Ariadne
Nucleotide Parts Editor
User’s Manual

Rev 1.1

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Ariadne Development Team

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Ariadne software constructs nucleic acids from three components: the nucleoside, the linker and the terminus. Over 100 default parts provided in Nucleotide Parts Table of the top page (http://ariadne.riken.jp/) can be chosen for the calculation / search. Additionally, with the Nucleotide Parts Editor, you can construct any nucleic acid or their analogues by defining your own parts in the database.

This manual describes the concept of parts and their specific MS/MS fragmentations, how to browse and activate parts, and how to use the editor for each part type as well as the requirements of the computational environment.

1. System requirements

See the system requirements for the Ariadne software for details (See Section 1.1.1 in the Ariadne Mass Calculator User's Manual). Over 100 default parts are available to anyone without the need to create a user account. However, a user account is required to define your own parts and to save them on the Ariadne server. How to register your account and how to login your account are described in the note. Account registration and login are described in Section 1.1 of the Ariadne Mass Calculator User's Manual.

2. The parts and their specific MS/MS fragmentation

2.1. What are the parts?

Nucleic acids are composed of a nucleoside, linker, and 5′ - and 3′ -termini, as shown in Figure 1. To simulate this structure, the Ariadne software allows you to create a nucleic acid from the 100+ default parts listed in Nucleotide Parts Table or from user-defined parts uploaded to the database.

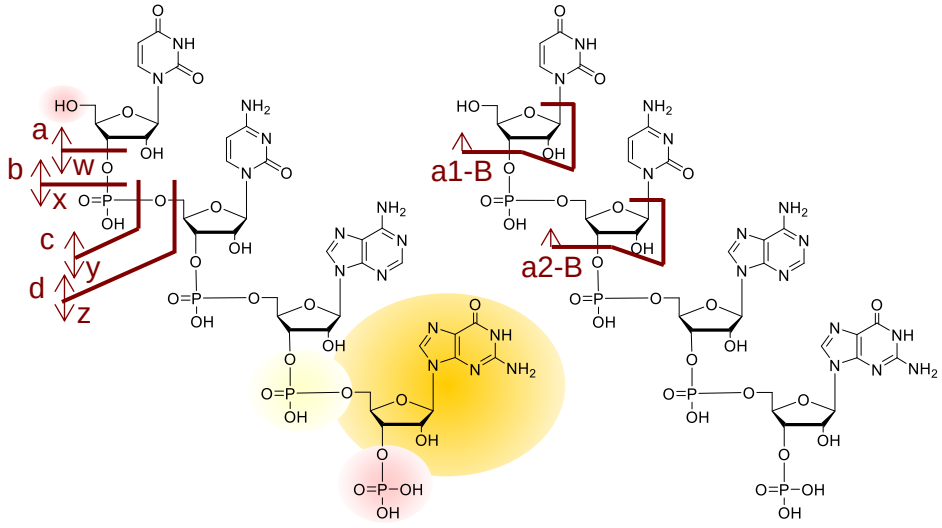


Figure 1. The structure of canonical nucleic acids

The mass of the ions is calculated by Ariadne after the user creates a nucleic acid from a combination of the three parts. The calculated mass values are used for searching sequence database. Figure 2 shows the schematic of the calculations.

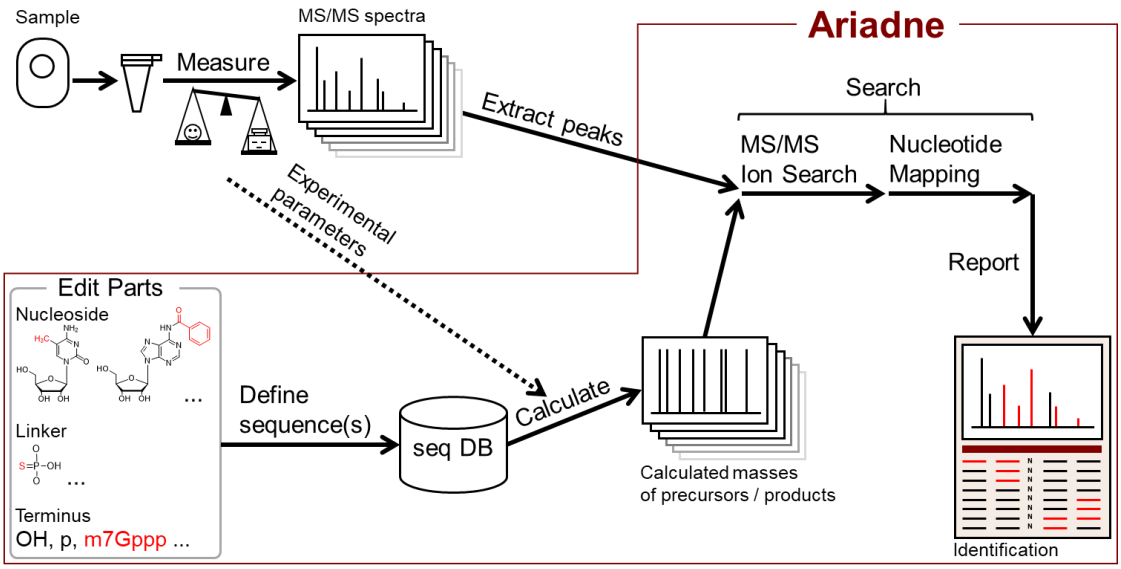


Figure 2. Schematic of Ariadne's calculation

Because parts are defined as the chemical formula of its typical subpart structures, the software enables you to calculate the mass values of various product ions upon gas-phase activation.

Nucleotide ions are cleaved at a single site on their backbone to produce a sequence ladder (See Figure 3). In the software, we call these fragmentations sequence-ladder ions. The gas-phase fragmentation of the nucleotide ions can also exhibit base losses and/or a double-site backbone cleavage resulting in an internal ion. Both of these scenarios are taken into account by the software. In addition, the software allows you to simulate stable isotope labeling experiments, not only for detecting/identifying a specific post-transcriptional modification of RNA but also for quantifying the modification with a single nucleotide resolution.

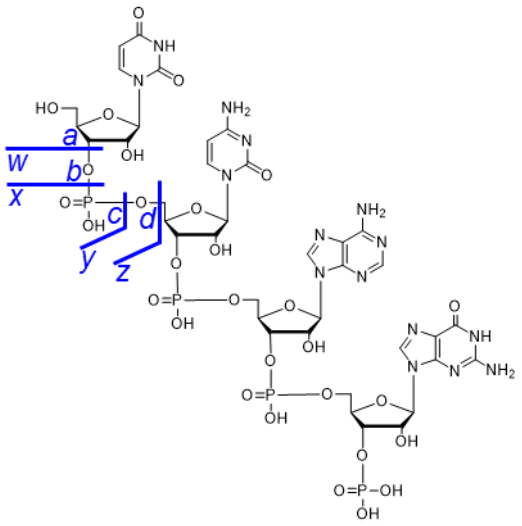


Figure 3. The gas-phase dissociation of nucleic acid and its nomenclature

To implement these functions, the software requires a chemical formula; it does not support direct input of its mass value. Suppose you use a highly accurate mass spectrometer like Q-TOF- or Orbitrap-type instrument to measure nucleic acids. In that case, probable chemical formulas can easily be obtained from the observed masses with the vender's software. For example, Thermo Xcalibur Qual Browser software can convert a mass value into chemical formula, as shown in Figure 4.

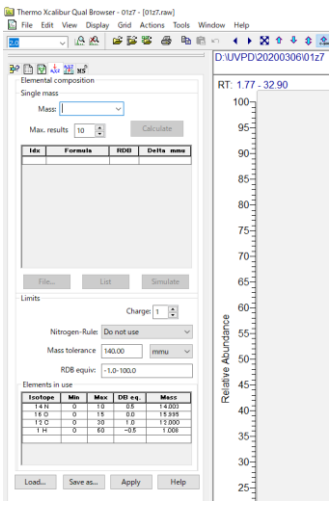


Figure 4. Obtaining chemical formula from observed mass in Thermo Xcalibur Qual Browser

2.2. Three types of parts

In this section, the three types of parts are illustrated. These three parts enable the software to calculate the masses of a nucleotide, its enzymatic digests, and their product ions, including sequence-ladder ions and internal ions.

2.2.1. Nucleoside

A nucleoside is a conjugate of a nucleobase and ribose or one of its derivatives. Except for pseudouridine and its derivatives, the 3-position nitrogen in a pyrimidine base or 9-position nitrogen in a purine base is conjugated to the 1′ -position of a ribose derivative. The nucleoside loses one water molecule during conjugation. According to this structure, a nucleotide ion is frequently dissociated at the C-N bond, resulting in the loss of neutral base(s) from molecular ion or sequence ions and the generation of base ion(s). To simulate the loss of a neutral base and the detection of the nucleobase ion, the software defines a nucleoside with two subparts: a base and sugar. The base and sugar should be defined as neutral molecules (BH and 1′ -OH sugar), as shown in Figure 5.

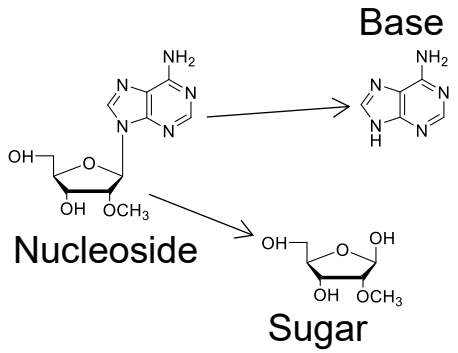


Figure 5. The structure of nucleoside parts and its subparts: (nucleo)base and sugar

A helpful function of the Ariadne software is the support of stable isotope labeling at the nucleoside parts. Figure 6 illustrate some examples of the available labeling methods. You can see the whole list if you click Isotope Set select box. Since the position format and bonding type of a nucleoside is complicated for a user to input correctly, the current parts editor does not support user defined isotope labeling. If you would like to use a part with stable isotope labeling, don’t hesitate to contact us via email (ariadne_dev_team@riken.jp). We are glad to set up a part and a labeling method used in an experiment.

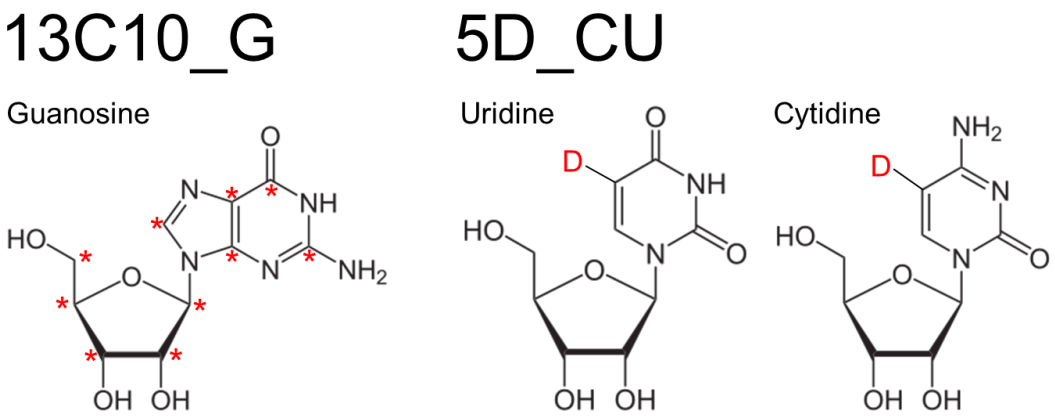


Figure 6. Examples of the Isotope Sets for the mass table. Left, 13C10G simulates full carbon labeled guanosine in RNAs. *: 13C. Right, 5D_CU simulates metabolic labeling with 5-D-uracil, which is labeled with D at the 5-position of uridine/cytidine and their modified nucleosides in RNAs.

