41.279070	804.926232	8	1	1	0	35	21	4	0
6	[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Leu]-[Val]-[NMe-Ala]-[NMe-Ala]-[NMe-Bmt]-[Leu]	C62H111N11O12	1201.841368	11	121	71	41.279070	804.926232	8	1	1	0	35	21	4	0
7	[Leu]-[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Leu]-[Val]-[NMe-Ala]-[NAC-Ile]-[Leu]	C62H111N11O12	1201.841368	11	126	67	38.953488	812.921414	3	1	1	0	37	19	5	0
8	[NMe-Leu]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Leu]-[NAC-Ile]-[Leu]	C62H111N11O12	1201.841368	11	125	67	38.953488	799.821893	5	1	1	0	35	21	3	0

Comparing theoretical spectra of candidates with the peak list...

.....10002000300040005000600070008000900010000

.....11000120001300014000150001600017000180001900020000

.....21000220002300024000250002600027000280002900030000

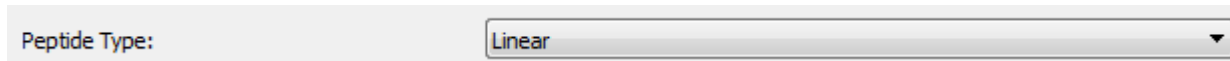
.....31000320003300034000350003600037000380003900040000

.....410004200043000440004500046000470004800049000ok

CycloBranch successfully finished at 10:15:00 (time elapsed: 0 hrs, 0 min, 57 sec).

Tutorial 4: How to determine a branched sequence ?

- Look for sequence tags in linear mode.

A screenshot of a web interface showing a dropdown menu for 'Peptide Type'. The menu is open, and the word 'Linear' is selected and displayed within the dropdown box. The background of the interface is light gray.

Peptide Type: Linear ▼

- If possible, look for a building block which may cause branching (lysine, ornithine, etc.).

Tutorial 4: How to determine a branched sequence ?

- Switch into the branched mode.



- Use a sequence tag:
 - [Asp]-[Gln] **sequence of blocks**
 - [Ser].*[Asp]-[Gln] **.* stands for unknown sequence**
 - \([Lys] **branching at [Lys]**
 - \([Lys].*\)[Asp]-[Gln] **partially known branched sequence**
- See also Tutorials 2 and 3.

Example 4a

Settings...

Peaklist

Peptide Type: Linear

Peaklist File: PeakLists/dau_677_recalibrated_10d.txt Select

Precursor m/z Ratio: 677.834850

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 2.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/20_amino_acids.txt Select

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Select

Miscellaneous

Action with Blind Paths in De Novo Graph: connect (allow detection of sequence tags)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: De Novo Search Engine

Sequence Database File: Select

Maximum Number of Threads: 1

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence: Edit

N-terminal Modification:

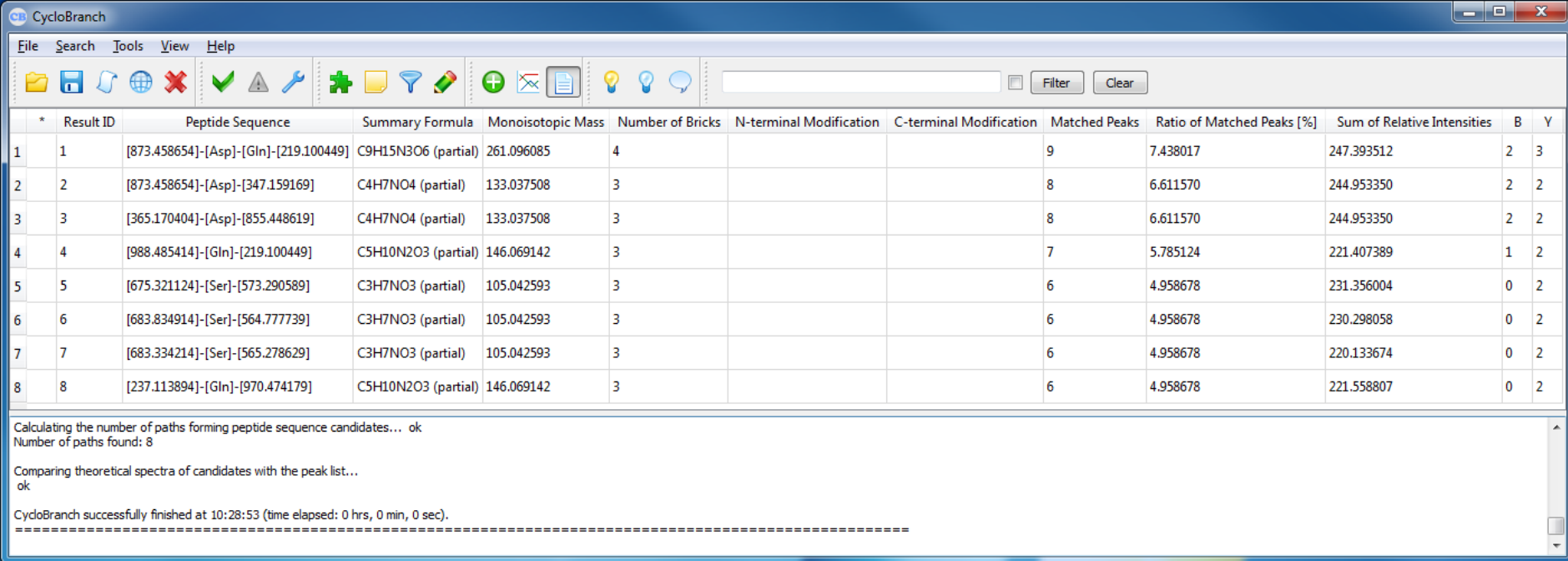
C-terminal Modification:

Branch Modification:

OK Cancel Apply Load Save 'tutorial4a.ini' Save As...

Example 4a

- Correct tag “[Asp]-[Gln]” was reported as a top hit.
- Correct tag “[Ser]” was also reported.



The screenshot shows the CycloBranch software interface. At the top is a menu bar with 'File', 'Search', 'Tools', 'View', and 'Help'. Below the menu is a toolbar with various icons for file operations, search, and analysis. A search bar with 'Filter' and 'Clear' buttons is located to the right of the toolbar. The main area contains a table with 12 columns: Result ID, Peptide Sequence, Summary Formula, Monoisotopic Mass, Number of Bricks, N-terminal Modification, C-terminal Modification, Matched Peaks, Ratio of Matched Peaks [%], Sum of Relative Intensities, B, and Y. The table lists 8 results. At the bottom, a status bar shows progress messages: 'Calculating the number of paths forming peptide sequence candidates... ok', 'Number of paths found: 8', 'Comparing theoretical spectra of candidates with the peak list... ok', and 'CycloBranch successfully finished at 10:28:53 (time elapsed: 0 hrs, 0 min, 0 sec)'.

	* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	N-terminal Modification	C-terminal Modification	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	B	Y
1	1	[873.458654]-[Asp]-[Gln]-[219.100449]	C9H15N3O6 (partial)	261.096085	4			9	7.438017	247.393512	2	3
2	2	[873.458654]-[Asp]-[347.159169]	C4H7NO4 (partial)	133.037508	3			8	6.611570	244.953350	2	2
3	3	[365.170404]-[Asp]-[855.448619]	C4H7NO4 (partial)	133.037508	3			8	6.611570	244.953350	2	2
4	4	[988.485414]-[Gln]-[219.100449]	C5H10N2O3 (partial)	146.069142	3			7	5.785124	221.407389	1	2
5	5	[675.321124]-[Ser]-[573.290589]	C3H7NO3 (partial)	105.042593	3			6	4.958678	231.356004	0	2
6	6	[683.834914]-[Ser]-[564.777739]	C3H7NO3 (partial)	105.042593	3			6	4.958678	230.298058	0	2
7	7	[683.334214]-[Ser]-[565.278629]	C3H7NO3 (partial)	105.042593	3			6	4.958678	220.133674	0	2
8	8	[237.113894]-[Gln]-[970.474179]	C5H10N2O3 (partial)	146.069142	3			6	4.958678	221.558807	0	2

Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 8

Comparing theoretical spectra of candidates with the peak list... ok

CycloBranch successfully finished at 10:28:53 (time elapsed: 0 hrs, 0 min, 0 sec).

Example 4b

Settings...

Peaklist

Peptide Type: Branched

Peaklist File: PeakLists/dau_677_recalibrated_10d.txt Select

Precursor m/z Ratio: 677.834850

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 2.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/20_amino_acids.txt Select

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 4

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/acetyl_amidated.txt Select

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: De Novo Search Engine

Sequence Database File: Select

Maximum Number of Threads: 6

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag: [Ser]. *[Asp]-[Gln]

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [Glu]-[Ser]-[Leu]\([Lys]-[Asn]-[Phe]-[Ile]\)[Asp]-[Gln]-[Tyr]-[Gly] Edit

N-terminal Modification: Acetyl

C-terminal Modification: Amidated

Branch Modification:

OK Cancel Apply Load Save 'tutorial4b.ini' Save As...

Example 4b

Modifications Editor

	Name	Summary Formula	Monoisotopic Mass	N-terminal	C-terminal
1	☐ Acetyl	H ₂ C ₂ O	42.010565	☒	☐
2	☐ Amidated	HNO-1	-0.984016	☐	☒

Close Add Row Remove Rows ☐ Filter Clear Load Save 'acetyl_amidated.txt' Save As...

Example 4b

CycloBranch											
File Search Tools View Help											
	* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	N-terminal Modification	Branch Modification	C-terminal Modification	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Rel
1	1	[Glu]-[Ser]-[Leu] ([Lys]-[Asn]-[Phe]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
2	2	[Glu]-[Ser]-[Leu] ([Lys]-[Leu]-[Asn]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
3	3	[Glu]-[Leu]-[Ser] ([Lys]-[Asn]-[Phe]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
4	4	[Glu]-[Leu]-[Ser] ([Lys]-[Leu]-[Phe]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
5	5	[Glu]-[Ser]-[Leu] ([Lys]-[Phe]-[Asn]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
6	6	[Glu]-[Ser]-[Leu] ([Lys]-[Phe]-[Leu]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
7	7	[Glu]-[Leu]-[Ser] ([Lys]-[Phe]-[Asn]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
8	8	[Glu]-[Ser]-[Leu] ([Lys]-[Leu]-[Phe]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
9	9	[Glu]-[Leu]-[Ser] ([Lys]-[Asn]-[Leu]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
10	10	[Glu]-[Leu]-[Ser] ([Lys]-[Leu]-[Asn]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
11	11	[Glu]-[Leu]-[Ser] ([Lys]-[Phe]-[Leu]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
12	12	[Glu]-[Ser]-[Leu] ([Lys]-[Asn]-[Leu]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
13	13	[Glu]-[Leu]-[Ser] ([Lys]-[Val]-[Gln]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
14	14	[Glu]-[Leu]-[Ser] ([Lys]-[Phe]-[Gln]-[Val]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
15	15	[Glu]-[Leu]-[Ser] ([Lys]-[Val]-[Phe]-[Gln]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
16	16	[Glu]-[Leu]-[Ser] ([Lys]-[Gln]-[Phe]-[Val]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
17	17	[Glu]-[Leu]-[Ser] ([Lys]-[Phe]-[Val]-[Gln]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
18	18	[Glu]-[Leu]-[Ser] ([Lys]-[Gln]-[Val]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
19	19	[Glu]-[Ser]-[Leu] ([Lys]-[Phe]-[Gln]-[Val]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
20	20	[Glu]-[Ser]-[Leu] ([Lys]-[Phe]-[Val]-[Gln]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
21	21	[Glu]-[Ser]-[Leu] ([Lys]-[Gln]-[Phe]-[Val]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
22	22	[Glu]-[Ser]-[Leu] ([Lys]-[Gln]-[Val]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
23	23	[Glu]-[Ser]-[Leu] ([Lys]-[Val]-[Phe]-[Gln]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
24	24	[Glu]-[Ser]-[Leu] ([Lys]-[Val]-[Gln]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23	19.008264	370.853312
25	25	[Glu]-[Leu]-[Ser] ([Leu]-[Lys]-[Phe]) [Asn]-[Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	21	17.355372	359.764118

Example 4b

- Correct sequence “^{Acetyl}[Glu]-[Ser]-[Leu]([Lys]-[Asn]-[Phe]-[Ile])[Asp]-[Gln]-[Tyr]-[Gly]_{Amidated}” was reported among 24 candidates with equal score (23 matched peaks).
- All 24 candidates have the same mask “^{Acetyl}[Glu]-[xxx]-[xxx]([Lys]-[xxx]-[xxx]-[xxx])[Asp]-[Gln]-[Tyr]-[Gly]_{Amidated}” → branching at [Lys]
- It is not easy to predict the numbers of combined blocks 2/4/1.
- Acetyl, Amidated must be listed in a list of modifications.

Tutorial 5: How to determine terminal modifications ?

- Open a file with modifications in “Tools -> Modifications Editor”.
- Remove unlikely modifications.
- Configure settings and click “Search -> Run” in the main menu.
- Detected modifications are shown in an output report.

Example 5

Modifications Editor

	Name	Summary Formula	Monoisotopic Mass	N-terminal	C-terminal
1 ☐	Acetyl	H ₂ C ₂ O	42.010565	☒	☐
2 ☐	Amidated	HNO-1	-0.984016	☐	☒
3 ☐	Ethanolamine	H ₅ C ₂ N	43.042199	☐	☒
4 ☐	Formyl	CO	27.994915	☒	☐

Close Add Row Remove Rows ☐ Filter Clear Load Save 'tutorial5.txt' Save As...

Example 5

Settings...

Peaklist

Peptide Type: Branched

Peaklist File: PeakLists/dau_677_recalibrated_10d.txt Select

Precursor m/z Ratio: 677.834850

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 2.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/20_amino_acids.txt Select

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 4

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/tutorial5.txt Select

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: De Novo Search Engine

Sequence Database File: Select

Maximum Number of Threads: 6

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag: [Ser].[Asp]-[Gln]

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [Glu]-[Ser]-[Leu] \ ([Lys]-[Asn]-[Phe]-[Ile] \) [Asp]-[Gln]-[Tyr]-[Gly] Edit

N-terminal Modification: Acetyl

C-terminal Modification: Amidated

Branch Modification:

OK Cancel Apply Load Save 'tutorial5.ini' Save As...

Example 5

CycloBranch

File Search Tools View Help

Filter Clear

	*	Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	N-terminal Modification	Branch Modification	C-terminal Modification	Ma
1		1	[Glu]-[Ser]-[Leu] ([Lys]-[Leu]-[Phe]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
2		2	[Glu]-[Leu]-[Ser] ([Lys]-[Leu]-[Phe]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
3		3	[Glu]-[Ser]-[Leu] ([Lys]-[Phe]-[Leu]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
4		4	[Glu]-[Ser]-[Leu] ([Lys]-[Phe]-[Asn]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
5		5	[Glu]-[Leu]-[Ser] ([Lys]-[Phe]-[Leu]-[Asn]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
6	*	6	[Glu]-[Ser]-[Leu] ([Lys]-[Asn]-[Phe]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
7		7	[Glu]-[Leu]-[Ser] ([Lys]-[Leu]-[Asn]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
8		8	[Glu]-[Ser]-[Leu] ([Lys]-[Leu]-[Asn]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
9		9	[Glu]-[Ser]-[Leu] ([Lys]-[Asn]-[Leu]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
10		10	[Glu]-[Leu]-[Ser] ([Lys]-[Asn]-[Phe]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
11		11	[Glu]-[Leu]-[Ser] ([Lys]-[Asn]-[Leu]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
12		12	[Glu]-[Leu]-[Ser] ([Lys]-[Phe]-[Asn]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23
13		13	[Glu]-[Leu]-[Ser] ([Lys]-[Val]-[Gln]-[Phe]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	23

Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 508
Number of edges: 5717

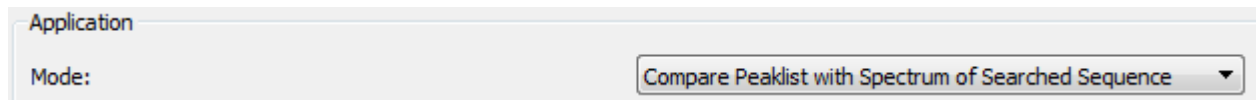
Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 2062

Comparing theoretical spectra of candidates with the peak list...
.....10002000 ok

CycloBranch successfully finished at 10:46:32 (time elapsed: 0 hrs, 0 min, 38 sec).

Tutorial 6: How to compare a theoretical spectrum with a peaklist ?

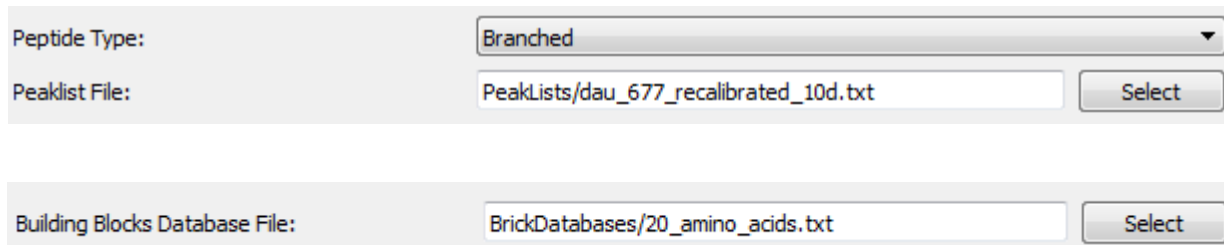
- Select mode – Compare Peaklist with Spectrum of Searched Sequence.



Application

Mode: Compare Peaklist with Spectrum of Searched Sequence ▼

- Select a peptide type, peaklist file and database of building blocks.



