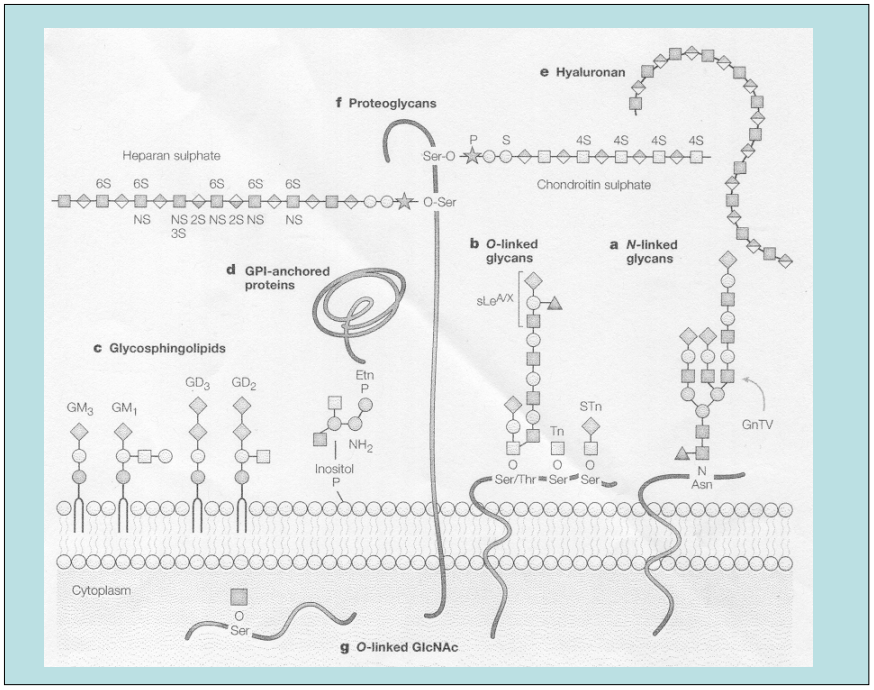
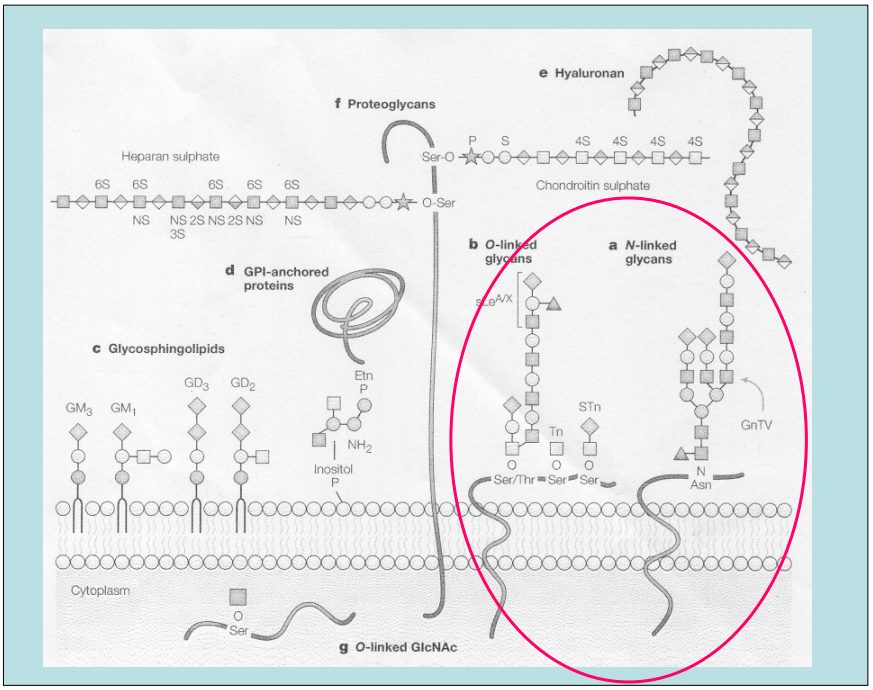
