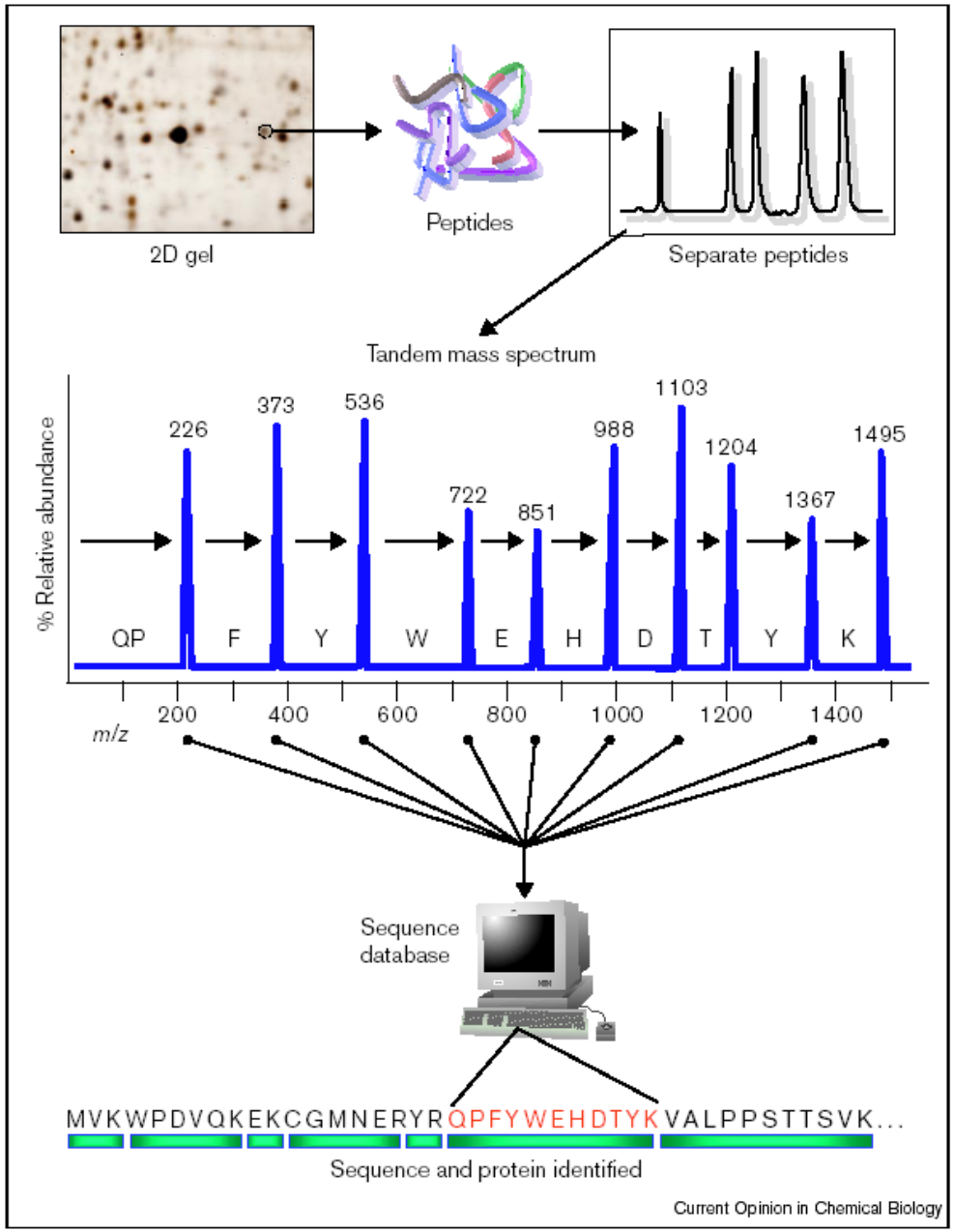
Part 2: Mass spectrometry

Structure of glycoproteins/peptides

Determination of glycosylation site

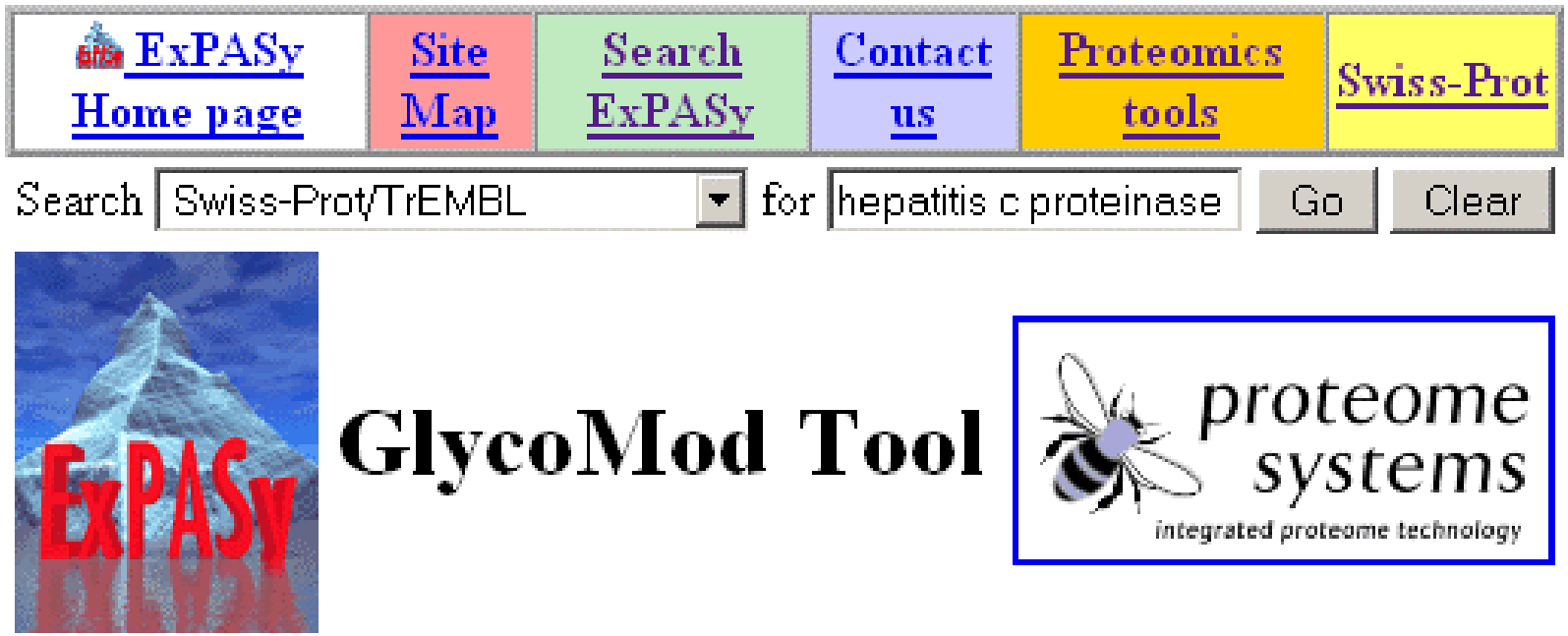
Determination of glycan structure



Schematic illustration of standard proteome analysis by 2DE–MS. Proteins are separated by 2DE. Stained spots are excised, subjected to in-gel digestion with trypsin, and the resulting peptides are separated by on-line HPLC. An eluting peptide is ionized by ESI, enters the mass spectrometer, and is fragmented to collect sequence information (tandem mass spectrum). The spectrum from the selected, ionized peptide is compared with predicted tandem mass spectra that are computer generated from a sequence database to identify the protein. Unambiguous protein identification is accomplished when multiple peptides from the same protein are matched. *m/z*, mass : charge ratio.

GlycoMod

www.expasy.org/tools/glycomod/



