SUPPORTING INFORMATION

CycloBranch 2: Molecular Formula Annotations Applied to imzML Data Sets in Bimodal Fusion and LC-MS Data Files

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Table of contents:

```
bool getcombination() {
    pos = 0;
    flag = 0;
    do {
        combination[pos]++;
        if (combination[pos] <= N) {
            if (flag > 0) {
                for (i = pos - 1; i >= 0; i--) {
                    combination[i] = combination[pos];
                }
            }
            return true;
        }
        else {
            flag = 1;
            pos++;
        }
    } while (pos < P);
    return false;
}
```

```
Example:
N = 4 ... number of items
P = 3 ... maximum number of combined items
combination = 000 ... input/output vector of length P

Output after 34 calls:
100                     411
200                     221
300                     321
400                     421
110                     331
210                     431
310                     441
410                     222
220                     322
320                     422
420                     332
330                     432
430                     442
440                     333
111                     433
211                     443
311                     444
```

Figure S1. The basic algorithm used to generate the combinations of elements with repetitions from an input list of *N* elements (e.g., 1 = ‘H’, 2 = ‘C’, 3 = ‘N’, 4 = ‘O’) is shown. The maximum number of elements in a formula is limited by *P*. We used *P* = 200, if not stated otherwise. In the real implementation, two further optimizations were used. The first was based on the idea that the input list of elements was sorted by mass. For example, if the cumulative mass corresponding to the combination 411 (OHH) exceeded a user-defined upper mass limit, the combinations 511, 611, etc. were not generated (for $N \geq 6$). Since CycloBranch keeps statistics about the number of elements in a currently computed combination and since the user can define the maximum number of occurrences of any element, a similar approach was used to prune out unnecessary combinations of elements in the second case.

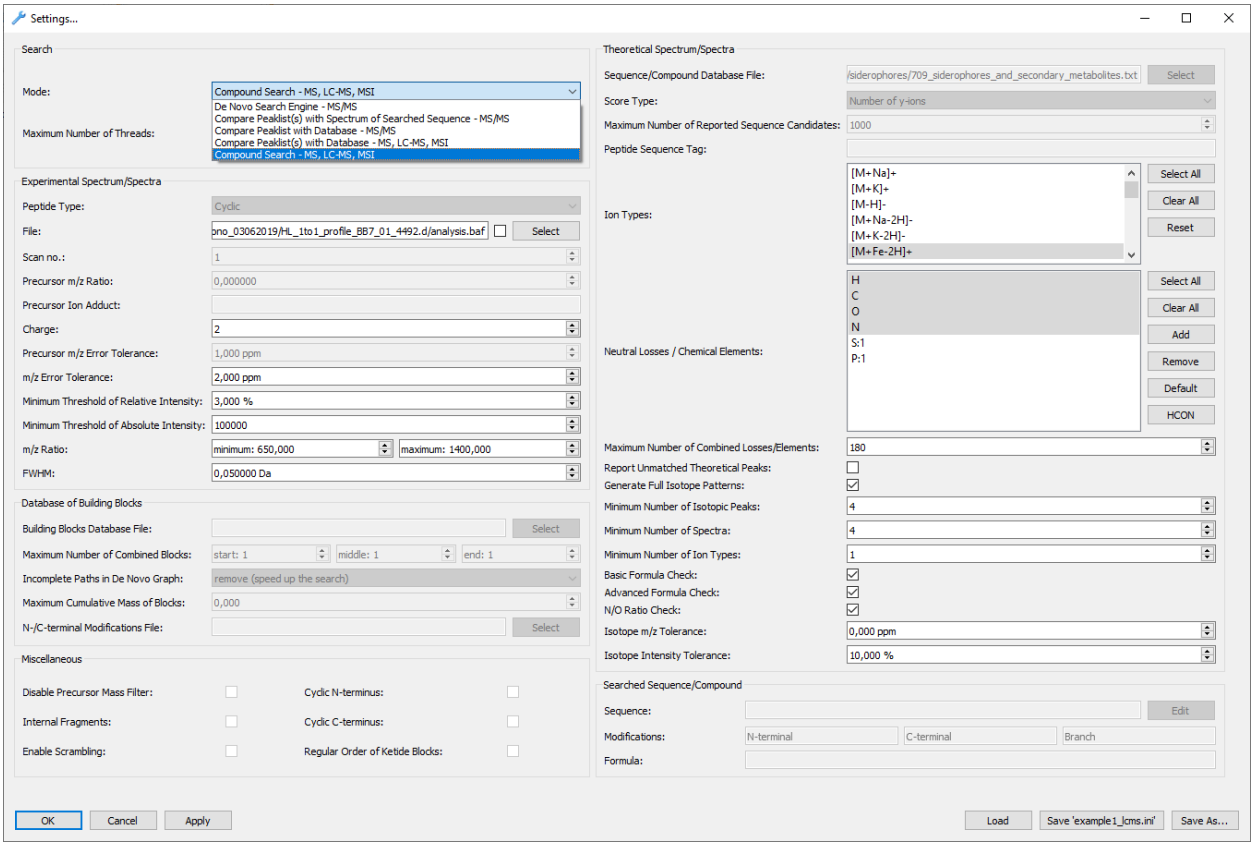


Figure S2. CycloBranch's settings dialog configured for analysis of LC-MS data using the database-free approach.