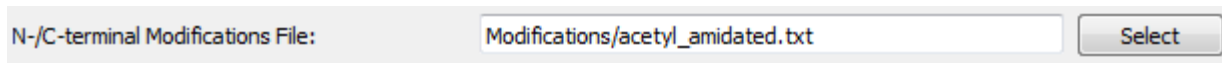
Peptide Type: Branched ▼

Peaklist File: PeakLists/dau_677_recalibrated_10d.txt Select

Building Blocks Database File: BrickDatabases/20_amino_acids.txt Select

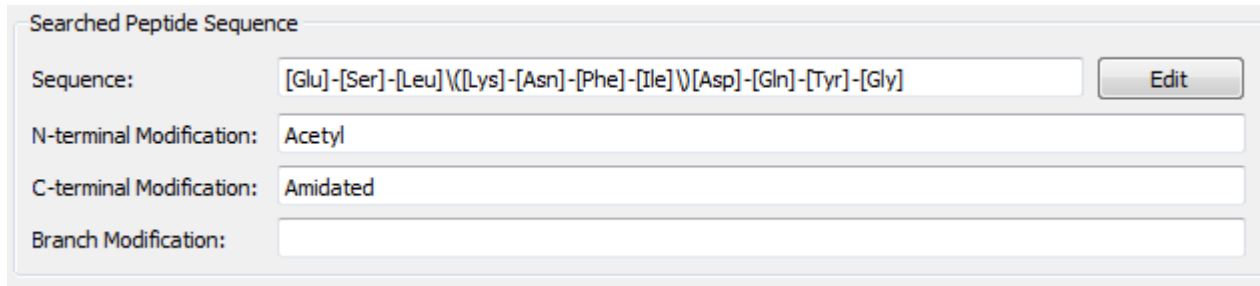
Tutorial 6: How to compare a theoretical spectrum with a peaklist ?

- Define “N-/C-terminal Modifications File”.



A screenshot of a software dialog box titled "N-/C-terminal Modifications File:". It features a text input field containing the file path "Modifications/acetyl_amidated.txt" and a "Select" button to the right.

- Define peptide sequence.



A screenshot of a software dialog box titled "Searched Peptide Sequence". It contains four input fields: "Sequence:" with the value "[Glu]-[Ser]-[Leu]\\([Lys]-[Asn]-[Phe]-[Ile]\\)[Asp]-[Gln]-[Tyr]-[Gly]", "N-terminal Modification:" with the value "Acetyl", "C-terminal Modification:" with the value "Amidated", and "Branch Modification:" which is empty. An "Edit" button is located to the right of the "Sequence:" field.

- Click “Search -> Run” in the main menu.

Example 6

Settings...

Peaklist

Peptide Type: Branched

Peaklist File: Peaklists/dau_677_recalibrated_10d.txt Select

Precursor m/z Ratio: 677.834850

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 2.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/20_amino_acids.txt Select

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 4

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/acetyl_amidated.txt Select

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: Compare Peaklist with Spectrum of Searched Sequence

Sequence Database File: Select

Maximum Number of Threads: 1

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [Glu]-[Ser]-[Leu]\([Lys]-[Asn]-[Phe]-[Ile]\[Asp]-[Gln]-[Tyr]-[Gly] Edit

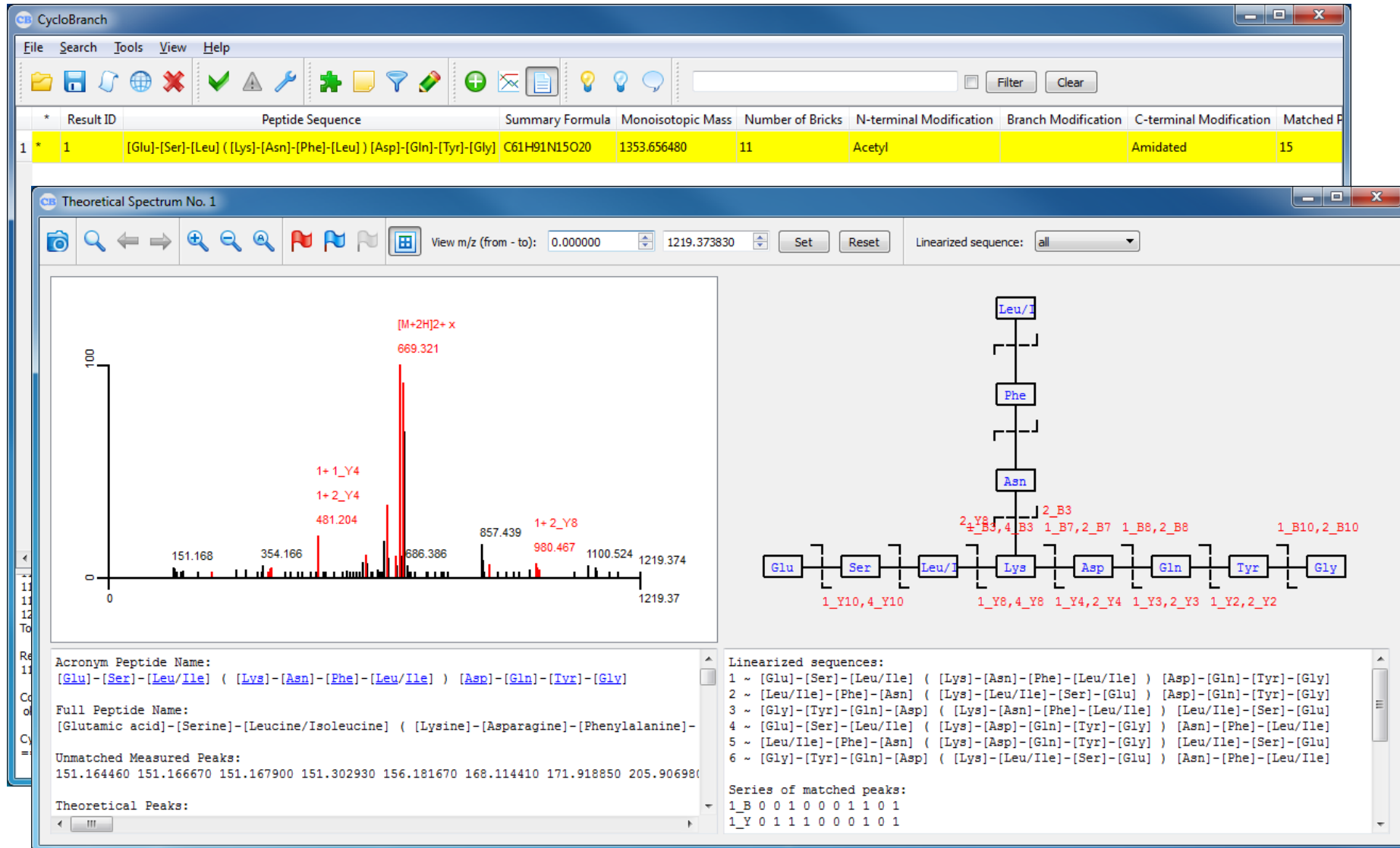
N-terminal Modification: Acetyl

C-terminal Modification: Amidated

Branch Modification:

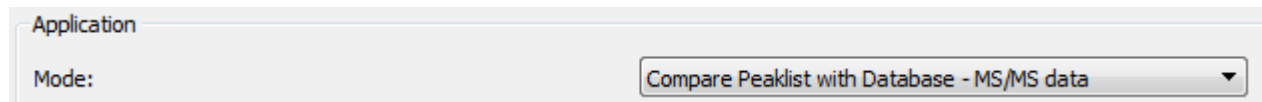
OK Cancel Apply Load Save 'tutorial6.ini' Save As...

Example 6



Tutorial 7: How to search in a database of MS/MS spectra ?

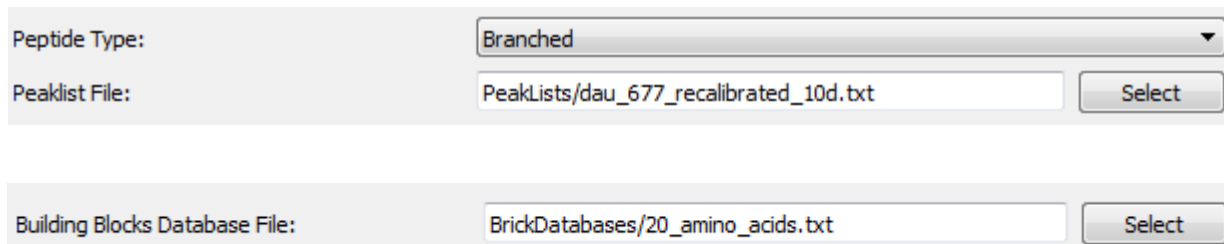
- Select mode – Compare Peaklist with Database – MS/MS data.



Application

Mode: Compare Peaklist with Database - MS/MS data

- Select a peptide type, peaklist file and database of building blocks.



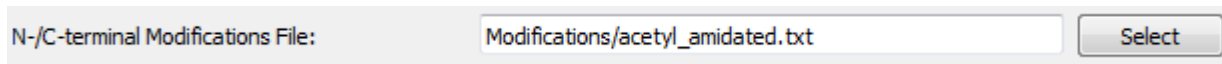
Peptide Type: Branched

Peaklist File: PeakLists/dau_677_recalibrated_10d.txt Select

Building Blocks Database File: BrickDatabases/20_amino_acids.txt Select

Tutorial 7: How to search in a database of MS/MS spectra ?

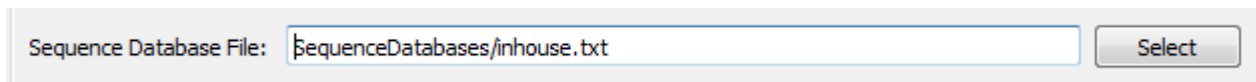
- Define “N-/C-terminal Modifications File”.



A screenshot of a software interface for selecting an N-/C-terminal Modifications File. It features a text input field containing the path "Modifications/acetyl_amidated.txt" and a "Select" button to its right.

N-/C-terminal Modifications File:

- Define database of peptide sequences.



A screenshot of a software interface for selecting a Sequence Database File. It features a text input field containing the path "SequenceDatabases/inhouse.txt" and a "Select" button to its right.

Sequence Database File:

- Click “Search -> Run” in the main menu.

Example 7

Settings...

Peaklist

Peptide Type: Branched

Peaklist File: PeakLists/dau_677_recalibrated_10d.txt

Precursor m/z Ratio: 677.834850

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance: 2.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 2.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db287_v2.txt

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 4

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/acetyl_amidated.txt

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: Compare Peaklist with Database - MS/MS data

Sequence Database File: SequenceDatabases/inhouse.txt

Maximum Number of Threads: 1

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

A
A*
Ax
A*x
B
B*
Bx
B*x
C
C*
Cx
C*x

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [Glu]-[Ser]-[Leu]\([Lys]-[Asn]-[Phe]-[Ile]\)[Asp]-[Gln]-[Tyr]-[Gly]

N-terminal Modification: Acetyl

C-terminal Modification: Amidated

Branch Modification:

Example 7

- Database of sequences.

Sequence Database Editor							
		Type	Name	Summary Formula	Monoisotopic Mass	Sequence	N-terminal Modification
1	☐	cyclic	beauverolide I	C27H41N3O5	487.304621	[C8:0-Me(4)-OH(3)]-[Phe]-[Ala]-[Leu]	
2	☐	cyclic	cyclosporin A	C62H111N11O12	1201.841368	[NMe-Bmt]-[Abu]-[NMe-Gly]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]	
3	☐	branched	dau 677 T2	C61H91N15O20	1353.656480	[Glu]-[Ser]-[Leu]\([Lys]-[Asn]-[Phe]-[Ile]\)[Asp]-[Gln]-[Tyr]-[Gly]	Acetyl
4	☐	linear	gramicidin C	C97H139N19O18	1858.054549	[Val]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Tyr]-[Leu]-[Trp]-[Leu]-[Trp]	Formyl
5	☐	branched	linearized pseudacyclin A	C39H63N7O8	757.473812	[Pro]-[Ile]-[Ile]\([Orn]-[NAC-Ile]\)[Phe]	
6	☐	cyclic	pseudacyclin A	C39H61N7O7	739.463247	[Phe]-[Pro]-[Ile]-[Ile]-[Orn]-[NAC-Ile]	
7	☐	branch-cyclic	pseudacyclin A	C39H61N7O7	739.463247	[Phe]-[Pro]-[Ile]-[Ile]\([Orn]-[NAC-Ile]\)	
8	☐	branch-cyclic	pyoverdin Pa A	C55H83N17O22	1333.589857	[Fo-OH-Orn]-[Thr]-[Thr]\([Lys]-[Fo-OH-Orn]-[Ser]-[Arg]-[Ser]-[ChrPaA]-[Suc]\)	
9	☐	cyclic	roseotoxin A	C31H53N5O7	607.394499	[C5:0-Me(4)-OH(2)]-[3Me-Pro]-[Ile]-[NMe-Val]-[NMe-Ala]-[bAla]	
10	☐	linear	substance P	C63H98N18O13S	1346.728146	[L-Arg]-[Pro]-[Lys]-[Pro]-[Gln]-[Gln]-[Phe]-[Phe]-[Gly]-[Leu]-[Met]	
11	☐	cyclic	surfactin C	C53H93N7O13	1035.683136	[C14:0-Me(13)-OH(3)]-[Glu]-[Leu]-[Leu]-[Val]-[Asp]-[Leu]-[Leu]	
12	☐	cyclic	valinomycin	C54H90N6O18	1110.631160	[Val]-[Lac]-[Val]-[Hiv]-[Val]-[Lac]-[Val]-[Hiv]-[Val]-[Lac]-[Val]-[Hiv]	

Close

Add Row

Remove Rows

☐ Filter

Clear

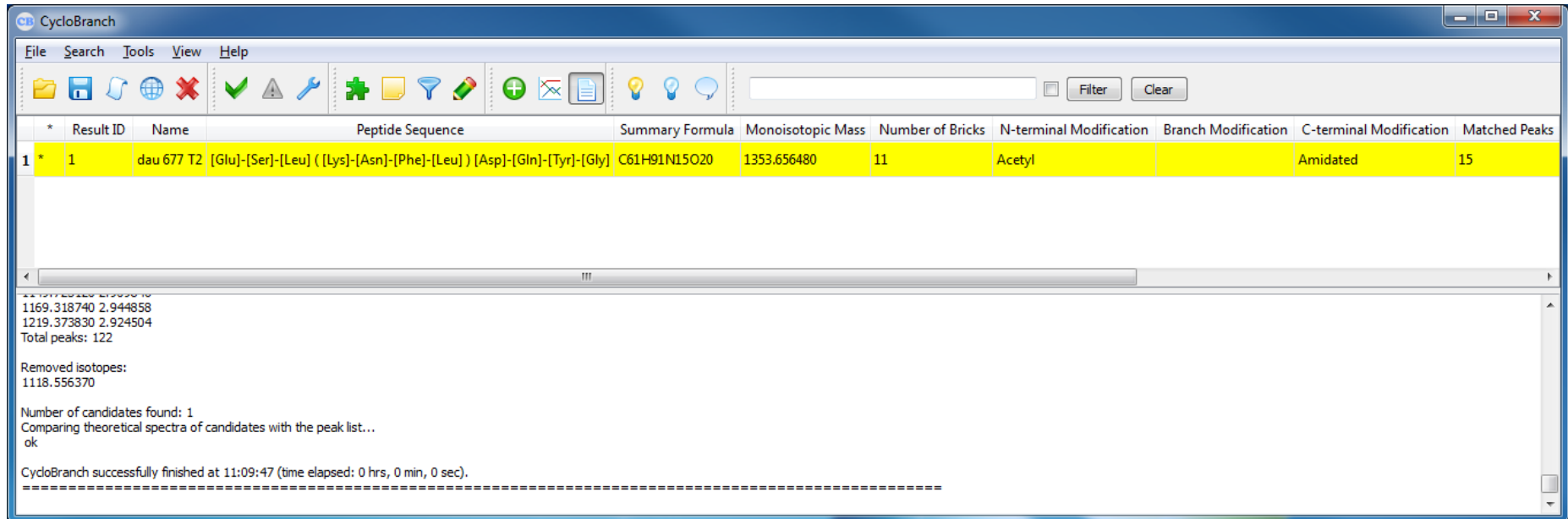
Load

Save 'inhouse.txt'

Save As...

Example 7

- Output.



The screenshot shows the CycloBranch software interface. The top menu bar includes File, Search, Tools, View, and Help. Below the menu is a toolbar with various icons for file operations, search, and analysis. A search bar with 'Filter' and 'Clear' buttons is located on the right. The main results table displays one entry with the following data:

* Result ID	Name	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	N-terminal Modification	Branch Modification	C-terminal Modification	Matched Peaks
1 *	dau 677 T2	[Glu]-[Ser]-[Leu] ([Lys]-[Asn]-[Phe]-[Leu]) [Asp]-[Gln]-[Tyr]-[Gly]	C61H91N15O20	1353.656480	11	Acetyl		Amidated	15

Below the table, a log window shows the following text:

```
1169.318740 2.944858
1219.373830 2.924504
Total peaks: 122

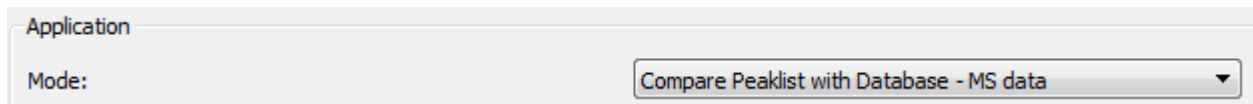
Removed isotopes:
1118.556370

Number of candidates found: 1
Comparing theoretical spectra of candidates with the peak list...
ok

CycloBranch successfully finished at 11:09:47 (time elapsed: 0 hrs, 0 min, 0 sec).
```

Tutorial 8: How to search MS spectrum against a database of compounds ?

