# Ariadne Nucleotide Parts Editor User's Manual

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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 gasphase 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.





## 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). 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).

Subpart1 Ó Subpart2 HO-P=S Subpart3 Ó

Figure 7. The structure of linker parts and its subparts

#### 2.2.3. Terminus

Many RNAs are phosphorylated or capped at the 5-terminus during maturation. The 3-terminus hydroxyl group of most 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 🗹	с 🗹	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	С
5	1493.2459	1605.2954	1623.3060	1685.2618	1703.2723	С
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	С
5	1328.1808	1440.2304	1458.2409	1520.1967	1538.2073	С
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'						-

**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.

## Ariadne: Database Search for RNA Identification Using Tandem Mass Spectrometry Data

#### Overview

Home

Arladne is a web-based database search service for the identification of RNAs and their post-transcriptional modifications using tandem mass spectrometry data. If you include results from Arladne in a publication, please cite the Arladne paper.

#### Manuals

Parts Editor Mass Calculator MS/MS Search

#### mormo ocaron

Demo and Example Data Browse demo search results of some of our published data including ribosomal RNA, transfer RNA, microRNA and other non-coding RNAs and more. Download and try example MS/MS data to search RNA sequence databases by yourself.

#### MS/MS Search

Searching an RNA sequence database (up to 50MB) with post-transcriptional modifications using up to 50MB of MS/MS data in Mascot generic format (MGF).

#### Browse Search Result

A result report can be browsed with 'search ID' issued on the time of search.
Browse

#### Mass Calculator

Calculating mass values of a nucleic acid, its RNase digests and their CID fragments from given sequence(s).

#### Nucleotide Parts Editor

Defining/editing user nucleotide parts such as Nucleosides, Linkers, and Termini as well as unusual part-specific MS/MS dissociations.

#### Nucleotide Parts Table

Table of the available parts that can be used in a calculation/search.

#### 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.

	lestide Deute Tek						Save A	ctivatio	n 🖌
NUC	leolide Paris Tac	Jie					eare /	ouruuu	
lucleosi	des / Linkers / Termini / Part-Specific MS	S/MS Fragments / Ato	mic Weights						
			inio moigino						
vucie	osides								
Symbol	Name	Nucleoside		Base		Sugar		Origin	Activatio
-,		Chemical Formula	Mass	Chemical Formula	Mass	Chemical Formula	Mass		
λ	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	<b>~</b>
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	<b>~</b>
A	depurination(1'H) of A	C5 H10 O4	134.05790878	H2	2.01565006	C5 H10 O5	150.05282340	A	<b>~</b>
	inosine	C10 H12 N4 O5	268.08076946	C5 H4 N4 O1	136.03851074	C5 H10 O5	150.05282340	A	<b>~</b>
m	2'-O-methylinosine	C11 H14 N4 O5	282.09641952	C5 H4 N4 O1	136.03851074	C6 H12 O5	164.06847346	A	<b>~</b>
RA	depurination(1'OH) of A	C5 H10 O5	150.05282340	H2 O1	18.01056468	C5 H10 O5	150.05282340	A	<b>~</b>
ac6A	N6-acetyladenosine	C12 H15 N5 O5	309.10731855	C7 H7 N5 O1	177.06505983	C5 H10 O5	150.05282340	A	<b>~</b>
t6A	cyclic N6-threonylcarbamoyladenosine	C15 H18 N6 O7	394.12369688	C10 H10 N6 O3	262.08143816	C5 H10 O5	150.05282340	A	<b>~</b>
6A	N6-formyladenosine	C11 H13 N5 O5	295.09166849	C6 H5 N5 O1	163.04940977	C5 H10 O5	150.05282340	A	<b>~</b>
16A	N6-glycinylcarbamoyladenosine	C13 H16 N6 O7	368.10804682	C8 H8 N6 O3	236.06578810	C5 H10 O5	150.05282340	A	<b>~</b>
m6A	N6-hydroxymethyladenosine	C11 H15 N5 O5	297.10731855	C6 H7 N5 O1	165.06505983	C5 H10 O5	150.05282340	A	<b>~</b>
nn6A	N6-hydroxynorvalylcarbamoyladenosine	C16 H22 N6 O8	426.14991162	C11 H14 N6 O4	294.10765290	C5 H10 O5	150.05282340	A	<b>~</b>
6A	N6-isopentenyladenosine	C15 H21 N5 O4	335.15935411	C10 H13 N5	203.11709539	C5 H10 O5	150.05282340	A	<b>V</b>
D6A	N6-(cis-hydroxyisopentenyl)adenosine	C15 H21 N5 O5	351.15426873	C10 H13 N5 O1	219.11201001	C5 H10 O5	150.05282340	A	<b>~</b>
n1A	1-methyladenosine	C11 H15 N5 O4	281.11240393	C6 H7 N5	149.07014521	C5 H10 O5	150.05282340	A	<b>~</b>
n1Am	1,2'-O-dimethyladenosine	C12 H17 N5 O4	295.12805399	C6 H7 N5	149.07014521	C6 H12 O5	164.06847346	A	<b>~</b>
ntl	1-methylinosine	C11 H14 N4 O5	282.09641952	C6 H6 N4 O1	150.05416080	C5 H10 O5	150.05282340	A	<b>~</b>
a1im	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.