GlycoMod

Useful for determining the glycosylation positions in a protein.

You enter:
protein sequence, type of digest (used to get peptides) and MS-data

You get:
possible glycopeptides – glycosylation positions – and suggested glycan structures

Enter a list of experimental masses:

```
3290.2
3266.2
3250.2
3225.2
```

All mass values are
○ average or ◉ monoisotopic.

Or upload a file, containing one mass per line, from your computer:
[ ] Browse...

Mass tolerance: +/- 0.2 Dalton

Ion mode and adducts:

positive	negative	neutral
◉ $[M+H]^+$	○ $[M-H]^-$	○ [M]
○ Na^+ or ○ K^+	○ acetate or ○ trifluoroacetic acid	
○ other: [ ] mass: [ ]	○ other: [ ] mass: [ ]	

A protein sequence or a Swiss-Prot/TrEMBL ID or AC:

```
TALYYCARRD GTYGNYFDYW GQGTTLTVSS
ESQSFPNVFP LVSCESPLSD KNLVAMGCLA
RDFLPSTISF TWNYQNNTEV IQGIRTFPTL
RTGGKYLATS QVLLSPKSIL EGSDEYLVCK
IHYGGKNRDL HVPIPAVAEM NPNVNVFVPP
```

Enzyme: Trypsin

max. 1 missed cleavage sites (MC).

