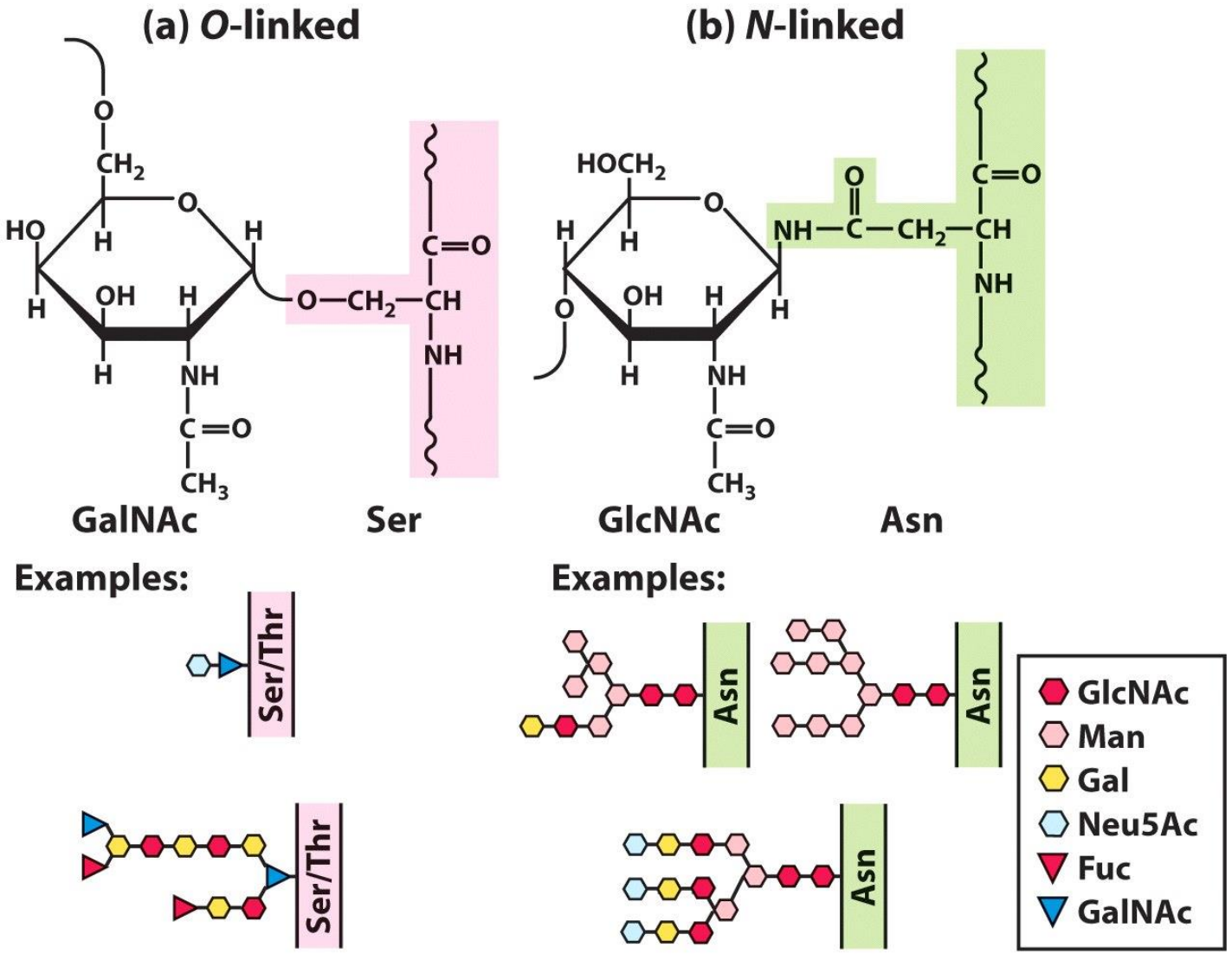
Analysis of Oligosaccharids by Mass Spectrometry


(a) O-linked
(b) N-linked
GalNAc
Ser
GlcNAc
Asn
Examples:
Examples:
Ser/Thr
Ser/Thr
Asn
Asn
Asn
GlcNAc
Man
Gal
Neu5Ac
Fuc
GalNAc

Working with Carbohydrate

- **Oligosaccharides removed from protein or lipid conjugates**
- **Stepwise degradations with specific reagents (eg. O- or N-glycosidase) that reveal bond position and stereochemistry**
- **Mixture separated by chromatography**
- **Overall composition and analysis by GC, Mass and NMR**

degradations with specific reagents

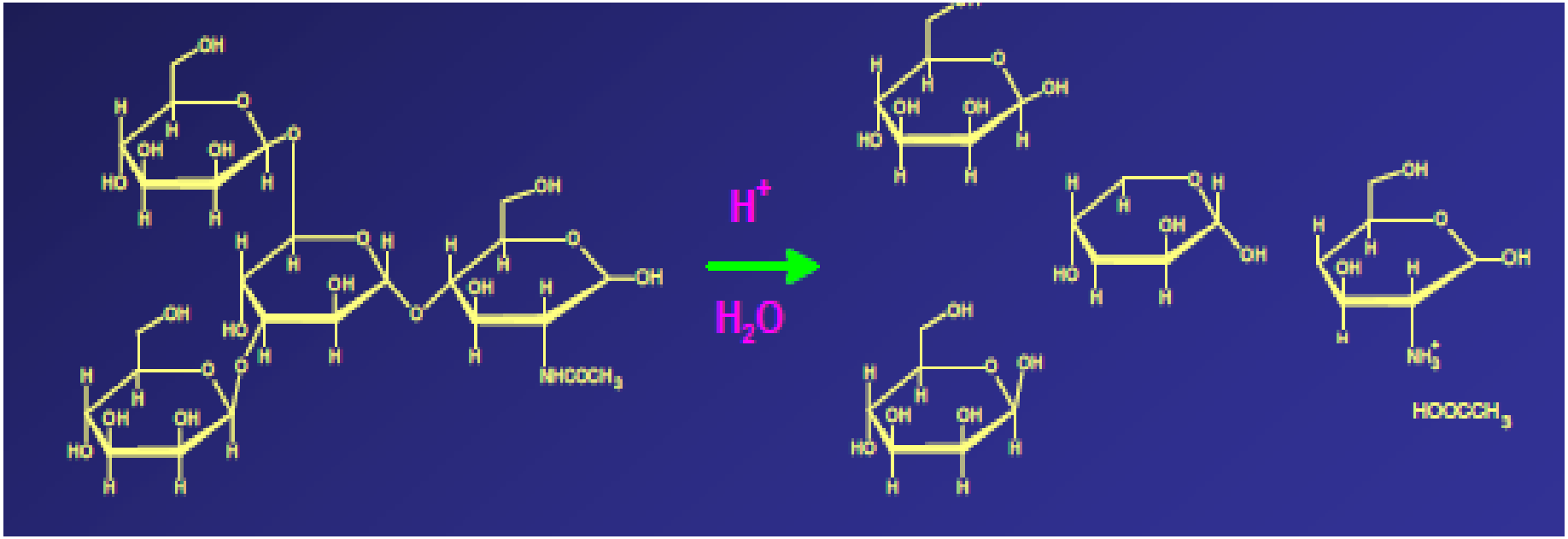
- Acid hydrolysis
- Methylation
- Smith degradation
- Chemical deglycolysation
- Glycosyl hydrolases
- Exoglycosidases
- endoglycosidases

Chemistry of Glycans

Acid Hydrolysis

- Many different conditions: acid concentration, temperature, time, solvent conditions
- General purpose to break oligosaccharide down into constituent monosaccharides
- Some monomers more susceptible to hydrolysis: weak conditions remove them

  sialic acids, fucose



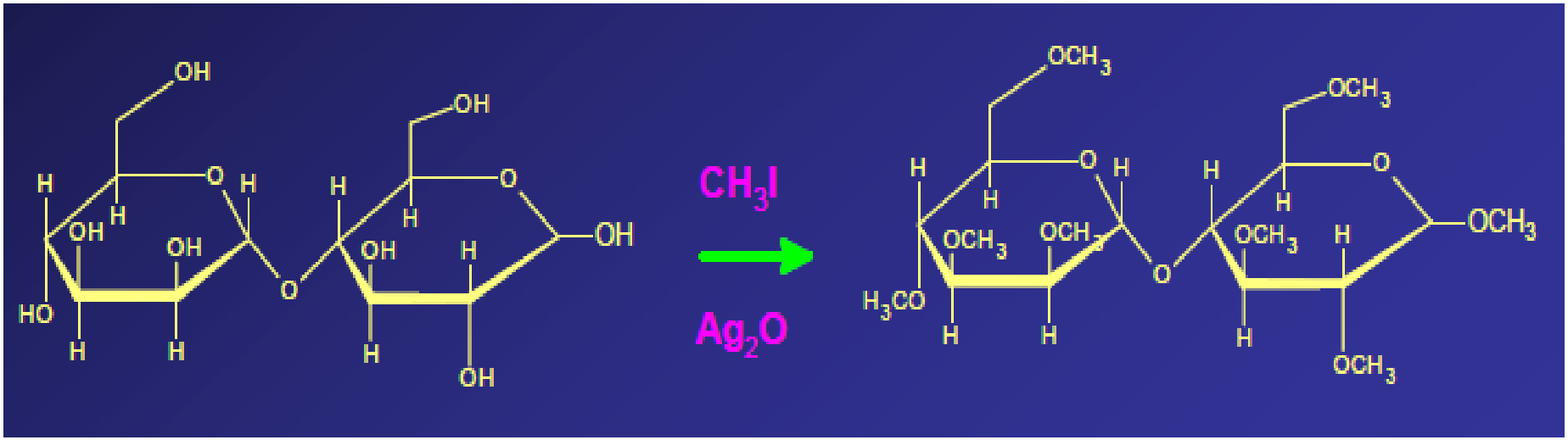
Ether and ester derivatization. Methylation