lenni	ni					
Symbol	Name	Chemical Formula	Mass	Origin	Activati 5' 3'	on
p	phosphate	H2 P1 O4	96.96907017	OH		2
s	phosphorothioate	H2 P1 O3 S1	112.94622655	OH		2
OH	hydroxy	H1 O1	17.00273965	OH	•	2
ср	cyclic phosphate	H0 P1 O3	78.95850549	ОН		2
DMTr	4,4'-dimethoxytrityl	C21 H19 O3	319.13341943	OH		1
m2,2,7Gpp	optrimethyl guanosine cap	C13 H21 N5 O14 P	3 564.0297852	OH		1
n2,7Gppp		C12 H19 N5 O14 P	3 550.01413514	m7Gppp		1
n7Gppp	monomethyl guanosine ca	pC11 H17 N5 O14 P3	3 535.99848508	OH		1
		_				
Tormi		-				
rermi						_
					A	0.0
Sumbol	Namo	Chemical Formula	Mace	Origin	Activati	1011
Symbol	Name	Chemical Formula	Mass	Origin	5' 3'	
Symbol p	Name phosphate	Chemical Formula	Mass 96.96907017	Origin OH	Activati	2
Symbol p s	Name phosphate phosphorothioate	Chemical Formula H2 P1 O4 H2 P1 O3 S1	Mass 96.96907017 112.94622655	Origin OH OH	S' 3'	2
Symbol p s OH	Name phosphate phosphorothioate hydroxy	Chemical Formula H2 P1 O4 H2 P1 O3 S1 H1 O1	Mass 96.96907017 112.94622655 17.00273965	Origin OH OH OH	Activati 5' 3' 0 0	2
Symbol p s OH cp	Name phosphate phosphorothioate hydroxy cyclic phosphate	Chemical Formula H2 P1 04 H2 P1 03 S1 H1 01 H0 P1 03	Mass 96.96907017 112.94622655 17.00273965 78.95850549	Origin OH OH OH OH	Activati 5' 3' 	2
Symbol p s OH cp DMTr	Name phosphate phosphorothioate hydroxy cyclic phosphate 4,4*-dimethoxytrityl	Chemical Formula H2 P1 O4 H2 P1 O3 S1 H1 O1 H0 P1 O3 C21 H19 O3	Mass 96.96907017 112.94622655 17.00273965 78.95850549 319.13341943	Origin OH OH OH OH OH	Activati 5' 3' 	2
Symbol p s OH cp DMTr m2,2,7Gpp	Name phosphate phosphorothioate hydroxy cyclic phosphate 4,4*-dimethoxytrityl primethyl guanosine cap	Chemical Formula H2 P1 O4 H2 P1 O3 S1 H1 O1 H0 P1 O3 C21 H19 O3 C13 H21 N5 O14 P3	Mass 96.96907017 112.94622655 17.00273965 78.95850549 319.13341943 3564.0297852	Origin OH OH OH OH OH OH	Activati 5' 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 3 3 3
s s OH cp DMTr m2,2,7Gpp m2,7Gppp	Name phosphate phosphorothioate hydroxy cyclic phosphate 4,4'-dimethoxytrityl sptrimethyl guanosine cap	Chemical Formula H2 P1 04 H2 P1 03 S1 H1 01 H0 P1 03 C21 H19 03 C13 H21 N5 014 P: C12 H19 N5 014 P:	Mass 96.96907017 112.94622655 17.00273965 78.95850549 319.13341943 3564.0297852 3550.01413514	Origin OH OH OH OH OH OH OH M7Gppp	Activati 5' 3' 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	

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).

Home Mass Calculator MS/MS Search Manual
Nucleotide Parts Editor
Editors Nucleoside Linker Terminus Part-Specific MS/MS Fragmentation
User Parts
Linkers
Termini
Part-Specific MS/MS Fragmentations
Sequence Type
Nucleoside Subtype
Linker Subtype
Terminus Subtype
Composition Type

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

Home MS/MS Search Mass	Calculator Nucleoti	de Parts Table		
Nucleotide Parts	Editor			
Editors Nucleoside Linker Terminus Known MS2 Fragment				
User Parts				
Nucleosides				
Symbol Name	Nucleoside Chemical Formula	Base+H Chemical Formula Posi	ition Mode Chemic Formula	Position Mode Origin
Bz_Am N6-benzoyl-2'-O-methylAdenosine	C18H19N5O5	C12H9N5O1	C6H12O5	A
Linkers				
Termini				
Known MS2 Fragments -Sequence-type Nucleoside-subtype				
Linker-subtype				
Terminus-subtype				
-Composition-type				

**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.

Nuc	cleoside Editor								×
Step1:	Input General Information			NH <sub>2</sub>					
Origin*	Av		N -						
Symbol*	A		li Ti	Ň					
	- description	1	N-	\.//					
Name	adenosine	но		N					
Chemica	Formula* C10 H13 N5 O4								
*: Reau	lired	l	$\langle \cdot \rangle$						
Choos	e How to Input/Edit Elemental Com	nosition							
01008		position	ОН ОН						
• Bas	e and Sugar Directly								
ODITE	erence from the Origin								
Next									
lser l	Nucleoside Table								
) of our	It Nucleosido Tablo								
Jelau									
symbol	Name	Nucleoside Formula	Base+H			Sugar		Origin	
		Hucieoside Formule	Formula	Position	Mode	Formula	Position M	ode	
	adenosine	C10 H13 N5 O4	C5 H5 N5			C5 H10 O5		A	
:6A	N6-acetyladenosine	C12 H15 N5 O5	C7 H7 N5 O1	N6	sub	C5 H10 O5		A	
m	2'-O-methyladenosine	C11 H15 N5 O4	C5 H5 N5			C6 H12 O5	2 su	ib A	
rp	2'-O-ribosyladenosine phosphate	C15 H22 N5 O11 P1	C5 H5 N5			C10 H19 O12 P1	12 su	ib A	

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: Difference from the Origin, or Base and Sugar Directly 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

Step1: Input	General Information	NH <sub>2</sub>
Origin*	A •	N N
Symbol*	Bz_Am	
Name	N6-benzoyl-2'-O-methylAdenosine	N N
Chemical Formula	* C18 H19 N5 O5	HO
*: Required		
Choose How	to Input/Edit Elemental Composi	tion OH OH
● Base and S ○ Difference fr Next	ugar Directly rom the Origin	

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).