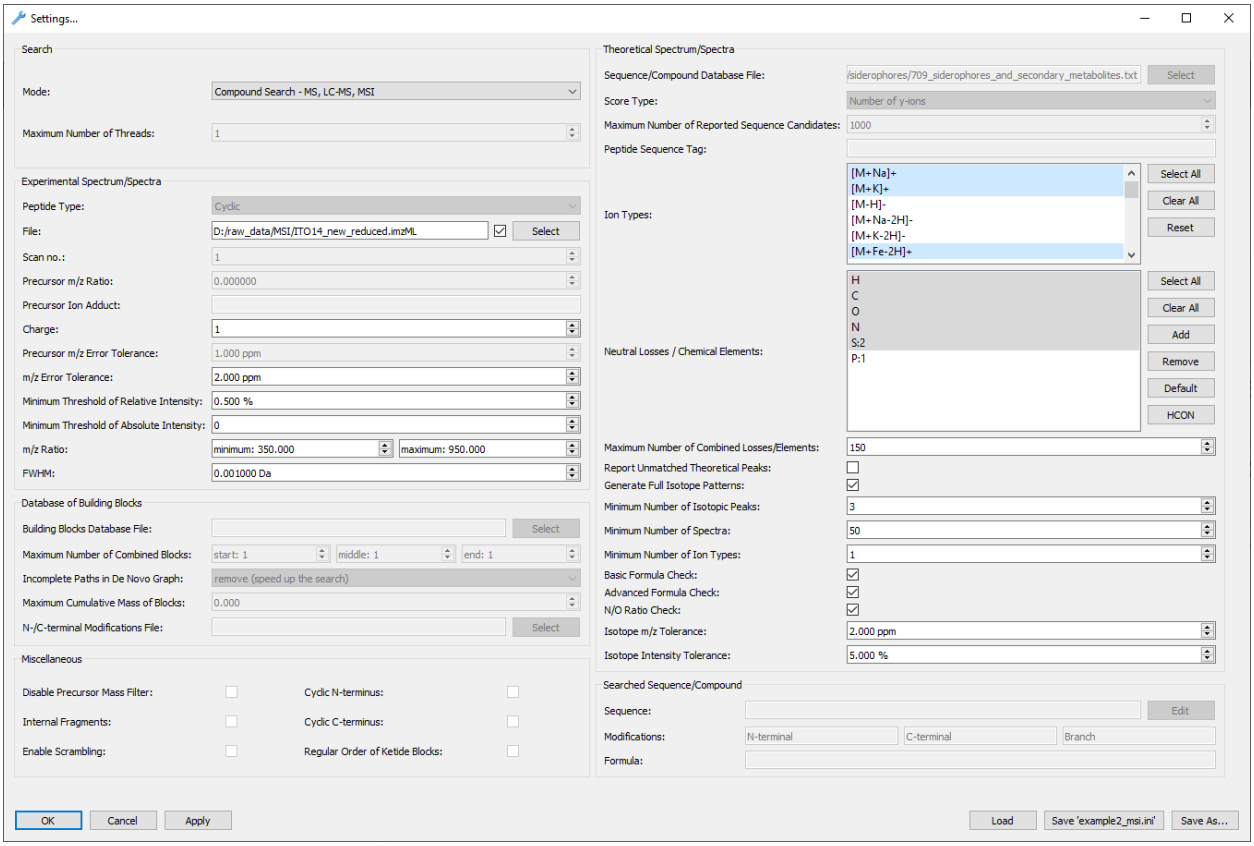


Figure S3. CycloBranch's settings dialog configured for analysis of MSI data using the database-free approach.

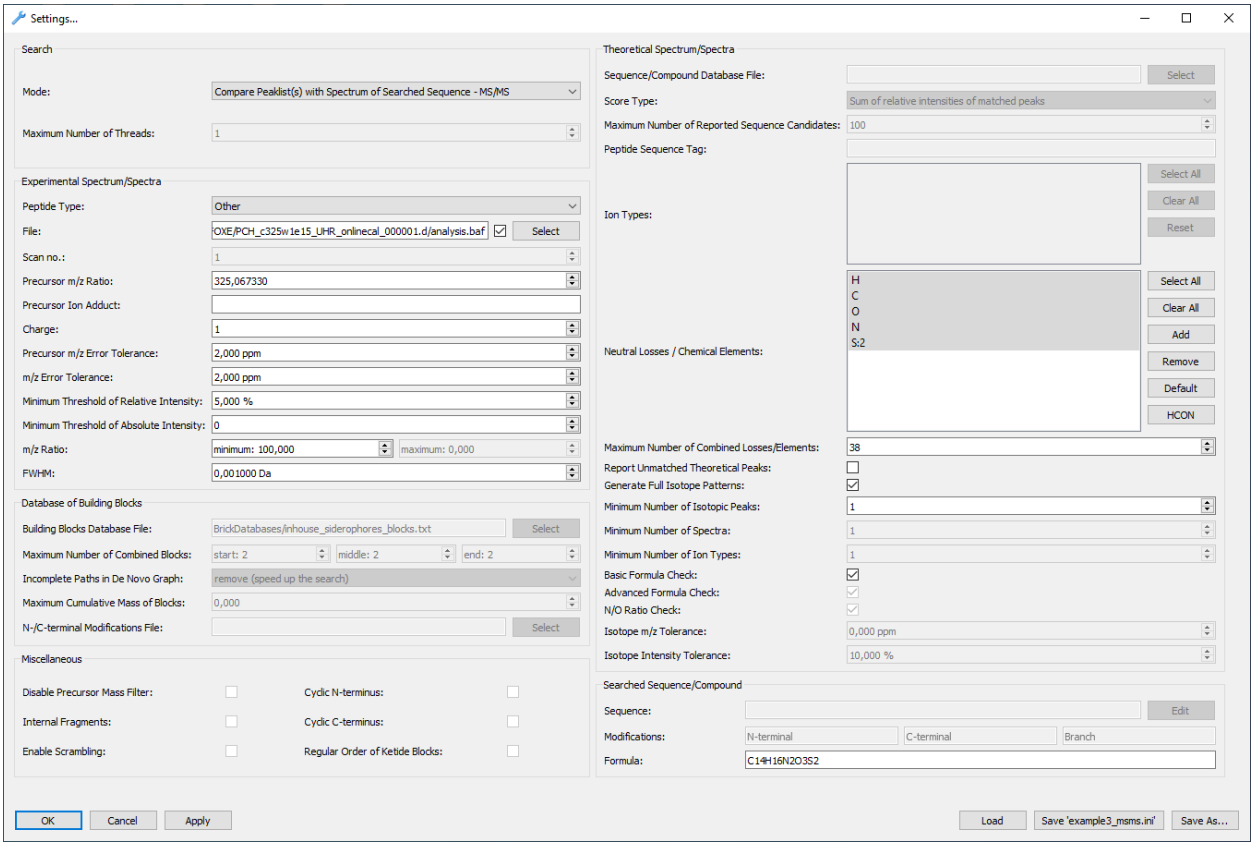
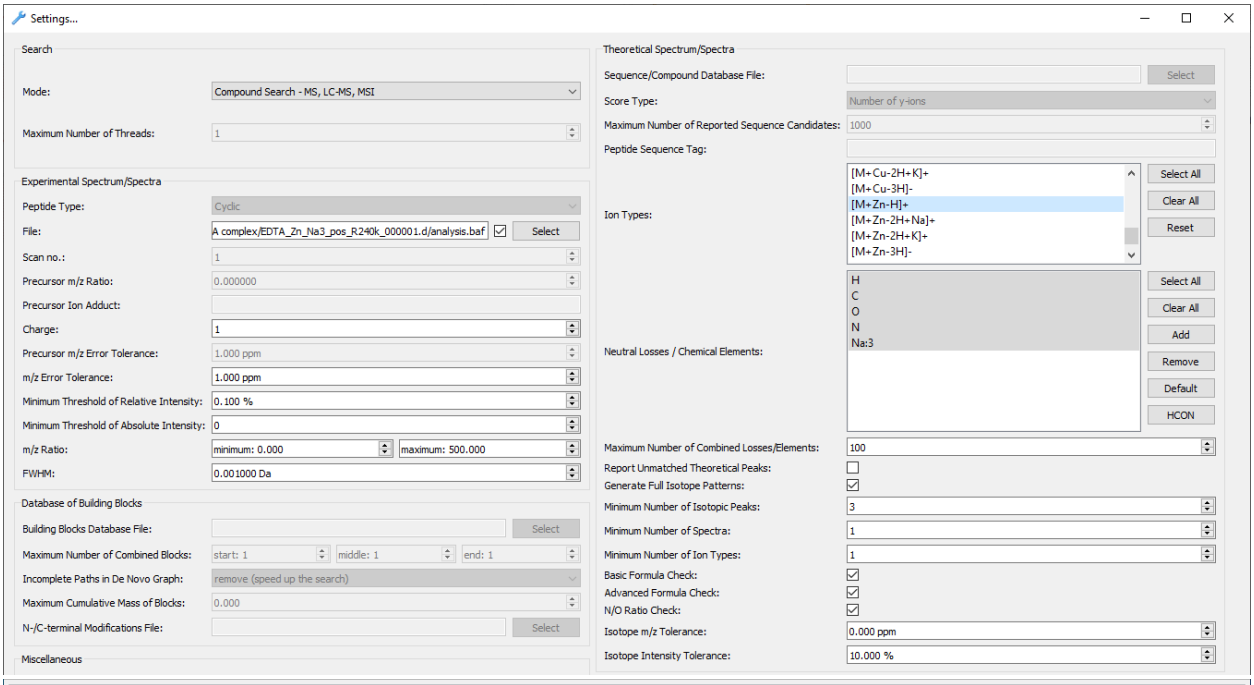
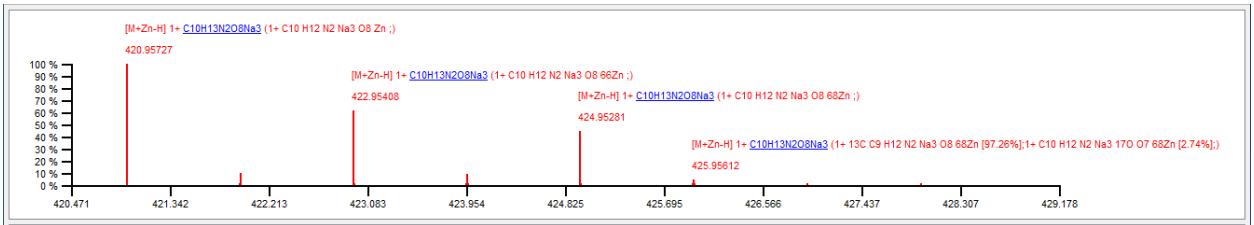