Esterification

- acid chlorides or acid hydrides
- in alkaline conditions (pyridine)

Ether derivatization (O-alkylation)

- Williamson ether synthesis: excess alkyl halide (R-X) + Ag_2O
- exhaustive ether formation, including the anomeric hydroxyl



Smith Degradation

- A technique useful in studying linkage analysis in smaller oligosaccharides
- Sample is processed in three preparative steps
  1. periodate oxidation
  2. borohydride reduction
  3. mild acid hydrolysis
- Products separated by (paper) chromatography referenced to known / predictable substances
- Standard set of rules are followed to deduce oligosaccharide composition and linkage

Chemical deglycosylation

Trifluoromethanesulfonic acid (TFMS)

- Extremely powerful acid
- Excellent tool for deglycosylation of glycoproteins
- Better than trying to deglycosylate enzymatically with glycosidase cocktail

- TFMS is used to remove oligosaccharides from glycoproteins so that the protein can be analyzed in later steps.

- Phosphate-modified amino acids in the protein are apparently unaffected

Anhydrous hydrogen fluoride deglycosylates glycoproteins without degrading either the protein. Monosaccharide structures of neutral and acidic sugars are retained

Glycosyl hydrolases

hydrolytic enzymes break down oligosaccharides into their monomers or smaller oligosaccharides

Exoglycosidases

- remove monosaccharides from a nonreducing terminal
- specific for monosaccharide and anomeric linkage
  - glucosidases
  - mannosidases
  - galactosidases
  - fucosidases
  - neuraminidases
- specific for α- and β-anomeric forms

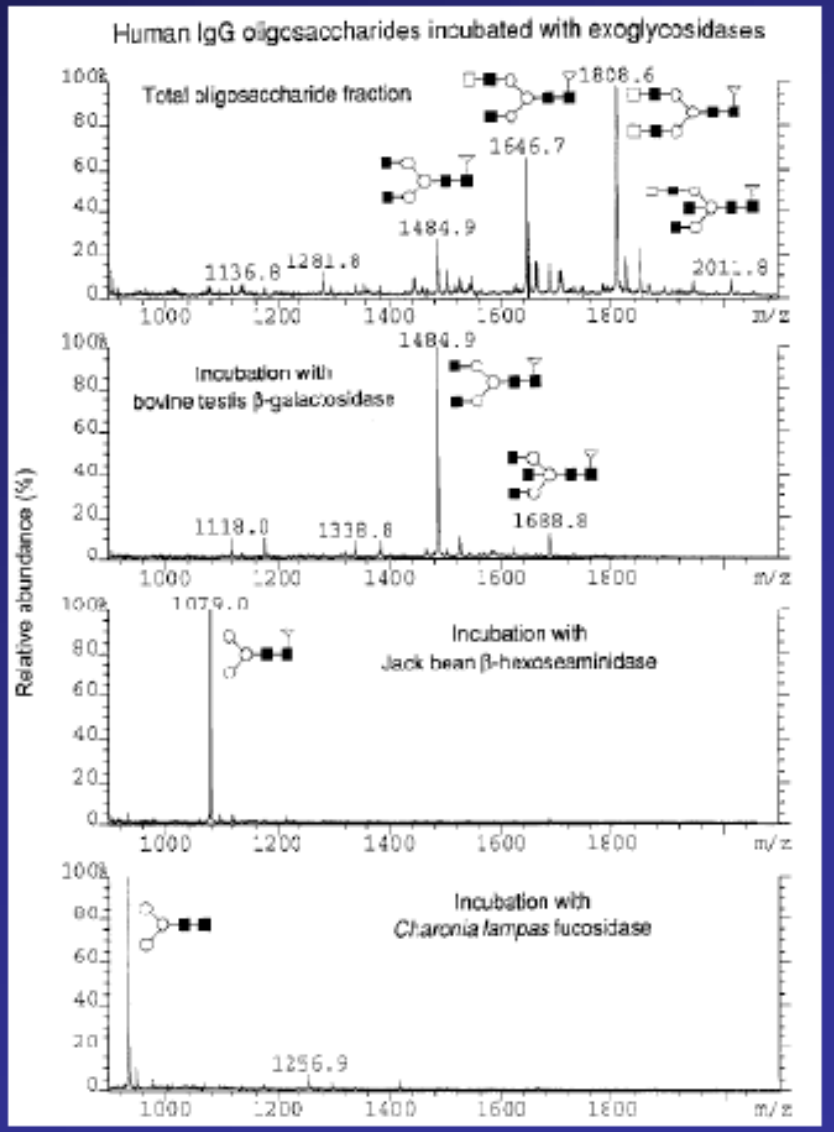
Endoglycosidases

- glycoside bond-hydrolyzing enzyme where bond broken does not produce a monosaccharide from a nonreducing terminus
- usually removes glycan from its aglycone

Sequencing/Structural Analyses

Mass Spectrometry (MS)

- MS excellent tool for structural determination
  - glycans (removed from glycopeptides)
  - intact glycoproteins
- MALDI-MS and ESI-MS/MS methods well described in literature reviews
- For MALDI-MS, special matrices for carbohydrate (e.g. “Super DHB”)
- Combined with exo- and endo-glycosidase digestions, sequence and linkage data can be obtained for glycan
- Neutral sugars ionized by Na^+ adduct formation

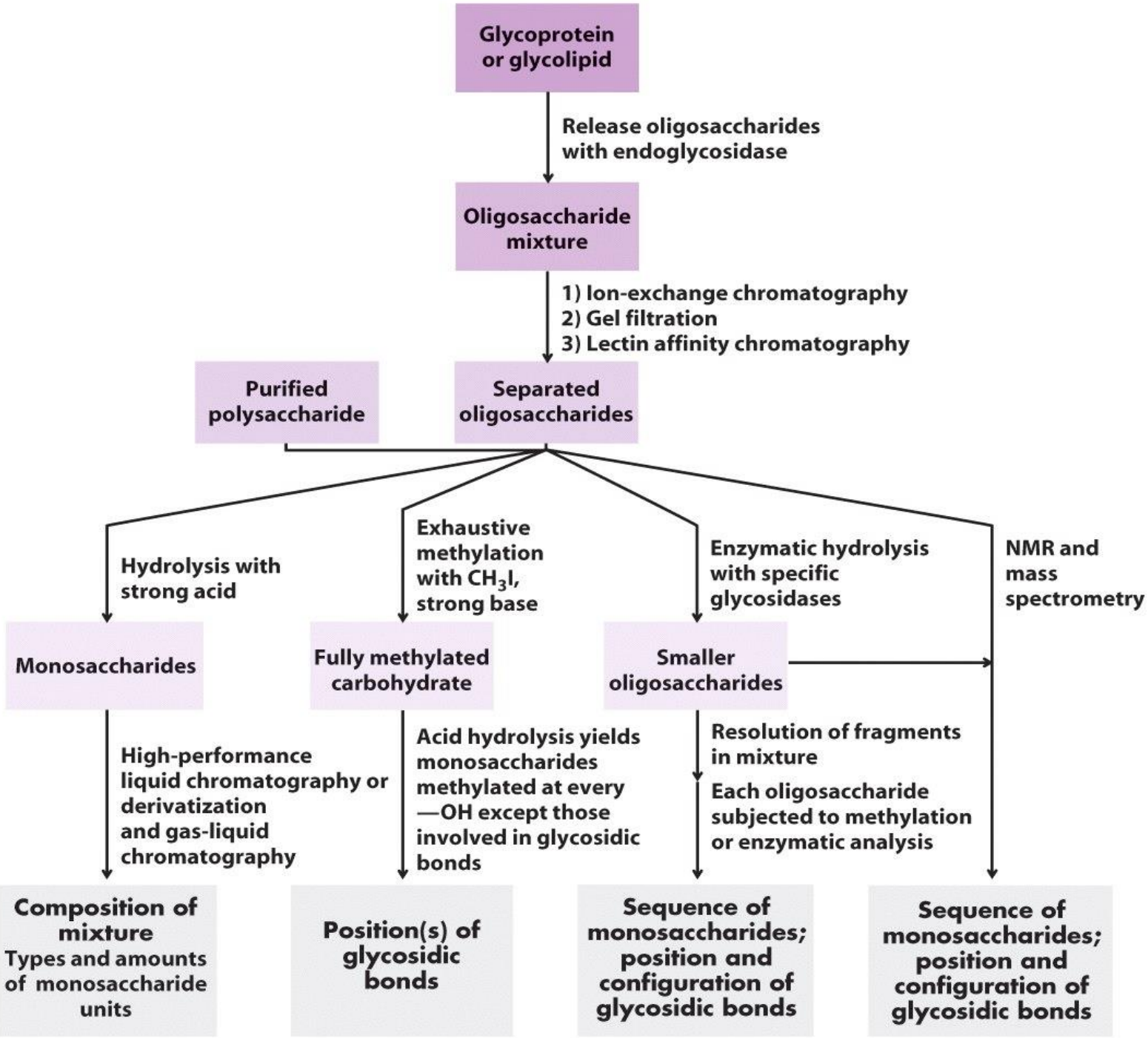


Some Conclusions

- Better to work with glycopeptides rather than the glycoprotein in the preparative (purification) steps if glycan analysis is a goal