2.2.2. Linker

Nucleotide ions are commonly dissociated at the linker by tandem mass spectrometry (MS/MS) which results in informative sequence-ladder product ions. Figure 3 shows the nomenclature of the product ions. To calculate these products, the linker consists of three subparts as shown in Figure 7. For example, *a* ion series contain no subpart, *b* ion series contain only subpart 1, *c* ion series subparts 1 and 2, *d* ion series subparts 1, 2, and 3 and so on. It is noted that linker parts are not a complete molecule; rather it is a fragment (See Figure 7).

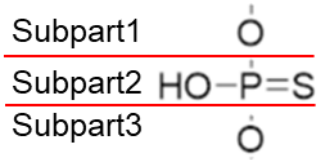


Figure 7. The structure of linker parts and its subparts

2.2.3. Terminus

Many cellular RNAs are phosphorylated or capped at the 5-terminus during maturation. The 3-terminus hydroxyl group of most cellular RNAs remains unmodified. For synthetic nucleotides, one or both of termini are sometimes modified with various functional group(s). To simulate all of them, the software allows the definition of the terminus parts. As default, OH and phosphate (p) for both 5′ - and 3′ -termini as well as cyclic phosphate (cp) for 3-terminus are available. In most cases the same functional groups are observed for both

termini, therefore you can assign the defined terminus to either 5′ , 3′ , or both ends through the Activation option in the editor.

2.3. Part-Specific MS/MS Fragmentation

2.3.1. What is Part-Specific MS/MS Fragmentation?

In addition to typical dissociations mentioned earlier, parts-specific fragmentations are observed for nucleic acid ions, which we term Part-Specific MS/MS Fragmentation. The fragmentations are non-typical gas-phase dissociations but provide the information on the part's existence at particular sites in the molecule or that in the whole molecule. The former is called Sequence-type fragmentations and the latter Composition-type.

As mentioned earlier, metabolic/chemical labeling with a stable-isotope-containing nucleosides or nucleobases are useful for not only detecting post-transcriptional modifications of RNA but also quantifying them. Although the Part-Specific MS/MS Fragmentation of an isotope-labeled part will provide valuable information, the current version of the software does not fully support it yet. This function will be supported in a future version.

2.3.2. Sequence Type fragmentations

Specific product ions that are generated by one of the sequence-type fragmentations can indicate the existence of the part at a particular site on a nucleotide sequence. For example, MS/MS of N7-methylguanosine (m7G)-containing nucleotide anions will easily lose neutral N7-methylguanine base from the molecular ion. The resulting ions suffer secondary fragmentations, producing abundant sequence-ladder ions with the loss of neutral N7-guanine base instead of the corresponding sequence-ladder ions, as shown in Figure 8. The facile cleavage by the non-typical neutral loss allows us to know m7G's existence and position in the molecule (Figure 8).

#	a-B	a	b	c	d	seq
5'						OH
1	113.0244	226.0580	244.0685	306.0243	324.0349	U
2	420.0560	585.1211	603.1316	665.0874	683.0980	m7G
3	779.1191	993.2003	1011.2109	1073.1666	1091.1772	acp3U
4	1187.1983	1299.2479	1317.2584	1379.2142	1397.2248	C
5	1493.2459	1605.2954	1623.3060	1685.2618	1703.2723	C
6	1799.2935	1912.3270	1930.3376	1992.2933	2010.3039	U
7	2106.3250	2219.3586	2237.3692	2299.3249	2317.3355	U
8						G
3'						P

#	a-B-B(m7G)	a-B(m7G)	b-B(m7G)	c-B(m7G)	d-B(m7G)	seq
5'						OH
1	113.0244	226.0580	244.0685	306.0243	324.0349	U
2	420.0560	420.0560	438.0666	500.0223	518.0329	m7G
3	614.0540	828.1353	846.1458	908.1016	926.1121	acp3U
4	1022.1333	1134.1828	1152.1934	1214.1491	1232.1597	C
5	1328.1808	1440.2304	1458.2409	1520.1967	1538.2073	C
6	1634.2284	1747.2620	1765.2725	1827.2283	1845.2389	U
7	1941.2600	2054.2935	2072.3041	2134.2599	2152.2704	U
8						G
3'						P

Figure 8. Product-ion assignment of m7G-containing oligonucleotide without or with the Part-Specific MS/MS Fragmentation

2.3.3. Composition Type fragmentations

Composition specific product ions with known MS/MS channels can indicate the existence of parts in the molecule.

For example, MS/MS of nucleotide anions with a relatively high energy will generate characteristic nucleobase anions, *e. g.* MS/MS spectrum of ACCG will provide the base ions of adenine (*m/z* 134), cytosine (*m/z* 110), and guanine (*m/z* 150) though they provide no sequence information. These nucleobase anions are especially useful for the detection of a modified nucleoside in the nucleotides. Another example would be pseudouridine (Y)-containing nucleotide anions, which generate no uracil base anion due to the stable C-C bond which connects the base and ribose, will instead spawn a characteristic anion at *m/z* 207.04 corresponding to the pseudouridine anion with double losses of water molecules (Pomerantz SC et al. 2005 Anal Chem; Yamauchi Y et al. 2006 Nucleic Acids Res.) (Figure 9).

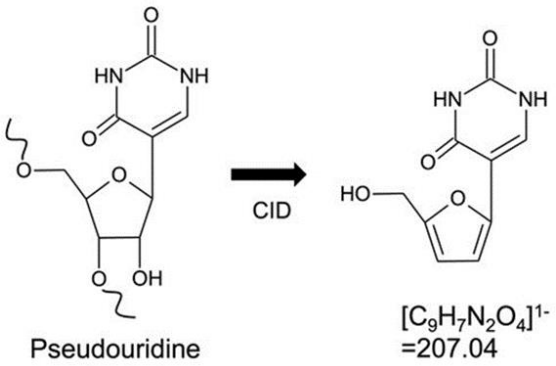


Figure 9. Specific product ion of pseudouridine-containing oligonucleotide

Similarly, MS/MS spectra of 2′ -O-methylated nucleotides will represent specifically the ion at *m/z* 225 that corresponds to methylribose phosphate (Qiu F et al. 1999 Nucleic Acids Res.). Those known composition-specific MS/MS product ions allow one to identify the existence of those parts.

Of course, these two types of characteristic gas-phase dissociation are observed in a linker or terminus as well as a nucleoside, which can locate the parts in the sequence or identify the existence of it in the molecules.

3. Browsing the Nucleotide Parts Table

3.1. Nucleotide Parts Table

You can browse all default parts in the Nucleotide Parts Table without logging-in your account. To see the table, click the Nucleotide Parts Table link on the top page or click on Nucleotide Parts Table link on the menu at the top of some of pages as shown in Figure 10. Guest users who do not have their user account cannot activate or deactivate parts.

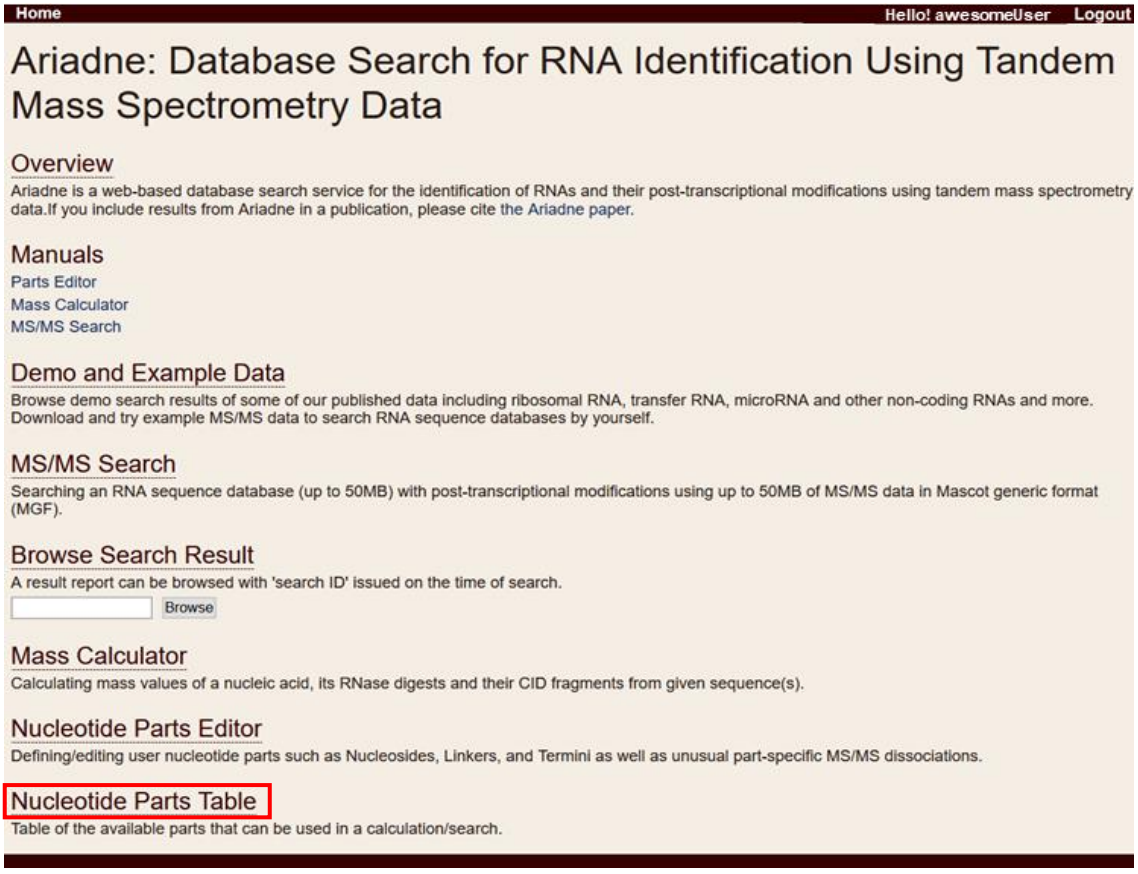
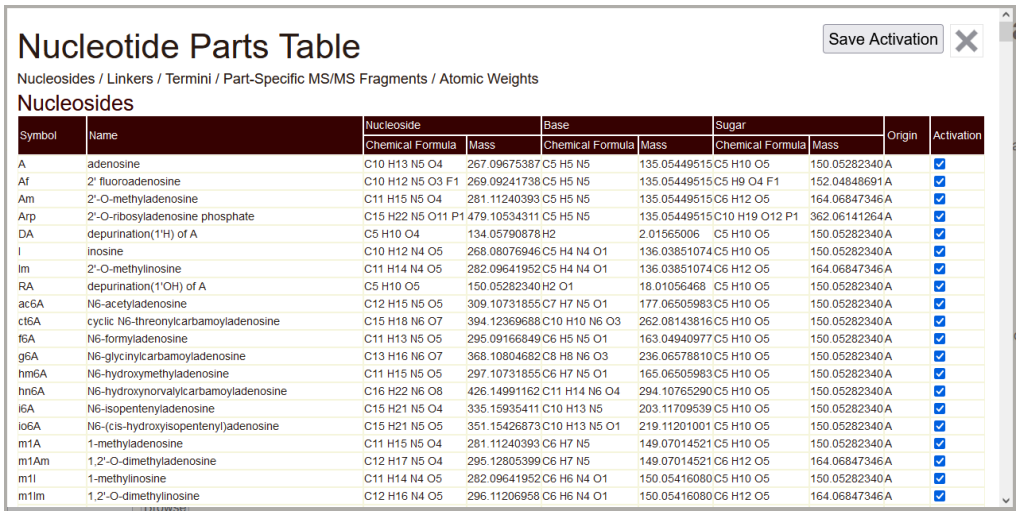


Figure 10. Link to Nucleotide Parts Table

When you login to your account, you can browse not only default parts but your own parts as well if you have already defined them. Account creation and login instructions can be found in the Mass Calculator User's Manual or MS/MS Search User's Manual. You can also activate / deactivate those parts when you have logged in your account.