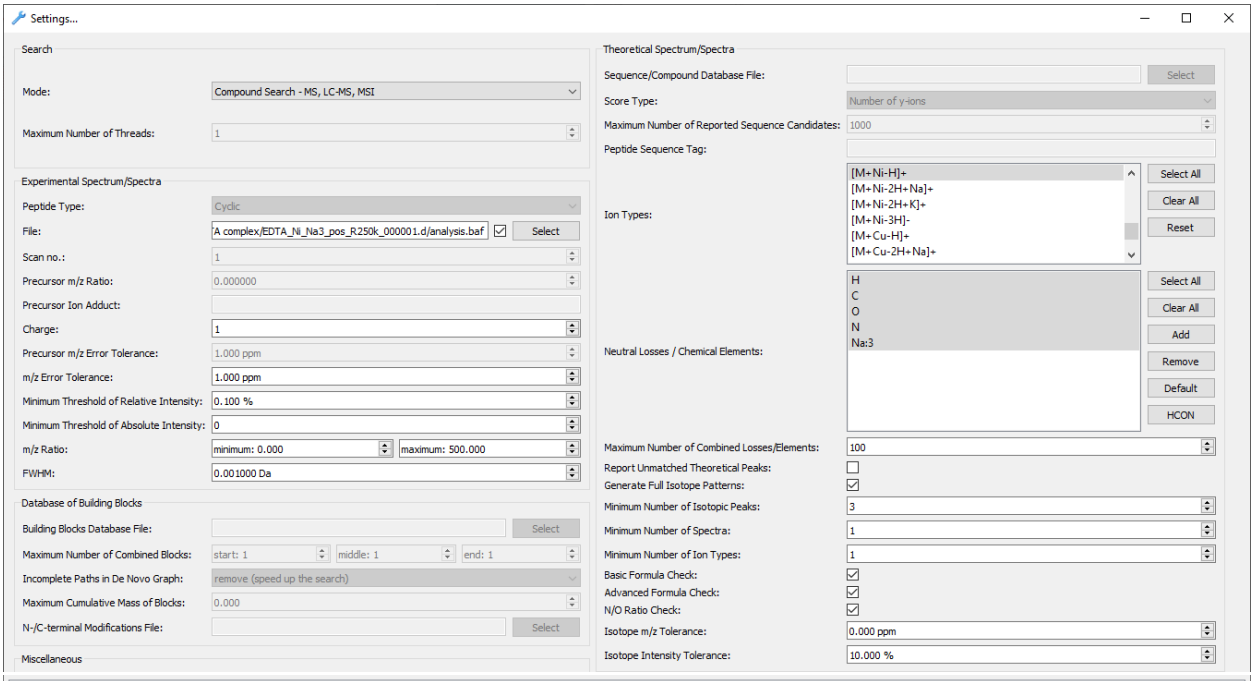
Figure S4. CycloBranch's settings dialog configured for the analysis of the fragment ion mass spectrum of pyochelin $[C_{14}H_{16}N_2O_3S_2+H]^+$.