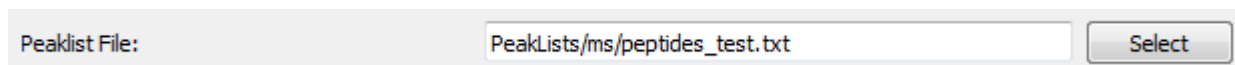
- Select mode – Compare Peaklist with Database – MS data.



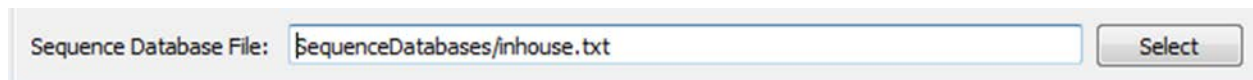
Application

Mode: Compare Peaklist with Database - MS data ▼

- Select a peaklist file and database of compounds.



Peaklist File: Select



Sequence Database File: Select

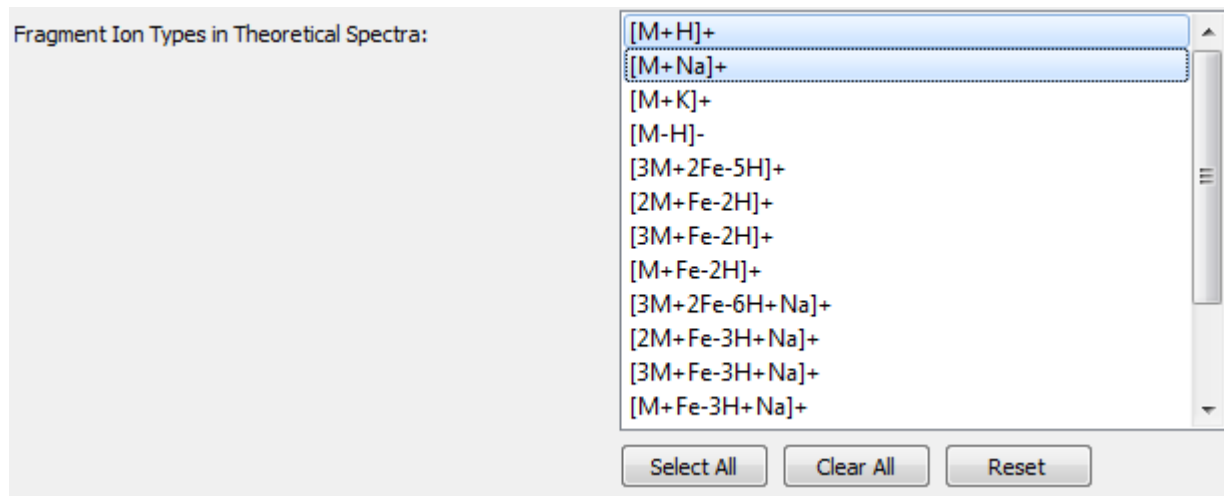
Tutorial 8: How to search MS spectrum against a database of compounds ?

- Set a maximum charge of ions.



Charge: 2

- Select types of ions to be detected.



Fragment Ion Types in Theoretical Spectra:

- [M+H]+
- [M+Na]+
- [M+K]+
- [M-H]-
- [3M+2Fe-5H]+
- [2M+Fe-2H]+
- [3M+Fe-2H]+
- [M+Fe-2H]+
- [3M+2Fe-6H+Na]+
- [2M+Fe-3H+Na]+
- [3M+Fe-3H+Na]+
- [M+Fe-3H+Na]+

Select All Clear All Reset

- Click “Search -> Run” in the main menu.

Example 8

Settings...

Peaklist

Peptide Type: Cyclic

Peaklist File: PeakLists/ms/peptides_test.txt

Precursor m/z Ratio: 0.000000

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File:

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: Compare Peaklist with Database - MS data

Sequence Database File: SequenceDatabases/inhouse.txt

Maximum Number of Threads: 1

Score Type: Number of b-ions

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- [M+H]⁺
- [M+Na]⁺
- [M+K]⁺
- [M-H]⁻
- [3M+2Fe-5H]⁺
- [2M+Fe-2H]⁺
- [3M+Fe-2H]⁺
- [M+Fe-2H]⁺
- [3M+2Fe-6H+Na]⁺
- [2M+Fe-3H+Na]⁺
- [3M+Fe-3H+Na]⁺
- [M+Fe-3H+Na]⁺

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence:

N-terminal Modification:

C-terminal Modification:

Branch Modification:

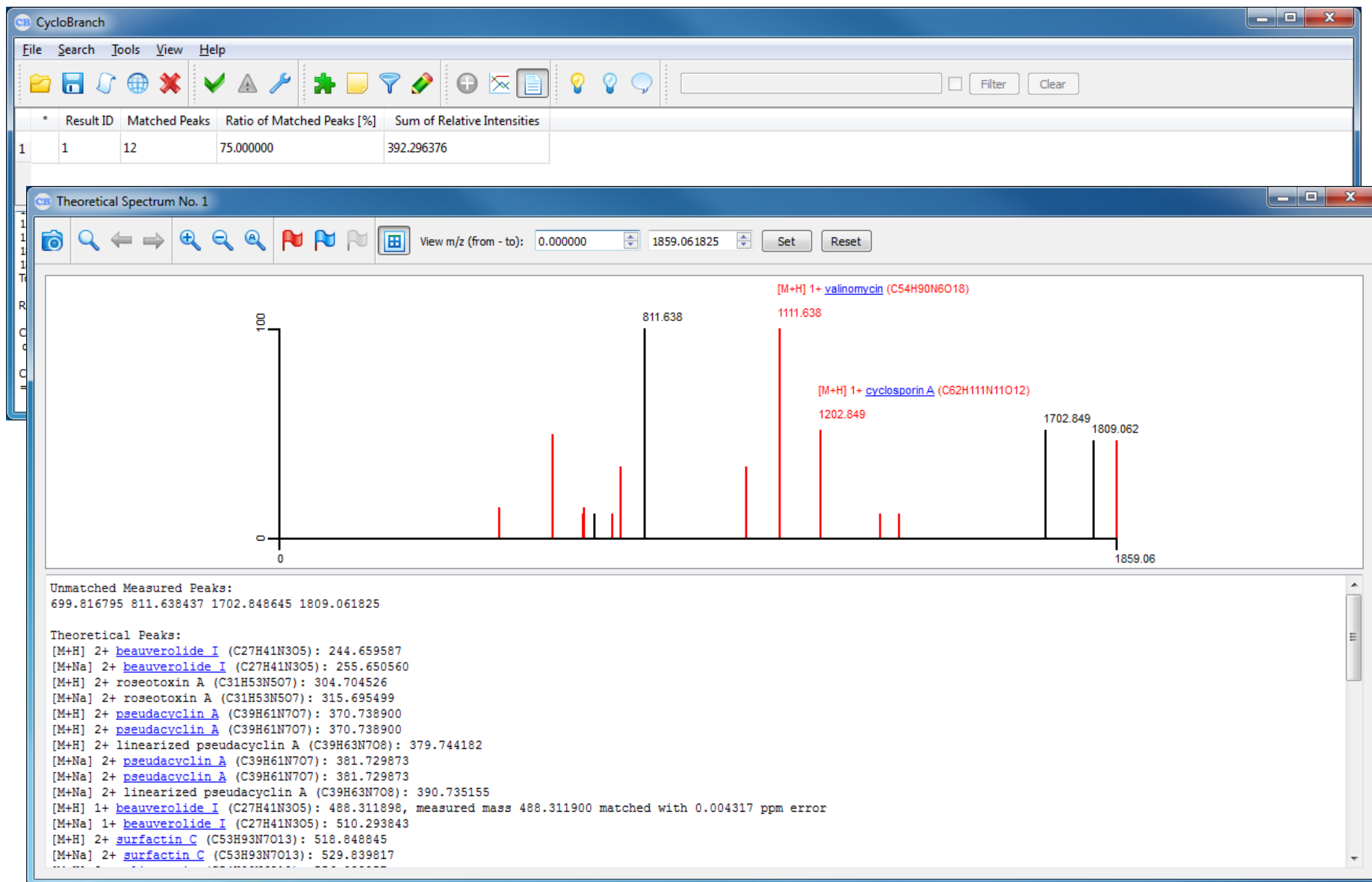
Example 8

- Database of compounds/sequences.

	Type	Name	Summary Formula	Monoisotopic Mass	Sequence	N-terminal Modification
1	☐ cyclic	beauverolide I	C27H41N3O5	487.304621	[C8:0-Me(4)-OH(3)]-[Phe]-[Ala]-[Leu]	
2	☐ cyclic	cyclosporin A	C62H111N11O12	1201.841368	[NMe-Bmt]-[Abu]-[NMe-Gly]-[NMe-Leu]-[Val]-[NMe-Leu]-[Ala]-[Ala]-[NMe-Leu]-[NMe-Leu]-[NMe-Val]	
3	☐ branched	dau 677 T2	C61H91N15O20	1353.656480	[Glu]-[Ser]-[Leu]\([Lys]-[Asn]-[Phe]-[Ile]\)[Asp]-[Gln]-[Tyr]-[Gly]	Acetyl
4	☐ linear	gramicidin C	C97H139N19O18	1858.054549	[Val]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Tyr]-[Leu]-[Trp]-[Leu]-[Trp]	Formyl
5	☐ branched	linearized pseudocyclin A	C39H63N7O8	757.473812	[Pro]-[Ile]-[Ile]\([Orn]-[NAC-Ile]\)[Phe]	
6	☐ cyclic	pseudocyclin A	C39H61N7O7	739.463247	[Phe]-[Pro]-[Ile]-[Ile]-[Orn]-[NAC-Ile]	
7	☐ branch-cyclic	pseudocyclin A	C39H61N7O7	739.463247	[Phe]-[Pro]-[Ile]-[Ile]\([Orn]-[NAC-Ile]\)	
8	☐ branch-cyclic	pyoverdin Pa A	C55H83N17O22	1333.589857	[Fo-OH-Orn]-[Thr]-[Thr]\([Lys]-[Fo-OH-Orn]-[Ser]-[Arg]-[Ser]-[ChrPaA]-[Suc]\)	
9	☐ cyclic	roseotoxin A	C31H53N5O7	607.394499	[C5:0-Me(4)-OH(2)]-[3Me-Pro]-[Ile]-[NMe-Val]-[NMe-Ala]-[bAla]	
10	☐ linear	substance P	C63H98N18O13S	1346.728146	[L-Arg]-[Pro]-[Lys]-[Pro]-[Gln]-[Gln]-[Phe]-[Phe]-[Gly]-[Leu]-[Met]	
11	☐ cyclic	surfactin C	C53H93N7O13	1035.683136	[C14:0-Me(13)-OH(3)]-[Glu]-[Leu]-[Leu]-[Val]-[Asp]-[Leu]-[Leu]	
12	☐ cyclic	valinomycin	C54H90N6O18	1110.631160	[Val]-[Lac]-[Val]-[Hiv]-[Val]-[Lac]-[Val]-[Hiv]-[Val]-[Lac]-[Val]-[Hiv]	

Close Add Row Remove Rows Filter Clear Load Save 'inhouse.txt' Save As...

Example 8



Tutorial 9: Draw Peptide Tool

Draw Peptide

Peptide Type: Linear

Sequence: [Ala]-[Leu]-[Tyr]-[Lys]-[Gln] correct Copy to Clipboard

Define Blocks Separately: ☒

Number of Blocks at Backbone: 5

Number of Blocks at Branch: 1

Branch Connection Block No.: 1

Backbone Blocks: Ala Leu Tyr Lys Gln

Branch Blocks:


```
graph LR; Ala[Ala] --- Leu[Leu]; Leu --- Tyr[Tyr]; Tyr --- Lys[Lys]; Lys --- Gln[Gln]
```

Tutorial 9: Draw Peptide Tool

Draw Peptide

Peptide Type: **Cyclic**

Sequence: correct [Copy to Clipboard](#)

Define Blocks Separately: ☒

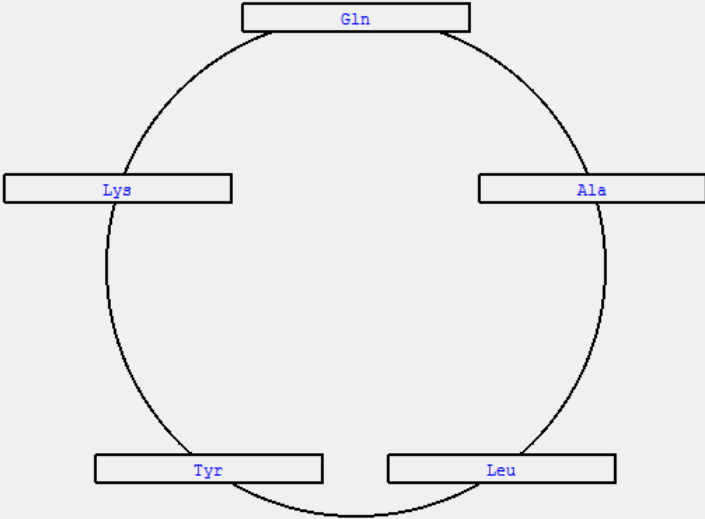
Number of Blocks at Backbone:

Number of Blocks at Branch:

Branch Connection Block No.:

Backbone Blocks:

Branch Blocks:



Tutorial 9: Draw Peptide Tool

Draw Peptide

Peptide Type: **Branched**

Sequence: correct [Copy to Clipboard](#)

Define Blocks Separately: ☒

Number of Blocks at Backbone:

Number of Blocks at Branch:

Branch Connection Block No.:

Backbone Blocks:

Branch Blocks:

```
graph LR; Ala[Ala] --- Leu[Leu]; Leu --- Tyr[Tyr]; Tyr --- Lys1[Lys]; Lys1 --- Gln[Gln]; Lys1 --- Ile[Ile]; Ile --- Lys2[Lys]; Lys2 --- Met[Met]
```

Tutorial 9: Draw Peptide Tool

Draw Peptide

Peptide Type: **Branch-cyclic**

Sequence: correct **Copy to Clipboard**

Define Blocks Separately: ☒

Number of Blocks at Backbone:

Number of Blocks at Branch:

Branch Connection Block No.:

Backbone Blocks:

Branch Blocks:

Copy to Clipboard

- Copy to Clipboard
- Set as Searched Sequence
- Set as Sequence Tag
- Insert into Sequence Database

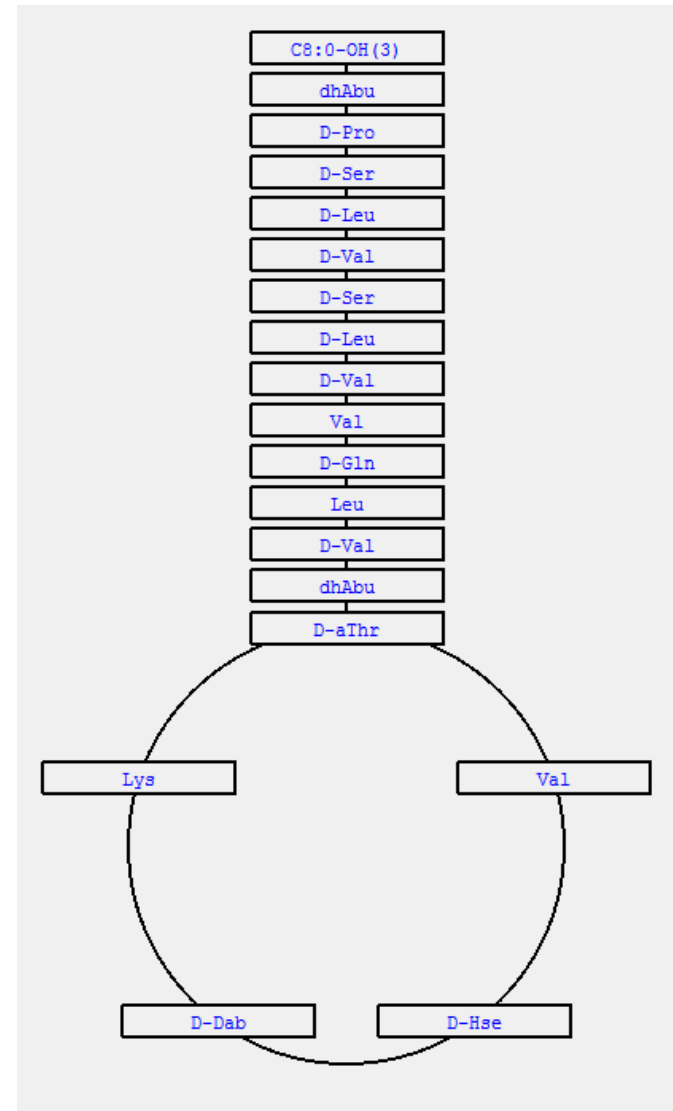
```
graph TD; Met[Met] --- Lys[Lys]; Lys --- Ile[Ile]; Ile --- Tyr[Tyr]; Tyr --- Ala[Ala]; Tyr --- Leu[Leu]; Tyr --- Lys2[Lys]; Tyr --- Gln[Gln]; Ala --- Gln;
```

Tutorial 10: Identification of Branch-Cyclic Peptide with a Long-sized Branch

- 3 ways of identification may be used for branch-cyclic peptides
 - short-sized branch (1-3 building blocks) → cyclic mode
 - long-sized branch (≥ 5 building blocks) → linear mode
 - medium-sized branch → branch-cyclic mode

Tutorial 10: Identification of Branch-Cyclic Peptide with a Long-sized Branch

- Tolaasin B
- linear mode
 - simple & fast identification of the branch
- branch-cyclic mode
 - more peaks are matched



Example 10a

- Comparison of experimental spectrum of Tolaasin B with theoretical spectrum in linear mode
- Select peptide type – linear

Peptide Type:

- Enable cyclic C-terminus
 - i.e., mass of H_2O is subtracted when C-terminal fragment ions are generated
 - mass of b-ion is equal to mass of (y- H_2O)-ion

Cyclic C-terminus: ☒

Example 10a

- Enter peptide sequence in “linear” format
 - “linear” format
 - the branch is red
 - [C8:0-OH(3)]-[dhAbu]-[D-Pro]-[D-Ser]-[D-Leu]-[D-Val]-[D-Ser]-[D-Leu]-[D-Val]-[Val]-[D-Gln]-[Leu]-[D-Val]-[dhAbu]-[D-aThr]-[Lys]-[D-Dab]-[D-Hse]-[Val]

Sequence:

[C8:0-OH(3)]-[dhAbu]-[D-Pro]-[D-Ser]-[D-Leu]-[D-Val]-[D-Ser]-[D-Leu]-[D-Val]-[Val]-[D-Gln]-[Leu]-[D-Val]-[dhAbu]-[D-aThr]-[Lys]-[D-Dab]-[D-Hse]-[Val]

Edit

- “branch-cyclic” format

- [Val]-[D-Hse]-[D-Dab]-[Lys]\([D-aThr]-[dhAbu]-[D-Val]-[Leu]-[D-Gln]-[Val]-[D-Val]-[D-Leu]-[D-Ser]-[D-Val]-[D-Leu]-[D-Ser]-[D-Pro]-[dhAbu]-[C8:0-OH(3)]\)

Sequence:

[Val]-[D-Hse]-[D-Dab]-[Lys]\([D-aThr]-[dhAbu]-[D-Val]-[Leu]-[D-Gln]-[Val]-[D-Val]-[D-Leu]-[D-Ser]-[D-Val]-[D-Leu]-[D-Ser]-[D-Pro]-[dhAbu]-[C8:0-OH(3)]\)

Edit

Example 10a

Settings...