Cysteines treated with: nothing (in reduced form)

☐ acrylamide adducts on cysteines ☐ methionines oxidized

Peptides containing the motif 'N-X-S/T/C (X not P)':

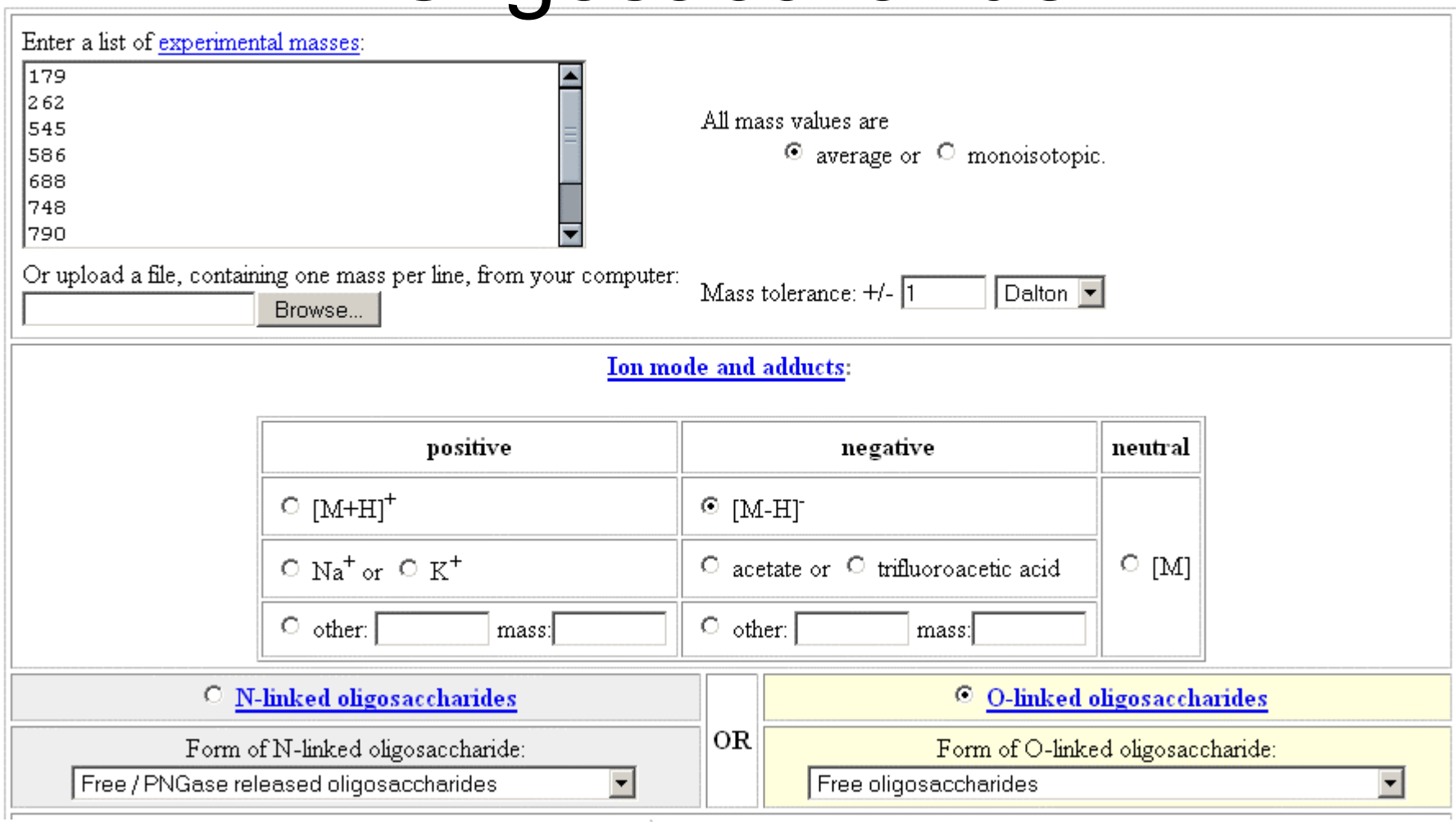
position	#MC	peptide mass [M]	peptide	modifications
1-10	0	1044.49883	SHPNGTFSAK	
1-18	1	1788.84644	SHPNGTFSAKGVASVCVE	

User mass: 3266.2

Adduct ($[M+H]^+$): 1.00727

glycoform mass	Δmass (Dalton)	structure	type	peptide mass [M]	peptide sequence	theoretical glycopeptide mass	mod.	Links
2220.767	-0.072	$(Hex)_1$ $(HexNAc)_2$ $(Deoxyhexose)_1$ $(NeuGc)_2$ + $(Man)_3(GlcNAc)_2$	hybrid/complex	1044.499	1-10 SHPNGTFSAK	3266.273		
2220.767	-0.072	$(Hex)_2$ $(HexNAc)_2$ $(NeuAc)_1$ $(NeuGc)_1$ + $(Man)_3(GlcNAc)_2$	hybrid/complex	1044.499	1-10 SHPNGTFSAK	3266.273		GlycoSuiteDB