Step2: Input/Edit Composition(s) Formula Nucleoside: C18 H19 N5 O5 Origin(A): C10 H13 N5 O4	
Base+H C12 H9 N5 O1	O OH
Sugar C6 H12 O5	OH OH
Prev Next	

**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).

Step3: Confirmation
Symbol: Bz_Am
Name: N6-benzoyl-2'-O-methylAdenosine
Nucleoside Formula: C18 H19 N5 O5
Base+H Formula: C12 H9 N5 O1
Sugar Formula: C6 H12 O5
Origin: A
Prev Add New Nucleoside

Figure 19. Confirming the input contents

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

"Saved at 20200609145943"	
ОК	

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).

Step1	: Input General Information			NH <sub>2</sub>					
Origin*			N	$\downarrow$					
Origin				Ň					
Symbol	A	_							
Name	adenosine	ЦО	N	Ň΄					
Chemica	al Formula* C10 H13 N5 O4	HO							
	uired	1							
			$\searrow$						
choos	se How to Input/Edit Elemental Con	position	ÓH ÓH						
Bas	e and Sugar Directly								
○ Diffe	erence from the Origin								
Next									
	J								
lser	Nucleoside Table								
		BasetH	Sugar						
ymdol N	ame Nucleoside Form	Base+H	Sugar	Desition mod	Origin	Delete			
ymbol N	ame Nucleoside Form	Base+H Formula Position	Sugar mode Formula	Position mod	e Origin	Delete			
<u>. Am</u> Ne	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5	Base+H Formula Position	Sugar mode Formula C6 H12 O5	Position mod	e Origin	Delete Delete			
Am Ne	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine,C18 H19 N5 O5	Base+H Formula Position C12 H9 N5 O1	Sugar mode Formula C6 H12 O5	Position mod	e Origin A	Delete			
z_Am Ne	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine,C18 H19 N5 O5 Ilt Nucleoside Table	Base+H Formula Position C12 H9 N5 O1	Sugar mode Formula C6 H12 O5	Position mod	e Origin A	Delete Delete			
Am Ne	ame Nucleoside Form 6-benzoyI-2'-O-methylAdenosine C18 H19 N5 O5 IIt Nucleoside Table	Base+H Formula Position C12 H9 N5 O1	Sugar Mode Formula I C6 H12 O5 Base+H	Position mod	e Origin A	Delete Delete Sugar			
Am Ne efau	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 III Nucleoside Table Name	Base+H Formula Position C12 H9 N5 O1 Nucleoside Formi	Sugar Mode Formula C6 H12 O5	Position mod	e Origin A Mode	Delete Delete Sugar	Position	Mode	Drigin
Am Ne efau mbol	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 Ilt Nucleoside Table Name adenosine	Ula Base+H Formula Position C12 H9 N5 O1 Nucleoside Formu C10 H13 N5 O4	Sugar mode Formula I C6 H12 O5	Position mod	e Origin A Mode	Delete Delete Sugar Formula C5 H10 Q5	Position	Mode A	Drigin
Am Ne	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 Ilt Nucleoside Table Name adenosine N6-acetyladenosine	Ula Base+H Formula Position C12 H9 N5 O1 Nucleoside Formu C10 H13 N5 O4 C12 H15 N5 O5	Sugar mode Formula 1 C6 H12 O5 Base+H Formula C5 H5 N5 C7 H7 N5 O1	Position mod	e Origin A Mode	Delete Delete Sugar Formula C5 H10 05 C5 H10 05	Position	Mode A	Drigin
(MDOI N (Am Ne (Am Ne (Am Ne (Am Ne) (Am Ne) (	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 IIT Nucleoside Table Name adenosine N6-acetyladenosine 2'-O-methyladenosine	Ula Base+H Formula Position C12 H9 N5 O1 Nucleoside Formi C10 H13 N5 O4 C12 H15 N5 O5 C11 H15 N5 O4	Sugar           mode         Formula         I           C6 H12 O5         G           JIa         Base+H           Formula         C           C5 H5 N5         C7 H7 N5 O1           C5 H5 N5         C7 H7 N5 O1	Position mod	e Origin A Mode sub	Delete Delete Sugar Formula C5 H10 O5 C5 H10 O5 C6 H12 O5	Position 2	Mode A sub A	Drigin
(mbol N     (mbol     (mbol     (mbol     (6A     (n     )     )	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 III Nucleoside Table Name adenosine N6-acetyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine	Uta Base+H Formula Position C12 H9 N5 O1 Nucleoside Formula C10 H13 N5 O4 C12 H15 N5 O4 C11 H15 N5 O4 C15 H22 N5 O11	Sugar           mode         Formula         1           C6 H12 O5         6           Ja         Base+H           Formula         C5 H5 N5           C7 H7 N5 O1         C5 H5 N5           C5 H5 N5         C7 H5 N5           C5 H5 N5         C7 H5 N5	Position mod Position N6	e Origin A Mode Sub	Delete Delete Sugar Formula C5 H10 O5 C5 H10 O5 C6 H12 O5 C10 H19 O12 P1	Position 2 s	Mode A sub A sub A	Drigin
ymbol Am Ne Defau ymbol 6A n p 5A	ame Nucleoside Form 5-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 III Nucleoside Table Name adenosine N6-acetyladenosine 2'-O-methyladenosine 2'-O-mithyladenosine 2'-O-mithyladenosine phosphate cyclic N6-threonylcarbamoyladenosine	Ula Base+H Formula Position C12 H9 N5 O1 Nucleoside Formu C10 H13 N5 O4 C12 H15 N5 O5 C11 H15 N5 O4 C15 H22 N5 O11 C15 H22 N5 O11	Bugar           mode         Formula         I           C6 H12 O5         C           Jala         Ease+H           Formula         C           C5 H5 N5         C           C7 H7 N5 O1         C           C5 H5 N5         C           C1 C5 H5 N5         C           C1 C5 H5 N5         C           C1 C4 H0 N6 O3         C	Position mod	e Origin A Mode sub	Delete Delete Sugar C5 H10 05 C5 H10 05 C6 H12 05 C10 H19 012 P1 C5 H10 05	Position	Mode A A Sub A Sub A A	Drigin