Peaklist

Peptide Type: Linear

Peaklist File: PeakLists/tutorial10_tolaasinB_gnps.mgf Select

Precursor m/z Ratio: 987.105901

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 11.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 0.000 ppm

Minimum Threshold of Relative Intensity: 1.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db287_v2.txt Select

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Select

Miscellaneous

Action with Blind Paths in De Novo Graph: connect (allow detection of sequence tags)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☒

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: Compare Peaklist with Spectrum of Searched Sequence

Sequence Database File: Select

Maximum Number of Threads: 1

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

A
A*
Ax
A*x
B
B*
Bx
B*x
C
Cx
C*x

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: u-[D-Val]-[Val]-[D-Gln]-[Leu]-[D-Val]-[dhAbu]-[D-aThr]-[Lys]-[D-Dab]-[D-Hse]-[Val] Edit

N-terminal Modification:

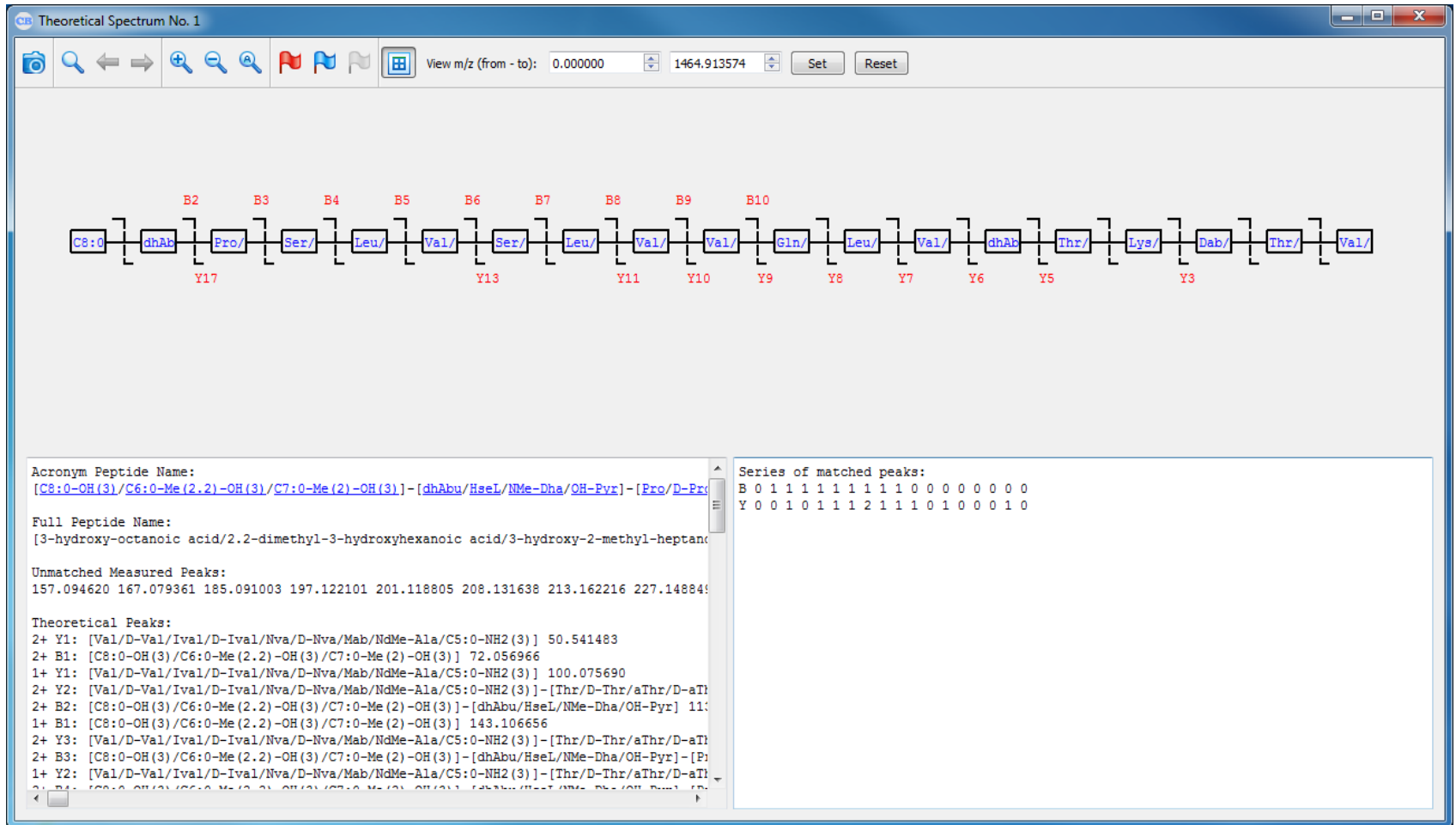
C-terminal Modification:

Branch Modification:

OK Cancel Apply Load Save 'tutorial10a.ini' Save As...

Example 10a

- 9 b-ions found + 11 (y-H₂O)-ions found



Example 10b

- Comparison of experimental spectrum of Tolaasin B with theoretical spectrum in branch-cyclic mode
- Select peptide type – branch-cyclic

Peptide Type:

Branch-cyclic ▼

- Enter peptide sequence in “branch-cyclic” format

Sequence:

[Val]-[D-Hse]-[D-Dab]-[Lys]\([D-aThr]-[dhAbu]-[D-Val]-[Leu]-[D-Gln]-[Val]-[D-Val]-[D-Leu]-[D-Ser]-[D-Val]-[D-Leu]-[D-Ser]-[D-Pro]-[dhAbu]-[C8:0-OH(3)]\)

Edit

Example 10b

Settings...

Peaklist

Peptide Type: Branch-cyclic

Peaklist File: PeakLists/tutorial10_tolaasinB_gnps.mgf

Precursor m/z Ratio: 987.105901

Precursor Ion Adduct:

Charge: 2

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 11.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 0.000 ppm

Minimum Threshold of Relative Intensity: 1.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db287_v2.txt

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Action with Blind Paths in De Novo Graph: connect (allow detection of sequence tags)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Application

Mode: Compare Peaklist with Spectrum of Searched Sequence

Sequence Database File:

Maximum Number of Threads: 1

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: a]-[D-Val]-[D-Leu]-[D-Ser]-[D-Val]-[D-Leu]-[D-Ser]-[D-Pro]-[dhAbu]-[C8:0-OH(3)]\

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Example 10a + 10b

CycloBranch

File Search Tools View Help

Filter Clear

* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	N-terminal Modification	C-terminal Modification	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	B	Y
1	[C8:0-OH(3)]-[dhAbu]-[Pro]-[Ser]-[Leu]-[Val]-[Ser]-[Leu]-[Val]-[Val]-[Gln]-[Leu]-[Val]-[dhAbu]-[Thr]-[Lys]-[Dab]-[Thr]-[Val]	C93H163N21O26	1990.207815	19			23	11.111111	188.291934	9	11

1165.736938 3.432877
1278.812988 1.241976
1351.827393 1.618755
1365.858032 2.344404
1366.854736 1.325705
1464.913574 2.079263
Total peaks: 207

Comparing theoretical spectra of candidates with the peak list...
ok

CycloBranch successfully finished at 11:25:14 (time elapsed: 0 hrs, 0 min, 0 sec).

CycloBranch

File Search Tools View Help

</

Example 10a + 10b

- Comparison in linear mode
 - 9 b-ions found
 - 11 (γ -H₂O)-ions found → 11 b-ions found
 - 20 b-ions found in total
- Comparison in branch-cyclic mode
 - 21 b-ions found
 - 1 γ -ion found
 - false hit (branch is N-terminated, γ -ions cannot be found)

Example 10c

- Detection of peptide sequence tag from the branch in linear mode

The screenshot shows the 'Settings...' dialog box with the following sections and values:

- Peaklist**
 - Peptide Type: Linear
 - Peaklist File: PeakLists/tutorial10_tolaasinB_gnps.mgf
 - Precursor m/z Ratio: 987.105901
 - Precursor Ion Adduct:
 - Charge: 2
 - Precursor m/z Error Tolerance: 10.000 ppm
 - Fragment m/z Error Tolerance: 15.000 ppm
 - Fragment m/z Error Tolerance for Deisotoping: 15.000 ppm
 - Minimum Threshold of Relative Intensity: 2.500
 - Minimum m/z Ratio: 150.000
- Database of Building Blocks**
 - Building Blocks Database File: BrickDatabases/gnps/gnps40.txt
 - Maximum Number of Combined Blocks (start): 1
 - Maximum Number of Combined Blocks (middle): 1
 - Maximum Number of Combined Blocks (end): 1
 - Maximum Cumulative Mass of Blocks: 0.000
 - Generate Permutations of Combined Blocks: ☒
 - N-/C-terminal Modifications File:
- Miscellaneous**
 - Action with Blind Paths in De Novo Graph: connect (allow detection of sequence tags)
 - Cyclic N-terminus: ☐
 - Cyclic C-terminus: ☒
 - Enable Scrambling: ☐
 - Enable Similarity Search: ☐
- Application**
 - Mode: De Novo Search Engine
 - Sequence Database File:
 - Maximum Number of Threads: 1
 - Score Type: Number of matched peaks
 - Maximum Number of Candidate Peptides Reported: 100
 - Peptide Sequence Tag:
 - Fragment Ion Types in Theoretical Spectra: A, A*, Ax, A*x, B, B*, Bx, B*x, C, C*, Cx, C*x
 - Remove Hits of Fragments without Hits of Parent Fragments: ☒
- Searched Peptide Sequence**
 - Sequence: [D-Pro]-[D-Ser]-[D-Leu]-[D-Val]-[D-Ser]-[D-Leu]-[D-Val]-[Val]
 - N-terminal Modification:
 - C-terminal Modification:
 - Branch Modification:

Buttons at the bottom: OK, Cancel, Apply, Load, Save 'tutorial10c.ini', Save As...

Example 10c

- Sequence tag was found [D-Pro]-[D-Ser]-[D-Leu]-[D-Val]-[D-Ser]-[D-Leu]-[D-Val]-[Val]

CycloBranch

File Search Tools View Help

Filter Clear

	* Result ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Matched Peaks	Sum of Relative Intensities	B	Y
1	1	[225.134310]-[Pro]-[Ser]-[Leu]-[Val]-[Ser]-[Leu]-[Val]-[Val]-[952.559933]	C38H68N8O11 (partial)	812.500755	13	164.401340	9	3
2	2	[952.559933]-[Val]-[Val]-[Leu]-[Ser]-[Val]-[Leu]-[Ser]-[Pro]-[225.134310]	C38H68N8O11 (partial)	812.500755	13	164.401340	3	9
3	3	[184.083727]-[Leu]-[Val]-[dhAbu]-[C8:0-OH(3)]-[Ser]-[Leu]-[Val]-[Val]-[952.559933]	C42H75N7O11 (partial)	853.552456	12	164.052470	9	2
4	4	[225.134310]-[Pro]-[NMe-Phe]-[Leu]-[Val]-[Pro]-[Lys]-[Val]-[952.559933]	C42H68N8O8 (partial)	812.516011	12	157.186715	8	3
5	5	[952.559933]-[Val]-[Lys]-[Pro]-[Val]-[Leu]-[NMe-Phe]-[Pro]-[225.134310]	C42H68N8O8 (partial)	812.516011	12	157.186715	3	8
6	6	[952.559933]-[Val]-[Val]-[Leu]-[Ser]-[C8:0-OH(3)]-[dhAbu]-[Val]-[Leu]-[184.083727]	C42H75N7O11 (partial)	853.552456	12	164.052470	2	9
7	7	[952.559933]-[Val]-[Val]-[Leu]-[Ser]-[C8:0-OH(3)]-[dhAbu]-[Val]-[297.165819]	C36H64N6O10 (partial)	740.468392	11	157.563494	2	8
8	8	[225.134310]-[Pro]-[Ser]-[Leu]-[Val]-[Ser]-[Leu]-[C12:0-OH(3)]-[952.559933]	C40H72N6O11 (partial)	812.525907	11	154.605080	8	2
9	9	[368.234148]-[Ala]-[dhAbu]-[Val]-[Ser]-[Leu]-[Val]-[Val]-[952.559933]	C31H55N7O9 (partial)	669.406126	11	141.110801	8	2
10	10	[225.134310]-[Pro]-[Ser]-[Leu]-[Val]-[Ser]-[Leu]-[Val]-[1051.636838]	C33H59N7O10 (partial)	713.432341	11	154.353893	8	2
11	11	[952.559933]-[Val]-[Val]-[Leu]-[Ser]-[Val]-[C8:0-OH(3)]-[Thr]-[279.158617]	C36H66N6O11 (partial)	758.478957	11	162.168574	2	8
12	12	[225.134310]-[Pro]-[NMe-Phe]-[Leu]-[Val]-[Val]-[Ac-OH-Orn]-[NMe-Ala]-[Leu]-[807.467587]	C48H79N9O11 (partial)	957.589904	11	71.560145	9	2
13	13	[225.134310]-[Pro]-[NMe-Phe]-[Bmt]-[Bmt]-[Val]-[Val]-[952.559933]	C43H68N6O9 (partial)	812.504778	11	142.673737	7	3
14	14	[299.185412]-[Val]-[Pro]-[Thr]-[Val]-[Pro]-[Lys]-[Val]-[952.559933]	C35H62N8O9 (partial)	738.463976	11	135.193972	8	2
15	15	[299.185412]-[Pro]-[dhAbu]-[C8:0-OH(3)]-[Ser]-[Leu]-[Val]-[Val]-[952.559933]	C36H62N6O10 (partial)	738.452742	11	143.594753	8	2

Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 1136

Comparing theoretical spectra of candidates with the peak list...
.....1000 . ok

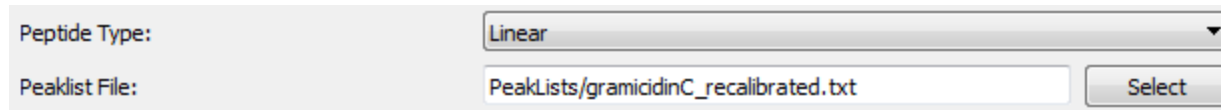
CycloBranch successfully finished at 09:02:50 (time elapsed: 0 hrs, 0 min, 18 sec).

Tutorial 11: Identification of Peptide Family using Similarity Search

- Suitable when a searched peptide is not included in a sequence database but its analogue is included (i.e., one or more building blocks are different)

Tutorial 11: Identification of Peptide Family using Similarity Search

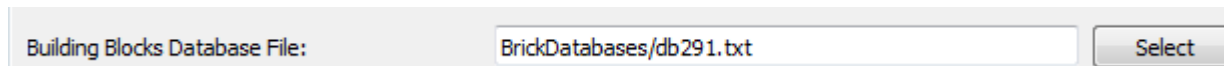
- Define peptide type and peaklist file



Peptide Type:

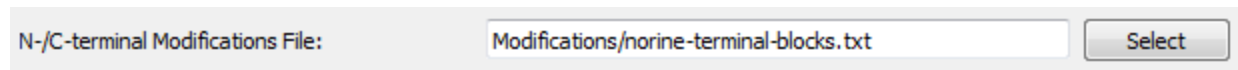
Peaklist File:

- Select a database of building blocks



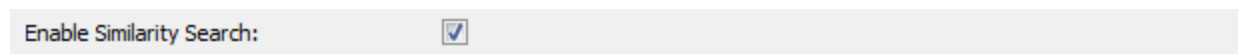
Building Blocks Database File:

- Select N-/C-terminal Modifications File



N-/C-terminal Modifications File:

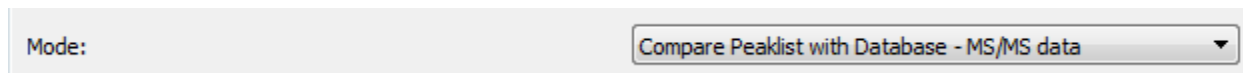
- Enable Similarity Search
 - this option disables filtering of peptide sequence candidates by precursor mass



Enable Similarity Search: ☒

Tutorial 11: Identification of Peptide Family using Similarity Search

- Select mode – Compare Peaklist with Database – MS/MS data



Mode: Compare Peaklist with Database - MS/MS data ▼

- Select a database of sequences



Sequence Database File: SequenceDatabases/norine-07-2013-linear.txt Select

Tutorial 11: Identification of Peptide Family using Similarity Search

Settings...