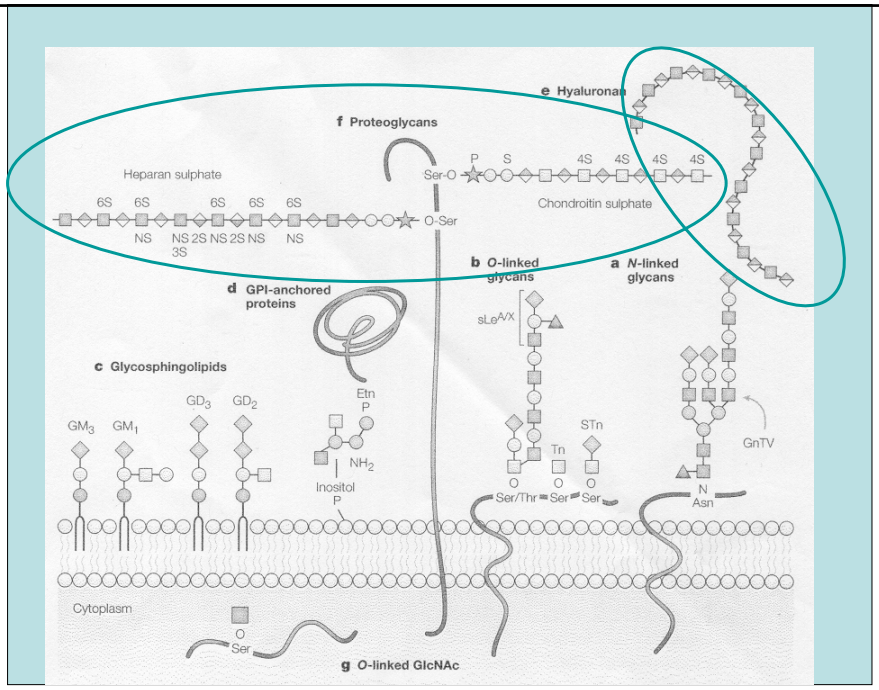
Carbohydrate structures in biopolymers