ymbol )efau ymbol 6A n p 6A A	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 III Nucleoside Table Name adenosine N6-acetyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine N6-formyladenosine	Ula Base+H Formula Position C12 H9 N5 O1 C10 H13 N5 O4 C12 H15 N5 O5 C11 H15 N5 O4 C15 H22 N5 O11 C15 H18 N6 O7 C11 H13 N5 O5	Base+H         Formula         I           Formula         I         C6         H12         O5           Base+H         Formula         C5         H5         N5         C7         H7         N5         O1         C5         H5         N5         C10         H10         N6         O3         C6         H5         N5         C1         C1         N6         O3         C6         H5         N5         C1         C1         C1         C5         C1         C1         C1         N6         O3         C6         H5         N5         C1         C1         C1         C1         C1         C1         C6         C1         C	Position mod	A A Mode sub sub sub	Delete Delete Sugar Formula C5 H10 05 C5 H10 05 C6 H12 05 C6 H12 05 C5 H10 05	Position 2 s 2 s	Mode A A Sub Sub A A A A	Drigin
ymbol Defau ymbol :6A m p 6A A 5A	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5  IIT Nucleoside Table Name adenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-ribosyladenosine 2'-O-ribosyladenosine 0.6-formyladenosine N6-agryladenosine N6-gryladenosine N6-gryladenosine N6-gryladenosine	Base+H         Position           Formula         Position           C12 H9 N5 O1         Position           C12 H9 N5 O1         C10 H13 N5 O4           C12 H15 N5 O5         C11 H15 N5 O4           C15 H22 N5 O11         C15 H18 N6 O7           C11 H13 N5 O5         C13 H16 N6 O7	Sugar           mode         Formula         I           C6 H12 O5         Genula         C           Base+H         Formula         C           C5 H5 N5         C7 H7 N5 O1         C5 H5 N5           C7 H7 N5 O1         C5 H5 N5         C10 H10 N6 O3           C6 H5 N5 O1         C8 H8 N6 O3         C3 H8 N6 O3	Position mod	A A Mode sub sub sub sub	Delete Delete Sugar Formula C5 H10 O5 C5 H10 O5 C5 H10 O5 C5 H10 O5 C5 H10 O5	Position 2 s 2 s	Mode A Sub A Sub A A A A	Drigin
ymbol N ymbol c6A m cp 6A A 6A A 6A 6A 6A	ame Nucleoside Form	Base+H           Formula         Position           C12 H9 N5 O1         C12 H9 N5 O1           Nucleoside Formula         C10 H13 N5 O4           C12 H15 N5 O5         C11 H15 N5 O4           C15 H18 N6 O7         C11 H13 N5 O5           C11 H13 N5 O5         C11 H13 N5 O5	Base+H           Formula         C6 H12 O5           Base+H         Formula           C5 H5 N5         C7 H7 N5 O1           C5 H5 N5         C10 H10 N6 O3           C6 H5 N5 O1         C3 H5 N5 O1           C6 H5 N5 O1         C3 H5 N6 O3           C6 H7 N5 O1         C4 H7 N5 O1	Position mod Position N6 N6 N6	e Origin A Mode sub sub sub sub sub	Delete Delete Sugar Formula C5 H10 05 C5 H10 05 C6 H12 05 C10 H19 012 P1 C5 H10 05 C5 H10 05 C5 H10 05	Position	Mode A Sub A Sub A A A A A A	Drigin
ymbol N z_Am Ne Defau ymbol :6A m rp 6A A 3A n6A 16A	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5 III Nucleoside Table Name adenosine N6-acetyladenosine 2'-O-methyladenosine 2'-O-methyladenosine phosphate 2'-O-methyladenosine 2'-O-methyladenosine N6-fylcinyladenosine N6-fylcinyladenosine N6-hydroxymethyladenosine N6-hydroxymethyladenosine N6-hydroxymethyladenosine	UIA Base+H Formula Position C12 H9 N5 O1 C12 H9 N5 O1 C12 H5 N5 O5 C11 H15 N5 O4 C15 H22 N5 O11 C15 H18 N6 O7 C11 H13 N5 O5 C13 H16 N6 O7 C11 H15 N5 O5 C13 H16 N6 O7 C11 H15 N5 O5 C16 H22 N6 O8	Bugar           mode         Formula         I           C6 H12 O5         C6 H12 O5           Base+H         Formula         C5 H5 N5           C5 H5 N5         C7 H7 N5 O1         C5 H5 N5           C1 C5 H5 N5         C10 H10 N6 O3         C6 H5 N5 O1           C8 H8 N6 O3         C6 H7 N5 O1         C11 H14 N6 O4	Position mod	e Origin A Mode sub sub sub sub sub sub	Delete Delete Sugar 5 Frmula C5 H10 05 C5 H10 05 C6 H12 05 C5 H10 05 C5 H10 05 C5 H10 05 C5 H10 05 C5 H10 05	Position 2 s 2 s	Mode A A Sub A Sub A A A A A A	
xymbol N z_Am Ne Defau xymbol c6A m rp 6A 6A 6A 6A 6A 6A 6A	ame Nucleoside Form 6-benzoyl-2'-O-methylAdenosine C18 H19 N5 O5  IIT Nucleoside Table Name adenosine N6-acetyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine 2'-O-methyladenosine N6-ghycinylcarbamoyladenosine N6-ghycinylcarbamoyladenosine N6-ghydroxynorvalylcarbamoyladenosine N6-hydroxymethyladenosine N6-hydroxynorvalylcarbamoyladenosine Inosine	Base+H         Position           Formula         Position           C12 H9 N5 O1         C12 H9 N5 O1           C10 H13 N5 O4           C12 H15 N5 O5         C11 H15 N5 O4           C15 H22 N5 O11         C15 H18 N6 O7           C11 H13 N5 O5         C13 H16 N6 O7           C11 H15 N5 O5         C13 H16 N6 O7           C11 H15 N5 O5         C13 H16 N2 O5           C16 H22 N6 O8         C10 H12 N4 O5	Sugar           mode         Formula         I           C6 H12 O5         Genula         C           Base+H         Formula         C           C5 H5 N5         C7 H7 N5 O1         C5 H5 N5           C10 H10 N6 O3         C6 H5 N5 O1         C8 H8 N6 O3           C6 H7 N5 O1         C1 H8 N6 O3         C6 H7 N5 O1           C1 H14 N6 O4         C5 H4 N4 O1         C5 H4 N4 O1	Position mod Position N6 N6 N6 N6 N6 N6 N6	e Origin A Mode sub sub sub sub sub sub sub	Delete Delete Sugar Formula C5 H10 05 C5 H10 05	Position 2 s 2 s	Mode A A Sub A Sub A A A A A A A	Drigin