Peaklist

Peptide Type: Linear

Peaklist File: PeakLists/gramicidinC_recalibrated.txt

Precursor m/z Ratio: 1859.061825

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance: 1.000 ppm

Fragment m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 0.000

Minimum m/z Ratio: 150.000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/db291.txt

Maximum Number of Combined Blocks (start): 3

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 5

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/norine-terminal-blocks.txt

Miscellaneous

Action with Blind Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☒

Application

Mode: Compare Peaklist with Database - MS/MS data

Sequence Database File: SequenceDatabases/norine-07-2013-linear.txt

Maximum Number of Threads: 1

Score Type: Number of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Fragment Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence:

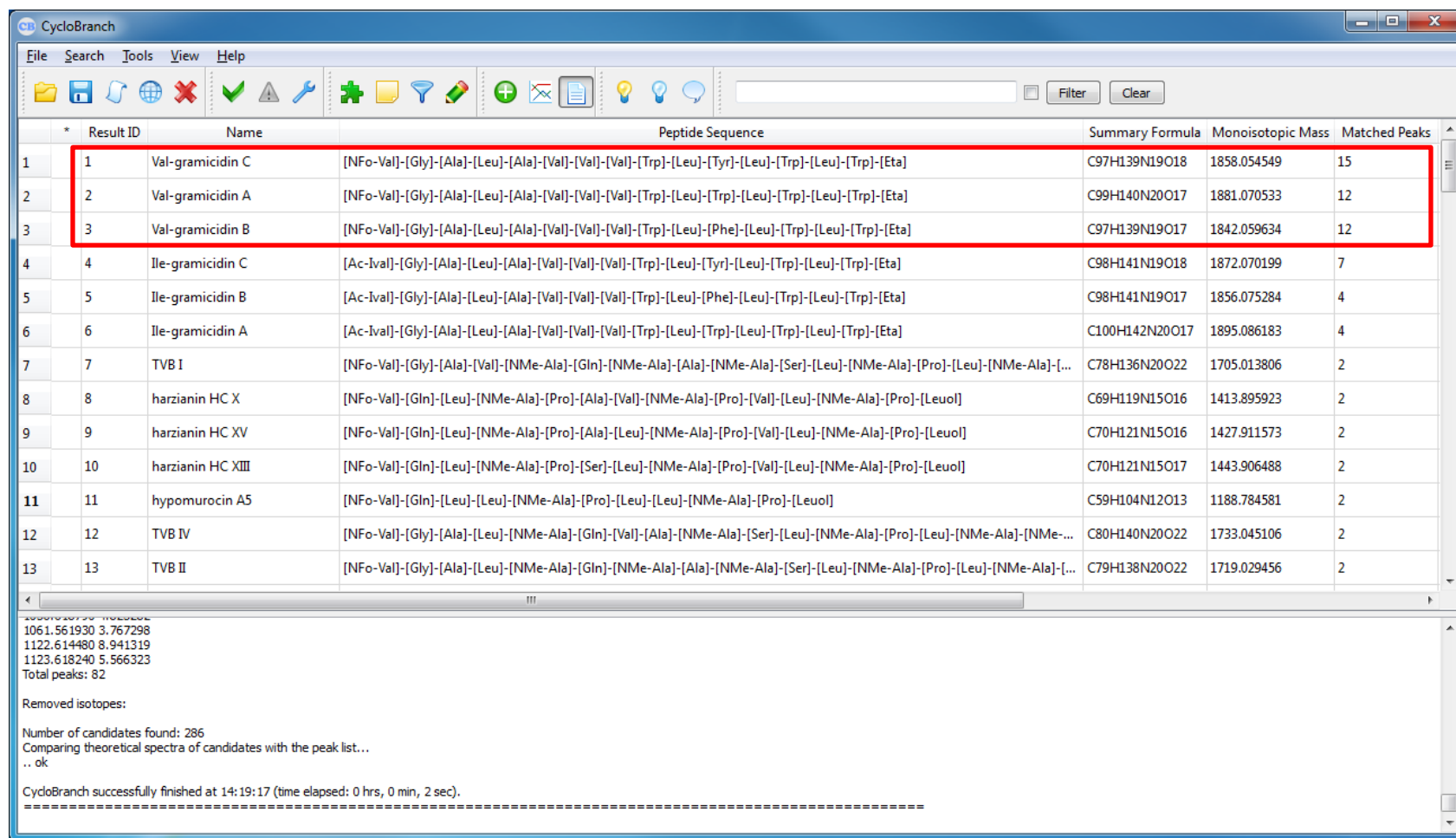
N-terminal Modification:

C-terminal Modification:

Branch Modification:

Tutorial 11: Identification of Peptide Family using Similarity Search

- Gramicidin C and 2 other analogs were found



	* Result ID	Name	Peptide Sequence	Summary Formula	Monoisotopic Mass	Matched Peaks
1	1	Val-gramicidin C	[NFo-Val]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Tyr]-[Leu]-[Trp]-[Leu]-[Trp]-[Eta]	C97H139N19O18	1858.054549	15
2	2	Val-gramicidin A	[NFo-Val]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Trp]-[Leu]-[Trp]-[Leu]-[Trp]-[Eta]	C99H140N20O17	1881.070533	12
3	3	Val-gramicidin B	[NFo-Val]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Phe]-[Leu]-[Trp]-[Leu]-[Trp]-[Eta]	C97H139N19O17	1842.059634	12
4	4	Ile-gramicidin C	[Ac-Ival]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Tyr]-[Leu]-[Trp]-[Leu]-[Trp]-[Eta]	C98H141N19O18	1872.070199	7
5	5	Ile-gramicidin B	[Ac-Ival]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Phe]-[Leu]-[Trp]-[Leu]-[Trp]-[Eta]	C98H141N19O17	1856.075284	4
6	6	Ile-gramicidin A	[Ac-Ival]-[Gly]-[Ala]-[Leu]-[Ala]-[Val]-[Val]-[Val]-[Trp]-[Leu]-[Trp]-[Leu]-[Trp]-[Leu]-[Trp]-[Eta]	C100H142N20O17	1895.086183	4
7	7	TVB I	[NFo-Val]-[Gly]-[Ala]-[Val]-[NMe-Ala]-[Gln]-[NMe-Ala]-[Ala]-[NMe-Ala]-[Ser]-[Leu]-[NMe-Ala]-[Pro]-[Leu]-[NMe-Ala]-[...]	C78H136N20O22	1705.013806	2
8	8	harzianin HC X	[NFo-Val]-[Gln]-[Leu]-[NMe-Ala]-[Pro]-[Ala]-[Val]-[NMe-Ala]-[Pro]-[Val]-[Leu]-[NMe-Ala]-[Pro]-[Leuol]	C69H119N15O16	1413.895923	2
9	9	harzianin HC XV	[NFo-Val]-[Gln]-[Leu]-[NMe-Ala]-[Pro]-[Ala]-[Leu]-[NMe-Ala]-[Pro]-[Val]-[Leu]-[NMe-Ala]-[Pro]-[Leuol]	C70H121N15O16	1427.911573	2
10	10	harzianin HC XIII	[NFo-Val]-[Gln]-[Leu]-[NMe-Ala]-[Pro]-[Ser]-[Leu]-[NMe-Ala]-[Pro]-[Val]-[Leu]-[NMe-Ala]-[Pro]-[Leuol]	C70H121N15O17	1443.906488	2
11	11	hypomurocin A5	[NFo-Val]-[Gln]-[Leu]-[Leu]-[NMe-Ala]-[Pro]-[Leu]-[Leu]-[NMe-Ala]-[Pro]-[Leuol]	C59H104N12O13	1188.784581	2
12	12	TVB IV	[NFo-Val]-[Gly]-[Ala]-[Leu]-[NMe-Ala]-[Gln]-[Val]-[Ala]-[NMe-Ala]-[Ser]-[Leu]-[NMe-Ala]-[Pro]-[Leu]-[NMe-Ala]-[NMe-...]	C80H140N20O22	1733.045106	2
13	13	TVB II	[NFo-Val]-[Gly]-[Ala]-[Leu]-[NMe-Ala]-[Gln]-[NMe-Ala]-[Ala]-[NMe-Ala]-[Ser]-[Leu]-[NMe-Ala]-[Pro]-[Leu]-[NMe-Ala]-[...]	C79H138N20O22	1719.029456	2

1061.561930 3.767298
1122.614480 8.941319
1123.618240 5.566323
Total peaks: 82
Removed isotopes:
Number of candidates found: 286
Comparing theoretical spectra of candidates with the peak list...
.. ok
CycloBranch successfully finished at 14:19:17 (time elapsed: 0 hrs, 0 min, 2 sec).

Tutorial 12: Identification of Siderophores in MS/MS data

- CycloBranch can identify siderophores in
 - MS/MS data
 - linear, cyclic, branched, and branch-cyclic NRP siderophores
 - linear and cyclic polyketide siderophores
 - MALDI-MS and LC-MS data
 - imaging mass spectra (MSI)
 - various biometals are supported - Li, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga)

Example 12a: Identification of Fe-TAFC

- ferri-form of triacetylfusarinine C (Fe-TAFC)
- precursor ion adduct must be defined
 - $[M+Fe-2H]^+ \rightarrow FeH-2$

Precursor Ion Adduct:

FeH-2

- other examples:
 - $[M+H]^+ \rightarrow \text{empty or H}$
 - $[M+Fe-3H+Na]^+ \rightarrow FeH-3Na$
 - $[M+Al-2H]^+ \rightarrow AlH-2$
 - $[M+Zn-H]^+ \rightarrow ZnH-1$

Example 12a: Identification of Fe-TAFC

Settings...

Spectrum

Peptide Type: Cyclic

File: PeakLists/siderophores/fe_tafc.txt

Precursor m/z Ratio: 906.330350

Precursor Ion Adduct: FeH-2

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance: 5.000 ppm

m/z Error Tolerance for Deisotoping: 5.000 ppm

Minimum Threshold of Relative Intensity: 2.500 %

Minimum m/z Ratio: 0.000

FWHM: 0.100000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/inhouse_siderophores_blocks.txt

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 2

Maximum Number of Combined Blocks (end): 2

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Incomplete Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Regular Order of Ketide Blocks: ☐

Application

Mode: De Novo Search Engine

Sequence Database File:

Maximum Number of Threads: 1

Score Type: Sum of relative intensities of matched peaks

Maximum Number of Candidate Peptides Reported: 500

Peptide Sequence Tag:

Ion Types in Theoretical Spectra:

- A
- A*
- Ax
- A*x
- B
- B*
- Bx
- B*x
- C
- C*
- Cx
- C*x

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Example 12a: Identification of Fe-TAFC

- Fe-TAFC was reported among candidates 1-8 belonging to 4 groups

CycloBranch

File Search Tools View Help

Filter Reset

	* Result ID	Group ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Matched Bricks	Matched Pe
1 *	1	1	[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]	C39FeH57N6O15	905.323130	6	54	15
2	2	1	[N2Ac-OH-Orn]-[OH-MP]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]	C39FeH57N6O15	905.323130	6	42	15
3	3	2	[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[N2Ac-OH-Orn]-[OH-MP]	C39FeH57N6O15	905.323130	6	42	15
4	4	2	[N2Ac-OH-Orn]-[OH-MP]-[OH-MP]-[N2Ac-OH-Orn]-[N2Ac-OH-Orn]-[OH-MP]	C39FeH57N6O15	905.323130	6	42	15
5	5	3	[OH-MP]-[N2Ac-OH-Orn]-[N2Ac-OH-Orn]-[OH-MP]-[OH-MP]-[N2Ac-OH-Orn]	C39FeH57N6O15	905.323130	6	42	15
6	6	3	[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[OH-MP]-[N2Ac-OH-Orn]	C39FeH57N6O15	905.323130	6	42	15
7	7	4	[OH-MP]-[N2Ac-OH-Orn]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]	C39FeH57N6O15	905.323130	6	42	15
8 *	8	4	[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]-[N2Ac-OH-Orn]-[OH-MP]	C39FeH57N6O15	905.323130	6	54	15

Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 6
Number of edges: 10

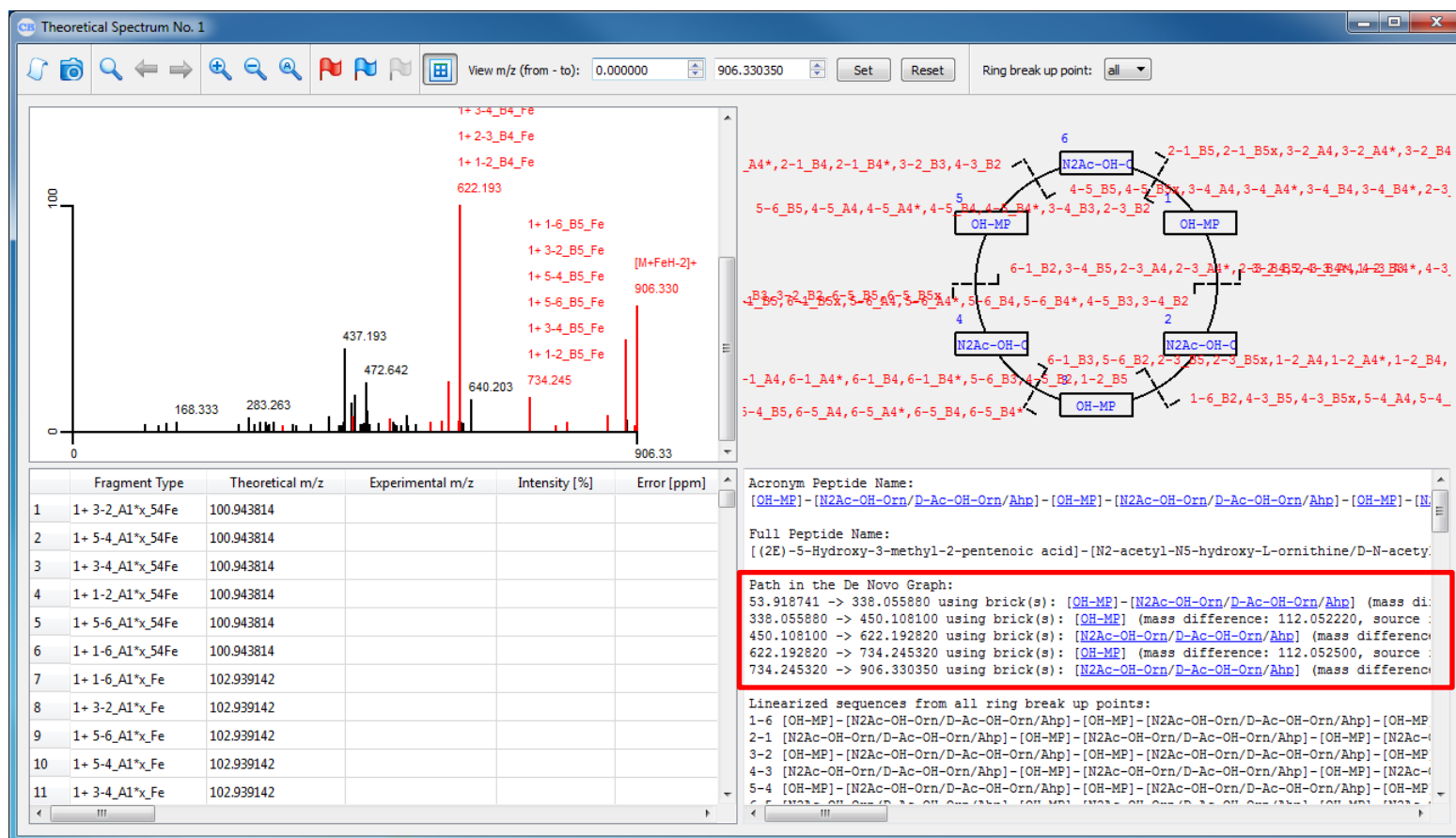
Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 4

Comparing theoretical spectra of candidates with the peak list... ok

CycloBranch successfully finished at 09:00:53 (time elapsed: 0 hrs, 0 min, 0 sec).

Example 12a: Identification of Fe-TAFC

- a group corresponds to a path in the *de novo* graph, i.e., the 8 sequence candidates were generated from 4 paths by permutations of building blocks



Example 12b: Identification of desferri-ferrioxamine B

- linear polyketide must be selected

Peptide Type:

- database of ketide blocks must be defined

Building Blocks Database File:

- ketide terminal modifications must be defined

N-/C-terminal Modifications File:

Example 12b: Identification of desferri-ferrioxamine B

Settings...