Oligosaccharide



Correct structure is $Hex_3HexNAc_2$

User mass: 909

Adduct ($[M-H]^-$): -1.00739
Derivative mass (Free reducing end): 18.01528

glycoform mass	Δmass (Dalton)	structure	Links
891.829	0.163	$(Deoxyhexose)_4\ (NeuGc)_1$	
891.829	0.163	$(Hex)_1\ (Deoxyhexose)_3\ (NeuAc)_1$	
892.817	-0.824	$(Hex)_3\ (HexNAc)_2$	GlycoSuiteDB
892.857	-0.864	$(Hex)_1\ (Deoxyhexose)_5$	

…but it takes some deduction to arrive at the solution

User mass: 586

Adduct ([M-H]$^-$): -1.00739
Derivative mass (Free reducing end): 18.01528

glycoform mass	Δmass (Dalton)	structure	Links
568.532	0.46	$(Hex)_1$ $(HexNAc)_2$	GlycoSuiteDB

User mass: 748

Adduct ([M-H]$^-$): -1.00739
Derivative mass (Free reducing end): 18.01528

glycoform mass	Δmass (Dalton)	structure	Links
730.675	0.317	$(Hex)_2$ $(HexNAc)_2$	GlycoSuiteDB
730.715	0.277	$(Deoxyhexose)_5$	