Figure 21. The User Nucleoside Table. The defined part (Bz\_Am) shown in the table.

Input option 2: Difference from the Origin

Step1: Input 0	General Information	NH <sub>2</sub>
Origin*	A ~	N N
Symbol*	Bz_Am	
Name	N6-benzoyl-2'-O-methylAdenosine	N N
Chemical Formula*	C18 H19 N5 O5	HO
*: Required Choose How • Base and Su • Difference fr Next	to Input/Edit Elemental Compo Igar Directly om the Origin	sition OH OH

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.

Input the S	Symbol, Name, Ch	emical I	Formula, a	and Origin	Cuba		-		
for the Lini	ker to be defined.				Supp	arti	<u> </u>		
Symbol*	s		1		Subp	art2 O=	P−OH		
Name	phosphorothio	ate	1		Subr	art3	Ó		
Chemical For	mula		_		Saph		ī		
Subpart1*	01		1						
Subpart2*	H1 01 S1 P1		า						
Subpart2*	01		1						
Supparts	01		4						
Origin*	S								
	-								
*: Required	t								
*: Required Add New Jser Lin	ker Table								
*: Required Add New Jser Lin )efault I	Linker ker Table Linker Table	Subpart1	Chemical F	ormula Subpart	2 Chemical For	nula Subpart3	6 Chemical Form	nula Origin	
*: Required Add New Jser Lin )efault I	ker Table	Subpart1	Chemical F	ormula Subpart: H1 O2 P	2 Chemical For	nula Subpart3	6 Chemical Form	nula Origin	
*: Required Add New Jser Lin )efault I ymbol Name phospt	ker Table	Subpart1 01 01	Chemical F	ormula Subpart H1 O2 P H1 O1 S	2 Chemical For	nula Subpart3 O1 O1	3 Chemical Form	nula Origin p p	
*: Required Add New Jser Lin )efault I ymbol Name phospt phospt phospt	ker Table Linker Table Linker Table	Subpart1 01 01 01	Chemical F	ormula Subpart H1 02 P H1 01 S H1 01 S	2 Chemical For 1 1 P1 1 P1	nula Subpart3 O1 O1 O1	Chemical Form	nula Origin p p s	
*: Required Add New Jser Lin Default I ymbol Name phospi ; phospi phospi	d / Linker ker Table _inker Table inker Table inker Table inte	Subpart1 01 01 01 01 01	Chemical F	ormula Subpart H1 02 P H1 01 S H1 01 S H1 02 P	2 Chemical For 1 1 P1 1 P1	nula Subpart3 O1 O1 O1 O1 O1	6 Chemical Form	nula Origin p p s s s	

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 nucleoside.

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).