3.2. Activation/deactivation of parts in the table

Activating/deactivating parts requires the user to be logged into their account. Guest users may browse the parts table without an account; however, they cannot activate/deactivate parts. To browse the parts table, click Nucleotide Parts Table link on the top page or Nucleotide Parts Table link as shown in Figure 11.



Symbol	Name	Nucleoside		Base		Sugar		Origin	Activation
		Chemical Formula	Mass	Chemical Formula	Mass	Chemical Formula	Mass		
A	adenosine	C10 H13 N5 O4	267.09675387	C5 H5 N5	135.05449515	C5 H10 O5	150.05282340	A	☑
Af	2' fluoroadenosine	C10 H12 N5 O3 F1	269.09241738	C5 H5 N5	135.05449515	C5 H9 O4 F1	152.04848691	A	☑
Am	2'-O-methyladenosine	C11 H15 N5 O4	281.11240393	C5 H5 N5	135.05449515	C6 H12 O5	164.06847346	A	☑
Arp	2'-O-ribosyladenosine phosphate	C15 H22 N5 O11 P1	479.10534311	C5 H5 N5	135.05449515	C10 H19 O12 P1	362.06141264	A	☑
DA	depurination(1'H) of A	C5 H10 O4	134.05790878	H2	2.01565006	C5 H10 O5	150.05282340	A	☑
I	inosine	C10 H12 N4 O5	268.08076946	C5 H4 N4 O1	136.03851074	C5 H10 O5	150.05282340	A	☑
Im	2'-O-methylinosine	C11 H14 N4 O5	282.09641952	C5 H4 N4 O1	136.03851074	C6 H12 O5	164.06847346	A	☑
RA	depurination(1'OH) of A	C5 H10 O5	150.05282340	H2 O1	18.01056468	C5 H10 O5	150.05282340	A	☑
ac6A	N6-acetyladenosine	C12 H15 N5 O5	309.10731855	C7 H7 N5 O1	177.06505983	C5 H10 O5	150.05282340	A	☑
ct6A	cyclic N6-threonylcarbamoyladenosine	C15 H18 N6 O7	394.12369688	C10 H10 N6 O3	262.08143816	C5 H10 O5	150.05282340	A	☑
f6A	N6-formyladenosine	C11 H13 N5 O5	295.09166849	C6 H5 N5 O1	163.04940977	C5 H10 O5	150.05282340	A	☑
g6A	N6-glycinylcarbamoyladenosine	C13 H16 N6 O7	368.10804682	C8 H8 N6 O3	236.06578810	C5 H10 O5	150.05282340	A	☑
hm6A	N6-hydroxymethyladenosine	C11 H15 N5 O5	297.10731855	C6 H7 N5 O1	165.06505983	C5 H10 O5	150.05282340	A	☑
hn6A	N6-hydroxynorvalylcarbamoyladenosine	C16 H22 N6 O8	426.14991162	C11 H14 N6 O4	294.10765290	C5 H10 O5	150.05282340	A	☑
i6A	N6-isopentenyladenosine	C15 H21 N5 O4	335.15935411	C10 H13 N5	203.11709539	C5 H10 O5	150.05282340	A	☑
io6A	N6-(cis-hydroxyisopentenyl)adenosine	C15 H21 N5 O5	351.15426873	C10 H13 N5 O1	219.11201001	C5 H10 O5	150.05282340	A	☑
m1A	1-methyladenosine	C11 H15 N5 O4	281.11240393	C6 H7 N5	149.07014521	C5 H10 O5	150.05282340	A	☑
m1Am	1,2'-O-dimethyladenosine	C12 H17 N5 O4	295.12805399	C6 H7 N5	149.07014521	C6 H12 O5	164.06847346	A	☑
m1I	1-methylinosine	C11 H14 N4 O5	282.09641952	C6 H6 N4 O1	150.05416080	C5 H10 O5	150.05282340	A	☑
m1Im	1,2'-O-dimethylinosine	C12 H16 N4 O5	296.11206958	C6 H6 N4 O1	150.05416080	C6 H12 O5	164.06847346	A	☑

Figure 11. The Nucleotide Parts Table view. The view contains 4 tables that show all the available nucleosides, linkers, and termini as well as their specific MS/MS fragmentations during MS/MS. An example of nucleoside table is shown in the figure.

In the table, you can browse all the parts including the defaults and those defined by the user. If a part is activated, the composing sequence database can be used for the calculation/search. Default parts are those for RNA, DNA and other artificial nucleotides and there are over 100 of them are listed in the table. Account users can deactivate unneeded parts, *e. g.* when you analyze RNA data, you would like to deactivate any parts of DNA and artificial nucleotides.

Activating/deactivating a part

Clicking the checkbox in the rightmost column of the parts tables will alter the activation status of the parts (See Figure 12). Note that you can use only the activated parts in the calculation and search.

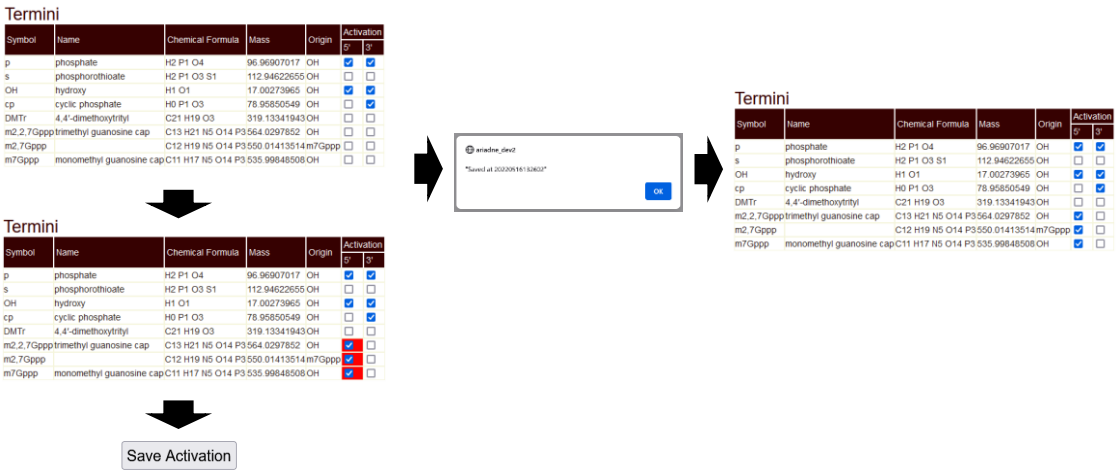


Figure 12. Activating some parts in the parts table

When clicking the Save Activation button in the right-top of the view, the change will be saved in the server. Leaving it unsaved with clicking the close button [x] will cause the change to not be saved. The cells changed will notify the status shown in red.
Click the Save Activation button and you will see an alert notifying you that the data was saved and the time in 14-digit format (Saved at YYYYMMDDHHMMSS). Click the OK button to unmark the column. The change is now saved to the server, and you can use your own parts (See Figure 12).
To re-activate the parts, click the checkbox again and then click the Save Activation button.

If you have changed the activation states of the terminus parts, 5′ Term and 3′ Term the select boxes list only activated termini as shown in Figure 13.
For the Nucleoside and Linker parts, if you include the deactivated parts in your sequence and run the calculation/search, the software will return an error.

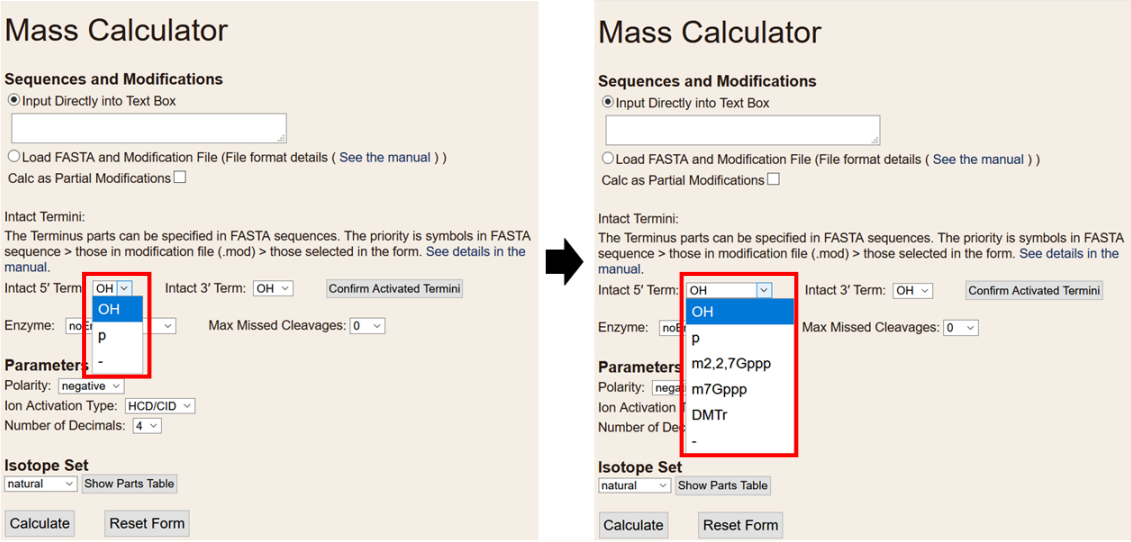


Figure 13. The activated terminus parts

4. Defining parts with the Nucleotide Parts Editor

4.1. Starting Nucleotide Parts Editor

Open Ariadne top page.

Login to your account.

If you do not have an account, you should create one and login before using the editor. The steps to create your account and log in are described in the Mass Calculator User's Manual or MS/MS Search User's Manual. If you do not log in to your account, you can neither define your parts nor activate/deactivate parts. User-defined parts are saved separately and securely on the server; therefore, you need to log in to your account to access them.

Click the Nucleotide Parts Editor link, and you will see the editor page as shown in Figure 14. The initial page of Nucleotide Parts Editor consists of Editors and User Parts sections. The Editors section is further divided into Nucleoside, Linker, Terminus, and Part-Specific MS/MS Fragmentation subsections. In those subsections, you can define three types of parts and their specific fragmentations. Clicking each link will pop up a modal window for defining a type using a wizard. After definition, your parts will appear in the corresponding subsection of the User Parts section (Figure 15).