  digest the glycoprotein

- remove the oligosaccharide from the peptide (enzymatically or chemically)
- combinations of exoglycosidases with mass spectrometry get sequence results


Glycoprotein or glycolipid
Release oligosaccharides with endoglycosidase
Oligosaccharide mixture
1) Ion-exchange chromatography
2) Gel filtration
3) Lectin affinity chromatography
Purified polysaccharide
Separated oligosaccharides
Hydrolysis with strong acid
Exhaustive methylation with CH_3I, strong base
Enzymatic hydrolysis with specific glycosidases
NMR and mass spectrometry
Monosaccharides
Fully methylated carbohydrate
Smaller oligosaccharides
High-performance liquid chromatography or derivatization and gas-liquid chromatography
Acid hydrolysis yields monosaccharides methylated at every —OH except those involved in glycosidic bonds
Resolution of fragments in mixture
Each oligosaccharide subjected to methylation or enzymatic analysis
Composition of mixture
Types and amounts of monosaccharide units
Position(s) of glycosidic bonds
Sequence of monosaccharides; position and configuration of glycosidic bonds
Sequence of monosaccharides; position and configuration of glycosidic bonds

Microanalysis of N-linked oligosaccharides in a glycoprotein by capillary liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry

Nana Kawasaki,* Satsuki Itoh, Miyako Ohta, and Takao Hayakawa

be also terminated with Gal by α1,3-linkage. CapLC/MS/MS and exoglycosidase followed by CapLC/MS were able to elucidate the detailed carbohydrate structure of HGF through yielding the information of monosaccharide sequence, and linkages.

Oligosaccharides

- Oligosaccharides are important group of polymeric carbohydrates that are found in all living organisms.
- Oligosaccharides composed of **2 to 10** monosaccharide residues.
- These monosaccharide's linked together by glycoside (α-1,4 or α-1,6) bonds.
- The discovery of new enzymes helps in developing other oligosaccharides of monosaccharide's with other linked bonds.

Trehalose (α,α 1,1), **Gentio-oligosaccharides** (β-1,6), **Nigero-oligosaccharides** (α-1,3), **Cyclodextrin** (α-1-4).

Oligosaccharides groups

- Sucrose-related oligosaccharides.
- Starch-related oligosaccharides.
- Lactose-related oligosaccharides.
- Others-oligosaccharides.

Oligosaccharides Substrates

Oligosaccharides	Substrate
• Fructo-oligosaccharide	Sucrose/Innulin.
• Malto- oligosaccharide	Starch.
• Isomalto-oligosaccharide	Starch.
• Galacto-oligosaccharide	Lactose.
• Lactosucrose	Lactose+ sucrose.
• Lactulose	Lactose.
• Xylo- oligosaccharide	Xylan.
• Soy- oligosaccharide	Soy.

Properties

- Low sweetness intensity (*1/3 of sucrose*)
- Calorie free.
- Resistance to hydrolysis by digestive enzymes.
- Non-cariogenic (*inhibit the growth of Streptococcus mutans*)
- Highly soluble than sucrose.
- Heat stable (*doesn't degrade by heating process*)
- Hydrolyze in high acid environment.

Benefits

- Prebiotic (e*nhance befidus bacteria in colon*).
- Increase digestion of lactose metabolism.
- Increase mineral absorption.
- Increase HDL/LDL ratio.
- Decrease serum lipids and blood cholesterol.
- Decrease blood pressure.
- Decrease glycemic response.
- Decrease fecal PH, toxic, and carcinogenic metabolites.

Mass spectrometry of oligosaccharides

- **Mass spectrometry is an important tool for the structural analysis of carbohydrates**, and offers precise results, analytical versatility, and very high sensitivity.
- Whereas mass spectrometric analysis options for **proteins** and **peptides** are well-defined **relative** to those for **carbohydrates**.

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1. An On-Line Platform for the Analysis of Permethylated **Oligosaccharides** Using Capillary Liquid Chromatography-Electrospray Ionization-Tandem **Mass** Spectrometry.
   Jang KS, Park HM, Kim YG, Kim BG, Kim JH.
   J Nanosci Nanotechnol. 2015 May;15(5):3962-6.
   PMID: 26505032
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Réka Balogh, Péter Jankovics, Szabolcs Béni
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Matrix-assisted laser desorption/ionization mass spectrometry analysis of glycans with co-derivatization of asparaginyl-oligosaccharides Original Research Article
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Wenjie Gao, Gaozhi Ou, Xiaojun Feng, Bi-Feng Liu, Houjin Zhang, Xin Liu
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Characterization of goat colostrum oligosaccharides by nano-liquid chromatography on chip quadrupole time-of-flight mass spectrometry and hydrophilic interaction liquid chromatography-quadrupole mass spectrometry Original Research Article
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A. Martín-Ortiz, J. Salcedo, D. Barile, A. Bunyatratchata, F.J. Moreno, I. Martin-García, A. Clemente, M.L. Sanz, A.I. Ruiz-Matute
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Profiling pneumococcal type 3-derived oligosaccharides by high resolution liquid chromatography–tandem mass spectrometry Original Research Article
Journal of Chromatography A, Volume 1397, 5 June 2015, Pages 43-51

Analytica Chimica Acta

Volume 896, 8 October 2015, Pages 102–110

Matrix-assisted laser desorption/ionization mass spectrometry analysis of glycans with co-derivatization of asparaginyl-oligosaccharides

Wenjie Gao[a], Gaozhi Ou[b], Xiaojun Feng[a], Bi-Feng Liu[a], Houjin Zhang[a,], Xin Liu[a,]

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Food Chemistry

Volume 176, 1 June 2015, Pages 487–492

Analytical Methods

Simultaneous determination of monosaccharides and oligosaccharides in dates using liquid chromatography–electrospray ionization mass spectrometry

Ayman A. Ghfar[a], Saikh M. Wabaidur[a], A. Yacine Badjah Hadj Ahmed[a], Zeid A. Alothman[a], Mohammad R. Khan[a], Nora H. Al-Shaalan[b]

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Analytical Biochemistry

Volume 433, Issue 1, 1 February 2013, Pages 28–35

Quantification of neutral human milk oligosaccharides by graphitic carbon high-performance liquid chromatography with tandem mass spectrometry

Yuanwu Bao, Ceng Chen, David S. Newburg

Journal of Chromatography A

Available online 21 September 2015

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Characterization of goat colostrum oligosaccharides by nano-liquid chromatography on chip quadrupole time-of-flight mass spectrometry and hydrophilic interaction liquid chromatography-quadrupole mass spectrometry

A. Martín-Ortiz[a], J. Salcedo[b], D. Barile[b], A. Bunyatratchata[b], F.J. Moreno[c], I. Martin-García[d], A. Clemente[d], M.L. Sanz[a,], , A.I. Ruiz-Matute[a]

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Journal of Chromatography A

Volume 1397, 5 June 2015, Pages 43–51

Profiling pneumococcal type 3-derived oligosaccharides by high resolution liquid chromatography–tandem mass spectrometry

Guoyun Li[a, b], Lingyun Li[b], Changhu Xue[a], Dustin Middleton[f], Robert J. Linhardt[b, c, d, e], Fikri Y. Avci[f]

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Analytica Chimica Acta

Volume 807, 7 January 2014, Pages 84–95

Deciphering the structure of isomeric oligosaccharides in a complex mixture by tandem mass spectrometry: Photon activation with vacuum ultra-violet brings unique information and enables definitive structure assignment

David Ropartz[a, 1], Jérôme Lemoine[b], Alexandre Giuliani[c, d], Yann Bittebière[a], Quentin Enjalbert[b, e], Rodolphe Antoine[e], Philippe Dugourd[e], Marie-Christine Ralet[a], Hélène Rogniaux[a, 1]