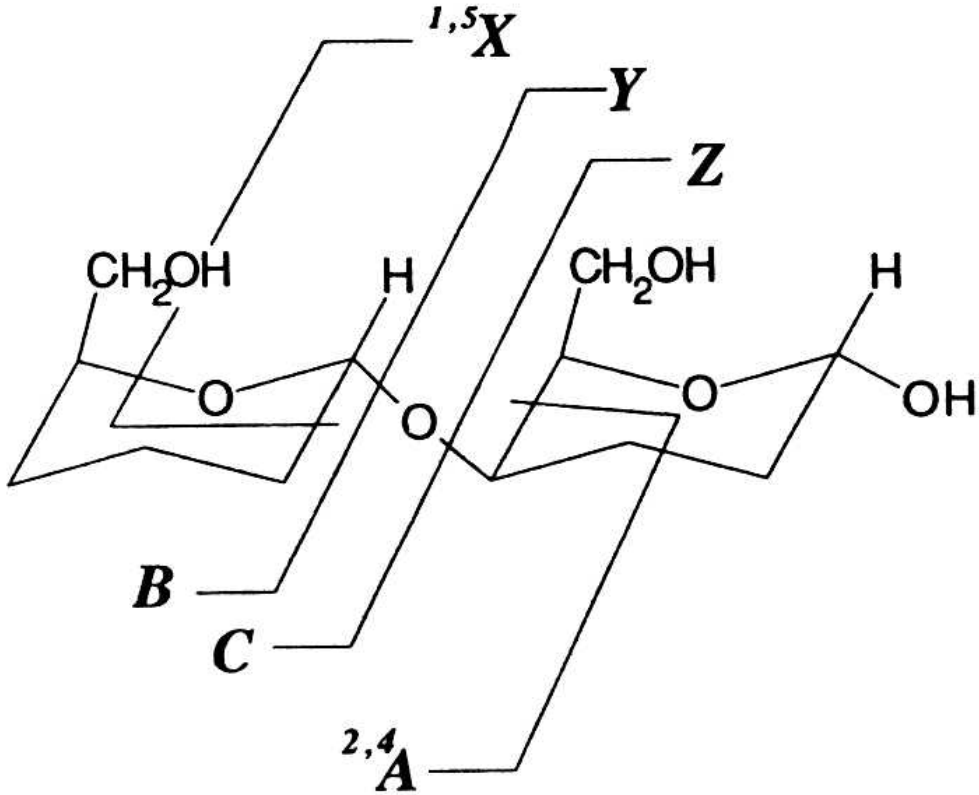
Glyco-search-MS
glycosciences.de

A database of *calculated* MS fragments of oligosaccharides in SweetDB

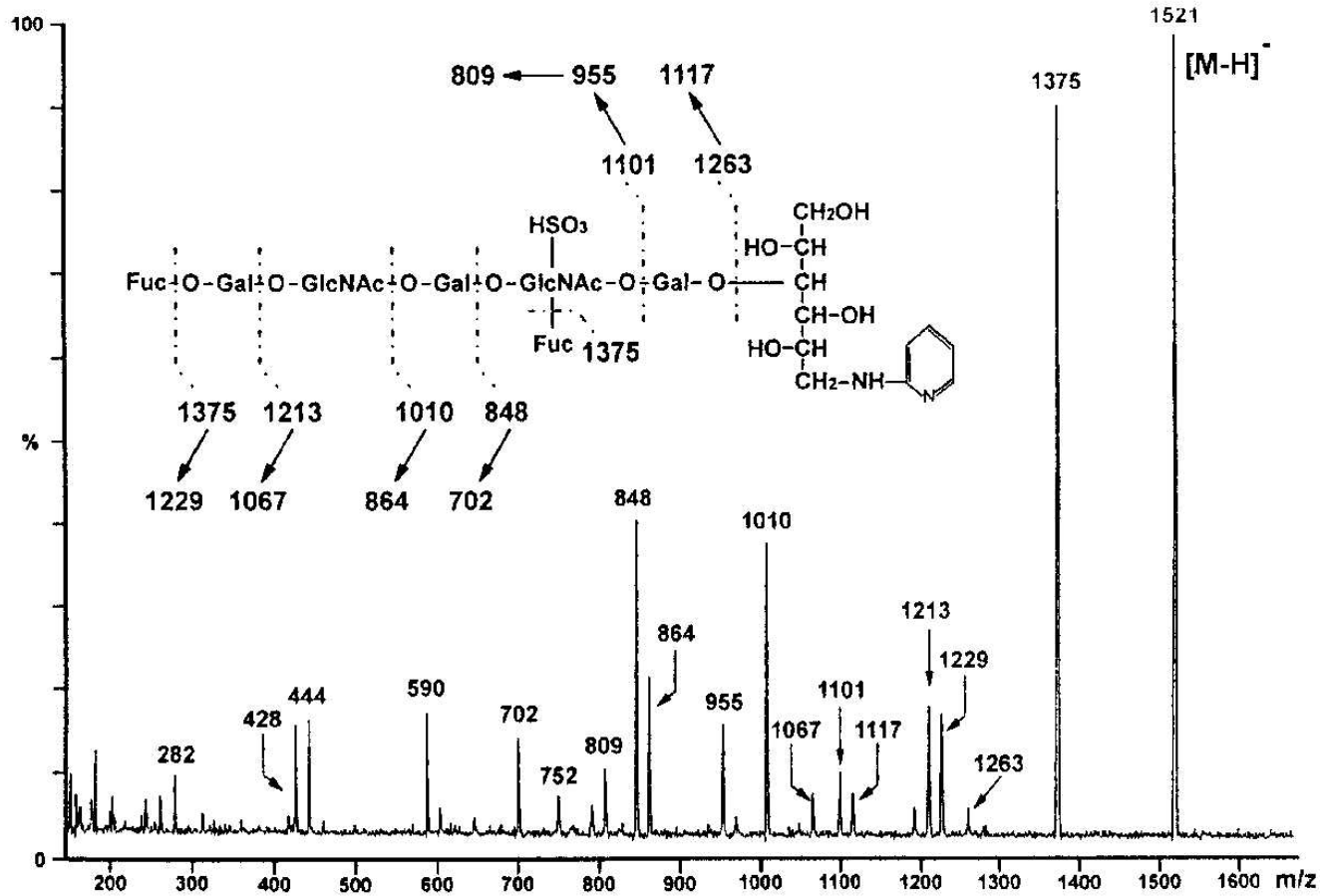
Determination of oligosaccharide structure is easier than in GlycoMod

Beware! There are few structures in the database!