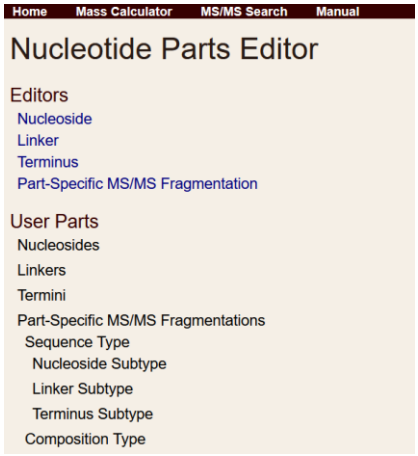


Figure 14. The Nucleotide Parts Editor initial page before defining a user part.

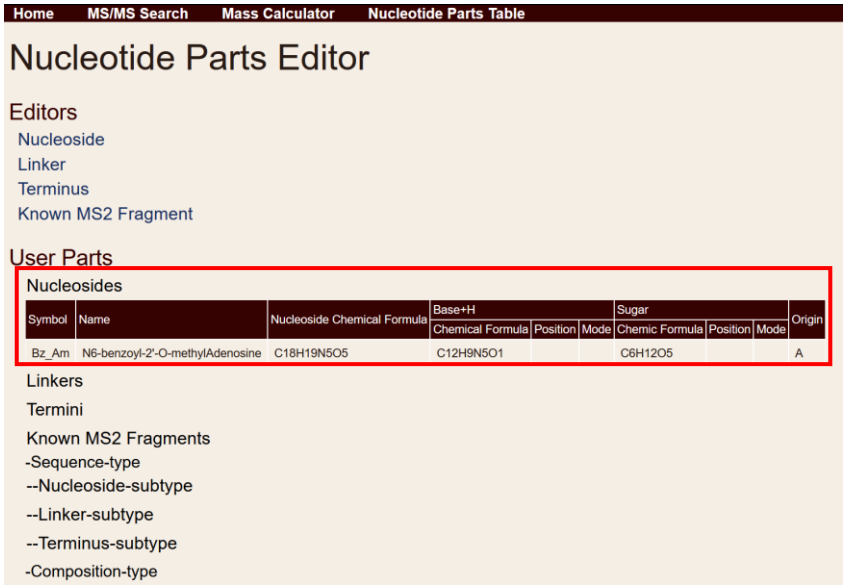


Figure 15. Nucleotide Parts Editor initial page after defining a user part. The defined part is added in the list of the User Parts tables.

4.2. Definition of three types of parts

On the editor page (See Figure 14), you can define all three types of parts and their specific MS/MS fragmentation(s). You can also browse and/or delete your own defined parts.

4.2.1. Nucleoside

Click the Nucleoside link in the Editor section, and you will see the pop-up window as shown in Figure 16. The page contains the input wizard for the Nucleoside Editor, User Nucleoside

Table, and Default Nucleoside Table. You can discard inputs during a definition by clicking the close button [x] at the right top of the wizard; you will see the initial page of the editor.

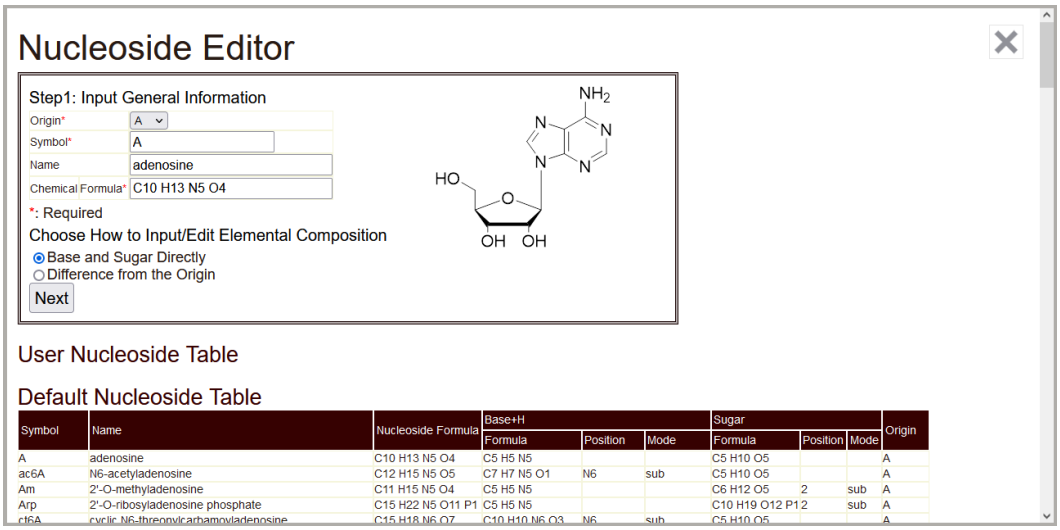


Figure 16. The Nucleoside Editor pop-up window

In the Nucleoside Editor page, you can choose from two different ways to input the chemical formula: Base and Sugar Directly, or Difference from the Origin radio buttons.

Step 1:

Choose one from the two ways to enter the chemical formula with the radio button and input the part information. Read the following explanation of each field.

Origin: Choose the original nucleoside from the select box. It is required for calculating modification (See Section 1.5 in the Mass Calculator User's Manual or Section 1.6 in the MS/MS Search User's Manual).

Symbol: Used to represent a sequence in the calculation/search form and result report. All alphanumeric characters and commas (',') can be used to define a nucleoside symbol; however, a symbol consisting of only lowercase letters is not allowed (The program regards a symbol consisting of only lowercase letters as a linker).

Name: Name and/or explanation of the nucleoside

Chemical Formula: The molecular formula of the nucleoside to be defined (Figure 17,23).

Input option 1: Base and Sugar Directly

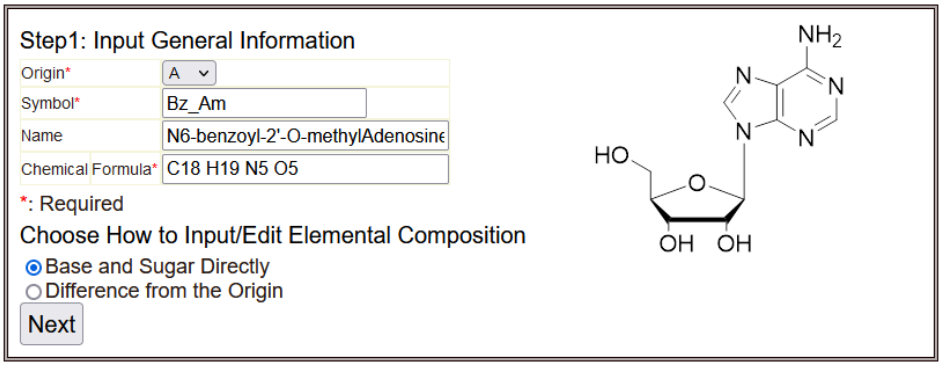


Figure 17. Step 1 of the form for inputting general information of the parts

Step 2:

Input the Chemical Formulas for Base+H (neutral nucleobase molecule) and Sugar (Figure 18).

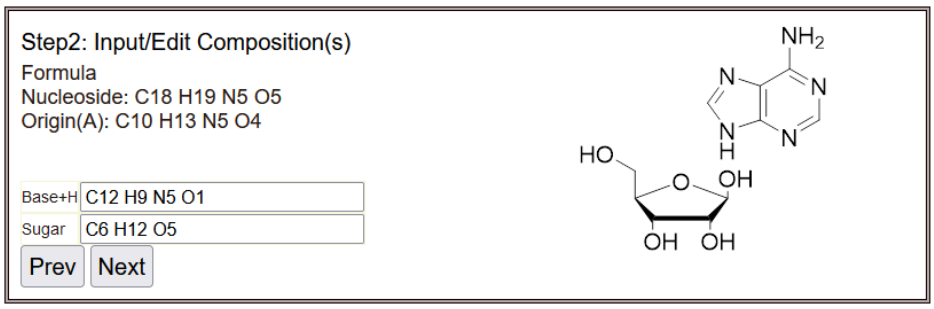


Figure 18. Step 2 of the form for inputting/editing the chemical formulas of base and sugar subparts

Step 3:

Confirm the input and click Add New Nucleoside button to save the parts on the server (Figure 19).

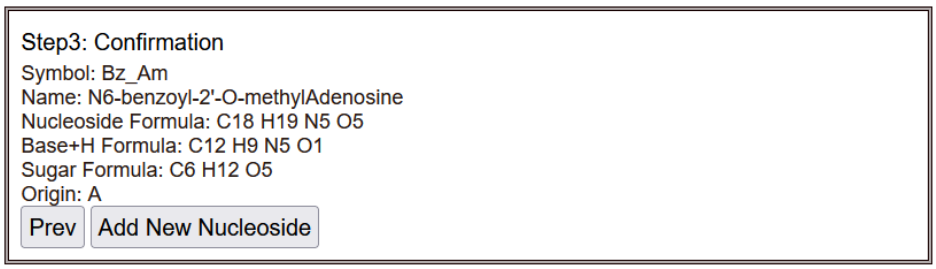


Figure 19. Confirming the input contents

You will see the dialog box that shows the save date and time and click OK (Figure 20).

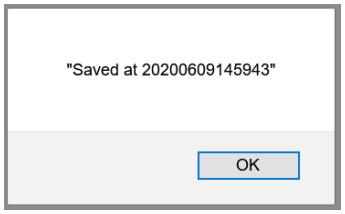


Figure 20. The saved data and time dialog box

Then you will see the parts appear in the User Nucleoside Table.

Clicking the Delete button at the rightmost column of the User Nucleoside Table will delete the corresponding user defined nucleoside. Default parts cannot be deleted (Figure 21).

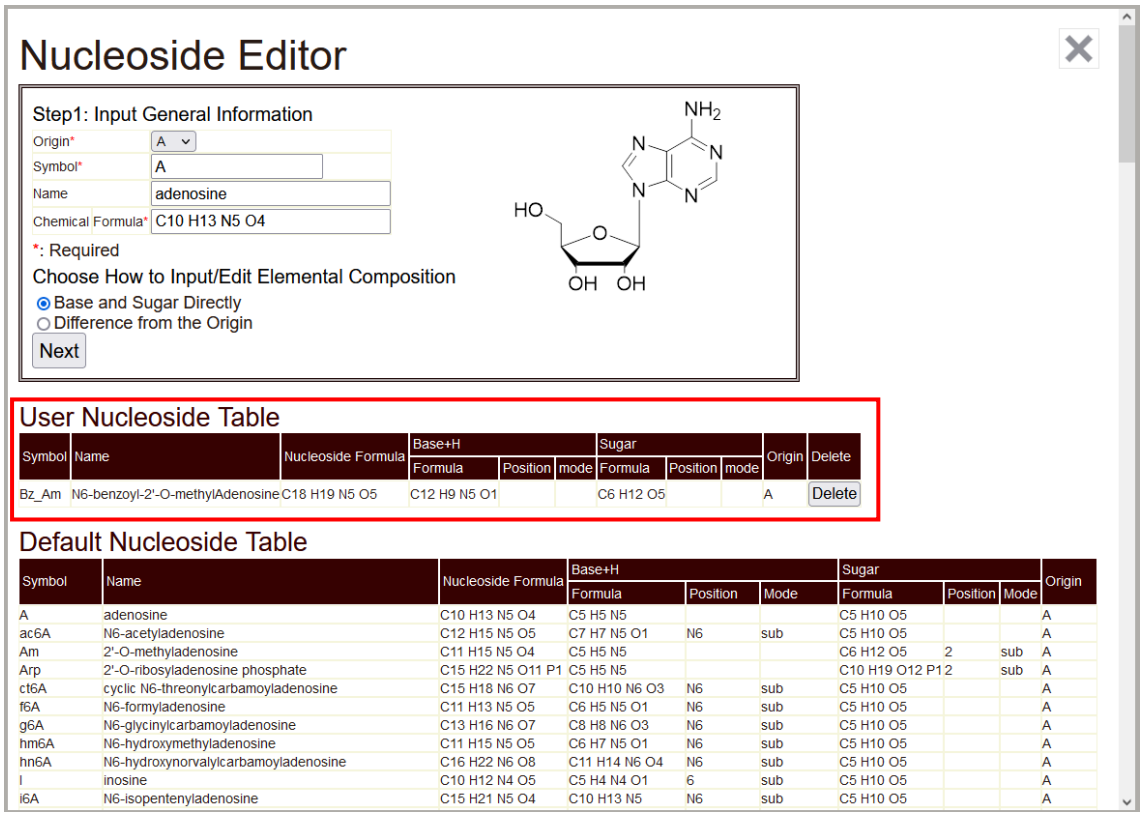


Figure 21. The User Nucleoside Table. The defined part (Bz_Am) shown in the table.