Received 28 August 2013, Revised 8 November 2013, Accepted 8 November 2013, Available online 16 November 2013

Analytica Chimica Acta

Volume 843, 16 September 2014, Pages 27–37

Structural analysis of isomeric chondroitin sulfate oligosaccharides using regioselective 6-*O*-desulfation method and tandem mass spectrometry

Shu-Ting Chen, Guor-Rong Her · ✉

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Journal of Chromatography A

Volume 1397, 5 June 2015, Pages 43–51

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Guoyun Li[a, b], Lingyun Li[b], Changhu Xue[a], Dustin Middleton[f], Robert J. Linhardt[b, c, d, e], , , Fikri Y. Avci[f],

Bioresource Technology

Volume 133, April 2013, Pages 221–231

Analysis of oligosaccharides in lignocellulosic biomass hydrolysates by high-performance anion-exchange chromatography coupled with mass spectrometry (HPAEC–MS)

Leon Coulier[a, b, 1], Ying Zha[b, c, 1], Richard Bas[d], Peter J. Punt[b, c]

Carbohydrate Research

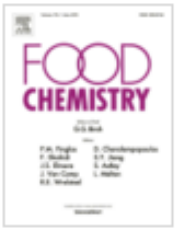
Volume 404, 2 March 2015, Pages 1–8

Identification of novel isomeric pectic oligosaccharides using hydrophilic interaction chromatography coupled to traveling-wave ion mobility mass spectrometry

Antonius G.M. Leijdekkers[a, b], Jie-Hong Huang[a], Edwin J. Bakx[a], Harry Gruppen[a], Henk A. Schols[a,]

Food Chemistry

Volume 176, 1 June 2015, Pages 487–492



Analytical Methods

Simultaneous determination of monosaccharides and oligosaccharides in dates using liquid chromatography–electrospray ionization mass spectrometry

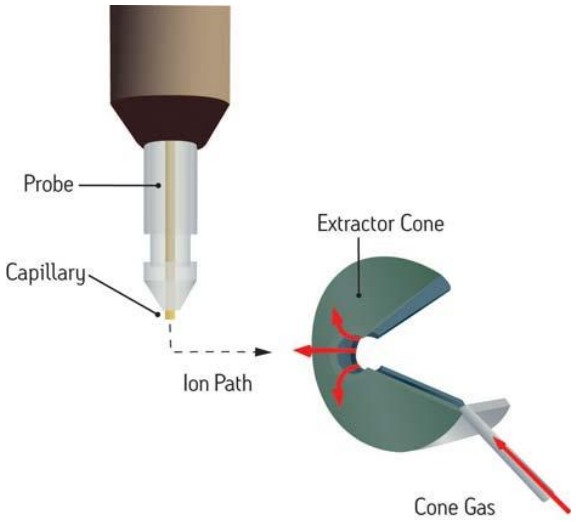
Characteristics of tandem mass spectra of **oligosaccharides**

1. **Electrospray Ionization (ESI)**

ESI MS: Conventional ESI MS involves the pumping of a solution (a forced flow) into the ion source, and has been observed to produce relatively weak ion signals for native oligosaccharides compared to those for peptides and proteins.

Nano ESI: on the other hand, produces ion signals that are comparable between the peptide and carbohydrate compound classes.

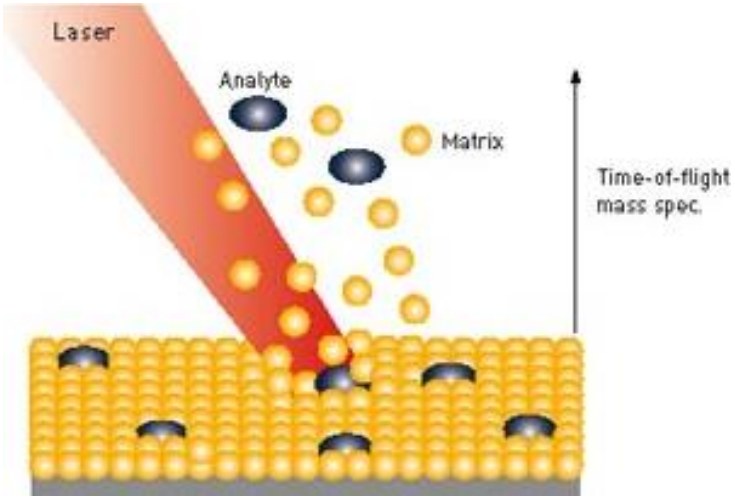
It, therefore, appears that the **hydrophilicity** of oligosaccharides **limits the surface activity** in ESI droplets and that, **with small droplets, their sensitivity is significantly enhanced**.



Characteristics of tandem mass spectra of **oligosaccharides**

2. **Matrix-Assisted Laser Desorption/Ionization (MALDI)**

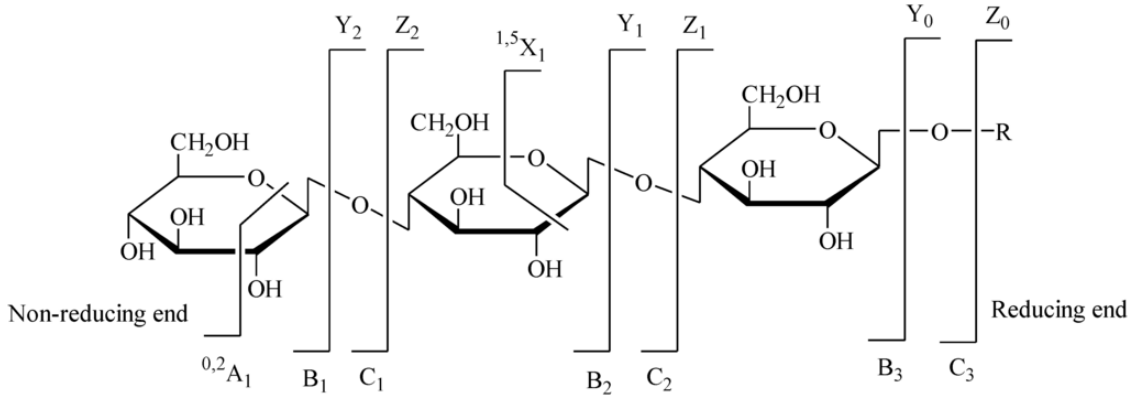
The MALDI-TOF ionization efficiency for neutral carbohydrates oligomers has been observed to **be constant as the size of the molecule increases**, in contrast to that for ESI, where the ionization efficiency decreases with an increasing molecular weight.



Characteristics of tandem mass spectra of **oligosaccharides**

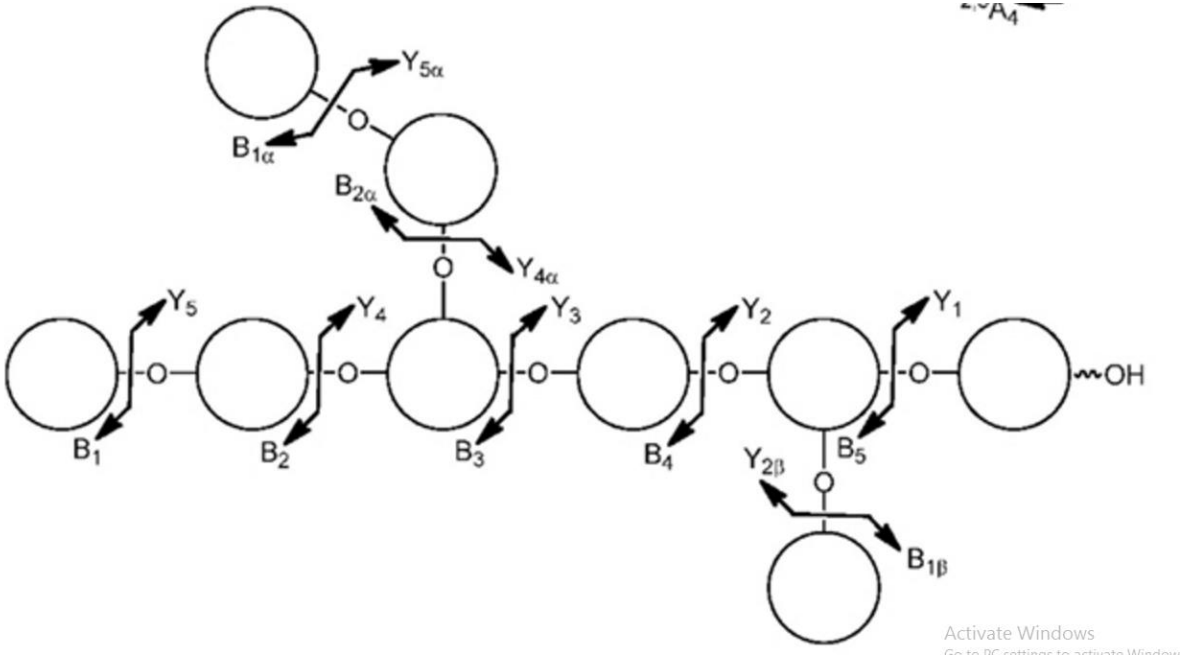
- **Note**: The **advantages** of MALDI in terms of ionization response have to be balanced against the **disadvantages** of the **metastable fragmentation** that is caused by the higher internal energies imparted to the ions relative to those resulting from ESI.