					]	1
Inpu for t	ut the Symbol, Na the Linker to be d	me, Chemical Form efined.	ula, and Origin	Subpart1	<u> \</u>	
Sym	bol* S			Subpart2 O=	₽́−OH	
Nam	e phosp	horothioate		Subpart3	0	
Cher	mical Formula	lorotinouto		ouspuito	ĭ	
Sub	part1* O1					
Subr	part2* H1 O1	S1 P1				
Sub	part3* O1					
Origi	in* e					
*: R Ad	Required					
*: R Ad	d New Linker	ble				
*: R Ad	equired Id New Linker er Linker Tal	기은 Subpart1 Cher	nical Formula Subpart2 Cher	mical Formula Subpart	3 Chemical Formula	rigin Dele
*: R Ad JSC	equired Id New Linker er Linker Tal In Name cyanoethyl phospho	DIE Subpart1 Cher rothioate 01	nical Formula Subpart2 Che C3 H4 N1 O1 F	mical Formula <mark>Subpart</mark> P1 S1 O1	3 Chemical Formula O S	rigin Dele Del
*: R Ad Jse ymbo s )ef	er Linker Tal	Subpart1 Cher rothioate01	nical Formula Subpart2 Cher C3 H4 N1 O1 F nical Formula Subpart2 Cher	nical Formula Subpart 11 S1 O1 nical Formula Subpart	3 Chemical Formula O 5 3 Chemical Formula O	rigin Dele Del
*: R Ad JSE /mbo s	tequired Id New Linker er Linker Tal I Name cyanoethyl phosphor al Name phosphate	DIE Subpart1 Cher rothioate 01 Table Subpart1 Cher O1	nical Formula Subpart2 Che C3 H4 N1 O1 F nical Formula Subpart2 Che H1 O2 P1	mical Formula Subpart P1 S1 O1 nical Formula Subpart O1	3 Chemical Formula O s 3 Chemical Formula O p	rigin Dele Del
*: R Ad ////Se //mbo	tequired Id New Linker er Linker Tal Name cyanoethyl phosphor phosphorothioate	Subpart1 Cher prothioate O1 Table Subpart1 Cher O1	nical Formula Subpart2 Che C3 H4 N1 O1 F nical Formula Subpart2 Che H1 O2 P1 H1 O1 S1 P1	nical Formula Subpart 14 S1 O1 mical Formula Subpart O1 O1	3 Chemical Formula O s 3 Chemical Formula O p p	rigin Dele Del
*: R Ad JSE ymbo s	Anter and a second seco	Subpart1 Cher rothioateO1 Table Subpart1 Cher O1 O1 O1	nical Formula C3 H4 N1 O1 F nical Formula H1 O2 P1 H1 O1 S1 P1 H1 O1 S1 P1 H1 O1 S1 P1	nical Formula Subpart 11 S1 O1 nical Formula Subpart O1 O1 O1	3 Chemical Formula O s 3 Chemical Formula P p s	rigin Dele Del
*: R Ad JSE ymbo s )ef	tequired Id New Linker er Linker Tal Name cyanoethyl phosphor ault Linker phosphorothioate phosphorothioate phosphorothioate phosphorothioate	Die Subpart1 Cher rothioate O1 Table O1 O1 O1 O1 O1 O1	nical Formula Subpart2 Cher C3 H4 N1 O1 F Nical Formula Subpart2 Cher H1 O2 P1 H1 O1 S1 P1 H1 O1 S1 P1 H1 O1 S1 P1 H1 O2 P1	nical Formula Subpart 11 S1 O1 nical Formula Subpart O1 O1 O1 O1	3 Chemical Formula O s 3 Chemical Formula O p 5 s s	rigin Dele Del
*: R Ad JSE ymbo s )ef	tequired Id New Linker er Linker Tal Name cyanoethyl phosphor phosphate phosphorothoate	DIE Subpart1 Cher rothioate 01 Table Subpart1 Cher 01	nical Formula Subpart2 Chet C3 H4 N1 O1 F nical Formula Subpart2 Chet H1 O2 P1 H1 O1 S1 P1 H1 O1 S1 P1	mical Formula Subpart 14 S1 O1 mical Formula Subpart O1 O1	3 Chemical Formula O s 3 Chemical Formula O p	rigin

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).

Home Mass Calculator MS/MS Search Manual

## **Nucleotide Parts Editor**

Editors Nucleoside Linker Terminus Part-Specific MS/MS Fragmentation

## User Parts

Nucleosides

Sumbol	Namo	Nucleoside Chemical Formula	Base+H			Sugar			Origin
Symbol	Name	Nucleoside Chemical Formula	Chemical Formula	Position	Mode	Chemic Formula	Position	Mode	Ongin
Bz_Am	N6-benzoyl-2'-O-methylAdenosine	C18 H19 N5 O5	C12 H9 N5 O1			C6 H12 O5			Α
Linke	rs								
Symbol	Name Subp	oart1 Chemical Formula Subpa	art2 Chemical Form	ula Subp	art3 Cł	nemical Formula	Drigin		
CSS	cyanoethyl phosphorothioate O1	C3 H4	N1 O1 P1 S1	01		S			
Term	ni							-	
Part-	Specific MS/MS Fragm	entations							
Seq	uence Type								
Nu	cleoside Subtype								
Lin	ker Subtype								
Ter	minus Subtype								
Con	position Type								

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.