Input option 2: Difference from the Origin

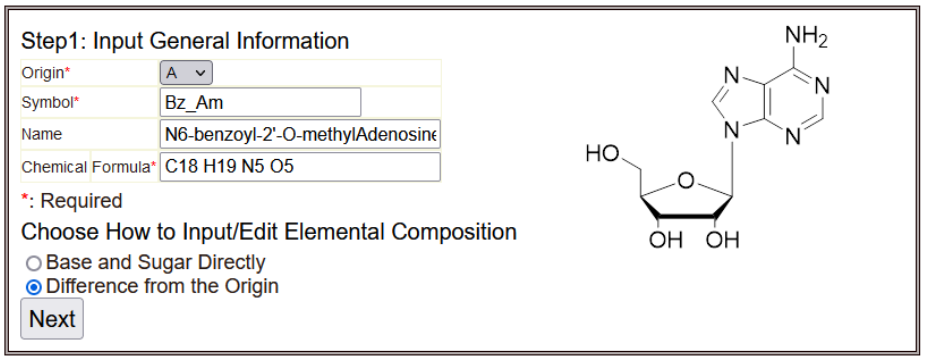


Figure 22. Step 1 of the form for inputting general information of the parts

Step 1':

Review the chemical formula differences between the parts to be defined and the origin. The difference is shown as Delta in the wizard.

Input the changes in the chemical formula for both the base and sugar subparts (Figure 23).

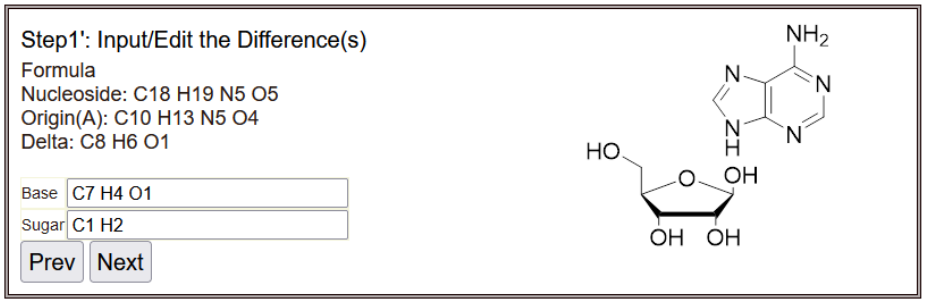


Figure 23. Step 1' of the form for inputting/editing the difference(s) in chemical formula(s)

Step2 and Step3 are same as the case of "Input option 1".

4.2.2. Linker

Click the Linker link in the Editor section, and you will see the pop-up window as shown in Figure 24. The page contains the web form of the Linker Editor and User Linker Table as well as Default Linker Table.

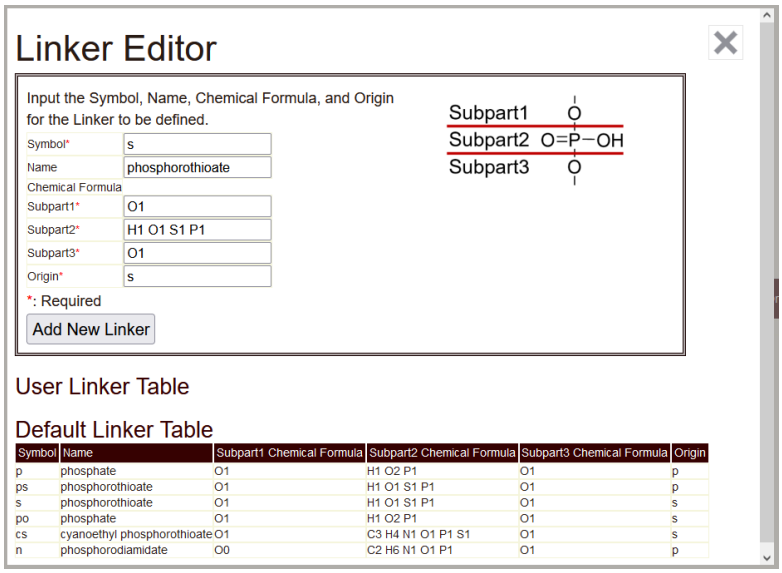


Figure 24. The Linker Editor pop-up window

Inputting the form of Linker Editor

A linker is defined as an assembly of three subparts as shown in Figure 24, which enables the software to calculate sequence-ladder product ions such as *a*, *b*, *c*, *d*, *w*, *x*, *y*, and *z* ions. Figure 25 shows the form of Linker Editor. The content of each field is explained using the definition of cyanoethyl phosphorothioate liker as an example.

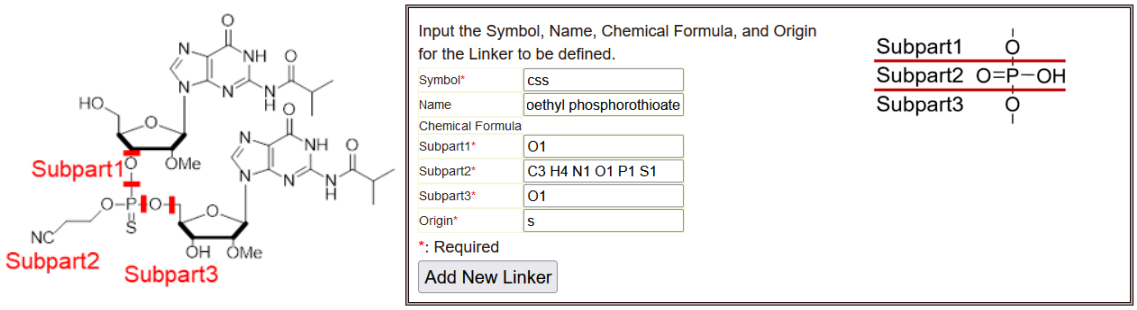


Figure 25. The definition of cyanoethyl phosphorothioate linker. The structure (left) and the form (right).

Symbol: This is used to represent a sequence in the calculation/search form and result report. All lowercase alphabetical characters can be used to define a linker symbol.

Name: Name and/or explanation of the linker.

Chemical Formulas of Subparts 1, 2, and 3: The elemental composition of the subparts of the linker to be defined.

Origin: Choose the originated linker from the select box. It is required for Modification function (See Section 1.5 in the Mass Calculator User's Manual or Section 1.6 in the MS/MS Search User's Manual) in the mass calculation.

Confirm all the input and click the Add New Linker button to save the part in the server (Figure 26).

Then you will see the part appeared in User Linker Table (Figure 26).

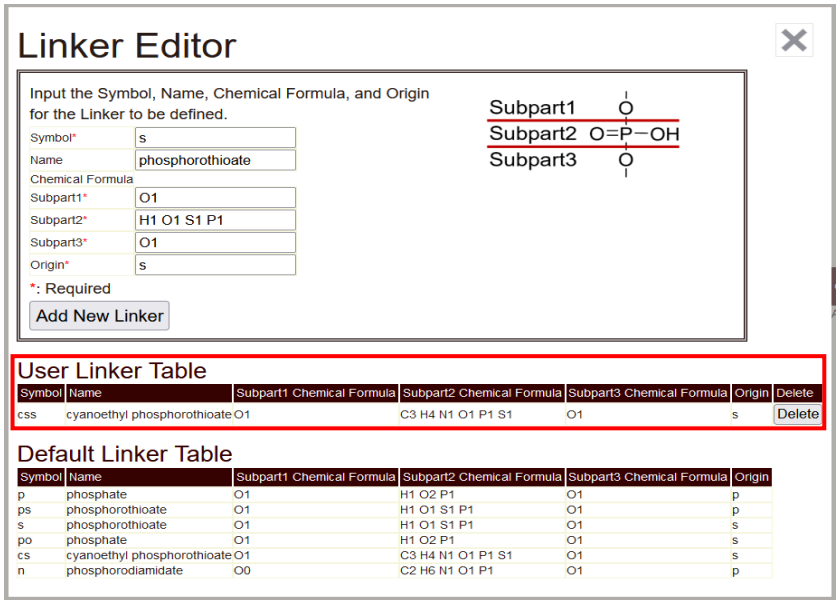


Figure 26. The user linker table. The defined part(s) appeared in the table.

Clicking the Delete button at the rightmost of the defined parts in User Linker Table will delete the corresponding linker. After definition, your own parts will appear in the corresponding subsection of User Parts section (Figure 27).