Spectrum

Peptide Type: Linear polyketide

File: PeakLists/siderophores/desferri_ferrioxamineB_18V.txt Select

Precursor m/z Ratio: 561.360500

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 3.000 %

Minimum m/z Ratio: 0.000

FWHM: 0.100000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/inhouse_siderophores_blocks.txt Select

Maximum Number of Combined Blocks (start): 2

Maximum Number of Combined Blocks (middle): 2

Maximum Number of Combined Blocks (end): 2

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/siderophores/ketide_modifications.txt Select

Miscellaneous

Incomplete Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Regular Order of Ketide Blocks: ☒

Application

Mode: De Novo Search Engine

Sequence Database File: Select

Maximum Number of Threads: 1

Score Type: Sum of relative intensities of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Ion Types in Theoretical Spectra:

- LB
- LB*
- LBx
- LB*x
- LB-CO
- LB*-CO
- LBx-CO
- LB*x-CO
- LB+2H
- LB*+2H
- LBx+2H
- LB*x+2H

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [Hpd]-[Suc]-[Hpd]-[Suc]-[Hpd] Edit

N-terminal Modification: Acetyl

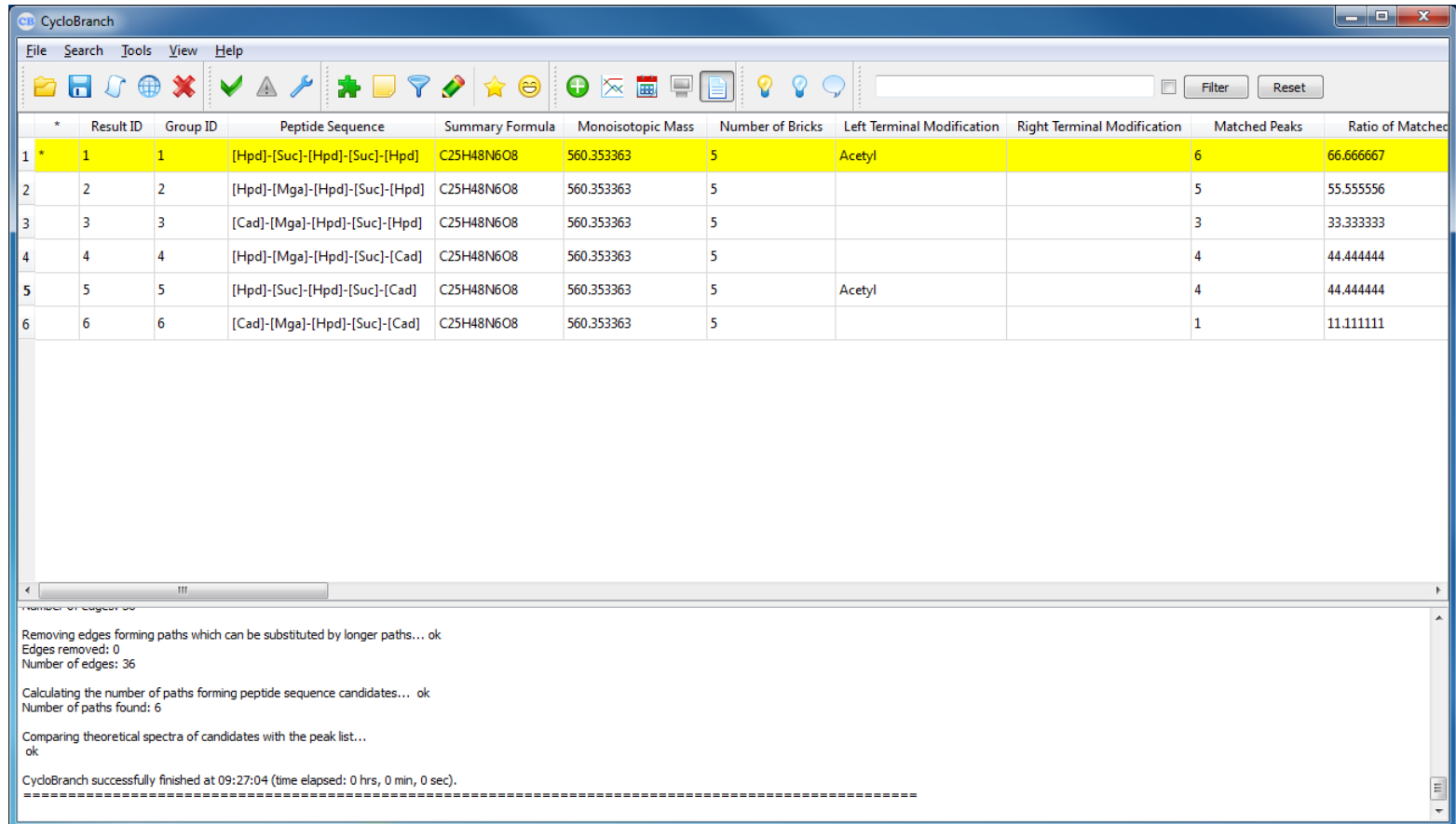
C-terminal Modification:

Branch Modification:

OK Cancel Apply Load Save 'tutorial12b.ini' Save As...

Example 12b: Identification of desferri-ferrioxamine B

- desferri-ferrioxamine B was reported as the top hit (6 peaks were matched)



The screenshot shows the CycloBranch software interface. The main window displays a table of search results. The top hit is highlighted in yellow. The table has the following columns: Result ID, Group ID, Peptide Sequence, Summary Formula, Monoisotopic Mass, Number of Bricks, Left Terminal Modification, Right Terminal Modification, Matched Peaks, and Ratio of Matched Peaks.

	* Result ID	Group ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Left Terminal Modification	Right Terminal Modification	Matched Peaks	Ratio of Matched
1	1	1	[Hpd]-[Suc]-[Hpd]-[Suc]-[Hpd]	C25H48N6O8	560.353363	5	Acetyl		6	66.666667
2	2	2	[Hpd]-[Mga]-[Hpd]-[Suc]-[Hpd]	C25H48N6O8	560.353363	5			5	55.555556
3	3	3	[Cad]-[Mga]-[Hpd]-[Suc]-[Hpd]	C25H48N6O8	560.353363	5			3	33.333333
4	4	4	[Hpd]-[Mga]-[Hpd]-[Suc]-[Cad]	C25H48N6O8	560.353363	5			4	44.444444
5	5	5	[Hpd]-[Suc]-[Hpd]-[Suc]-[Cad]	C25H48N6O8	560.353363	5	Acetyl		4	44.444444
6	6	6	[Cad]-[Mga]-[Hpd]-[Suc]-[Cad]	C25H48N6O8	560.353363	5			1	11.111111

Below the table, the software status is displayed:

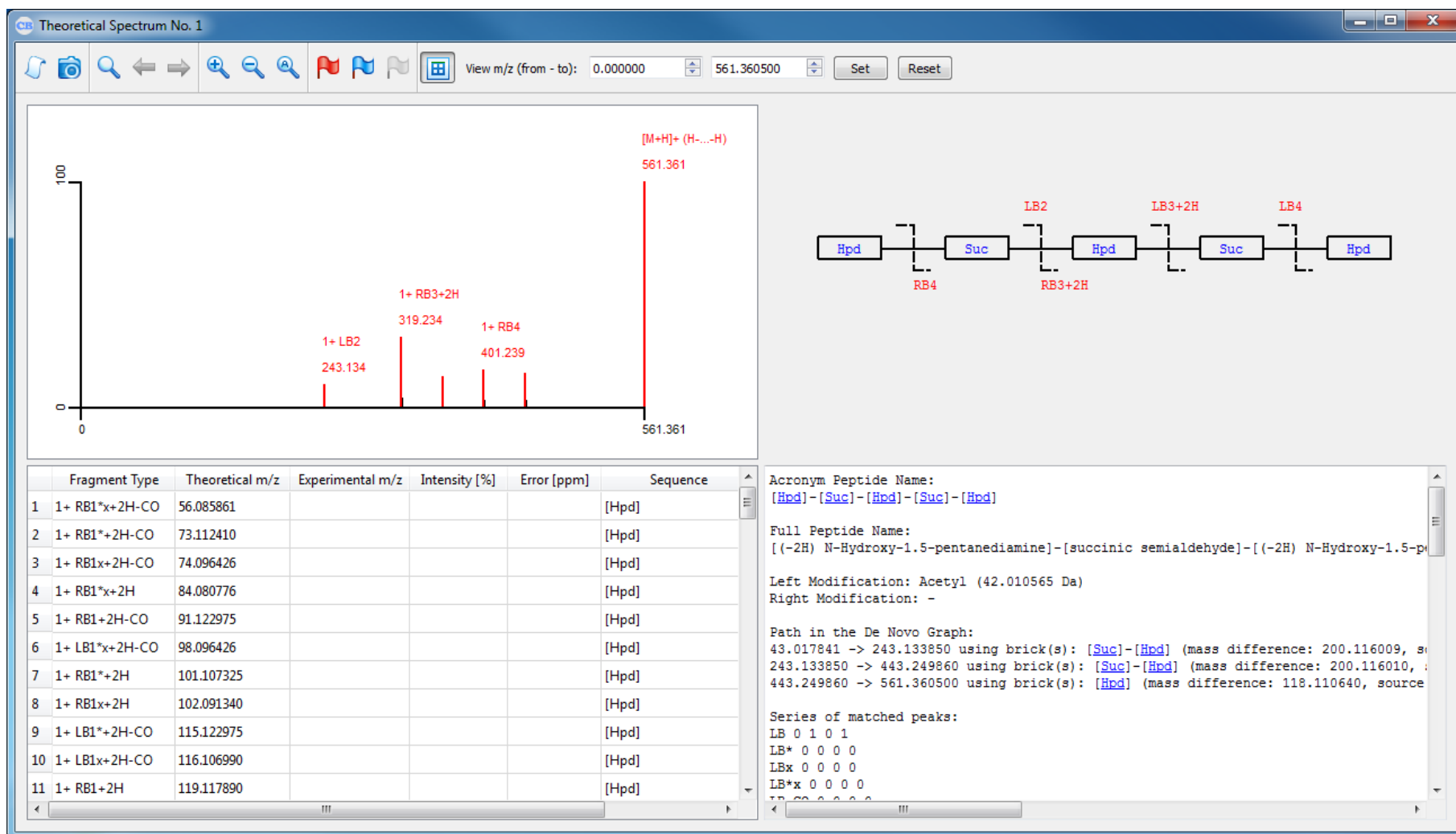
Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 0
Number of edges: 36

Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 6

Comparing theoretical spectra of candidates with the peak list... ok

CycloBranch successfully finished at 09:27:04 (time elapsed: 0 hrs, 0 min, 0 sec).

Example 12b: Identification of desferri-ferrioxamine B



Example 12c: Identification of ferri-ferrioxamine B

- precursor ion adduct must be defined

Precursor Ion Adduct:

FeH-2

Example 12c: Identification of ferri-ferrioxamine B

Settings...

Spectrum

Peptide Type: Linear polyketide

File: PeakLists/siderophores/ferri_ferrioxamineB.txt Select

Precursor m/z Ratio: 614.271980

Precursor Ion Adduct: FeH-2

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance for Deisotoping: 1.000 ppm

Minimum Threshold of Relative Intensity: 2.000 %

Minimum m/z Ratio: 0.000

FWHM: 0.100000

Database of Building Blocks

Building Blocks Database File: BrickDatabases/inhouse_siderophores_blocks.txt Select

Maximum Number of Combined Blocks (start): 4

Maximum Number of Combined Blocks (middle): 4

Maximum Number of Combined Blocks (end): 4

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File: Modifications/siderophores/ketide_modifications.txt Select

Miscellaneous

Incomplete Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Regular Order of Ketide Blocks: ☒

Application

Mode: De Novo Search Engine

Sequence Database File: Select

Maximum Number of Threads: 1

Score Type: Sum of relative intensities of matched peaks

Maximum Number of Candidate Peptides Reported: 100

Peptide Sequence Tag:

Ion Types in Theoretical Spectra:

- LB
- LB*
- LBx
- LB*x
- LB-CO
- LB*-CO
- LBx-CO
- LB*x-CO
- LB+2H
- LB*+2H
- LBx+2H
- LB*x+2H

Select All Clear All Reset

Remove Hits of Fragments without Hits of Parent Fragments: ☒

Searched Peptide Sequence

Sequence: [Hpd]-[Suc]-[Hpd]-[Suc]-[Hpd] Edit

N-terminal Modification: Acetyl

C-terminal Modification:

Branch Modification:

OK Cancel Apply Load Save 'tutorial12c.ini' Save As...

Example 12c: Identification of ferri-ferrioxamine B

CycloBranch

File Search Tools View Help

Filter Reset

	*	Result ID	Group ID	Peptide Sequence	Summary Formula	Monoisotopic Mass	Number of Bricks	Left Terminal Modification	Right Terminal Modification	Matched Peaks	
1	*	1	1	[Hpd]-[Suc]-[Hpd]-[Suc]-[Hpd]	C25FeH45N6O8	613.264827	5	Acetyl		14	3
2	*	2	2	[Hpd]-[Suc]-[Hpd]-[Suc]-[Hpd]	C25FeH45N6O8	613.264827	5	Acetyl		14	3
3		3	3	[Cad]-[Suc]-[Hpd]-[Suc]-[Hpd]	C25FeH45N6O8	613.264827	5		Acetyl	13	3
4		4	4	[Hpd]-[Suc]-[Hpd]-[Suc]-[Cad]	C25FeH45N6O8	613.264827	5	Acetyl		13	3
5		5	5	[Hpd]-[Suc]-[Hpd]-[Suc]-[Cad]	C25FeH45N6O8	613.264827	5	Acetyl		13	3
6		6	6	[Hpd]-[Mga]-[Hpd]-[Suc]-[Cad]	C25FeH45N6O8	613.264827	5			12	2
7		7	7	[Suc]-[Put]-[C4:0-OH(3)]-[Put]-[Suc]-[Hpd]	C25FeH45N6O8	613.264827	6			12	2
8		8	7	[C4:0-OH(3)]-[Put]-[Suc]-[Put]-[Suc]-[Hpd]	C25FeH45N6O8	613.264827	6			12	2
9		9	8	[Cad]-[Suc]-[Hpd]-[Mga]-[Hpd]	C25FeH45N6O8	613.264827	5			12	2
10		10	9	[Hpd]-[Mga]-[Hpd]-[Suc]-[Cad]	C25FeH45N6O8	613.264827	5			12	2
11		11	10	[Hpd]-[Mga]-[Hpd]-[Suc]-[Hpd]	C25FeH45N6O8	613.264827	5			12	2
12		12	11	[Suc]-[Put]-[C4:0-OH(3)]-[Put]-[Suc]-[Cad]	C25FeH45N6O8	613.264827	6			12	2
13		13	11	[C4:0-OH(3)]-[Put]-[Suc]-[Put]-[Suc]-[Cad]	C25FeH45N6O8	613.264827	6			12	2

Number of edges: 214

Removing edges forming paths which can be substituted by longer paths... ok
Edges removed: 28
Number of edges: 214

Calculating the number of paths forming peptide sequence candidates... ok
Number of paths found: 128

Comparing theoretical spectra of candidates with the peak list...
.. ok

CycloBranch successfully finished at 09:40:12 (time elapsed: 0 hrs, 0 min, 4 sec).

Tutorial 13: Identification of Siderophores in MALDI-MS and LC-MS data

- select MS mode

Mode: Compare Peaklist(s) with Database - MS or MSI data

- select a database of compounds

Sequence Database File: ases/siderophores/inhouse_siderophores.txt Select

- select ion types to be detected

Ion Types in Theoretical Spectra:

[M+H] ⁺
[M+Na] ⁺
[M+K] ⁺
[M-H] ⁻
[M+Fe-2H] ⁺
[M+Fe-3H+Na] ⁺
[2M+Fe-2H] ⁺
[2M+Fe-3H+Na] ⁺
[3M+Fe-2H] ⁺
[3M+Fe-3H+Na] ⁺
[3M+2Fe-5H] ⁺
[3M+2Fe-6H+Na] ⁺

Tutorial 13a: Identification of Siderophores in MALDI-MS data

Settings...

Spectrum

Peptide Type: Linear

File: PeakLists/siderophores/dereplication/ferrioxamines.txt

Precursor m/z Ratio: 0.000000

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 5.000 ppm

m/z Error Tolerance: 2.000 ppm

m/z Error Tolerance for Deisotoping: 0.000 ppm

Minimum Threshold of Relative Intensity: 1.000 %

Minimum m/z Ratio: 150.000

FWHM: 0.100000

Database of Building Blocks

Building Blocks Database File:

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Incomplete Paths in De Novo Graph: connect (allow detection of sequence tags)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Regular Order of Ketide Blocks: ☐

Application

Mode: Compare Peaklist(s) with Database - MS or MSI data

Sequence Database File: ases/siderophores/inhouse_siderophores.txt

Maximum Number of Threads: 1

Score Type: Number of b-ions

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag:

Ion Types in Theoretical Spectra:

- [M+H]⁺
- [M+Na]⁺
- [M+K]⁺
- [M-H]⁻
- [M+Fe-2H]⁺
- [M+Fe-3H+Na]⁺
- [2M+Fe-2H]⁺
- [2M+Fe-3H+Na]⁺
- [3M+Fe-2H]⁺
- [3M+Fe-3H+Na]⁺
- [3M+2Fe-5H]⁺
- [3M+2Fe-6H+Na]⁺