Input th	ne Symbol, Name, Ch	nemical Formula	, and Origin	NH <sub>2</sub>	
	Terminus to be define	su.		, i	
Symbol	p			S'term N O	
Name	phosphoric ac	cid		HO-T_O_	
Chemica	Formula <sup>•</sup> H2P1O4				
Origin	OH			O OH	
5' Term A				O=P-OH	
3' Term A				OH 3'term	
*: Requ	lired				
*: Requ Add N Jser 7	lired Iew Terminus Terminus Table It Terminus Ta	e			
*: Requ Add N Jser 7 Defau	lired Iew Terminus Ferminus Table It Terminus Ta	e ble			
*: Requ Add N Jser T Jser T Oefau	lired lew Terminus Terminus Table It Terminus Ta Name Natrov	e ble Chemical Formu	la Origin		
*: Requ Add N Jser T Jefau	Ired Iew Terminus Terminus Table It Terminus Ta Name Indrovy polosohate	Chemical Formu H1 01 H2 P1 04	la Origin OH OH		
*: Requ Add N Jser 7 )efau <sup>ymbol</sup>	Ired Iew Terminus Ierminus Table It Terminus Ta Name Name phosphorobinate	Chemical Formu H1 01 H2 P1 04 H2 P1 03 S1	ia <mark>Origin</mark> ОН ОН ОН		
*: Requ Add N Jser 7 Defau ymbol	Ired Iew Terminus Ierminus Table It Terminus Ta Vance Phosphate phosphate phosphate	Chemical Formu (Chemical Formu H1 01 H2 P1 04 H2 P1 03 S1 H0 P1 03	ia <mark>Origin</mark> Он Он Он Он		
*: Requ Add N Jser 7 )efau ymbol H 2,2,7Gpp	Ired Iew Terminus Ierminus Table It Terminus Table It Terminus Ta Name phosphorbioate cyclic phosphate phorembry quashate phosphorbiate cyclic phosphate phorembry quashate phosphorbiate cyclic phosphate phorembry quashate	Chemical Formu H1 01 H2 P1 04 H2 P1 03 H0 P1 03 C13 H21 N5 012	la Orgin ОН ОН ОН ОН РЗОН		
*: Requi	Ired Iew Terminus Ierminus Table It Terminus Table It Terminus Ta Manc yanc yanc yhosphate phosphorbhioate cyclic phosphate ptrimethy guanosine cap	Chemical Formu H1 01 H2 P1 04 H2 P1 03 S1 H0 P1 03 C13 H21 N5 014 apC11 H17 N5 014	Ia Origin ОН ОН ОН ОН РЗОН РЗОН		

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 and comma (,) can be used to define a nucleoside symbol; however, symbols consisted of only lowercase letters are not allowed.

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).

			NH2	1	
Input	the Symbol, Name, Cr		and Origin		
for the	e Terminus to be define	ed.			
Symbo	l* p		5'term N		
Name	phosphoric a	cid	HO-1 -O		
Chemi	al Formula* H2P104				
Oninin			<mark>о</mark> о́н		
Origin	UH		O=P-OH		
5' iem			ÓH 3'term		
3º Term	Activation				
Add	New Terminus	e	7		
Add Jser Symbol h2,7Gpp	New Terminus Terminus Table Name Chemical Formula P C12 H19 N5 O14 P Ult Terminus Ta	e Origin Delete 3m7Gppp Delete	]		
Add Jser Symbol 12,7Gpp Defa Symbol	New Terminus Terminus Table Name Chemical Formula P C12 H19 N5 O14 P Ult Terminus Ta Name	Chemical Formu	) Origin		
Add Jser Symbol 12,7Gpp Defa Symbol	New Terminus Terminus Table Name Chemical Formula p. C12 H19 N5 O14 P ult Terminus Ta Name hydroxy	Chemical Formu	1 Origin OH		
Add Jser ymbol 12,7Gpp )efa ymbol	New Terminus Terminus Table Name Chemical Formula p C12 H19 N5 O14 P Ult Terminus Ta Name hydroxy phosphate	e Origin Delete 3m7Gppp Delete ble Chemical Formu H1 01 H2 P1 04	Origin OH OH		
Add Jser 2,7Gpp Defa	New Terminus Terminus Table Name Chemical Formula p C12 H19 N5 O14 P Ult Terminus Ta Name Nydroxy phosphate phosphorothioate	Origin         Delete           3m7Gppp         Delete           Ible         Chemical Formulation           H1 01         H2 P1 04           H2 P1 03 S1         H2 P1 03 S1	2 <mark>Origin</mark> ОН ОН ОН		
Add Jser 2,7Gpp Defa	New Terminus Terminus Table Name Chemical Formula p C12 H19 N5 O14 P Ult Terminus Ta Name Nydroxy phosphate phosphate cyclic phosphate	Crigin         Delete           3m7Gppp         Delete           Chemical Formul         H1 01           H1 01         H2 P1 04           H2 P1 03 S1         H0 P1 03	Crigin OH OH OH OH		
Add Jser symbol i2,7Gpp Defa symbol iH	New Terminus Terminus Table Name Chemical Formula P C12 H19 N5 O14 P C12 H19 N5 O14 P Ult Terminus Ta Name hydroxy phosphate phosphate phosphate popptrimethyl guanosine cap	Chemical Formu H1 01 H2 P1 04 H2 P1 03 C13 H21 N5 01	2 Origin OH OH OH OH OH OH OH		

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.