- *N*- and *O*- glycosylation of proteins
  - N-glycosylation (Asn)
  - O-glycosylation (Thr, Ser, Hyp)
  - non-enzymatic „glycation“ (Lys)
- Proteoglycanes
  - O-linked glucosaminoglycans (Ser)
    e.g. Chondroitan, Dematan, Heparan, Hyaluronan
- Free glucosaminoglycanes (GAGs)
  - Hyaluronan, Heparan
- GPI-anchor
- Glycolipids
  - Ganglioside




e Hyaluronan
f Proteoglycans
Heparan sulphate
Chondroitin sulphate
Ser-O
O-Ser
P S 4S 4S 4S 4S
6S 6S 6S 6S 6S
NS NS 2S NS 2S NS NS
3S
b O-linked glycans
a N-linked glycans
d GPI-anchored proteins
c Glycosphingolipids
GM3 GM1 GD3 GD2
Etn P
NH2
Inositol P
sLeA/X
Tn
STn
GnTV
O Ser/Thr
O Ser
O Ser
N Asn
Cytoplasm
O Ser
g O-linked GlcNAc


e Hyaluronan
f Proteoglycans
Heparan sulphate
Chondroitin sulphate
Ser-O
O-Ser
P S 4S 4S 4S 4S
6S 6S 6S 6S 6S
NS NS 2S NS 2S NS NS
3S
b O-linked glycans
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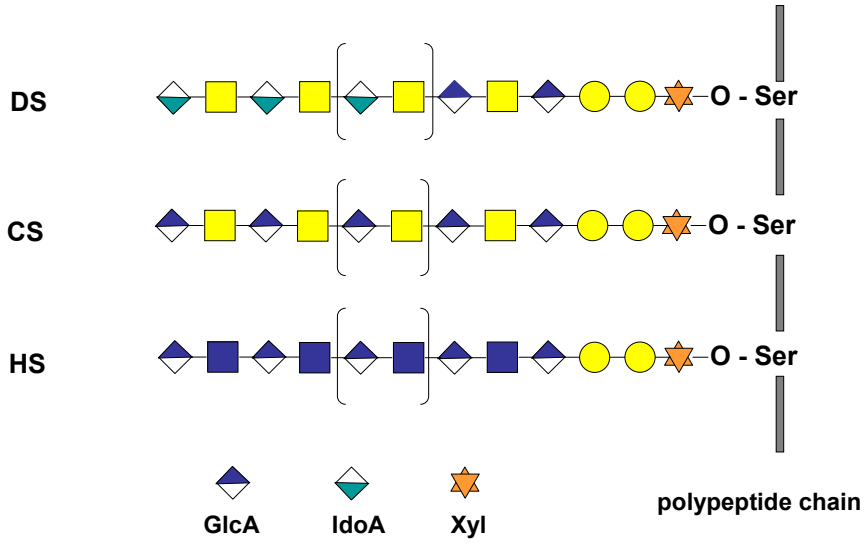
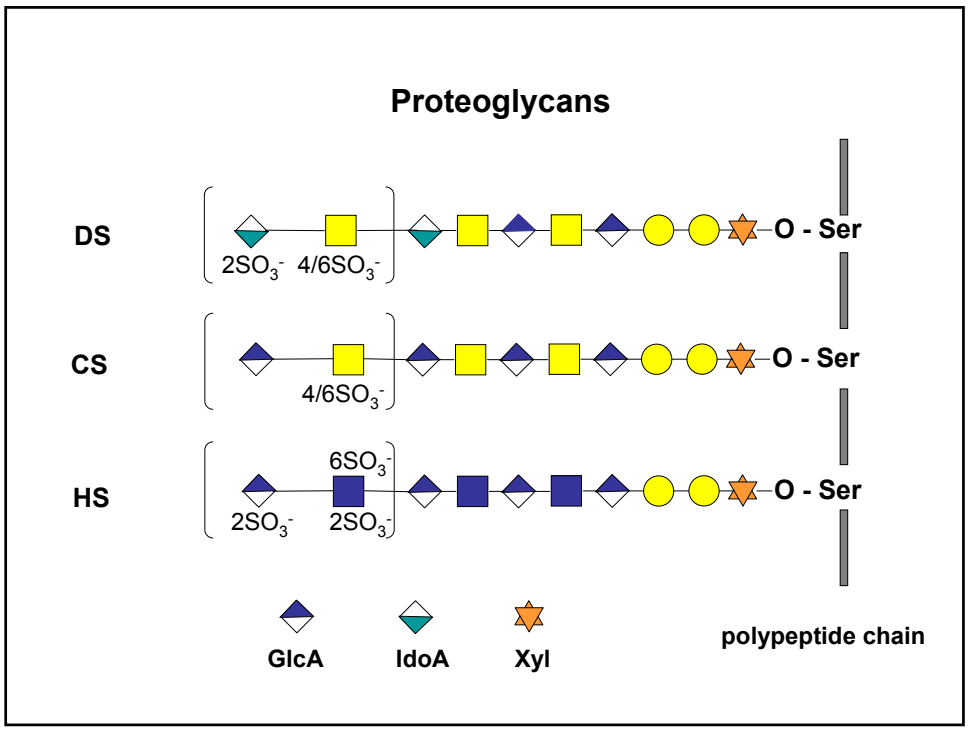
c Glycosphingolipids
GM3 GM1 GD3 GD2
Etn P
NH2
Inositol P
sLeA/X
Tn
STn
GnTV
O Ser/Thr
O Ser
O Ser
N Asn
Cytoplasm
O Ser
g O-linked GlcNAc