Fragmentation



FAB-MS/MS



On-line tools for the interpretation of NMR and MS-spectra

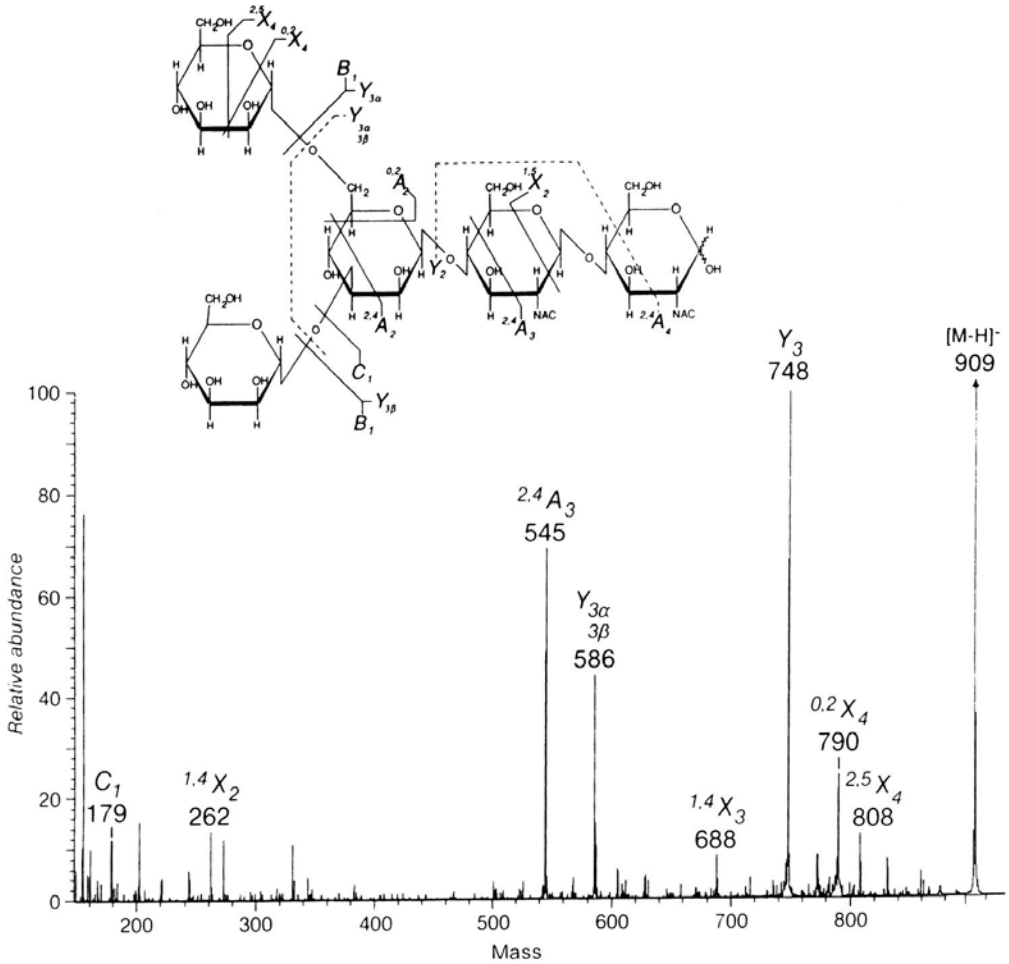
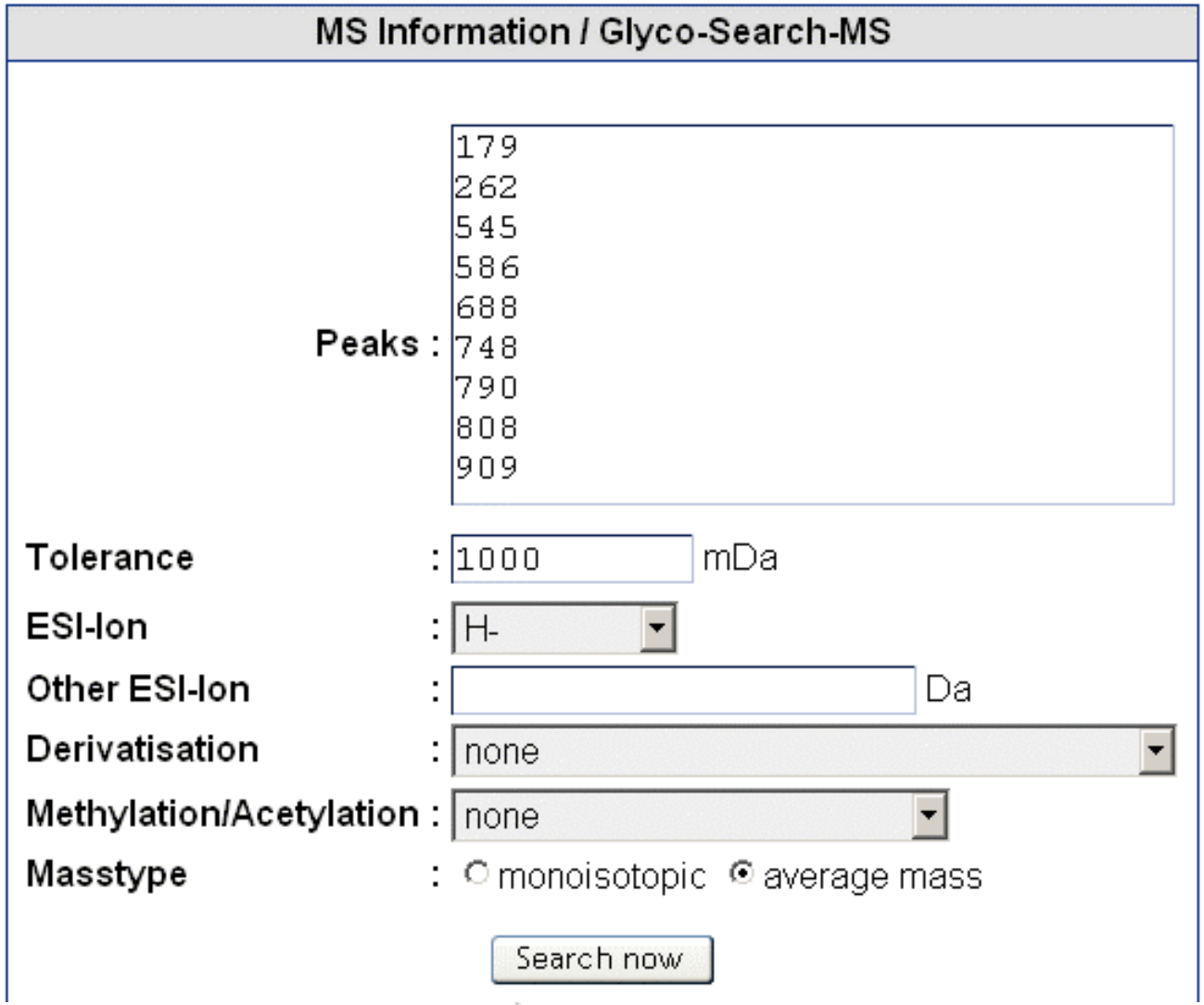