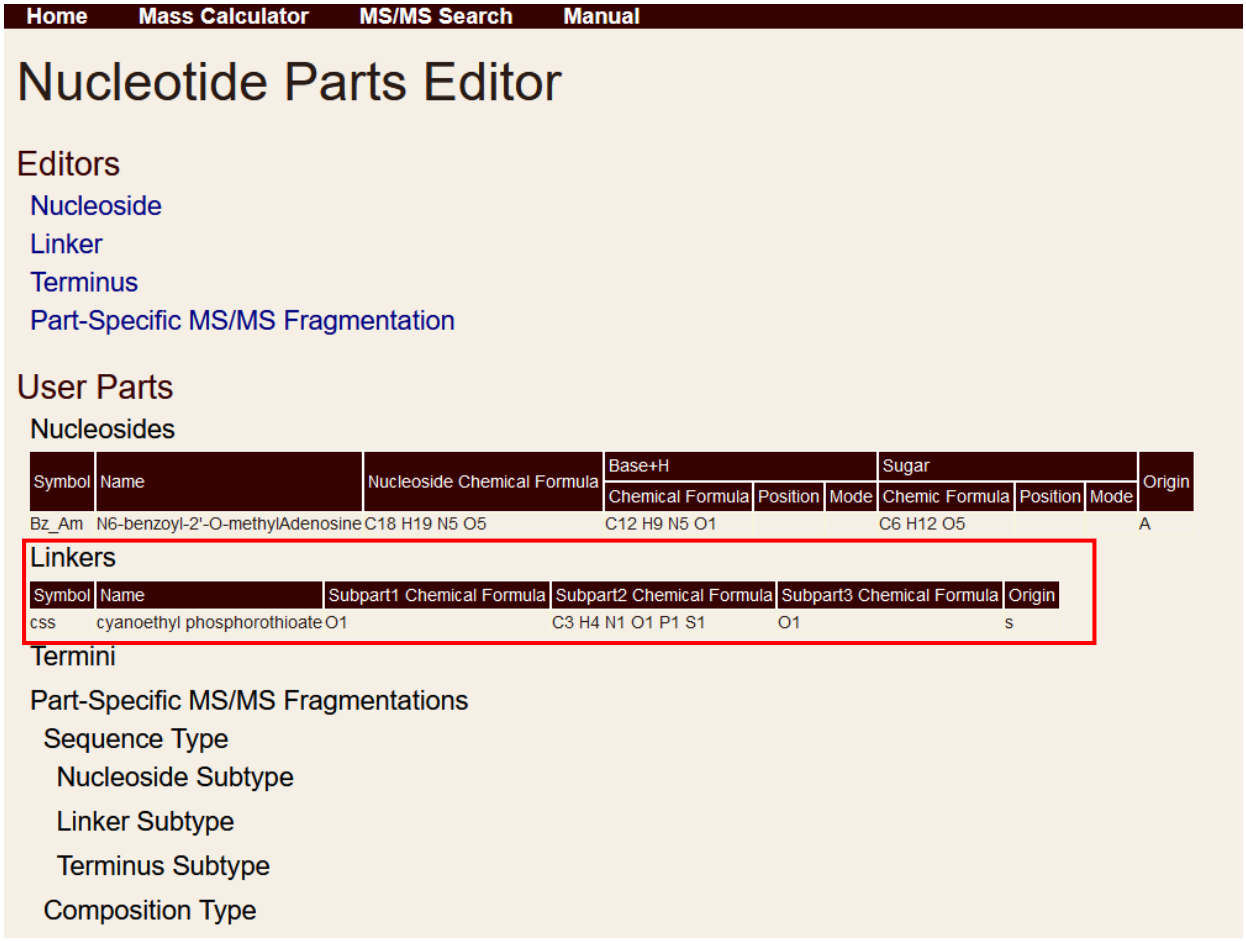


Figure 27. Nucleotide Parts Editor initial page after defining the linker. The defined “cs” linker part appeared in the Linkers table.

4.2.3. Terminus

Click the Terminus link in the Editors section, and you will see the pop-up window as shown in Figure 28. The page contains the web form of Terminus Editor and User Terminus Table as well as Default Terminus Table.

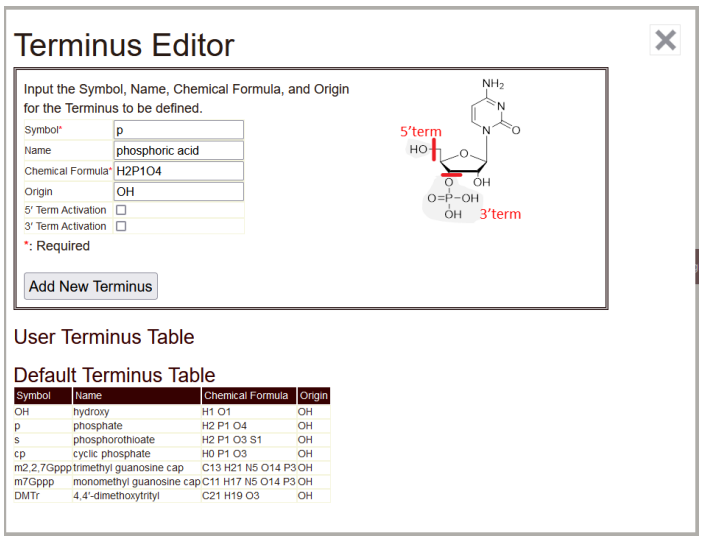


Figure 28. The Terminus Editor pop-up window

Inputting the form of Terminus Editor

Figure 29 shows the form of Terminus Editor. The content of each field is explained using the definition of terminus as an example. Whether the to-be-defined part is used as the 5′ -, 3′ -terminus or both termini is specified by using the activation checkbox.

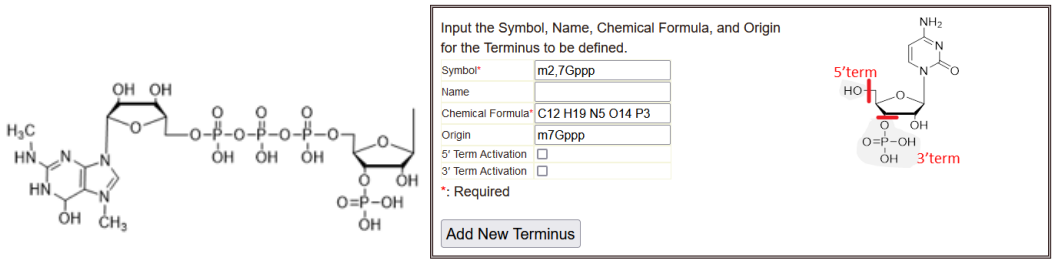


Figure 29. The definition of m2,7Gppp terminus. The structure (left) and the form (right).

Symbol: This is used to represent a sequence in the calculation/search form and result report. Unlike the nucleosides and linkers, termini are selected in the special fields in the web form called 5′ Term and 3′ Term. All alphanumeric characters, comma (‘,’) and parenthesis (‘(‘ and ‘)’)can be used to define a nucleoside symbol.

Name: Name and/or explanation of the terminus

Chemical Formula: The elemental composition of the terminus to be defined

Origin: Choose the originated terminus from the select box. It is required for calculating the partially modified terminus in the calculation/search (See Section 1.5 in the Mass Calculator User’s Manual or Seaction 1.6 in the MS/MS Search User’s Manual).

Activation: Choose which terminus the parts is for. You can choose 5′ , 3′ , or both using checkboxes.

Confirm all the input and click Add New Terminus button to save the parts in the server (Figure 29).

Then you will see the parts appeared in User Terminus Table (Figure 30).

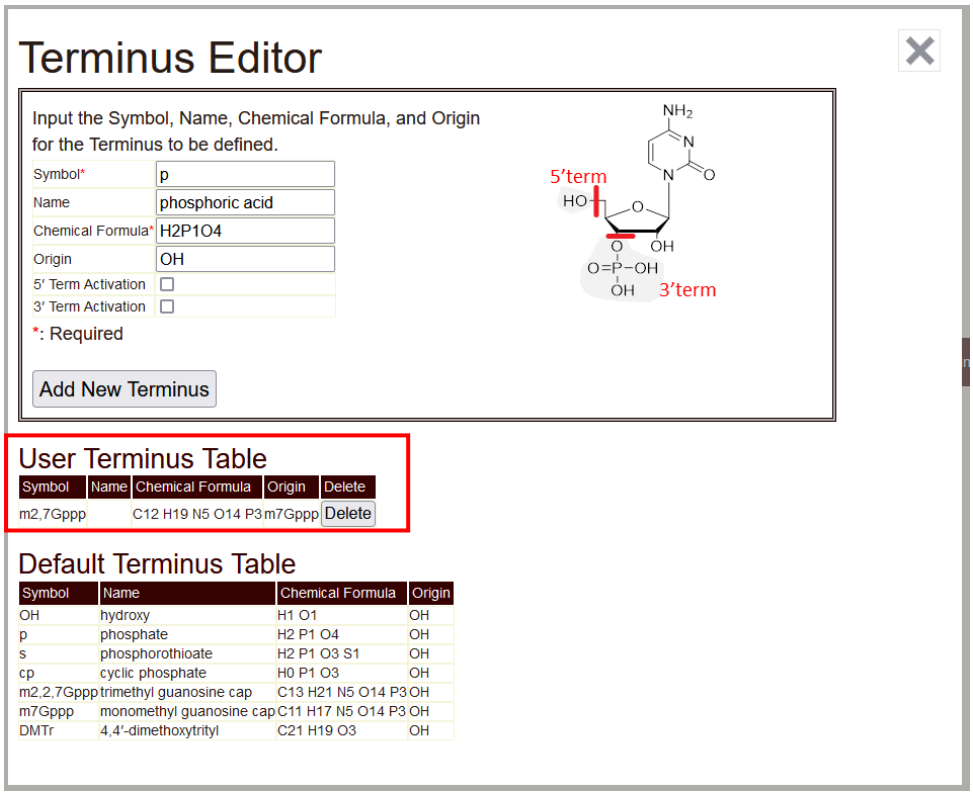


Figure 30. The user terminus table. The parts appeared in User Terminus Table.

Clicking the Delete button at the rightmost of the defined parts in User Terminus Table will delete the corresponding terminus.

4.3. Definition of Part-specific MS/MS Fragmentation

Click the Part-Specific MS/MS Fragmentation link in the Editors section (Figure 14), and you will see the pop-up window as shown in Figure 31. This page contains the Part-Specific MS/MS Fragmentation Editor, the User Part-Specific MS/MS Fragmentation table, and the Default Part-Specific MS/MS Fragmentation table.