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

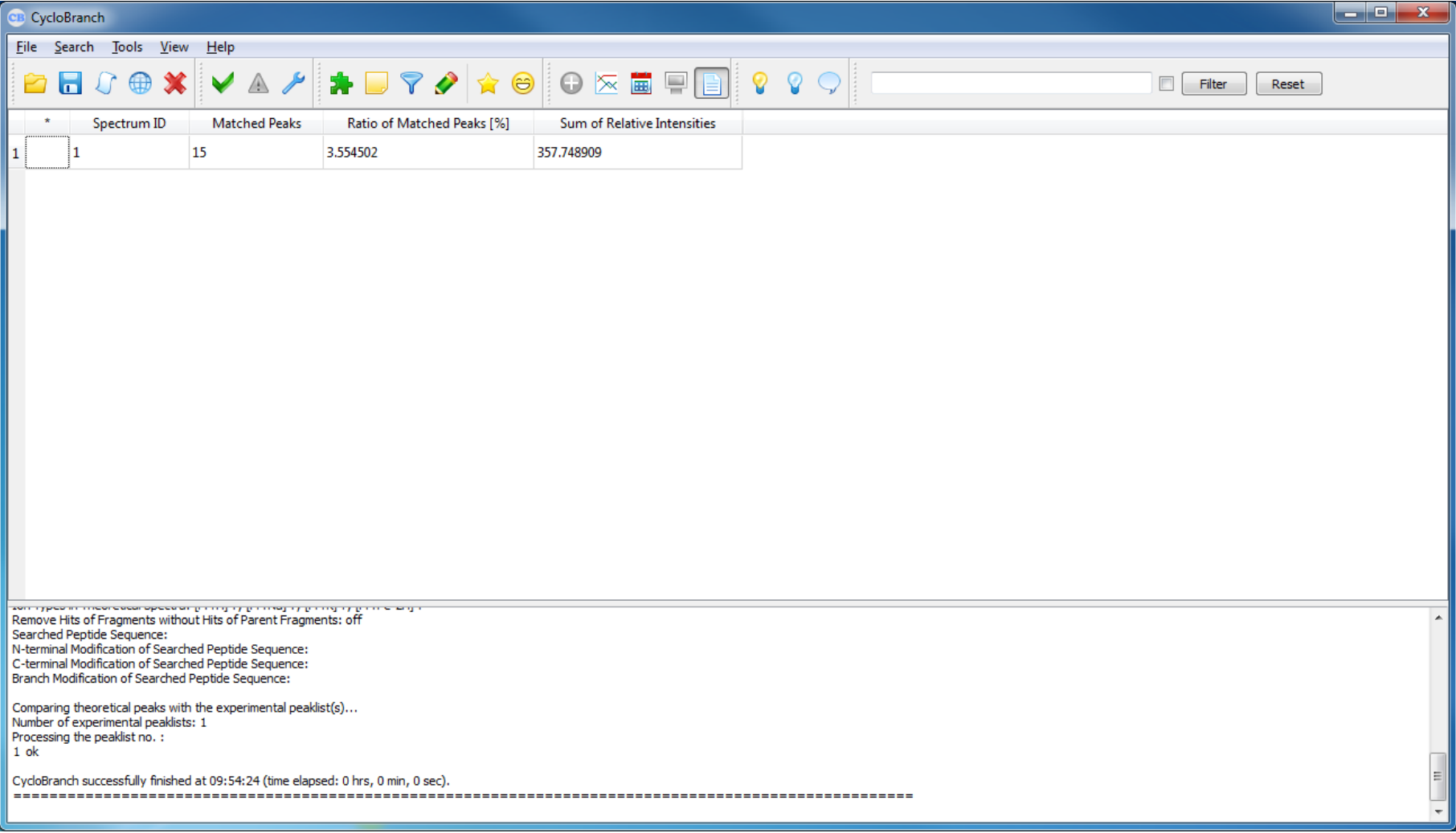
Sequence:

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Tutorial 13a: Identification of Siderophores in MALDI-MS data



The screenshot displays the CycloBranch software interface. At the top is a menu bar with 'File', 'Search', 'Tools', 'View', and 'Help'. Below the menu is a toolbar containing various icons for file operations, search, and analysis. To the right of the toolbar are 'Filter' and 'Reset' buttons. The main area features a table with the following data:

*	Spectrum ID	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities
1	1	15	3.554502	357.748909

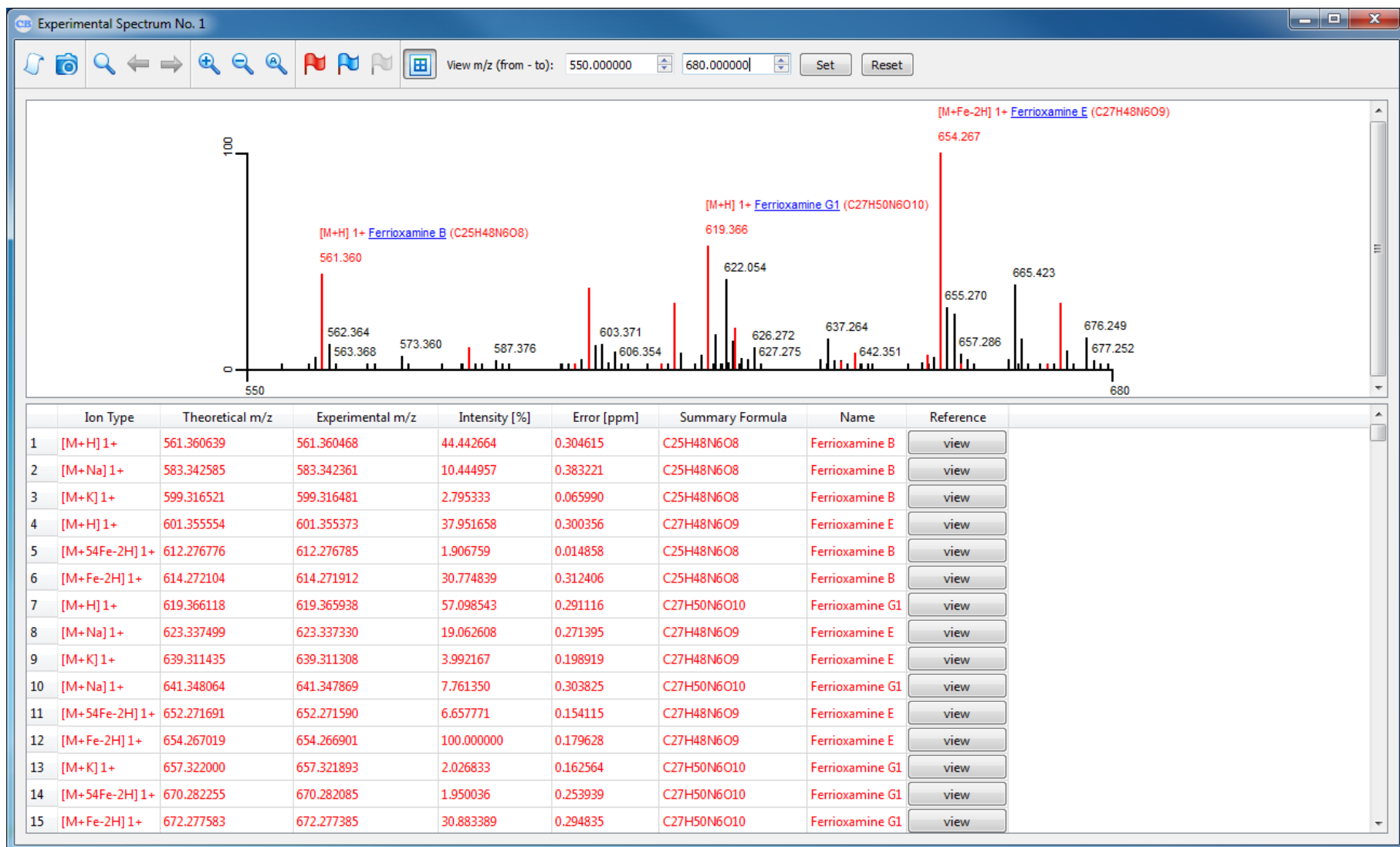
Below the table, a status log provides additional information:

Remove Hits of Fragments without Hits of Parent Fragments: off
Searched Peptide Sequence:
N-terminal Modification of Searched Peptide Sequence:
C-terminal Modification of Searched Peptide Sequence:
Branch Modification of Searched Peptide Sequence:

Comparing theoretical peaks with the experimental peaklist(s)...
Number of experimental peaklists: 1
Processing the peaklist no. :
1 ok

CycloBranch successfully finished at 09:54:24 (time elapsed: 0 hrs, 0 min, 0 sec).
=====

Tutorial 13a: Identification of Siderophores in MALDI-MS data



Tutorial 13b: Identification of Siderophores in LC-MS data

- select a file with LC-MS data

File:

Tutorial 13b: Identification of Siderophores in LC-MS data

Settings...

Spectrum

Peptide Type: Cyclic

File: datasets/cms_data/sid_mixture_10x_BA2_01_17.mgf

Precursor m/z Ratio: 0.000000

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance: 5.000 ppm

m/z Error Tolerance for Deisotoping: 0.000 ppm

Minimum Threshold of Relative Intensity: 1.000 %

Minimum m/z Ratio: 150.000

FWHM: 0.100000

Database of Building Blocks

Building Blocks Database File:

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Incomplete Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Regular Order of Ketide Blocks: ☐

Application

Mode: Compare Peaklist(s) with Database - MS or MSI data

Sequence Database File: ases/siderophores/inhouse_siderophores.txt

Maximum Number of Threads: 1

Score Type: Number of b-ions

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag:

Ion Types in Theoretical Spectra:

- [M+H]⁺
- [M+Na]⁺
- [M+K]⁺
- [M-H]⁻
- [M+Fe-2H]⁺
- [M+Fe-3H+Na]⁺
- [2M+Fe-2H]⁺
- [2M+Fe-3H+Na]⁺
- [3M+Fe-2H]⁺
- [3M+Fe-3H+Na]⁺
- [3M+2Fe-5H]⁺
- [3M+2Fe-6H+Na]⁺

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence:

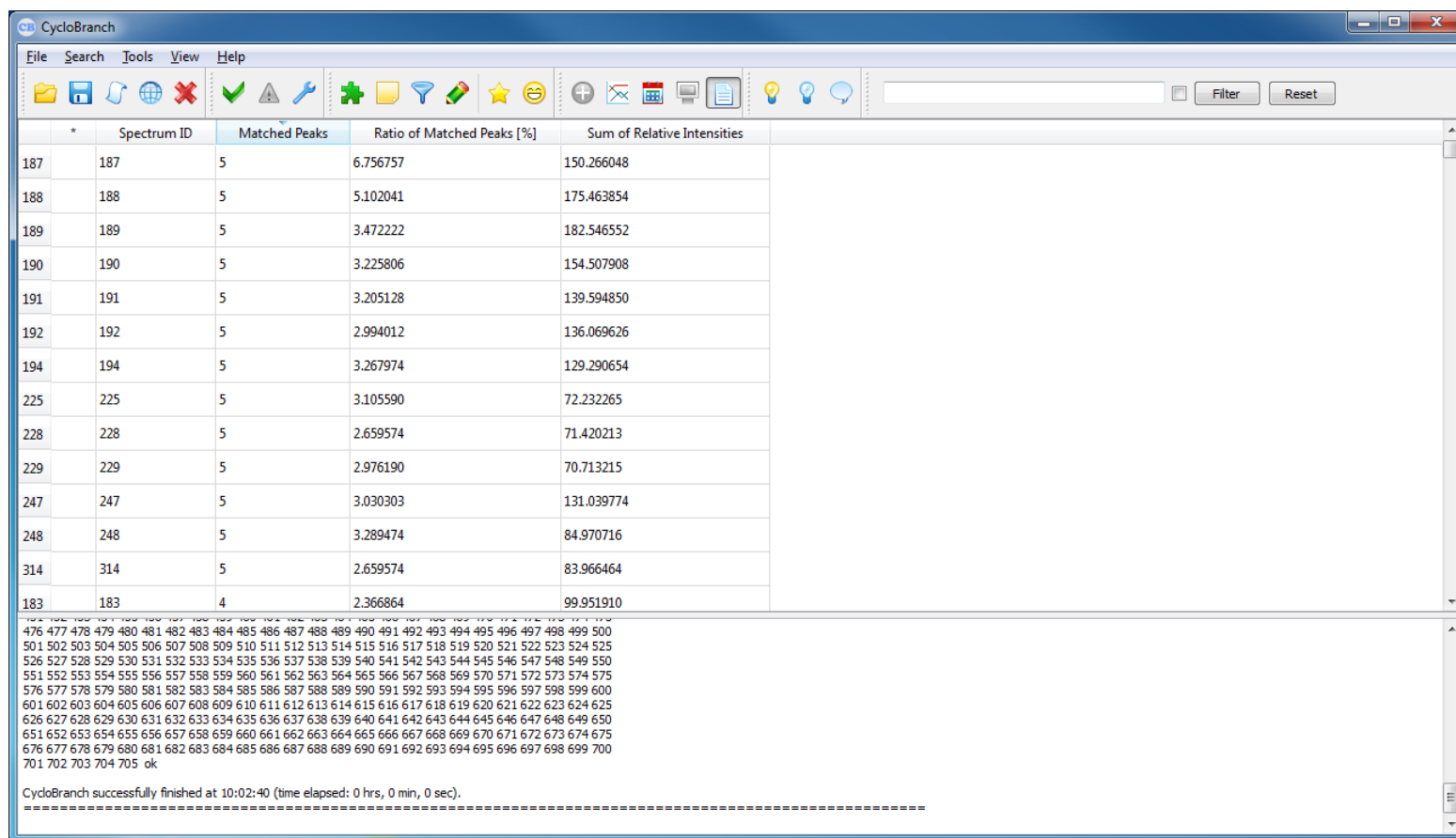
N-terminal Modification:

C-terminal Modification:

Branch Modification:

Tutorial 13b: Identification of Siderophores in LC-MS data

- each row corresponds to a scan
- spectrum ID = scan ID



The screenshot shows the CycloBranch software window. The title bar reads 'CycloBranch'. The menu bar includes 'File', 'Search', 'Tools', 'View', and 'Help'. Below the menu bar is a toolbar with various icons for file operations, search, and analysis. To the right of the toolbar are 'Filter' and 'Reset' buttons. The main area contains a table with the following columns: 'Spectrum ID', 'Matched Peaks', 'Ratio of Matched Peaks [%]', and 'Sum of Relative Intensities'. The table lists data for scans 187 through 183. At the bottom of the window, a status bar indicates 'CycloBranch successfully finished at 10:02:40 (time elapsed: 0 hrs, 0 min, 0 sec.)'.

	Spectrum ID	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities
187	187	5	6.756757	150.266048
188	188	5	5.102041	175.463854
189	189	5	3.472222	182.546552
190	190	5	3.225806	154.507908
191	191	5	3.205128	139.594850
192	192	5	2.994012	136.069626
194	194	5	3.267974	129.290654
225	225	5	3.105590	72.232265
228	228	5	2.659574	71.420213
229	229	5	2.976190	70.713215
247	247	5	3.030303	131.039774
248	248	5	3.289474	84.970716
314	314	5	2.659574	83.966464
183	183	4	2.366864	99.951910

CycloBranch successfully finished at 10:02:40 (time elapsed: 0 hrs, 0 min, 0 sec.)

Tutorial 13b: Identification of Siderophores in LC-MS data

- Summary Table of Matched Peaks – matched peaks from all scans are listed (ID = scan ID)