FIG. 3. Negative ion CID spectrum of the free core heptasaccharide obtained from N-linked glycans. Glycosidic bond cleavages (*Y* and *B* ions) as well as ring cleavages (*A* and *X*) occur in this molecule. Charge is not preferentially retained on either terminus as demonstrated by the formation of both *A*/*B* and *X*/*Y* product ions. In addition, more than one bond appears to cleave in the same molecule to form ions such as the one at *m*/*z* 586, which involves two *Y* rearrangement losses of both mannose termini.


MS Information / Glyco-Search-MS
Peaks :
179
262
545
586
688
748
790
808
909
Tolerance : 1000 mDa
ESI-Ion : H-
Other ESI-Ion : Da
Derivatisation : none
Methylation/Acetylation : none
Masstype : monoisotopic average mass
Search now

Searched for ms information. Results: 1 - 10 of 10

Score: 35
Total Mass:
1405.2866

Neup		Hex	
NeuAc	1	Hex	3
		HexNAc	3

Details | Glycofragment | Explore

Score: 35
Total Mass:
1567.4291

Neup		Hex	
NeuAc	1	Hex	4
		HexNAc	3

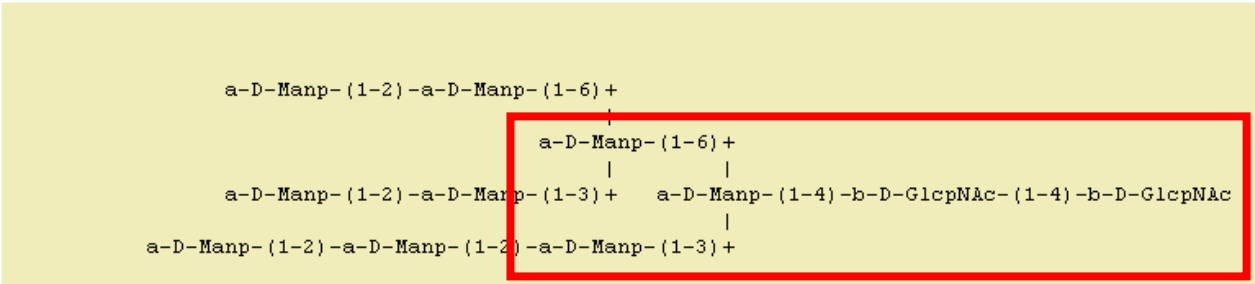
Details | Glycofragment | Explore

Score: 35
Total Mass:
1567.4291

Neup		Hex	
NeuAc	1	Hex	4
		HexNAc	3

Details | Glycofragment | Explore

Structure for LinucsID 23947



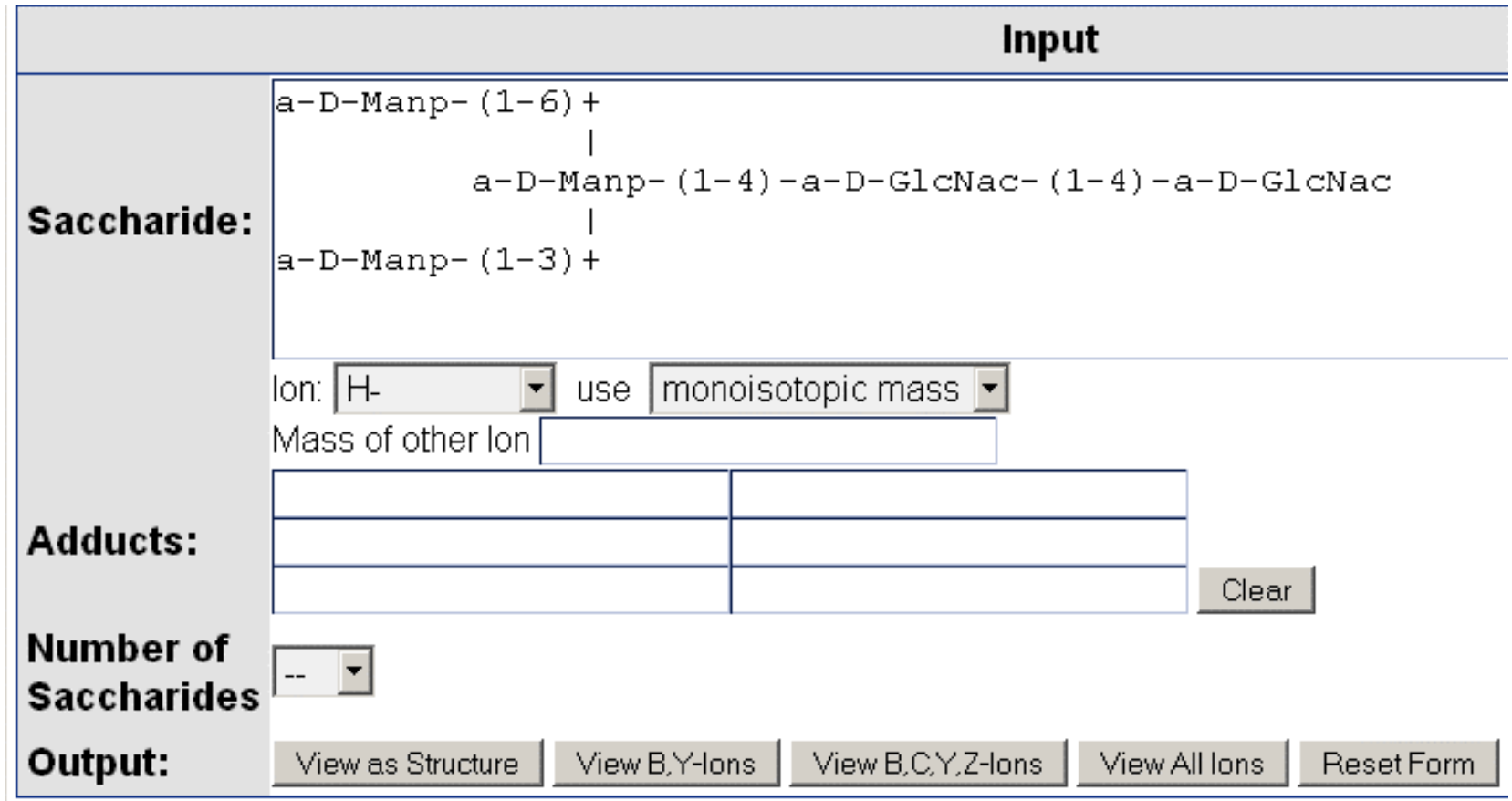
Composition for LinucsID 23947

Hex	
Hex	9
HexNAc	2

Theoretical masspeaks for LinucsID 23947