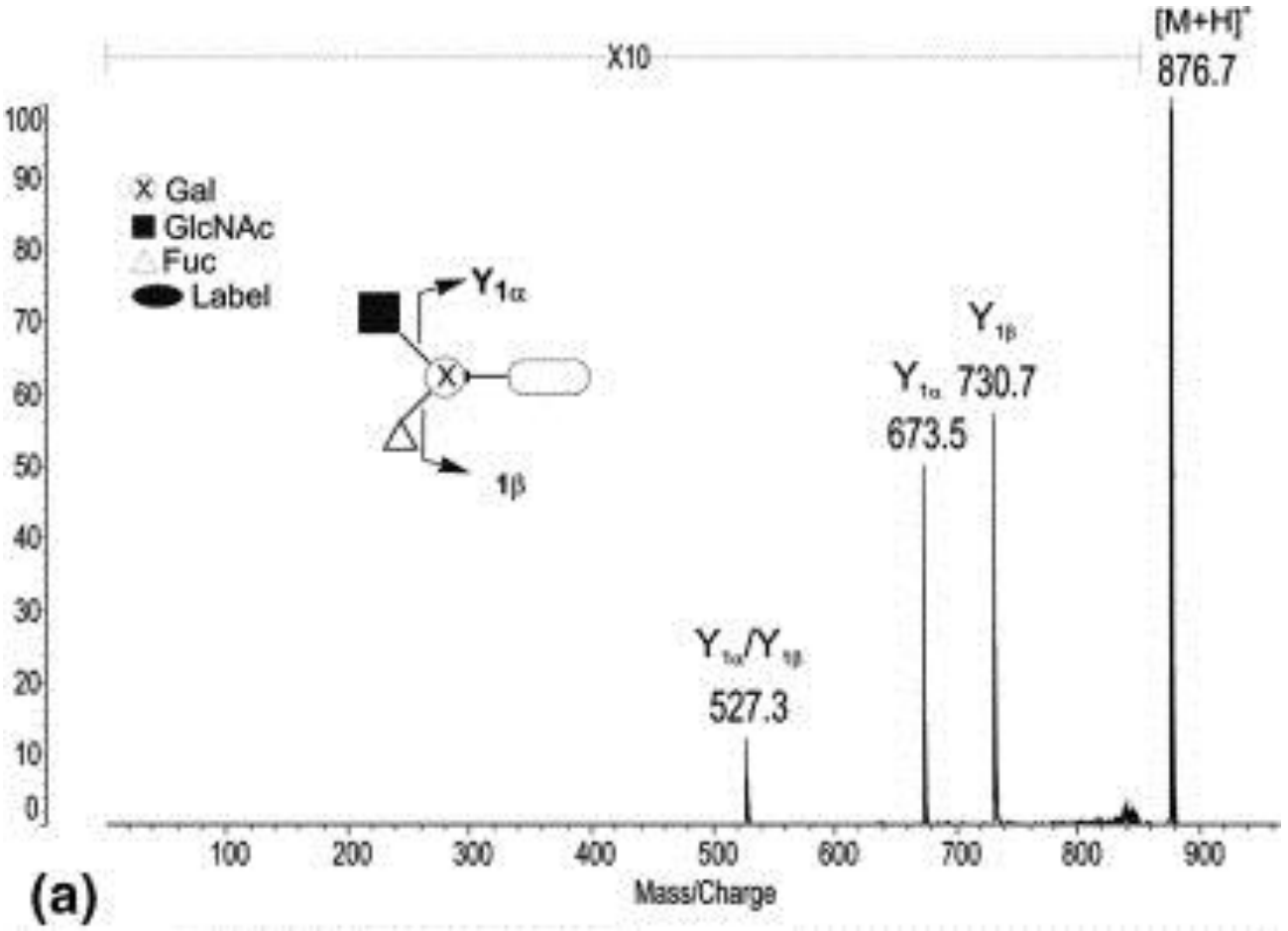
Nomenclature for the Fragmentation of Glycoconjugates

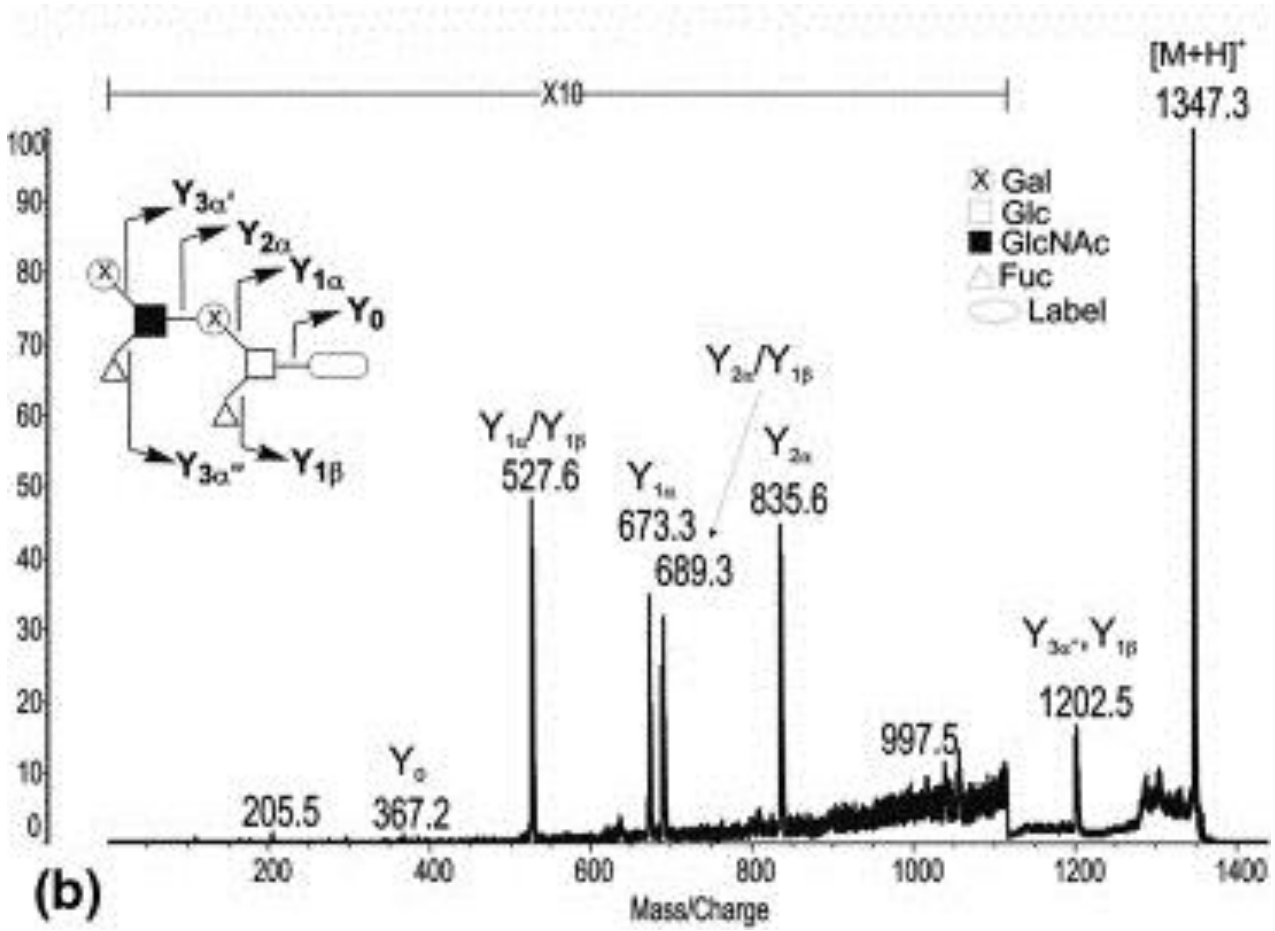


- Fragment ions that contain **a non-reducing terminus** are labeled with uppercase letters from the beginning of the alphabet (A, B, C),
- and those that contain **the reducing end** of the oligosaccharide or the aglycon are labeled with letters from the end of the alphabet (X, Y, Z); subscripts indicate the cleaved ions.
- The A and X ions are produced by cleavage across the glycosidic ring, **and are labeled by assigning each ring bond a number and counting clockwise.**

Nomenclature for the Fragmentation of Glycoconjugates




X10
[M+H]+
876.7
X Gal
GlcNAc
Fuc
Label
Y1α
1β
Y1β
Y1α 730.7
673.5
Y1α/Y1β
527.3
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600
700
800
900
Mass/Charge
(a)