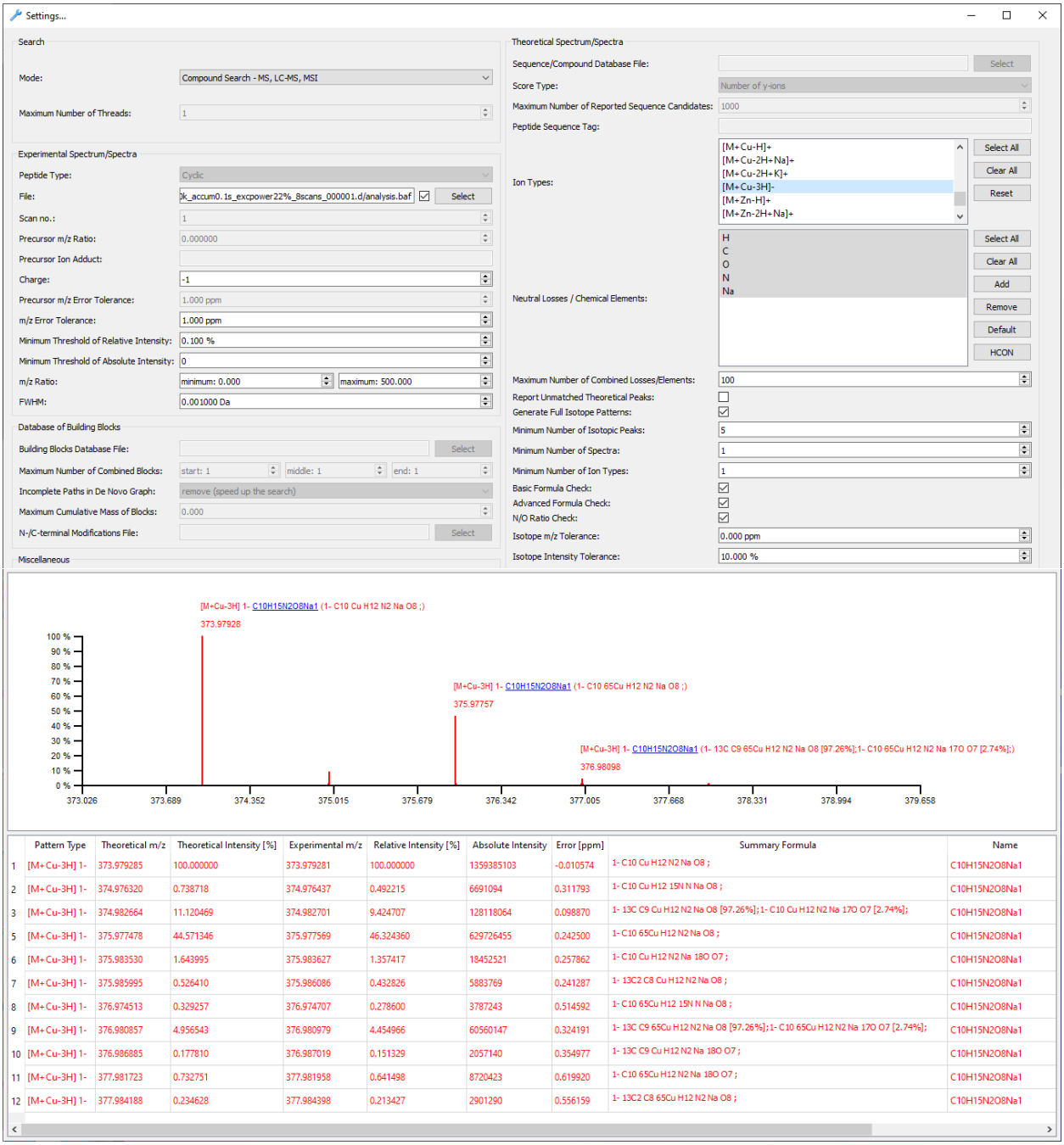
	Pattern Type	Theoretical m/z	Theoretical Intensity [%]	Experimental m/z	Relative Intensity [%]	Absolute Intensity	Error [ppm]	Summary Formula	Name
1	[M+Zn-H] 1+	420.957276	100.000000	420.957267	100.000000	324407327	-0.020882	1+ C10 H12 N2 Na3 O8 Zn ;	C10H13N2O8Na3
2	[M+Zn-H] 1+	421.954311	0.738718	421.954333	0.806750	2617156	0.051775	1+ C10 H12 15N N Na3 O8 Zn ;	C10H13N2O8Na3
3	[M+Zn-H] 1+	421.960654	11.120469	421.960594	10.762039	34912843	-0.142997	1+ 13C C9 H12 N2 Na3 O8 Zn [97.26%];1+ C10 H12 N2 Na3 17O O7 Zn [2.74%];	C10H13N2O8Na3
5	[M+Zn-H] 1+	422.954164	57.371993	422.954085	62.201094	201784907	-0.187223	1+ C10 H12 N2 Na3 O8 66Zn ;	C10H13N2O8Na3
6	[M+Zn-H] 1+	422.961521	1.643995	422.961448	1.585230	5142601	-0.172989	1+ C10 H12 N2 Na3 18O O7 Zn ;	C10H13N2O8Na3
7	[M+Zn-H] 1+	422.963986	0.526410	422.963767	0.547469	1776029	-0.516137	1+ 13C2 C8 H12 N2 Na3 O8 Zn ;	C10H13N2O8Na3
8	[M+Zn-H] 1+	423.951199	0.423818	423.951187	0.477537	1549165	-0.026753	1+ C10 H12 15N N Na3 O8 66Zn ;	C10H13N2O8Na3
9	[M+Zn-H] 1+	423.955259	8.431010	423.955181	9.269733	30071694	-0.183443	1+ C10 H12 N2 Na3 O8 67Zn ;	C10H13N2O8Na3
10	[M+Zn-H] 1+	423.957542	6.380035	423.957376	6.890778	22354188	-0.393516	1+ 13C C9 H12 N2 Na3 O8 66Zn [97.26%];1+ C10 H12 N2 Na3 17O O7 66Zn [2.74%];	C10H13N2O8Na3
11	[M+Zn-H] 1+	423.964876	0.177810	423.964743	0.166790	541080	-0.314116	1+ 13C C9 H12 N2 Na3 18O O7 Zn ;	C10H13N2O8Na3
12	[M+Zn-H] 1+	424.952976	38.556447	424.952813	44.647402	144839444	-0.382861	1+ C10 H12 N2 Na3 O8 68Zn ;	C10H13N2O8Na3
13	[M+Zn-H] 1+	424.958510	1.855068	424.958340	2.007796	6513436	-0.398617	1+ C10 H12 N2 Na3 18O O7 66Zn [50.84%];1+ 13C C9 H12 N2 Na3 O8 67Zn [49.16%];	C10H13N2O8Na3
15	[M+Zn-H] 1+	425.950011	0.284824	425.949936	0.304414	987542	-0.174500	1+ C10 H12 15N N Na3 O8 68Zn ;	C10H13N2O8Na3
16	[M+Zn-H] 1+	425.956354	4.287658	425.956124	4.685914	15201447	-0.541353	1+ 13C C9 H12 N2 Na3 O8 68Zn [97.26%];1+ C10 H12 N2 Na3 17O O7 68Zn [2.74%];	C10H13N2O8Na3
18	[M+Zn-H] 1+	425.961764	0.102013	425.961570	0.190501	618000	-0.454861	1+ 13C C9 H12 N2 Na3 18O O7 66Zn ;	C10H13N2O8Na3
19	[M+Zn-H] 1+	426.953455	1.274933	426.953201	1.544104	5009185	-0.595509	1+ C10 H12 N2 Na3 O8 70Zn ;	C10H13N2O8Na3
20	[M+Zn-H] 1+	426.957221	0.633866	426.957002	0.762328	2473048	-0.514075	1+ C10 H12 N2 Na3 18O O7 68Zn ;	C10H13N2O8Na3
21	[M+Zn-H] 1+	426.959686	0.202965	426.959302	0.265221	860395	-0.898909	1+ 13C2 C8 H12 N2 Na3 O8 68Zn ;	C10H13N2O8Na3
22	[M+Zn-H] 1+	427.956810	0.137893	427.956475	0.132425	429596	-0.782076	1+ 13C C9 H12 N2 Na3 O8 70Zn ;	C10H13N2O8Na3

Figure S5. Analysis of spectrum containing ethylenediaminetetraacetic acid (EDTA) with zinc. CycloBranch's settings dialog configured for the analysis of direct infusion electrospray mass spectrum (ESI-MS) containing EDTA with three atoms of sodium and one atom of zinc (top); and fine isotope structure annotated using the database-free approach (bottom). The spectrum was collected in positive ion mode.