Total Mass: 1882.6446 M[+]: 1883.6525 M[Na+]: 1905.6336 M[K+]: 1921.7426

Mass in amu	Ion	Linkage-Path
163.0606	B(6)-Ion	4, 4, 3, 2, 2
	B(3)-Ion	4, 4, 6, 3, 2
	B(1)-Ion	4, 4, 6, 6, 2
179.0556	C(6)-Ion	4, 4, 3, 2, 2
	C(3)-Ion	4, 4, 6, 3, 2
	C(1)-Ion	4, 4, 6, 6, 2


Input
Saccharide:
a-D-Manp-(1-6)+
|
a-D-Manp-(1-4)-a-D-GlcNac-(1-4)-a-D-GlcNac
|
a-D-Manp-(1-3)+
Ion: H-
use
monoisotopic mass
Mass of other Ion
Adducts:
Clear
Number of Saccharides
--
Output:
View as Structure
View B,Y-Ions
View B,C,Y,Z-Ions
View All Ions
Reset Form

[M-H]- 909.3207 Mass: 910.3277 [M+H]+: 911.3356 [M+Na+]: 933.3167 [M+K+]: 949.4257

Mass in amu	Ion
161.0450	$B_{4,4,3}$ + H-
	$B_{4,4,6}$ + H-
179.0556	$C_{4,4,3}$ + H-
	$C_{4,4,6}$ + H-
202.0715	Z_4 + H-
220.0821	Y_4 + H-
405.1509	$Z_{4,4}$ + H-
423.1615	$Y_{4,4}$ + H-
485.1506	$B_{4,4}$ + H-
503.1612	$C_{4,4}$ + H-
688.2300	B_4 + H-
706.2406	C_4 + H-
729.2565	$Z_{4,4,3}$ + H-
	$Z_{4,4,6}$ + H-
747.2671	$Y_{4,4,3}$ + H-
	$Y_{4,4,6}$ + H-

Conclusion

Many of the tools are difficult to use
- *bad interfaces, lack of manuals*

Some will/can not give appropriate answers
- *technical limitations*
- *sometimes limitations in the experiments*

They are useful if used the right way
- *they often give useful hints*

If you can't get them to solve a problem within ca 30 min you are probably attempting the impossible