[M+H]+
1347.3
X10
Gal
Glc
GlcNAc
Fuc
Label
Y3α'
Y2α
Y1α
Y0
Y3α''
Y1β
Y1α/Y1β
527.6
Y1α
673.3
689.3
Y2α/Y1β
Y2α
835.6
Y3α''+Y1β
1202.5
997.5
Y0
205.5
367.2
100
90
80
70
60
50
40
30
20
10
0
200
400
600
800
1000
1200
1400
Mass/Charge
(b)

Tandem MS of Native Oligosaccharide Molecular Ions

- 1. Protonated Ions: [M+H]+
- 2. Deprotonated Ions: [M–H]- ions
- 3. Alkali and Alkaline Earth Adducted Ions: [M(Li)+Li]+ ions

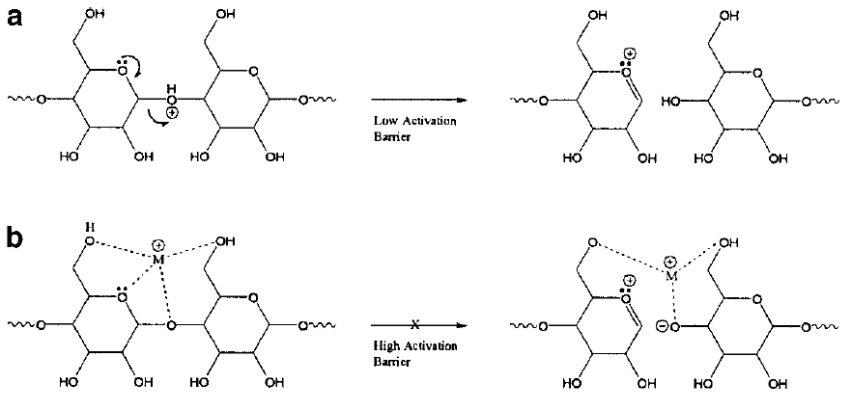
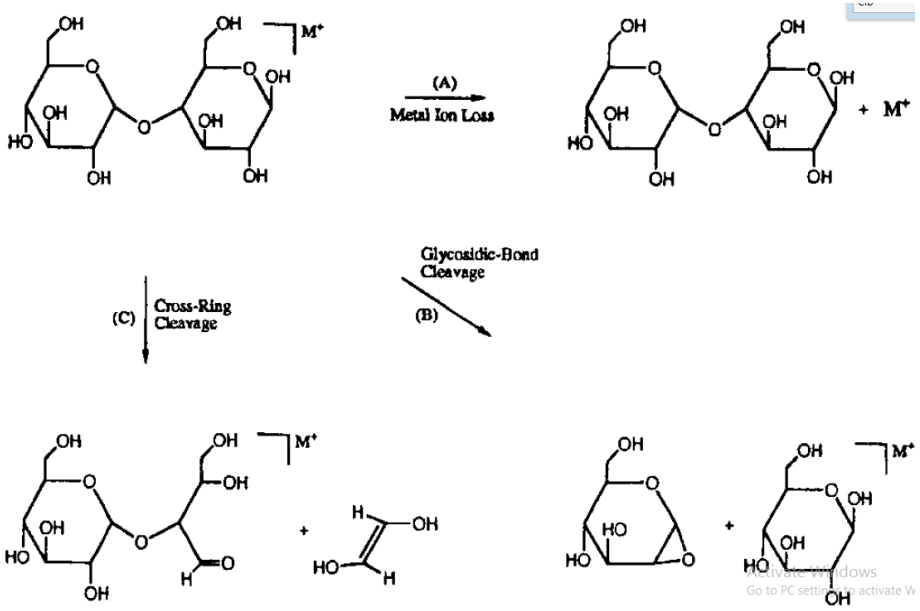


FIGURE 2. Fragmentation of (**a**) protonated and (**b**) alkali-cationized glycosidic bonds (Modified from Cancilla et al., 1996).

1996). Fragmentation yields were highest for oligomers with the least branching, and were inversely related to cation size, following the order $H^+ > Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. Mechanism of fragmentation of protonated ions is likely to be charge-induced, whereas that for cesiated ions is likely to be charge-remote. Charge-remote fragmentation requires more energy than charge-induced fragmentation, and the degree to which this occurs increases with increasing cation size.

Three possible fragmentation pathways for metal-cationized oligosaccharides



Computer-Based Approaches for Interpretation of Oligosaccharide Product-Ion Mass Spectra

- (1) monosaccharide residue loss from the non-reducing termini
- (2) subsequent monosaccharide residue losses from the aforementioned ions, generating a Y_n ion series
- (3) the complementaryBm ions
- (4) formation of internal fragment ions from loss of pyridylamidated residues from the Y_n Ions
- (5) formation of internal fragments from $_{Bm}$ ions
- (6) formation of Z_n, as well as the complementary C_m ions.

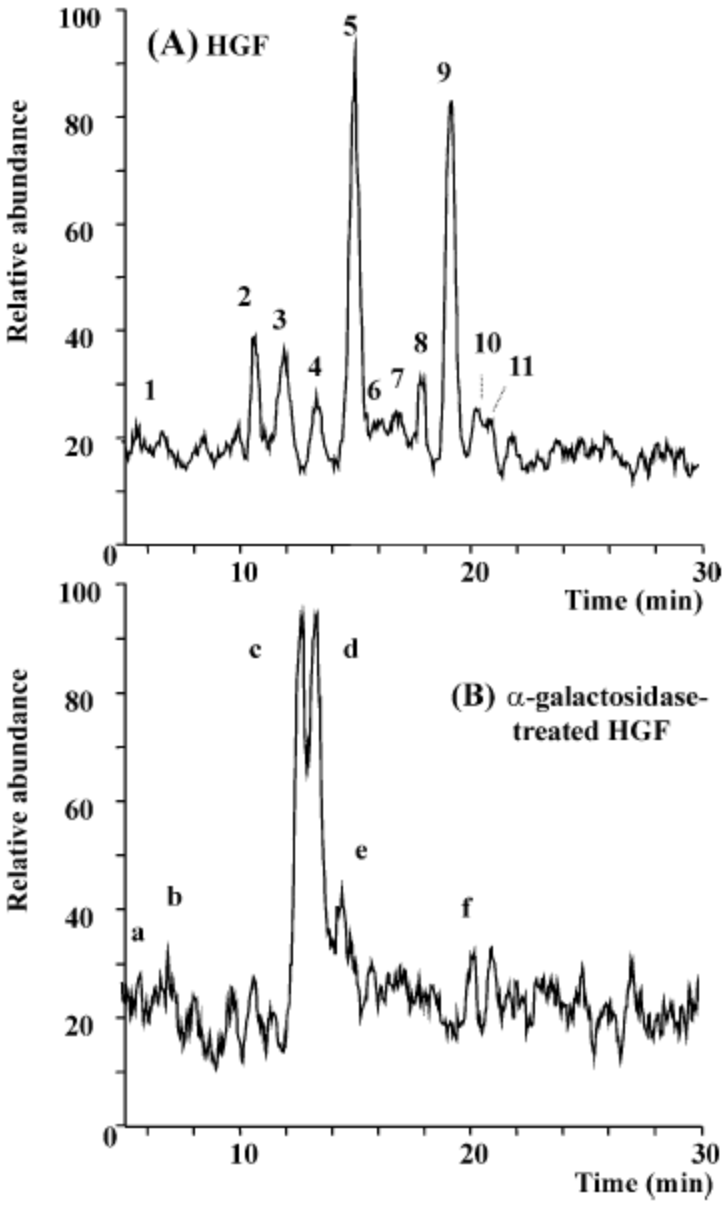
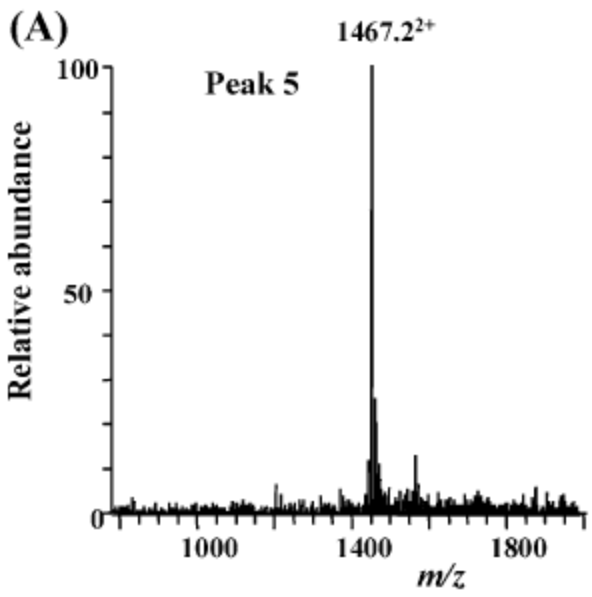
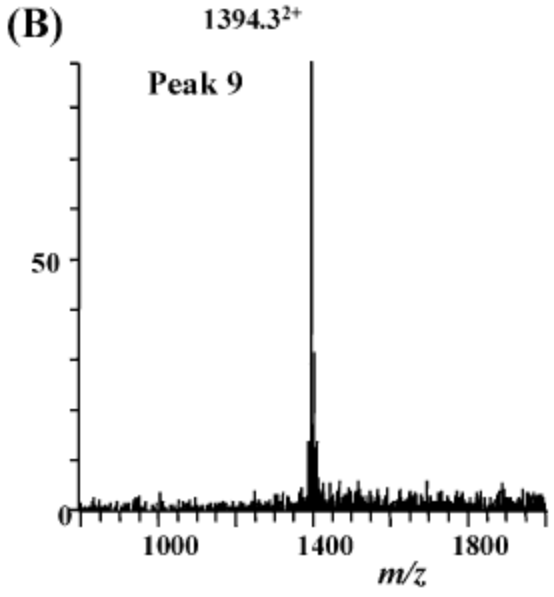


Fig. 2. Full-scan mass chromatography of borohydride-reduced oligosaccharides from HGF (200 ng) using CapLC/MS (A). Full-scan mass chromatography of α-galactosidase-treated borohydride-reduced oligosaccharides from HGF (200 ng) (B).