	Pattern Type	Theoretical m/z	Theoretical Intensity [%]	Experimental m/z	Relative Intensity [%]	Absolute Intensity	Error [ppm]	Summary Formula	Name
1	[M+Ni-H] 1+	414.963479	100.000000	414.963467	100.000000	463679282	-0.028413	1+ C10 H12 N2 Na3 Ni O8 ;	C10H13N2O8Na3
2	[M+Ni-H] 1+	415.960514	0.738718	415.960484	0.733541	3401278	-0.070461	1+ C10 H12 15N N Na3 Ni O8 ;	C10H13N2O8Na3
3	[M+Ni-H] 1+	415.966857	11.120469	415.966766	10.696249	49596292	-0.218857	1+ 13C C9 H12 N2 Na3 Ni O8 [97.26%];1+ C10 H12 N2 Na3 Ni 17O O7 [2.74%];	C10H13N2O8Na3
4	[M+Ni-H] 1+	415.969756	0.138016	415.969459	0.187394	868908	-0.713229	1+ C10 2H H11 N2 Na3 Ni O8 ;	C10H13N2O8Na3
5	[M+Ni-H] 1+	416.958918	38.519821	416.958835	42.230689	195814956	-0.198734	1+ C10 H12 N2 Na3 60Ni O8 ;	C10H13N2O8Na3
6	[M+Ni-H] 1+	416.967724	1.643995	416.967646	1.663340	7712563	-0.187632	1+ C10 H12 N2 Na3 Ni 18O O7 ;	C10H13N2O8Na3
7	[M+Ni-H] 1+	416.970189	0.526410	416.969939	0.540912	2508096	-0.599133	1+ 13C2 C8 H12 N2 Na3 Ni O8 ;	C10H13N2O8Na3
8	[M+Ni-H] 1+	417.955953	0.284553	417.955917	0.291430	1351301	-0.086640	1+ C10 H12 15N N Na3 60Ni O8 ;	C10H13N2O8Na3
9	[M+Ni-H] 1+	417.959190	1.674430	417.959088	1.804021	8364871	-0.244656	1+ C10 H12 N2 Na3 61Ni O8 ;	C10H13N2O8Na3
10	[M+Ni-H] 1+	417.962296	4.283585	417.962139	4.301250	19944006	-0.375492	1+ 13C C9 H12 N2 Na3 60Ni O8 [97.26%];1+ C10 H12 N2 Na3 60Ni 17O O7 [2.74%];	C10H13N2O8Na3
11	[M+Ni-H] 1+	417.971079	0.177810	417.970938	0.183380	850296	-0.338114	1+ 13C C9 H12 N2 Na3 Ni 18O O7 ;	C10H13N2O8Na3
12	[M+Ni-H] 1+	418.956476	5.338815	418.956323	5.545471	25713202	-0.364192	1+ C10 H12 N2 Na3 62Ni O8 ;	C10H13N2O8Na3
13	[M+Ni-H] 1+	418.963026	0.814366	418.963076	0.624571	2896005	0.120139	1+ C10 H12 N2 Na3 60Ni 18O O7 [77.76%];1+ 13C C9 H12 N2 Na3 61Ni O8 [22.24%];	C10H13N2O8Na3
14	[M+Ni-H] 1+	418.965628	0.202772	418.965385	0.240732	1116225	-0.579452	1+ 13C2 C8 H12 N2 Na3 60Ni O8 ;	C10H13N2O8Na3
15	[M+Ni-H] 1+	419.959831	0.577432	419.959609	0.558533	2589804	-0.527699	1+ 13C C9 H12 N2 Na3 62Ni O8 ;	C10H13N2O8Na3

Figure S6. Analysis of spectrum containing EDTA with nickel. CycloBranch's settings dialog configured for the analysis of direct infusion ESI-MS containing EDTA with three atoms of sodium and one atom of nickel (top); and fine isotope structure annotated using the database-free approach (bottom). The spectrum was collected in positive ion mode.



	Pattern Type	Theoretical m/z	Theoretical Intensity [%]	Experimental m/z	Relative Intensity [%]	Absolute Intensity	Error [ppm]	Summary Formula	Name
1	[M+Cu-3H] 1-	373.979285	100.000000	373.979281	100.000000	1359385103	-0.010574	1- C10 Cu H12 N2 Na O8 ;	C10H15N2O8Na1
2	[M+Cu-3H] 1-	374.976320	0.738718	374.976437	0.492215	6691094	0.311793	1- C10 Cu H12 15N N Na O8 ;	C10H15N2O8Na1
3	[M+Cu-3H] 1-	374.982664	11.120469	374.982701	9.424707	128118064	0.098870	1- 13C C9 Cu H12 N2 Na O8 [97.26%];1- C10 Cu H12 N2 Na 17O O7 [2.74%];	C10H15N2O8Na1
5	[M+Cu-3H] 1-	375.977478	44.571346	375.977569	46.324360	629726455	0.242500	1- C10 65Cu H12 N2 Na O8 ;	C10H15N2O8Na1
6	[M+Cu-3H] 1-	375.983530	1.643995	375.983627	1.357417	18452521	0.257862	1- C10 Cu H12 N2 Na 18O O7 ;	C10H15N2O8Na1
7	[M+Cu-3H] 1-	375.985995	0.526410	375.986086	0.432826	5883769	0.241287	1- 13C2 C8 Cu H12 N2 Na O8 ;	C10H15N2O8Na1
8	[M+Cu-3H] 1-	376.974513	0.329257	376.974707	0.278600	3787243	0.514592	1- C10 65Cu H12 15N N Na O8 ;	C10H15N2O8Na1
9	[M+Cu-3H] 1-	376.980857	4.956543	376.980979	4.454966	60560147	0.324191	1- 13C C9 65Cu H12 N2 Na O8 [97.26%];1- C10 65Cu H12 N2 Na 17O O7 [2.74%];	C10H15N2O8Na1
10	[M+Cu-3H] 1-	376.986885	0.177810	376.987019	0.151329	2057140	0.354977	1- 13C C9 Cu H12 N2 Na 18O O7 ;	C10H15N2O8Na1
11	[M+Cu-3H] 1-	377.981723	0.732751	377.981958	0.641498	8720423	0.619920	1- C10 65Cu H12 N2 Na 18O O7 ;	C10H15N2O8Na1
12	[M+Cu-3H] 1-	377.984188	0.234628	377.984398	0.213427	2901290	0.556159	1- 13C2 C8 65Cu H12 N2 Na O8 ;	C10H15N2O8Na1

Figure S7. Analysis of spectrum containing EDTA with copper. CycloBranch's settings dialog configured for the analysis of direct infusion ESI-MS containing EDTA with one atom of sodium and one atom of copper (top); and fine isotope structure annotated using the database-free approach (bottom). The spectrum was collected in negative ion mode.