Summary Table of Matched Peaks

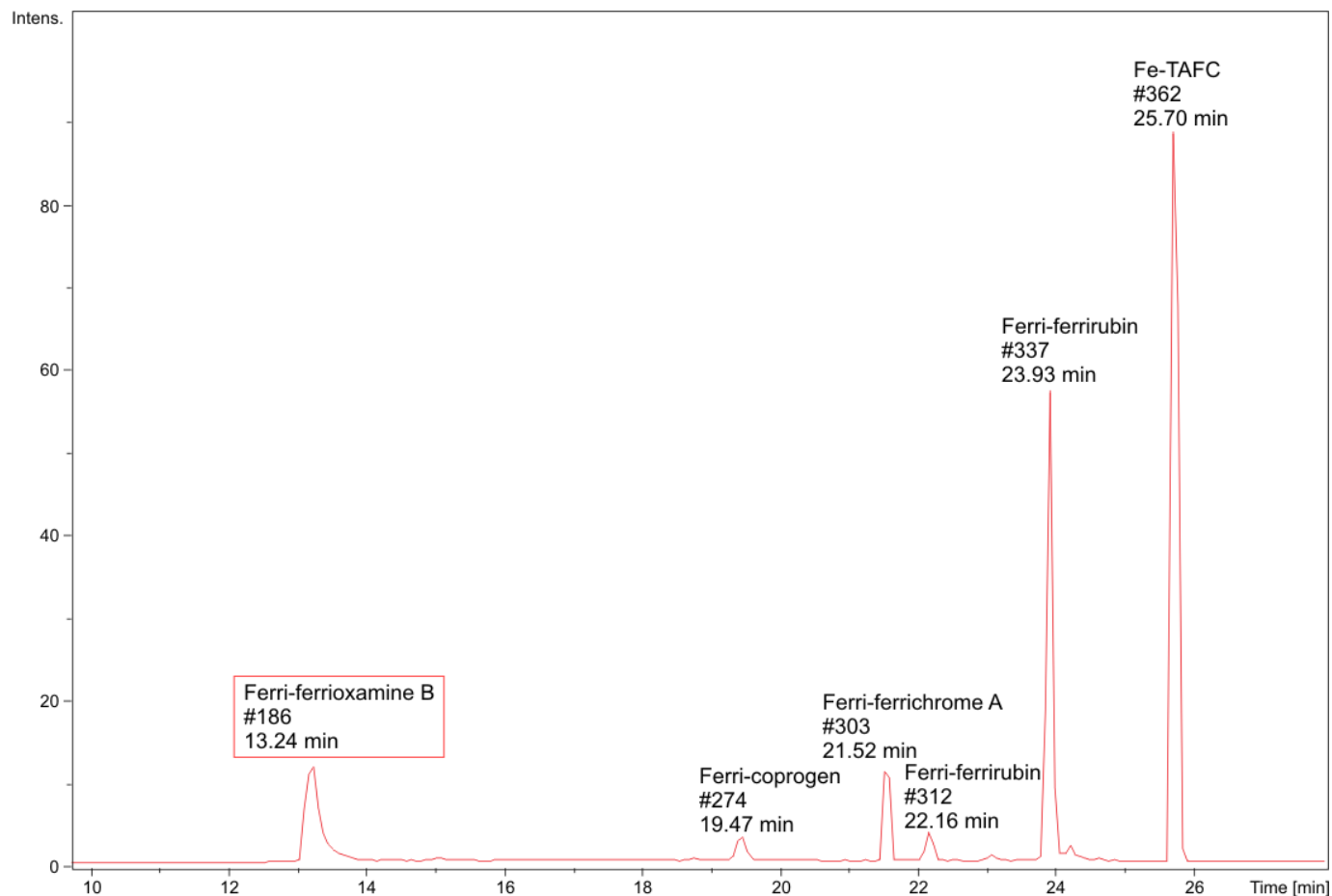
File Help

☒

ID	Ion Type	Theoretical m/z	Experimental m/z	Intensity [%]	Error [ppm]	Summary Formula	Name	Reference
56 181	[M+Fe-2H] ¹⁺	614.272104	614.274416	3.529314	3.763949	C25H48N6O8	Ferrioxamine B	view
58 182	[M+Fe-2H] ¹⁺	614.272104	614.271833	10.264801	0.441014	C25H48N6O8	Ferrioxamine B	view
60 183	[M+54Fe-2H] ¹⁺	612.276776	612.277625	2.899630	1.386785	C25H48N6O8	Ferrioxamine B	view
61 183	[M+Fe-2H] ¹⁺	614.272104	614.272568	40.626409	0.755524	C25H48N6O8	Ferrioxamine B	view
63 184	[M+54Fe-2H] ¹⁺	612.276776	612.277308	5.807795	0.869047	C25H48N6O8	Ferrioxamine B	view
64 184	[M+Fe-2H] ¹⁺	614.272104	614.272528	100.000000	0.690406	C25H48N6O8	Ferrioxamine B	view
65 185	[M+54Fe-2H] ¹⁺	612.276776	612.277200	5.916531	0.692656	C25H48N6O8	Ferrioxamine B	view
66 185	[M+Fe-2H] ¹⁺	614.272104	614.272538	100.000000	0.706686	C25H48N6O8	Ferrioxamine B	view
68 186	[M+54Fe-2H] ¹⁺	612.276776	612.277174	5.839689	0.650192	C25H48N6O8	Ferrioxamine B	view
69 186	[M+Fe-2H] ¹⁺	614.272104	614.272535	100.000000	0.701802	C25H48N6O8	Ferrioxamine B	view
71 187	[M+54Fe-2H] ¹⁺	612.276776	612.277155	5.898152	0.619160	C25H48N6O8	Ferrioxamine B	view
72 187	[M+Fe-2H] ¹⁺	614.272104	614.272556	100.000000	0.735988	C25H48N6O8	Ferrioxamine B	view
75 188	[M+54Fe-2H] ¹⁺	612.276776	612.277198	6.419116	0.689390	C25H48N6O8	Ferrioxamine B	view
76 188	[M+Fe-2H] ¹⁺	614.272104	614.272515	100.000000	0.669243	C25H48N6O8	Ferrioxamine B	view
79 189	[M+54Fe-2H] ¹⁺	612.276776	612.277411	5.899160	1.037271	C25H48N6O8	Ferrioxamine B	view
80 189	[M+Fe-2H] ¹⁺	614.272104	614.272513	100.000000	0.665987	C25H48N6O8	Ferrioxamine B	view
83 190	[M+54Fe-2H] ¹⁺	612.276776	612.276983	5.975054	0.338242	C25H48N6O8	Ferrioxamine B	view
84 190	[M+Fe-2H] ¹⁺	614.272104	614.272519	100.000000	0.675755	C25H48N6O8	Ferrioxamine B	view
87 191	[M+54Fe-2H] ¹⁺	612.276776	612.276838	5.900065	0.101421	C25H48N6O8	Ferrioxamine B	view
88 191	[M+Fe-2H] ¹⁺	614.272104	614.272553	100.000000	0.731105	C25H48N6O8	Ferrioxamine B	view
91 192	[M+54Fe-2H] ¹⁺	612.276776	612.276679	6.948608	0.158266	C25H48N6O8	Ferrioxamine B	view
92 192	[M+Fe-2H] ¹⁺	614.272104	614.272523	100.000000	0.682266	C25H48N6O8	Ferrioxamine B	view
95 193	[M+54Fe-2H] ¹⁺	612.276776	612.276758	6.296950	0.029239	C25H48N6O8	Ferrioxamine B	view
96 193	[M+Fe-2H] ¹⁺	614.272104	614.272487	100.000000	0.623661	C25H48N6O8	Ferrioxamine B	view

Tutorial 13b: Identification of Siderophores in LC-MS data

- chromatogram



Tutorial 13: Identification of Siderophores in MALDI-MS and LC-MS data

- List of currently supported ions:
 - $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M-H]^-$, $[M+Fe-2H]^+$, $[M+Fe-3H+Na]^+$, $[2M+Fe-2H]^+$, $[2M+Fe-3H+Na]^+$, $[3M+Fe-2H]^+$, $[3M+Fe-3H+Na]^+$, $[3M+2Fe-5H]^+$, $[3M+2Fe-6H+Na]^+$, $[M+Fe-4H]^-$, $[2M+Fe-4H]^-$, $[3M+Fe-4H]^-$, $[3M+2Fe-7H]^-$, $[M+Li]^+$, $[M+Mg-H]^+$, $[M+Mg-2H+Na]^+$, $[M+Mg-3H]^-$, $[M+Al-2H]^+$, $[M+Al-3H+Na]^+$, $[M+Al-4H]^-$, $[M+Ca-H]^+$, $[M+Ca-2H+Na]^+$, $[M+Ca-3H]^-$, $[M+Cr-2H]^+$, $[M+Cr-3H+Na]^+$, $[M+Cr-4H]^-$, $[M+Mn-H]^+$, $[M+Mn-2H+Na]^+$, $[M+Mn-3H]^-$, $[M+Co-H]^+$, $[M+Co-2H+Na]^+$, $[M+Co-3H]^-$, $[M+Ni-H]^+$, $[M+Ni-2H+Na]^+$, $[M+Ni-3H]^-$, $[M+Cu-H]^+$, $[M+Cu-2H+Na]^+$, $[M+Cu-3H]^-$, $[M+Zn-H]^+$, $[M+Zn-2H+Na]^+$, $[M+Zn-3H]^-$, $[M+Ga-2H]^+$, $[M+Ga-3H+Na]^+$, $[M+Ga-4H]^-$

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- standard imzML file format is supported
 - processed or continuous data format
 - m/z values and intensities must be stored as 32/64-bit floats
 - no compression
 - profile or centroided spectra
 - profile spectra are automatically converted to centroided spectra using OpenMS (www.openms.de)
 - see also www.imzml.org

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- the single spectra are batch-processed
- X and Y coordinates are reported for each single spectrum
- Summary Table of Matched Peaks – matched peaks from all single spectra are listed
- Image Window – visualization of matched compounds

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- select imzML file

File:

- set up the minimum threshold of rel. int.

Minimum Threshold of Relative Intensity:

- set up FWHM (for profile spectra only)

FWHM:

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- select MSI mode

Mode: Compare Peaklist(s) with Database - MS or MSI data ▼

- select a database of compounds

Sequence Database File: ases/siderophores/inhouse_siderophores.txt Select

- select ion types to be detected

Ion Types in Theoretical Spectra:

- [M+H]⁺
- [M+Na]⁺
- [M+K]⁺
- [M-H]⁻
- [M+Fe-2H]⁺
- [M+Fe-3H+Na]⁺
- [2M+Fe-2H]⁺
- [2M+Fe-3H+Na]⁺
- [3M+Fe-2H]⁺
- [3M+Fe-3H+Na]⁺
- [3M+2Fe-5H]⁺
- [3M+2Fe-6H+Na]⁺

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

Settings...

Spectrum

Peptide Type: Cyclic

File: _Siderophores_mixture_agar_converted_fwhm_0.020000.imzML

Precursor m/z Ratio: 0.000000

Precursor Ion Adduct:

Charge: 1

Precursor m/z Error Tolerance: 1.000 ppm

m/z Error Tolerance: 5.000 ppm

m/z Error Tolerance for Deisotoping: 0.000 ppm

Minimum Threshold of Relative Intensity: 1.000 %

Minimum m/z Ratio: 150.000

FWHM: 0.020000

Database of Building Blocks

Building Blocks Database File:

Maximum Number of Combined Blocks (start): 1

Maximum Number of Combined Blocks (middle): 1

Maximum Number of Combined Blocks (end): 1

Maximum Cumulative Mass of Blocks: 0.000

Generate Permutations of Combined Blocks: ☒

N-/C-terminal Modifications File:

Miscellaneous

Incomplete Paths in De Novo Graph: remove (speed up the search)

Cyclic N-terminus: ☐

Cyclic C-terminus: ☐

Enable Scrambling: ☐

Enable Similarity Search: ☐

Regular Order of Ketide Blocks: ☐

Application

Mode: Compare Peaklist(s) with Database - MS or MSI data

Sequence Database File: ases/siderophores/inhouse_siderophores.txt

Maximum Number of Threads: 1

Score Type: Number of b-ions

Maximum Number of Candidate Peptides Reported: 1000

Peptide Sequence Tag:

Ion Types in Theoretical Spectra:

- [M+H]⁺
- [M+Na]⁺
- [M+K]⁺
- [M-H]⁻
- [M+Fe-2H]⁺
- [M+Fe-3H+Na]⁺
- [2M+Fe-2H]⁺
- [2M+Fe-3H+Na]⁺
- [3M+Fe-2H]⁺
- [3M+Fe-3H+Na]⁺
- [3M+2Fe-5H]⁺
- [3M+2Fe-6H+Na]⁺

Remove Hits of Fragments without Hits of Parent Fragments: ☐

Searched Peptide Sequence

Sequence:

N-terminal Modification:

C-terminal Modification:

Branch Modification:

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

CycloBranch

File Search Tools View Help

Filter Reset

	*	Spectrum ID	Matched Peaks	Ratio of Matched Peaks [%]	Sum of Relative Intensities	Coordinate X	Coordinate Y
3748		3748	3	2.255639	8.953002	106	124
3756		3756	3	2.290076	10.888000	114	124
3786		3786	3	2.564103	8.668370	114	125
3857		3857	3	2.380952	8.510702	120	127
3939		3939	3	2.272727	6.874270	100	130
4010		4010	3	2.290076	6.020711	102	132
4195		4195	3	1.875000	6.441054	118	137
4352		4352	3	1.960784	7.224380	121	142
3584		3584	2	0.749064	4.898325	115	116
3586		3586	2	0.947867	4.310938	117	116
3587		3587	2	0.836820	5.855754	118	116
3588		3588	2	0.913242	3.328617	108	117
3589		3589	2	0.970874	3.354194	109	117
3590		3590	2	0.913242	3.944298	110	117

11376 11377 11378 11379 11380 11381 11382 11383 11384 11385 11386 11387 11388 11389 11390 11391 11392 11393 11394 11395 11396 11397 11398 11399 11400
11401 11402 11403 11404 11405 11406 11407 11408 11409 11410 11411 11412 11413 11414 11415 11416 11417 11418 11419 11420 11421 11422 11423 11424 11425
11426 11427 11428 11429 11430 11431 11432 11433 11434 11435 11436 11437 11438 11439 11440 11441 11442 11443 11444 11445 11446 11447 11448 11449 11450
11451 11452 11453 11454 11455 11456 11457 11458 11459 11460 11461 11462 11463 11464 11465 11466 11467 11468 11469 11470 11471 11472 11473 11474 11475
11476 11477 11478 11479 11480 11481 11482 11483 11484 11485 11486 11487 11488 11489 11490 11491 11492 11493 11494 11495 11496 11497 11498 11499 11500
11501 11502 11503 11504 11505 11506 11507 11508 11509 11510 11511 11512 11513 11514 11515 11516 11517 11518 11519 11520 11521 11522 11523 11524 11525
11526 11527 11528 11529 11530 11531 11532 11533 11534 11535 11536 11537 11538 11539 11540 11541 11542 11543 11544 11545 11546 11547 11548 11549 11550
11551 11552 11553 11554 11555 11556 11557 11558 11559 11560 11561 11562 11563 11564 11565 11566 11567 11568 11569 11570 11571 11572 11573 11574 11575
11576 11577 11578 11579 11580 11581 11582 11583 11584 11585 11586 11587 11588 11589 11590 11591 11592 11593 11594 11595 11596 11597 11598 11599 11600
11601 11602 11603 11604 11605 11606 11607 11608 11609 11610 11611 11612 ok

CycloBranch successfully finished at 13:13:59 (time elapsed: 0 hrs, 0 min, 18 sec).

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

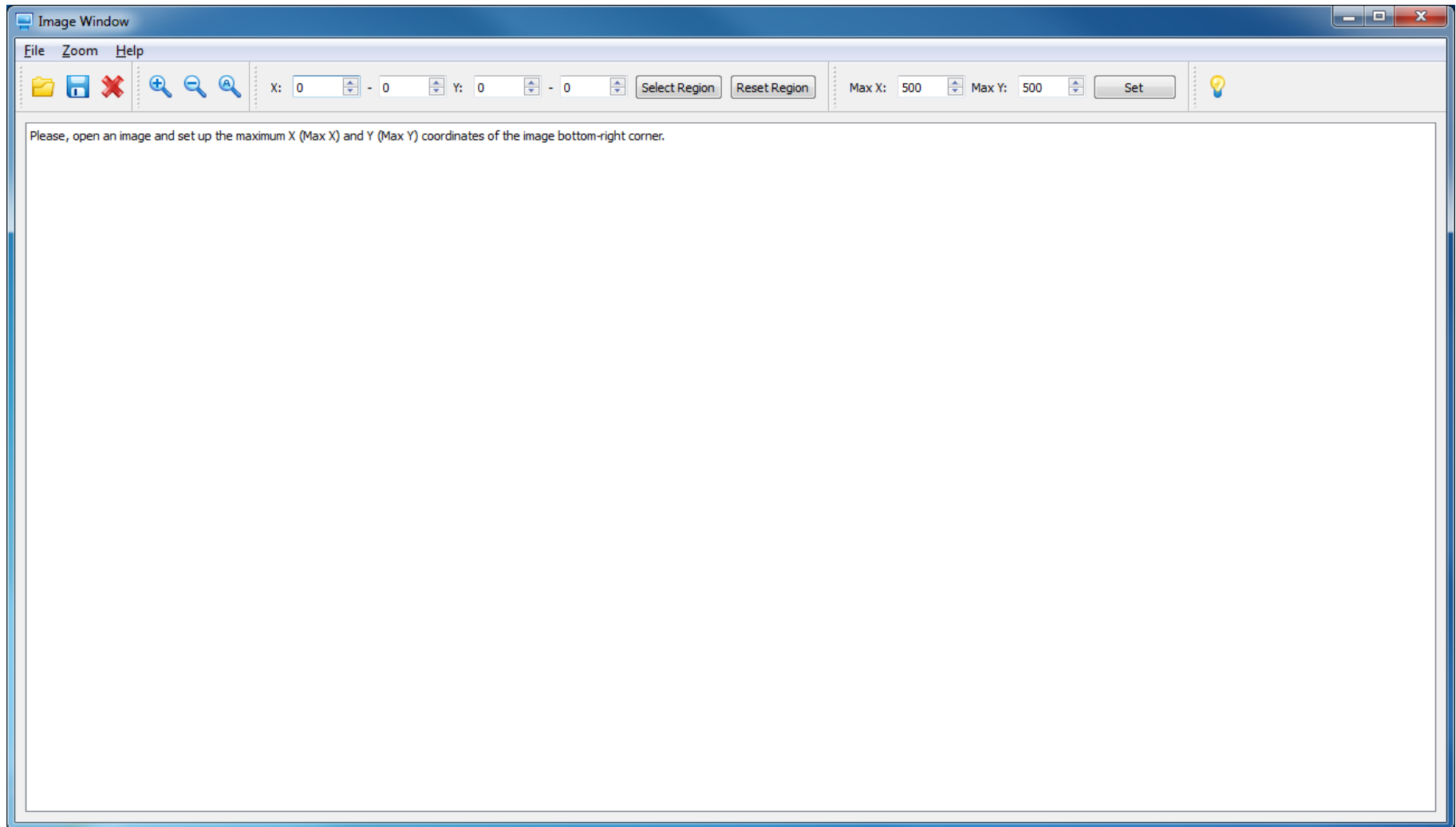
Summary Table of Matched Peaks

File

Help

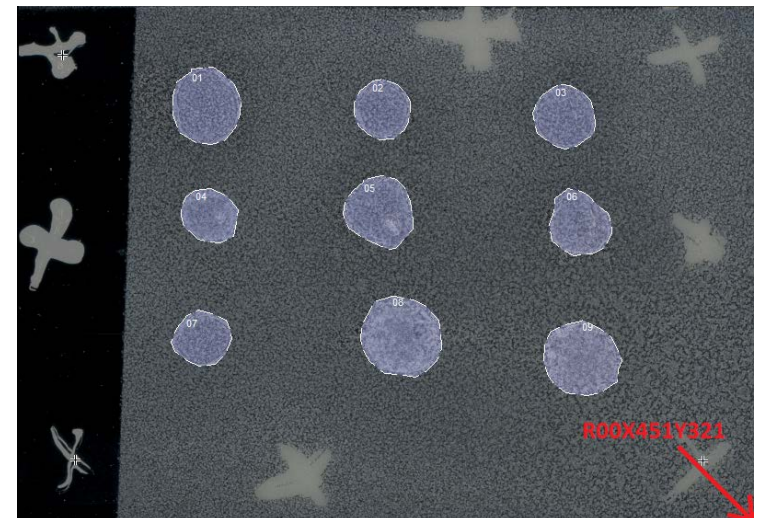
<

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra



Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- Image Window
 - open image
 - set up “Max X” and “Max Y”
 - coordinates of the image bottom-right corner
 - the values must be determined manually in FlexImaging (Bruker) to correctly map the spectra into the image
 - R00X451Y321 means
 - Max X = 451
 - Max Y = 321



Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- Image Window

- point corresponds to single mass spectrum
- standard HSL (hue, saturation, lightness) color model



- the most intensive point in a selected region is red
- zero intensity points are violet

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

- Image Window
 - color represents
 - sum of intensities of matched peaks in single mass spectrum
 - intensity of a specific compound if it was filtered by its name in “Summary Table of Matched Peaks”

Tutorial 14: Identification of Siderophores in Imaging Mass Spectra