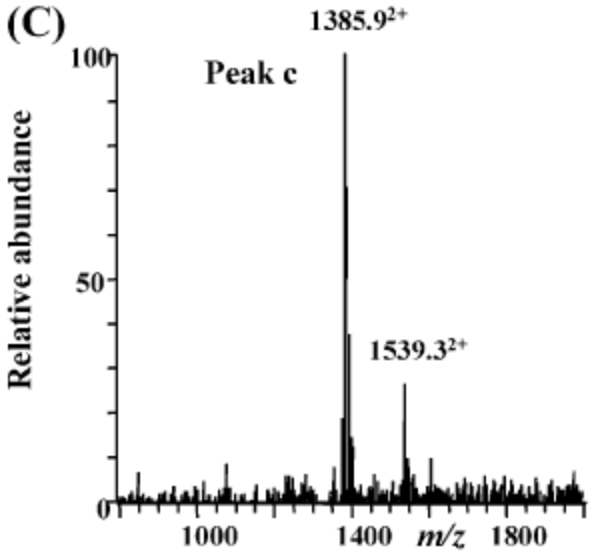
Exoglycosidase digestion followed by mass spectrometric sugar mapping was performed to determine the Hex and its linkage. Treatment with α-galactosidase, which cleaves Galα1–3,4,6Gal/Glc, resulted in new

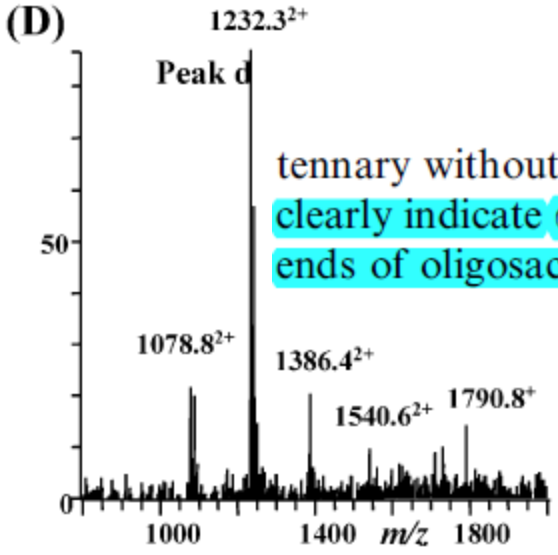




(peak 5) by losing one Gal (

(peak 9) by losing two Gal





tennary without additional Hex (Table 2). These results clearly indicate α-linked galactosylation on nonreducing ends of oligosaccharides in HGF.

Fig. 3. Mass spectra of peak 5 (A), peak 9 (B), peak c (C), and peak d (D) in Fig. 2.

Carbohydrate compositions and theoretical and calculated masses of peaks in Fig. 2A

Peak	Carbohydrate composition	Deduced carbohydrate structure	Theoretical mass[a]	Calculated mass	Charge state	Observed *m/z*
1	$[dHex]_1[Hex]_5[HexNAc]_4[NeuGc]_2$	FucBi-$NeuGc_2$[b]	2402.8	2403.6	2+	1202.8
2	$[dHex]_1[Hex]_6[HexNAc]_4[NeuGc]_1$	FucBi-Gal_1NeuGc_1	2257.8	2258.9	2+	1130.4
3	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_2$	FucTri-$NeuGc_2$[c]	2768.0	2769.8	2+	1385.9
	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_3$	FucTri-$NeuGc_3$	3075.1	3077.8	2+	1539.9
4	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_1$	FucTri-$NeuGc_1$	2460.9	2461.6	2+	1231.8
	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_3$	FucTri-$NeuGc_3$	3075.1	3077.2	2+	1539.6
5	$[dHex]_1[Hex]_7[HexNAc]_5[NeuGc]_2$	FucTri-Gal_1NeuGc_2	2930.0	2932.4	2+	1467.2
6	$[dHex]_1[Hex]_7[HexNAc]_4$	FucBi-Gal_2	2112.8	2114.6	2+	1058.3
	$[dHex]_1[Hex]_7[HexNAc]_5[NeuGc]_2$	FucTri-Gal_1NeuGc_2	2930.0	2932.0	2+	1467.0
7	$[dHex]_1[Hex]_7[HexNAc]_5[NeuGc]_1$	FucTri-Gal_1NeuGc_1	2622.9	2624.4	2+	1313.2
	$[dHex]_1[Hex]_7[HexNAc]_5[NeuGc]_2$	FucTri-Gal_1NeuGc_2	2930.0	2931.6	2+	1466.8
8	$[dHex]_1[Hex]_7[HexNAc]_5[NeuGc]_2$	FucTri-Gal_1NeuGc_2	2930.0	2931.6	2+	1466.8
9	$[dHex]_1[Hex]_8[HexNAc]_5[NeuGc]_1$	FucTri-Gal_2NeuGc_1	2785.0	2786.6	2+	1394.3
10	$[dHex]_1[Hex]_7[HexNAc]_6[NeuGc]_1$	FucTetra-$NeuGc_1$[d]	2826.0	2827.8	2+	1414.9
11	$[dHex]_1[Hex]_9[HexNAc]_5$	FucTri-Gal_3	2640.0	2641.0	2+	1321.5
	$[dHex]_1[Hex]_8[HexNAc]_5[NeuGc]_1$	FucTri-Gal_2NeuGc_1	2785.0	2787.4	2+	1394.7

[a] Monoisotopic mass value.

[b] FucBi–$Gal_m$$NeuGc_n$, Gal_m / $NeuGc_n$ { Gal–GlcNAc–Man, Gal–GlcNAc–Man } > Man–GlcNAc–GlcNAc–OH. (Fuc on GlcNAc)

[c] FucTri–$Gal_m$$NeuGc_n$, Gal_m / $NeuGc_n$ { Gal–GlcNAc–Man, Gal–GlcNAc–Man, Gal–GlcNAc } > Man–GlcNAc–GlcNAc–OH. (Fuc on GlcNAc)

[d] FucTetra–$NeuGc_1$, $NeuGc_1$ { Gal–GlcNAc, Gal–GlcNAc–Man, Gal–GlcNAc–Man, Gal–GlcNAc } > Man–GlcNAc–GlcNAc–OH. (Fuc on GlcNAc)

Carbohydrate compositions and theoretical and calculated masses of peaks in Fig. 2B

Peak	Carbohydrate composition	Deduced carbohydrate structure	Theoretical mass[a]	Calculated mass	Charge state	Observed *m/z*
a	$[dHex]_1[Hex]_5[HexNAc]_4[NeuGc]_2$	FucBi-$NeuGc_2$	2402.8	2405.4	2+	1203.7
b	$[dHex]_1[Hex]_5[HexNAc]_4[NeuGc]_1$	FucBi-$NeuGc_1$	2095.8	2098.2	2+	1050.1
c	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_2$	FucTri-$NeuGc_2$	2768.0	2769.8	2+	1385.9
	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_3$	FucTri-$NeuGc_3$	3075.1	3076.6	2+	1539.3
d	$[dHex]_1[Hex]_5[HexNAc]_4$	FucBi	1788.7	1789.8	1+	1790.8
	$[dHex]_1[Hex]_6[HexNAc]_5$	FucTri	2153.8	2155.6	2+	1078.8
	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_1$	FucTri-$NeuGc_1$	2460.9	2462.6	2+	1232.3
	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_2$	FucTri-$NeuGc_2$	2768.0	2770.8	2+	1386.4
	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_3$	FucTri-$NeuGc_3$	3075.1	3079.2	2+	1540.6
e	$[dHex]_1[Hex]_6[HexNAc]_5[NeuGc]_3$	FucTri-$NeuGc_3$	3075.1	3077.6	2+	1539.8
f	$[dHex]_1[Hex]_7[HexNAc]_6[NeuGc]_1$	FucTetra-$NeuGc_1$	2826.0	2825.2	2+	1413.6

[a] Monoisotopic mass value.