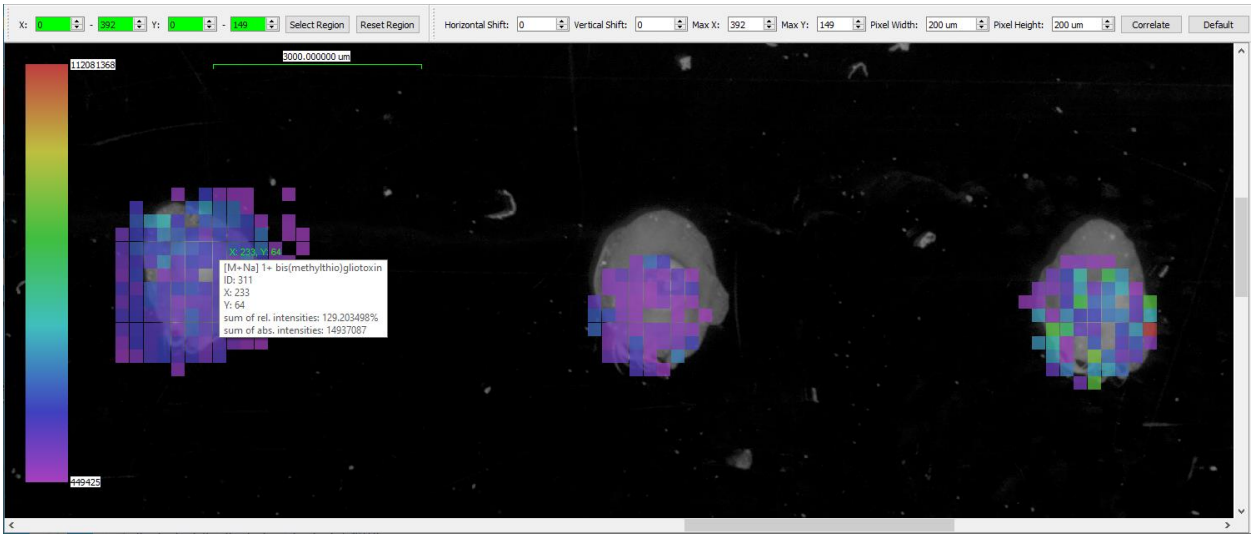
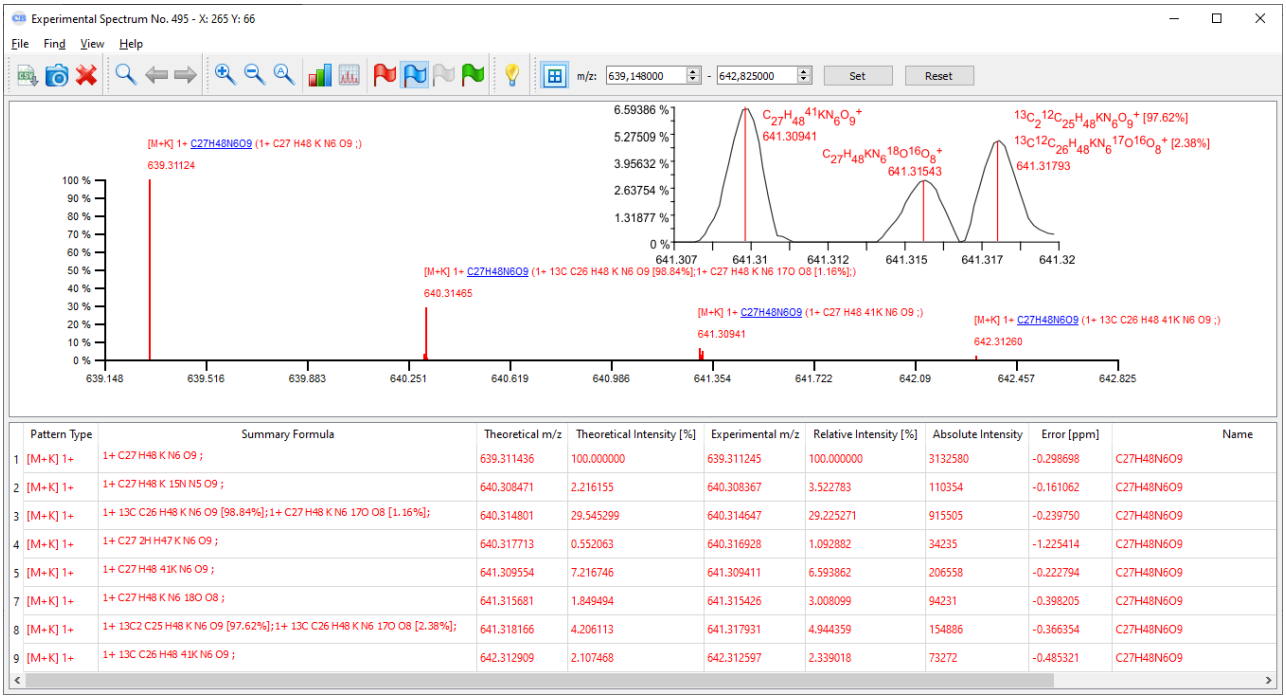
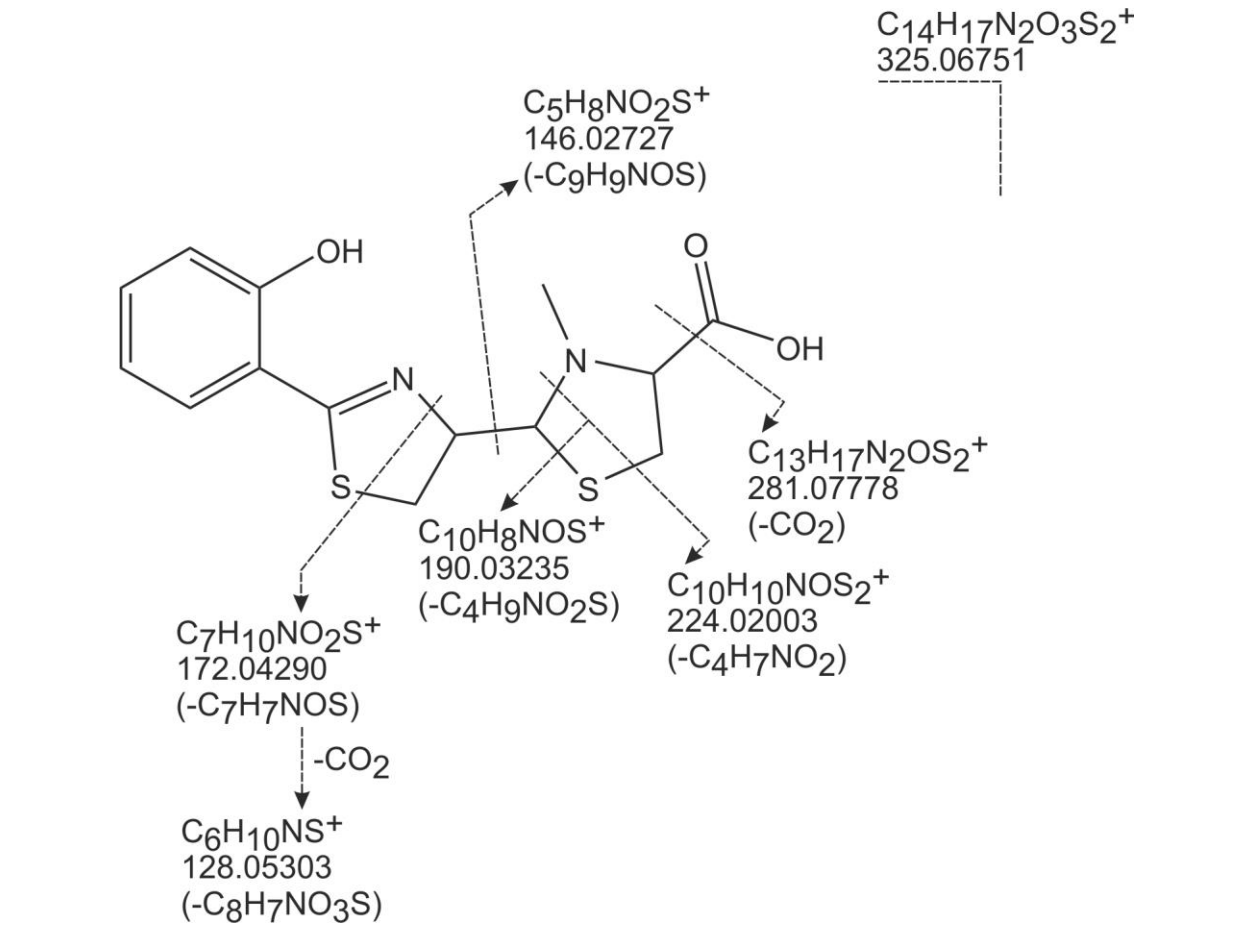