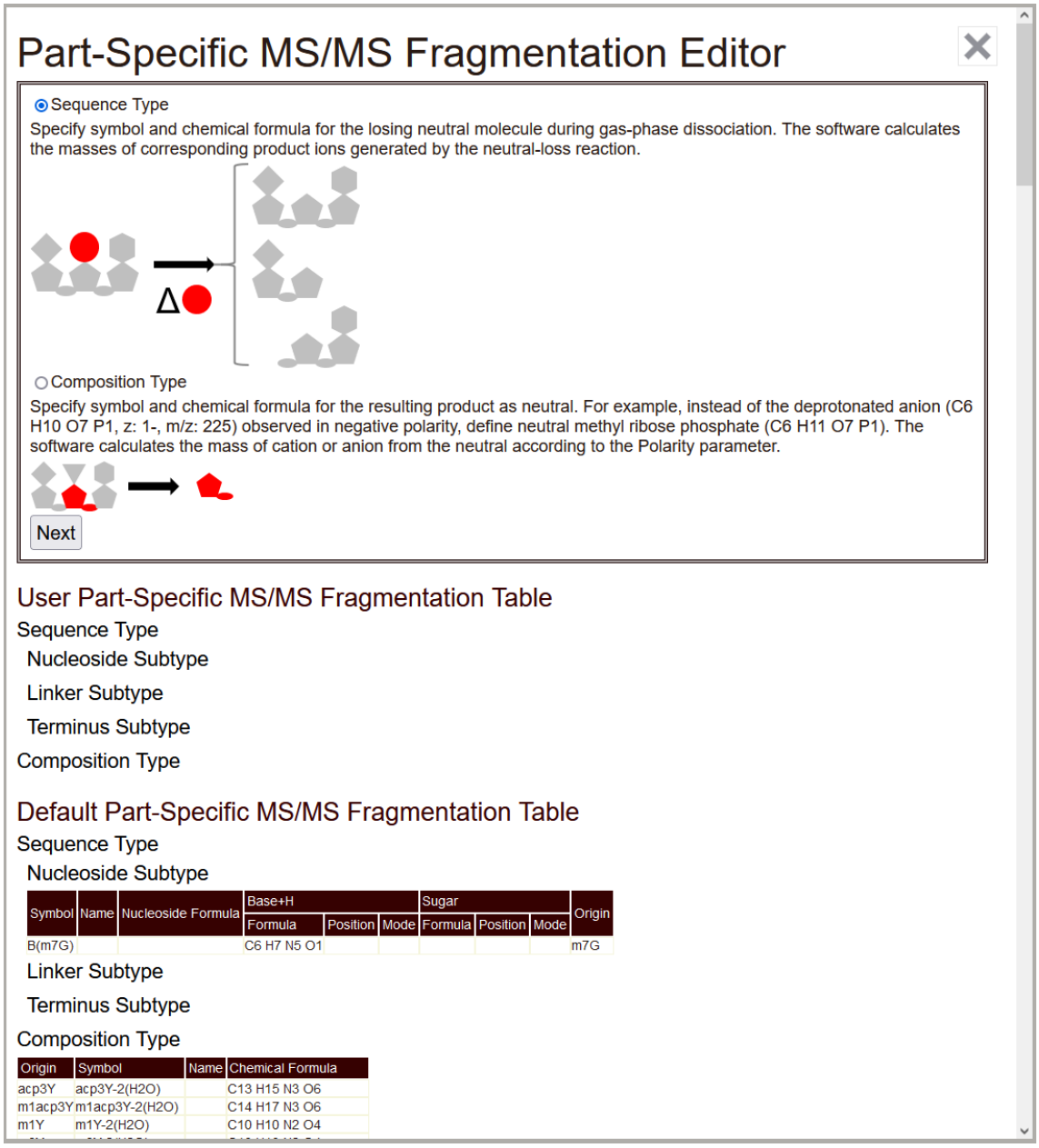


Figure 31. The Part-Specific MS/MS Fragmentation pop-up window

Inputting data into the Part-Specific MS/MS Fragmentation

In addition to typical gas-phase dissociations, some part-specific fragmentations are reported to pinpoint the modifications (Qiu F et al. 1999 Nucleic Acids Res; Pomerantz SC et al. 2005 Anal Chem; Yamanuchi Y et al. 2006 Nucleic Acids Res; Nakayama H et al. 2019 Anal Chem.). They are important for identification of nucleotides containing those parts unambiguously. There are two types of such fragmentations. One type allows the identification of the position of the parts of a given sequence and is thus called Sequence-

type Part-Specific MS/MS Fragmentation. The other allows identification of the parts existing in the nucleotide molecule and is therefore called Composition-type fragmentations. The Sequence-type is further divided into three (Nucleoside, Linker, and Terminus) subtypes. Each subtype is generated from the loss of a neutral molecule from the molecular ion, sequence-ladder product ions, and/or internal product ions.

4.3.1. Defining Sequence Type fragmentations

4.3.1.1. Nucleoside Subtype

Step 1:

Choose the type of fragmentations with the radio button as shown in Figure 32. If you choose Sequence-type, you will then select Nucleoside in the subtype select box, as shown in Figure 34.

Choose the origin in the select box.

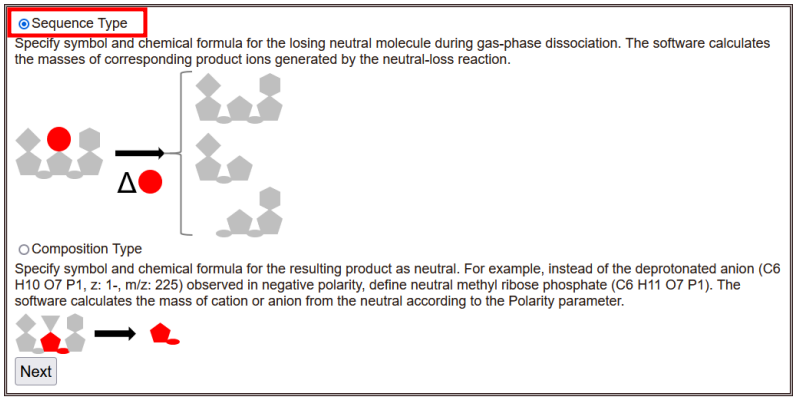


Figure 32. Step 0: choosing Sequence-type of the fragmentations with the radio button

For example, if you would like to define the neutral loss of a 7-methylguanine base from N7-methylguanosine (m7G), you choose the radio button: Sequence-type and then click the Next button. As shown in Figure 33, the Step 1 form will appear. Select Nucleoside for the Subtype field and “m7G” for the Origin field in the select boxes. Note that the loss of nucleobases from the precursor ion(s) and that of the 3′ -end nucleobase from *a* ion series (termed *a*-B ion series) are considered in the software as default. Non-typical base loss from product ions other than *a* type should be defined if you would like the software to consider them.

Click the Next button to proceed (Figure 33).

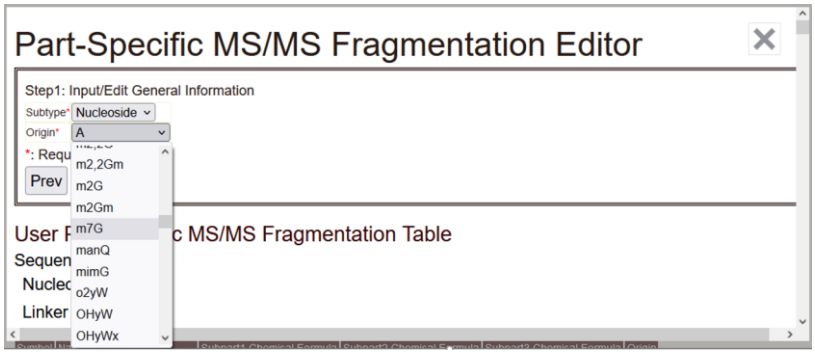


Figure 33. Step 1 of the form for inputting/editing general information

Step 2:

Define symbol for the neutral moiety that is lost from the molecular ions, sequence-ladder product ions, and internal ions. The available characters are the same as those in the Origin. In the case of m7G, define the N7-methylguanine base. Input the chemical formula of the base (C6H7N5O1) in the Base field on the form. Leave Sugar blank because it has no neutral loss (Figure 34).

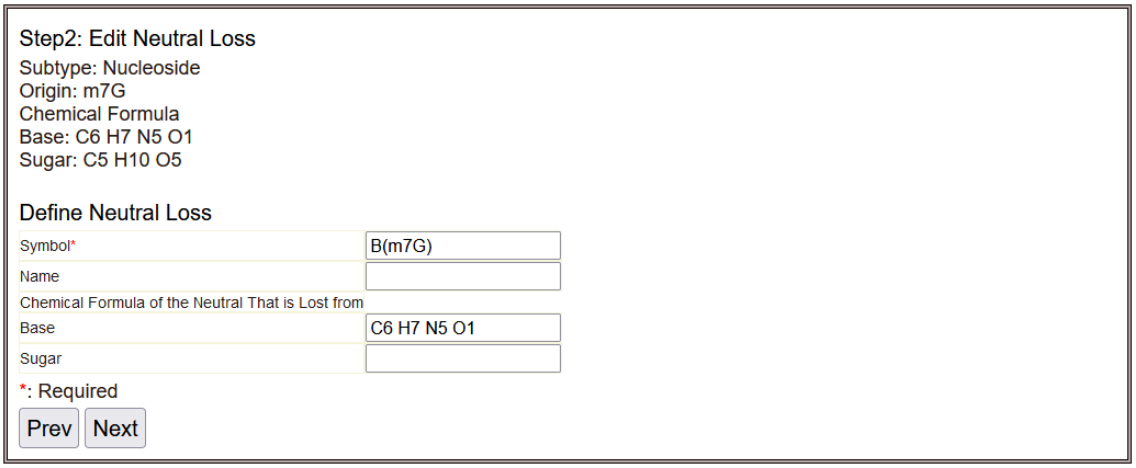


Figure 34. Step 2 of the form for editing neutral loss

Step 3:

Confirm the data and click Add New Part-Specific MS/MS Fragmentation button to save the fragmentation on the server (Figure 35).

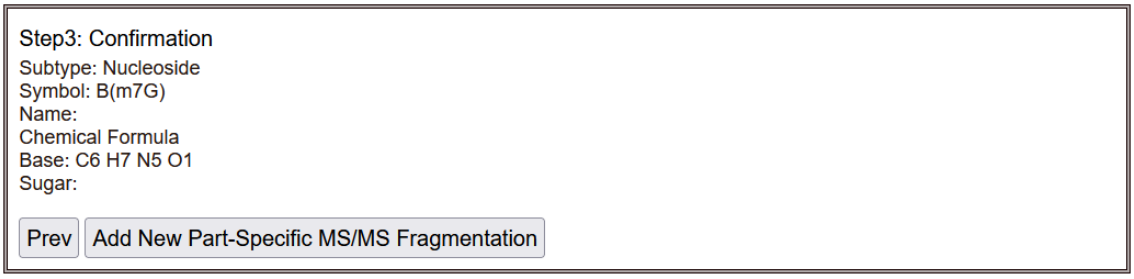


Figure 35. Step 3 Confirmation form

Then you will see the parts appear in the Sequence-type –Nucleoside-subtype of the User Part-Specific MS/MS Fragmentation Table (Figure 36).

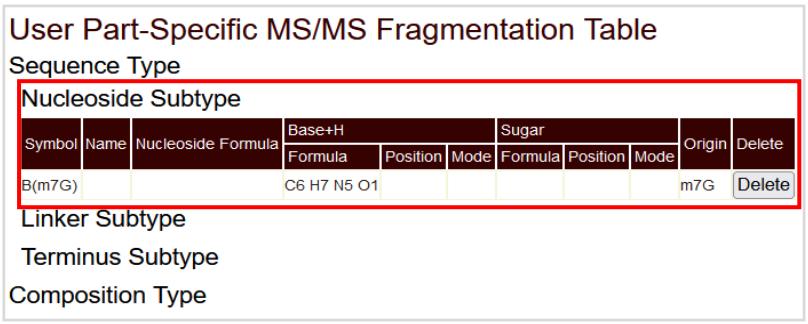


Figure 36. The user Part-Specific MS/MS Fragmentation Table. The defined fragmentation appeared in the table.

4.3.1.2. the Linker subtype

Step 1:

Choose type of the fragmentation with the radio button as shown in Figure 32. If you choose the Sequence type, you will then select Linker in the subtype select box as shown in Figure 37.

Choose the origin in the select box.

For example, if you define the neutral loss of cyanoethyl group from cyanoethyl phosphorothioate liker, you choose the radio button: Sequence-type, Subtype: Linker, and Origin: “cs”.

Click Next button to proceed (Figure 37).

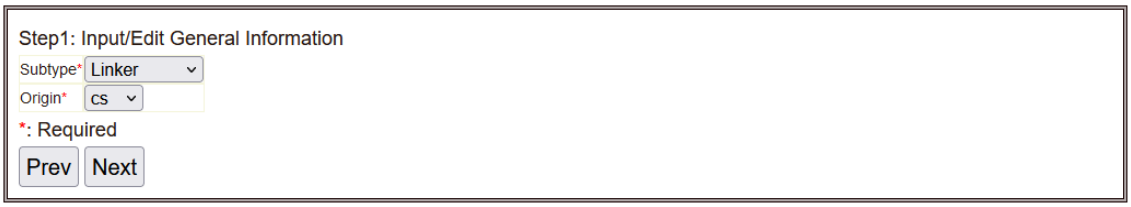


Figure 37. Step1 of the form for inputting/editing general information

Step2:

In the case of “cs”, the cyanoethyl group is lost from the subpart 2 of the typical ions. Input the chemical formula of the group (C3H4N1) at the Subpart 2 (See Figure 38). Leave Subparts 1 and 3 blanks because they no neutral losses (Figure 38).