Part-Specific MS/MS Fragmentation Editor
<ul> <li>Sequence Type</li> <li>Specify symbol and chemical formula for the losing neutral molecule during gas-phase dissociation. The software calculates the masses of corresponding product ions generated by the neutral-loss reaction.</li> </ul>
O Composition Type Specify symbol and chemical formula for the resulting product as neutral. For example, instead of the deprotonated anion (C6 H10 O7 P1, z: 1-, m/z: 225) observed in negative polarity, define neutral methyl ribose phosphate (C6 H11 O7 P1). The software calculates the mass of cation or anion from the neutral according to the Polarity parameter.
User Part-Specific MS/MS Fragmentation Table
Sequence Type
Linker Subtype
Terminus Subtype
Composition Type
Default Part-Specific MS/MS Fragmentation Table
Sequence Type
Nucleoside Subtype
Symbol         Name         Nucleoside Formula         Base+H         Sugar         Origin           Formula         Position         Mode         Formula         Position         Mode         Origin           B(m7G)         C6 H7 N5 O1         Mode         m7G         m7G         Mode         Mode<
Linker Subtype
Origin Symbol Name Chemical Formula
acp3Y         acp3Y-2(H2O)         C13 H15 N3 O6           m1acp3Ym1acp3Y-2(H2O)         C14 H17 N3 O6           m1Y         m1Y-2(H2O)         C10 H10 N2 O4

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 N7methylguanosine (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).

Par	t-Spec	cit	fic MS/MS Fragmentation Editor	^
Step1: Subtype* Origin* *: Requ Prev	A m2,2Gm m2G	v ^	al Information	
User F Sequen Nucleo Linker	m7G manQ mimG o2yW OHyW		c MS/MS Fragmentation Table	
	OHyWx	~	Outreadd Otensiael Earmula Outreadd Otensiael Earmula Outreadd Otensiael Earmula Oceaid	ř

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).

Step2: Edit Neutral Loss Subtype: Nucleoside Origin: m7G Chemical Formula Base: C6 H7 N5 O1	
Sugar: C5 H10 O5	
Define Neutral Loss	
Symbol*	B(m7G)
Name	
Chemical Formula of the Neutral That is	s Lost from
Base	C6 H7 N5 O1
Sugar	
*: Required	
Prev Next	

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).

```
Step3: Confirmation
Subtype: Nucleoside
Symbol: B(m7G)
Name:
Chemical Formula
Base: C6 H7 N5 O1
Sugar:
Prev Add New Part-Specific MS/MS Fragmentation
```

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).

User	Par	t-Specific N	/IS/MS	Fragi	men	Itatio	n Tab	le		
Seque	ence <sup>.</sup>	Туре								
Nucle	eosid	e Subtype								
Sumbo	Namo	Nuclear data Estatuta	Base+H			Sugar			Osisia	Delete
Symbo	Mame	Nucleoside Formula	Formula	Position	Mode	Formula	Position	Mode	Ongin	Delete
B(m7G	)		C6 H7 N5 O1						m7G	Delete
Linke	er Sul	otype								
Term	inus	Subtype								
Comp	ositio	n Type								

**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).

```
Step1: Input/Edit General Information
Subtype* Linker 
Orgin* cs 
*: Required
Prev Next
```

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).

Step2: Edit Neutral Loss Subtype: Linker Origin: cs Chemical Formula Subpart1: 01 Subpart2: C3 H4 N1 01 P1 S1 Subpart3: 01 Define Neutral Loss	
Define Neutral Loss	s2(ce)
Name	
Chemical Formula of the Neutral That is	Lost from
Subpart1	
Subpart2	C3 N4 H1
Subpart3	
*: Required	
Prev Next	

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).

User Part-Specific M Sequence Type Nucleoside Subtype	IS/MS I	Fragr	ner	itatioi	n Tab	le			
Symbol Name Nucleoside Formula	Base+H			Sugar			Origin	Delete	
Symbol Name Nucleoside Formula	Formula	Position	Mode	Formula	Position	Mode	Ongin	Delete	
B(m7G)	C6 H7 N5 O1						m7G	Delete	
Linker Subtype									
Symbol Name Subpart1 Chemical F	ormula Subp	art2 Che	mical F	ormula S	Subpart3 (	Chemic	al Forn	nula Orig	in Delete
s2(ce)	C3 N4	4 H1						cs	Delete
Terminus Subtype									
Composition Type									

**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).

```
Step1: Input/Edit General Information

Subtype* Terminus v

Origin* m2,2,7Gppp v

*: Required

Prev Next
```

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.

Step2: Edit N Subtype: Term Origin: m2,2,7 Chemical Form	leutral Loss inus Gppp nula: C13 H21 N5 O14 P3
Define Neutra	al Loss
Symbol*	m2,2,7Gpp
Name	
Chemical Formula	C13 H21 N5 O12 P2
*: Required	
Prev Next	

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).

User Part-Speci Sequence Type Nucleoside Subtyp	fic MS/MS e	Fragr	ner	itation	n Tab	le			
	Base+H			Sugar			0-1-1-1-	Delete	
Symbol Name Nucleoside F	Formula	Position	Mode	Formula	Position	Mode	Origin	Delete	
B(m7G)	C6 H7 N5 O1	1					m7G	Delete	
Linker Subtype									
Symbol Name Subpart1 Ch	emical Formula Sub	part2 Che	mical F	Formula S	Subpart3 (	Chemic	al Form	nula Origi	n Delete
s2(ce)	C3 N	I4 H1						cs	Delete
Terminus Subtype			٦.						
Symbol Name Chemical	Formula Origin	Delete							
m2,2,7Gpp C13 H21 N	15 O12 P2 m2,2,7Gp	pDelete	•						

**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.





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.

Step1: Input/Ec	dit General Information	
Origin*	Υ	]
Symbol(for ion)*	Y-2H2O	
Name		]
Chemical Formula*	C9 H8 N2 O4	]
X: Wildcard(Or	igin)	
*: Required		
Prev Add New Part-Specific MS/MS Fragmentation		

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.