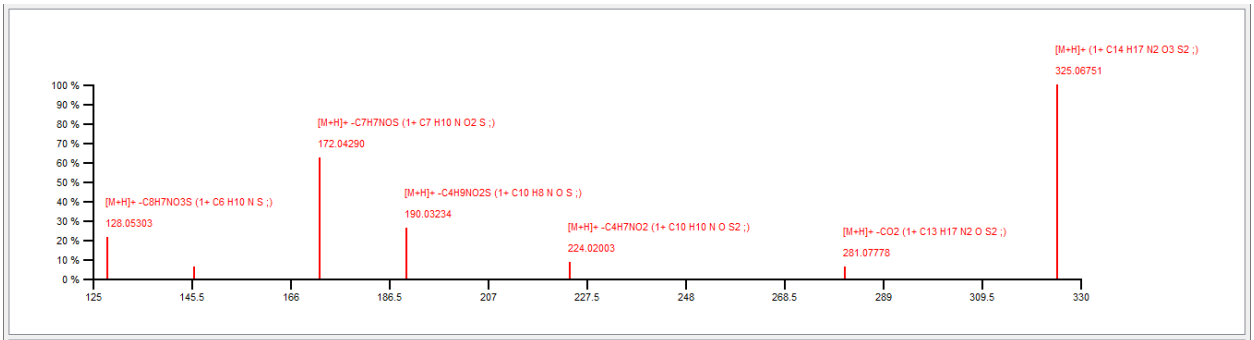
Figure S8. Fusion of identified compounds with an optical image. From left to right—droplet-generated circles of bmGTX ($[C_{15}H_{20}N_2O_4S_2+Na]^+$, *m/z* 379.07567), FOXE ($[C_{27}H_{48}N_6O_9+K]^+$, *m/z* 639.31144), and TAFC ($[C_{39}H_{60}N_6O_{15}+Fe-2H]^+$, *m/z* 906.33041).



	Pattern Type	Summary Formula	Theoretical m/z	Theoretical Intensity [%]	Experimental m/z	Relative Intensity [%]	Absolute Intensity	Error [ppm]	Name
1	[M+K] 1+	1+ C27 H48 K N6 O9 ;	639.311436	100.000000	639.311245	100.000000	3132580	-0.298698	C27H48N6O9
2	[M+K] 1+	1+ C27 H48 K 15N N5 O9 ;	640.308471	2.216155	640.308367	3.522783	110354	-0.161062	C27H48N6O9
3	[M+K] 1+	1+ 13C C26 H48 K N6 O9 [98.84%];1+ C27 H48 K N6 17O O8 [1.16%];	640.314801	29.545299	640.314647	29.225271	915505	-0.239750	C27H48N6O9
4	[M+K] 1+	1+ C27 2H H47 K N6 O9 ;	640.317713	0.552063	640.316928	1.092882	34235	-1.225414	C27H48N6O9
5	[M+K] 1+	1+ C27 H48 41K N6 O9 ;	641.309554	7.216746	641.309411	6.593862	206558	-0.222794	C27H48N6O9
7	[M+K] 1+	1+ C27 H48 K N6 18O O8 ;	641.315681	1.849494	641.315426	3.008099	94231	-0.398205	C27H48N6O9
8	[M+K] 1+	1+ 13C2 C25 H48 K N6 O9 [97.62%];1+ 13C C26 H48 K N6 17O O8 [2.38%];	641.318166	4.206113	641.317931	4.944359	154886	-0.366354	C27H48N6O9
9	[M+K] 1+	1+ 13C C26 H48 41K N6 O9 ;	642.312909	2.107468	642.312597	2.339018	73272	-0.485321	C27H48N6O9

Figure S9. Dissecting ^{41}K, ^{18}O and $^{13}C_2$ (inset) from the $[M+K+2]^+$ ion of FOXE at FWHM = 0.001.





	Fragment Type	Summary Formula	Theoretical m/z	Theoretical Intensity [%]	Experimental m/z	Relative Intensity [%]	Absolute Intensity	Error [ppm]
1	[M+H]+ -C8H7NO3S	1+ C6 H10 N S ;	128.052846	21.531049	128.053030	21.531049	60145045	1.433515
2	[M+H]+ -C9H9NOS	1+ C5 H8 N O2 S ;	146.027026	6.468297	146.027273	6.468297	18068604	1.694108
3	[M+H]+ -C7H7NOS	1+ C7 H10 N O2 S ;	172.042676	62.536821	172.042902	62.536821	174690976	1.315496
4	[M+H]+ -C4H9NO2S	1+ C10 H8 N O S ;	190.032111	26.565667	190.032345	26.565667	74208797	1.231411
5	[M+H]+ -C4H7NO2	1+ C10 H10 N O S2 ;	224.019832	8.898911	224.020032	8.898911	24858306	0.893910
6	[M+H]+ -CO2	1+ C13 H17 N2 O S2 ;	281.077681	6.481809	281.077780	6.481809	18106350	0.352301
7	[M+H]+	1+ C14 H17 N2 O3 S2 ;	325.067510	100.000000	325.067511	100.000000	279340992	0.002399

Figure S10. Structure of pyochelin and formulas of fragment ions suggested by CycloBranch.

Table S1. Number of generated combinations of elements for different *m/z* ranges [All = no filtering was applied; Sen. = Senior's filtering rules were applied; Adv. = advanced filtering rules (including the N/O rule) were used]. The maximum number of elements in a formula was limited to 200. S:1 and P:1 indicate that only one occurrence of sulfur and phosphorus was allowed, respectively.

	m/z ≤ 500			*m/z* ≤ 1,000 Da			*m/z* ≤ 2,000 Da		
Elements	All	Sen.	Adv.	All	Sen.	Adv.	All	Sen.	Adv.
HCON	934,192	120,383	29,959	8,101,535	1,730,838	399,894	46,132,961	17,758,042	3,331,530
HCONS	3,757,914	620,212	122,476	65,355,150	16,723,250	2,962,328	796,949,136	352,440,391	29,991,863
HCONSP	13,306,477	2,496,101	308,917	444,850,486	121,964,934	8,596,749	11,113,584,055	5,140,835,916	82,784,488
HCONS:1	1,681,008	225,403	55,511	15,438,135	3,350,810	775,599	90,392,945	35,076,565	6,604,713
HCONS:1P:1	3,024,190	416,600	101,546	29,436,430	6,446,384	1,495,144	177,165,660	69,119,426	13,068,349

Table S2. Time required to generate combinations of elements for different *m/z* ranges [mm:ss] (MALDI-MS or direct infusion ESI).

	m/z ≤ 500 Da			*m/z* ≤ 1,000 Da			*m/z* ≤ 2,000 Da		
Elements	All	Sen.	Adv.	All	Sen.	Adv.	All	Sen.	Adv.
HCON	0:00	0:00	0:00	0:02	0:02	0:02	0:21	0:26	0:26
HCONS	0:00	0:00	0:00	0:17	0:20	0:22	5:34	6:53	7:29
HCONSP	0:02	0:02	0:02	1:27	1:47	2:06	52:05	69:38	86:24
HCONS:1	0:00	0:00	0:00	0:07	0:07	0:08	2:03	2:09	2:15
HCONS:1P:1	0:00	0:00	0:00	0:09	0:10	0:11	2:16	2:37	2:39