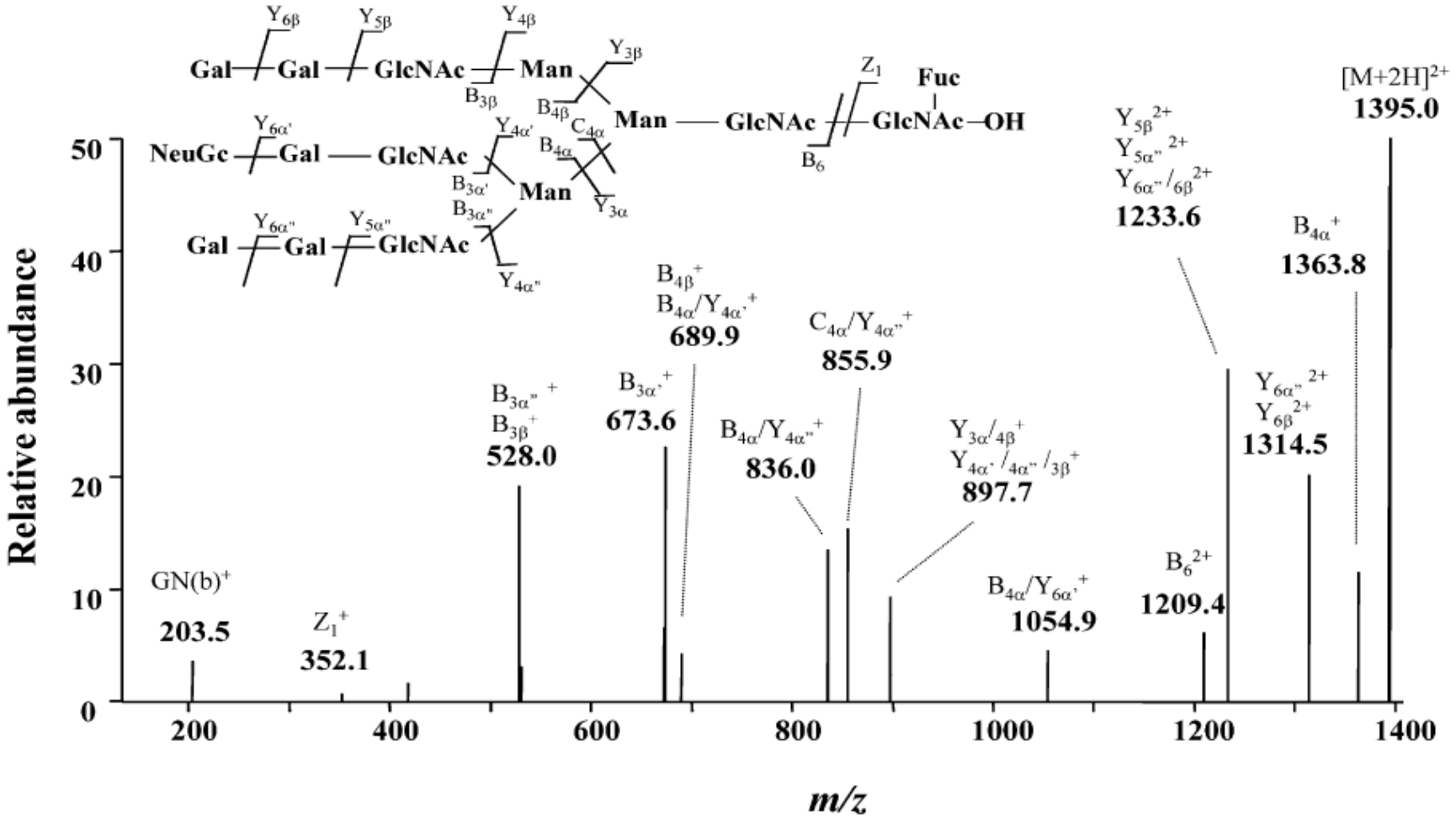


Fig. 4. MS/MS spectrum of the FucTri-$Gal_2NeuGc_1^{2+}$ at *m/z* 1395.

$[HexNAc]_4^{2+}$. These fragment ions suggest the monosaccharide sequence of FucTri-Gal_2NeuGc_1 shown in

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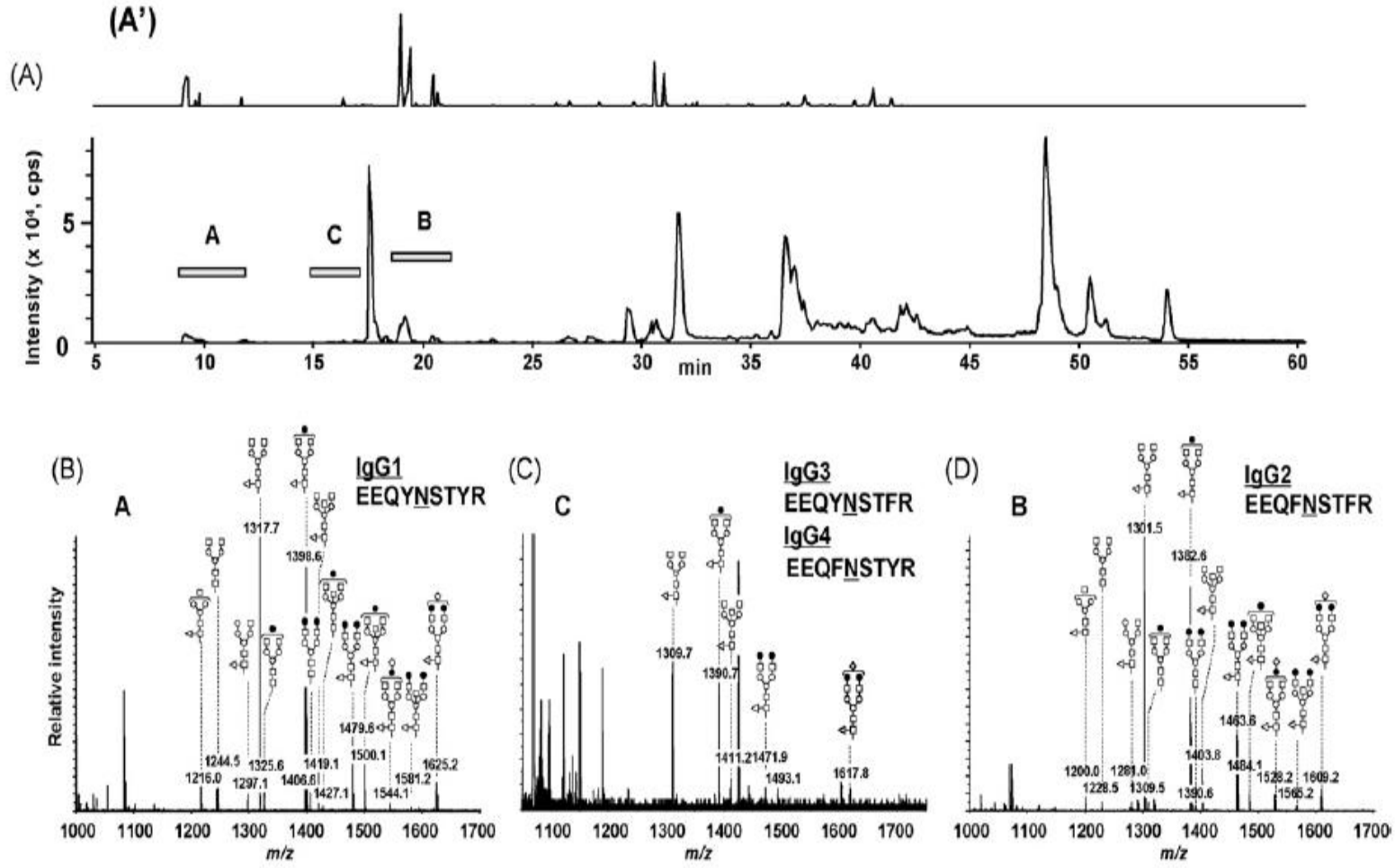

Fig. 5. Peptide map of commercially available human polyclonal IgG. (A) TIC (*m/z* 1000–2000) obtained by LC/MS/MS of trypsin-digested IgG. (A′) EIC (*m/z* 204.05–204.15) obtained by data-dependent MS/MS. (B) Mass spectrum of peak A, which was assigned as glycopeptides of EEQYNSTYR of IgG.1 (P01857). (C) Mass spectrum of peak C, which would be glycopeptides of EEQYNSTFR of IgG3 (CAA67886) and/or EEQFNSTYR of IgG4 (P01861). (D) Mass spectrum of peak B, which was assigned as glycopeptides of EEQFNSTFR of IgG2 (P01859).