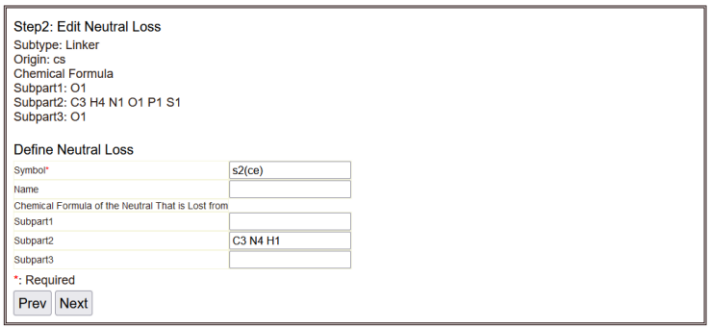


Figure 38. Step2 of the form for editing the neutral loss

Step3:

Confirm all the input and click the Add New Part-Specific MS/MS Fragmentation button to save the parts in the server (Figure 39).

Figure 39. Step3 Confirmation form

Click the OK button, and you will see the parts appeared in Sequence-type –Linker-subtype of User Part-Specific MS/MS Fragmentation Table (Figure 40).

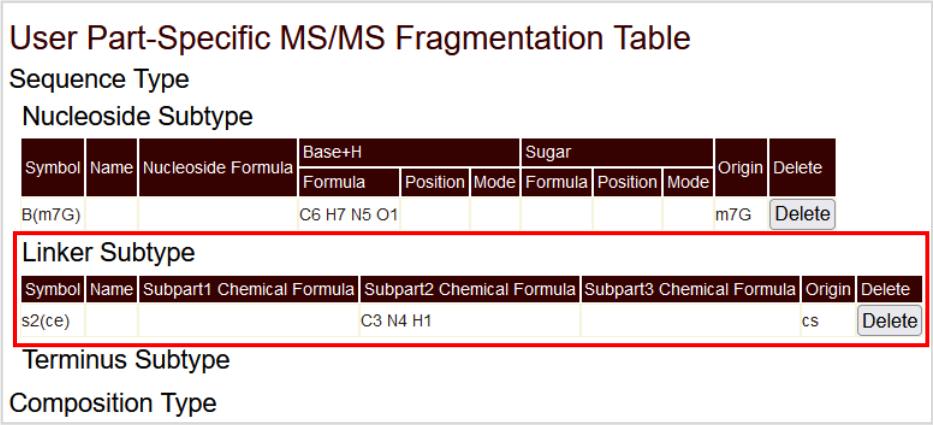


Figure 40. The user Part-Specific MS/MS Fragmentation Table. The defined fragmentation appeared in the table.

4.3.1.3. the Terminus subtype

Step 1:

Choose type of the fragmentations with the radio button as shown in Figure 32. If you choose the Sequence type, you will then select Terminus in the Subtype select box as shown in Figure 41.

Choose the origin in the select box.

For example, if you define the neutral loss of 2,2,7-trimethylguanosine diphosphate molecule from 2,2,7-trimethylguanosine triphosphate terminus, you choose the radio button: Sequence-type, Subtype: Terminus, and Origin: “m2,2,7Gppp” (trimethyl G cap).

Click the Next button to proceed (Figure 41).

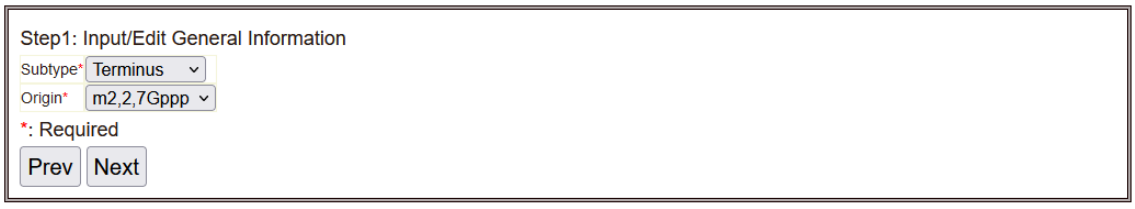


Figure 41. Step1 of the form for inputting/editing general information

Step2:

In the case of m2,2,7Gppp, define symbol for the 2,2,7-trimethylguanosine diphosphate molecule as m2,2,7Gpp. Input the chemical formula of the molecule (C13H21N5O12P2) as shown in Figure 42.

Figure 42. Step2 of the form for editing the neutral loss from the m2,2,7Gppp terminus

Step3:

Confirm all the input and click the Add New Part-Specific MS/MS Fragmentation button to save the parts in the server (Figure 43).

Figure 43. Step3 Confirming the input contents

Then you will see the parts appeared in Sequence-type –Terminus-subtype of User Known MS2 Fragmentation Table (Figure 44).

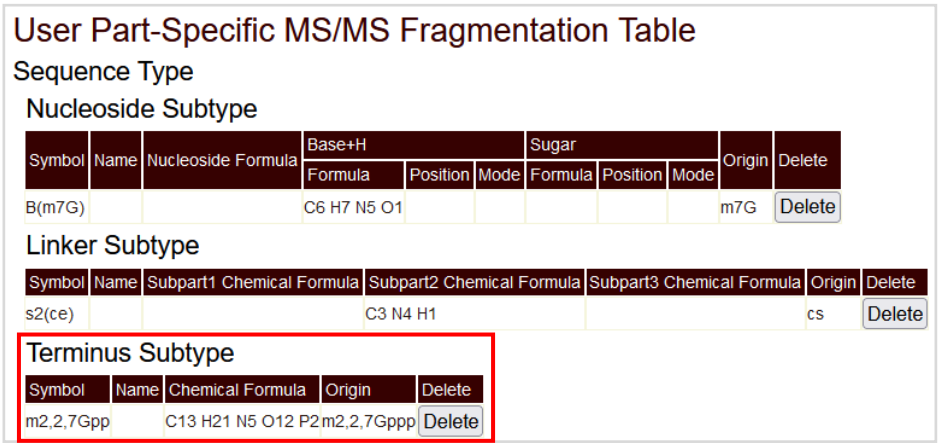


Figure 44. The user Part-Specific MS/MS Fragmentation Table. The defined fragmentation appeared in the table.

4.3.2. Defining Composition-type fragmentations

When a Composition type fragmentation is defined for a part or parts, the software will try to find the origin of the part in a sequence. If it is found, the mass of the part will be calculated and, in MS/MS Search, the calculated mass will be searched.

Choose Composition-type of the fragmentations with the radio button as shown in Figure 45. Click Next button to proceed.

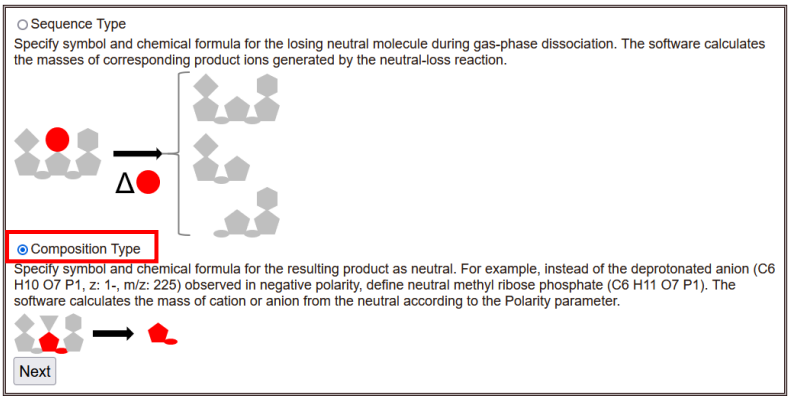


Figure 45. Composition-type of the fragmentations with the radio button

To define a Composition-type fragmentation, input the form, as shown in Figure 45,
Origin: Choose one of the Nucleoside, Linker, or Terminus part in the select box. If the fragmentation you would like to define is composed of more than one type of parts, *e. g.* ribose phosphate (sugar: a subpart of Nucleoside + Linker), select the major one. You can use “X” as a wildcard which indicates all of parts (All RNA, all DNA, etc.).
Symbol: It represents the resulting product ion in result reports. All alphanumeric characters and commas (‘,’) can be used to define the symbols.
Name: Name or explanation of the nucleoside
Chemical Formula: The elemental composition of the parts to be defined. To calculate the masses of both positive and negative ions, the composition should be a neutral molecule or fragment.

For example, defining a pseudouridine (Y)-specific fragmentation is illustrated as follows.
Choose “Y” in the Origin select box.
Input “Y-2H2O” in the Symbol text box; in the case of “Y”, the uracil ion should not be detected due to the stable C-C bond that binds the uracil base and ribose. Instead of the uracil nucleobase ion, the neutral loss of 2 water molecules from the nucleoside ion is frequently detected as signature ion.

Input “C9H8N2O4” in the Chemical Formula text box.

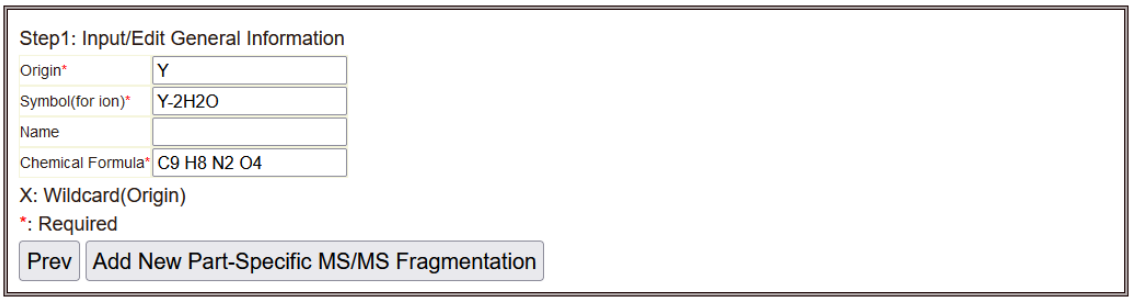


Figure 46. Step 1 of the form for inputting/editing general information

Confirm all of the input and click Add New Part-Specific MS/MS Fragmentation button to save the parts on the server (Figure 46).

Click the OK button, and you will see the parts appear in the Composition of User Part-Specific MS/MS Fragmentation Table.

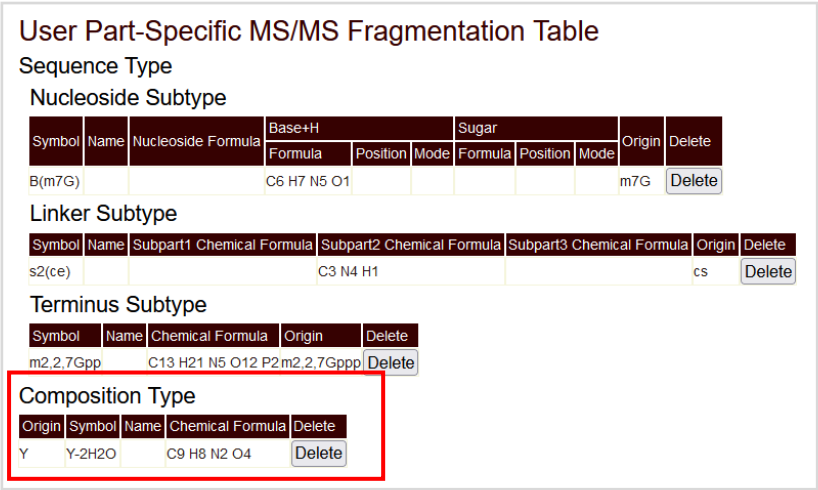


Figure 47. The user Part-Specific MS/MS Fragmentation Table. The defined fragmentation appeared in the table.