Comments on Conventional Mass Spectra Settings:

Go to the CycloBranch's settings dialog (i.e., click on "Search" → "Settings" in the main window) and select the mode "Compound Search – MS, LC-MS, MSI". Define the minimum and maximum *m/z* ratios to limit the number of generated molecular formulas. The smaller the difference between the maximum and minimum *m/z* ratio is, the faster the search. Click on the HCON button to define the chemical elements H, C, O, and N in the field "Neutral Losses/Chemical Elements". Any custom element can be added. Heteroatoms such as Na or K need not be added if $[M+Na]^+$ or $[M+K]^+$ ions are searched. The heteroatoms used in the names of ion types are included automatically, and thus, computational time can be saved. Define the maximum number of atoms in a generated molecular formula (e.g., 150 or 180) in the field "Maximum Number of Combined Losses/Elements". The smaller the number is, the faster the search. See the sample configuration in **Figure S2** for LC-MS data and that in **Figure S3** for MSI data. The following options can be used to reduce the number of false-positive annotations of peaks:

(1) Enable the option "Generate Full Isotope Patterns" and define the "Minimum Number of Isotopic Peaks" (e.g., 2). The theoretical isotopic patterns of compounds are generated and compared with the experimental spectrum. The "Minimum Number of Isotopic Peaks" (i.e., the minimum number of annotated peaks in an isotopic pattern used to report a given compound) is used to remove false-positive formulas.

(2) If LC-MS data are processed, define the minimum number of consecutive scans in which an ion must be detected to be considered a match in the field "Minimum Number of Spectra" (e.g., 2). If MSI data are processed, the field determines the minimum number of single-pixel spectra that must be detected for every compound (e.g., 50). Note that this criterion can be combined with the "Minimum Number of Isotopic Peaks".

(3) Define a minimum number of ions that must be matched to annotate a given compound. For example, if $[M+H]^+$ and $[M+Na]^+$ ions are selected, "Charge" = 2, and "Minimum Number of Ion Types" = 2, any pair of ions from the set of ions $[M+H]^+$, $[M+Na]^+$, $[M+2H]^{2+}$, and $[M+Na+H]^{2+}$ must be matched, otherwise the formula is discarded. This option can be combined with the "Minimum Number of Isotopic Peaks" and "Minimum Number of Spectra". If MALDI-MS, direct infusion ESI-MS, and MSI data are processed, the ions must be detected in the same spectrum. If LC-MS data are processed, the ions may occur at different retention times, which is advantageous, especially if $[M+H]^+$ and $[M+Fe-2H]^+$ ions are searched.

(4) Enable the option "Basic Formula Check" to discard formulas that do not meet Senior's rules.

(5) Enable the option "Advanced Formula Check" to check the advanced filtering rules, as stated in the manuscript.

(6) Enable the option "N/O Ratio Check" to remove formulas with a number of nitrogen atoms larger than the number of oxygen atoms.

(7) Define "Isotope *m/z* Tolerance" τ_{mz} in ppm.

(8) Define "Isotope Intensity Tolerance" τ_{int} in %.

Comments on Standard Product Ion Mass Spectra Settings:

In the settings dialog, select the mode "Compare Peaklist(s) with Spectrum of Searched Sequence - MS/MS" and define the peptide type "Other". In this mode, a single theoretical spectrum of a compound is compared with all experimental MS/MS spectra in an input file. The result of each comparison corresponds to one row in the output report. To perform the comparison, enter the molecular formula of a neutral compound into the field "Searched Sequence/Compound - Formula". Define an input list of chemical elements in the field "Neutral Losses/Chemical Elements". Click on the HCON button to use the default chemical elements and set the "Maximum Number of Combined Losses/Elements". The upper bound on the number of combined elements is the number of elements in the formula corresponding to the precursor ion of the analyzed spectrum. For example, if the precursor corresponds to $[C_{14}H_{16}N_2O_3S_2+H]^+$, use a value of 38 or lower. Note that the maximum number of occurrences of single elements can be limited by a colon. For example, S:1 and P:1 can be used to allow only one occurrence of sulfur or phosphorus, respectively. Furthermore, define the minimum *m/z* ratio to be as large as possible (see the impact of m_{min} below). See the sample configuration in **Figure S4**.

To perform a comparison of a single experimental MS/MS spectrum with multiple theoretical spectra (i.e., database compounds), select the mode "Compare Peaklist with Database – MS/MS" and define the peptide type as "Other". In this case, the database of compounds "Sequence/Compound Database File" does not have to include the sequence of building blocks for any compound. The mandatory items for every compound in the database are only the name and molecular formula. Because the database may contain many compounds, a precursor mass filter is enabled by default. This filter can optionally be disabled by the checkbox "Disable Precursor Mass Filter" to make a comparison of the experimental spectrum with all compounds in the database. Note that the type of peptide in the database is ignored in this search mode, and all the compounds are used.

The algorithm for annotation of peaks in product ion mass spectra works a similar way like the algorithm for conventional mass spectra:

(1) The input list of elements (e.g., H, C, O, and N) is loaded, and combinations of elements are generated. The number of generated combinations is limited by the maximum number of elements in a formula according to the "Maximum Number of Combined Losses/Elements" (e.g., 150).

(2) A combination of elements is discarded if $m_{total} > m_{1+} - m_{min}$ where m_{total} is the total mass of the combination, m_{1+} is the mass of singly charged precursor ion, and m_{min} is the minimum *m/z* ratio.

(3) If a theoretical product ion mass spectrum of a single compound, e.g., $C_aH_bN_cO_d$, is generated, any combination of elements $C_wH_xN_yO_z$ is limited by the condition $w \leq a$, $x \leq b$, $y \leq c$, and $z \leq d$. If theoretical spectra of multiple compounds from a database are generated (within a specified precursor *m/z* error tolerance), the maximum numbers of elements are used. For example, given another database compound, $C_eH_fN_gO_h$, then $w \leq \max(a,e)$, $x \leq \max(b,f)$, $y \leq \max(c,g)$, and $z \leq \max(d,h)$.

(4) Senior's rules no. 1 and 3 are checked. In this case, the sum of valences of all atoms in a formula had to be even and greater than or equal to 2 * ($n - \alpha$) where α was the maximum number of independent molecules of neutral losses. We did not use $\alpha = 1$ but an empirically determined value $\alpha = 10$ what means that at the most ten neutral losses could occur simultaneously (i.e., a molecular graph could include up to α components). The reason was that multiple neutral losses of molecules such as water of ammonia did not meet the criterion 2 * ($n - 1$).

(5) The remaining combinations of elements are applied as hypothetical neutral losses to a single molecular formula or a database of molecular formulas. Note that atoms in the formula of an annotated fragment ion form a subset of atoms in the formula of the precursor ion, what is an important difference from the MS^1 approach. Similar to the MS^1 approach, the presence of a monoisotopic peak is checked in the experimental spectrum while the theoretical spectrum is being generated to save the computer's main memory (i.e., unnecessary isotopic patterns of fragment ions are not generated).