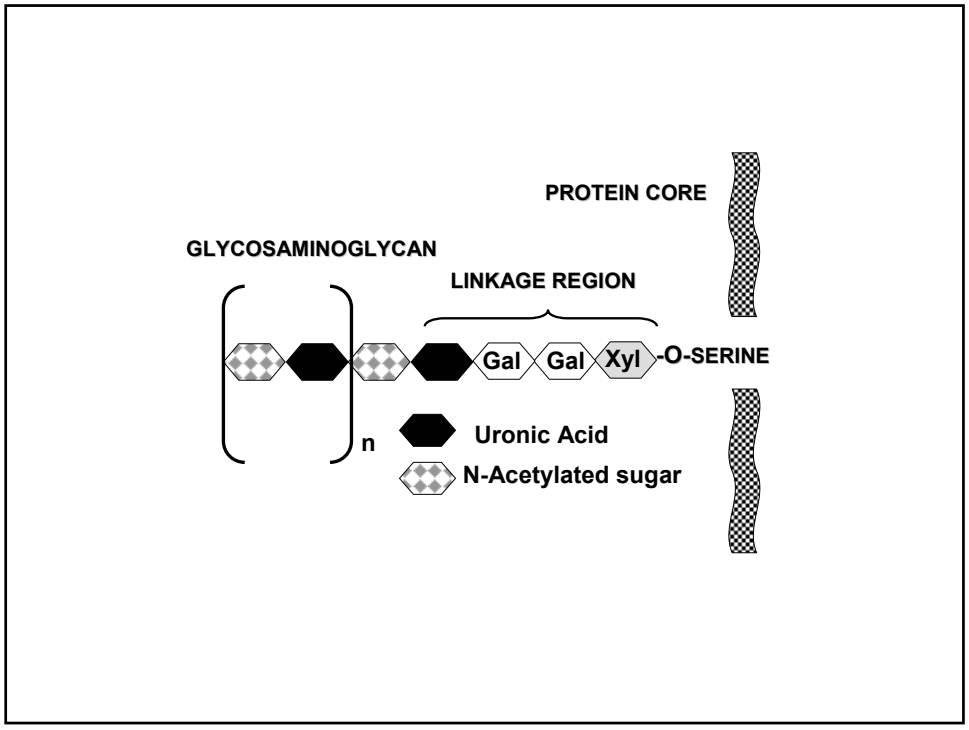
Glycosaminoglycane

GAGs

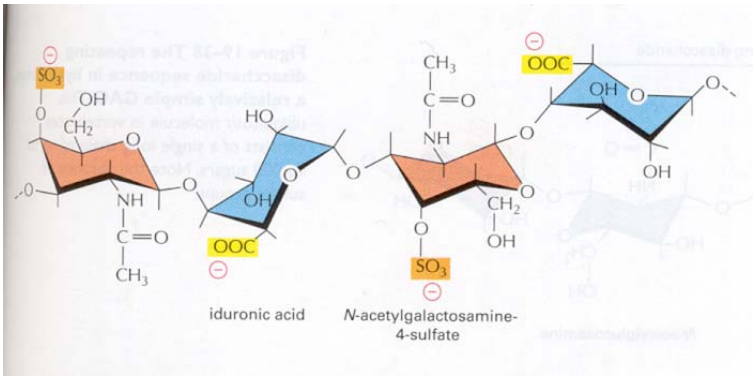
Proteoglycans




Proteoglycans
DS
2SO3- 4/6SO3-
O - Ser
CS
4/6SO3-
O - Ser
HS
6SO3-
2SO3- 2SO3-
O - Ser
GlcA
IdoA
Xyl
polypeptide chain

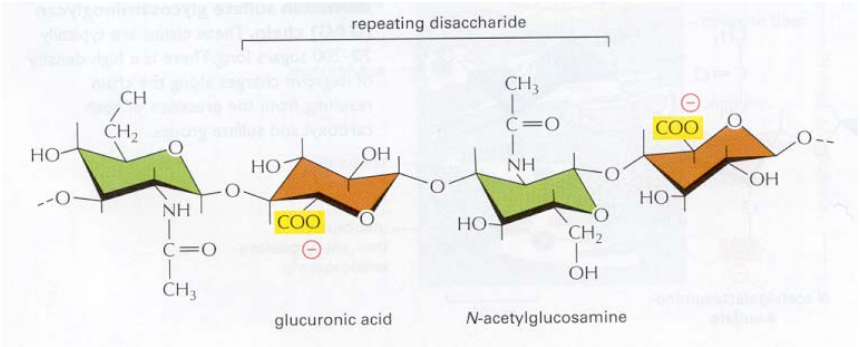

PROTEIN CORE
GLYCOSAMINOGLYCAN
LINKAGE REGION
Gal
Gal
Xyl
-O-SERINE
n
Uronic Acid
N-Acetylated sugar

Dermatansulfat

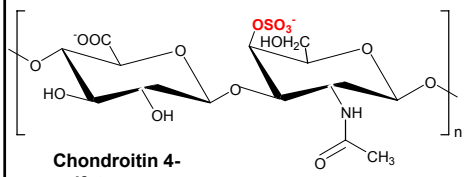


IdoA **GalNAc(4S)**

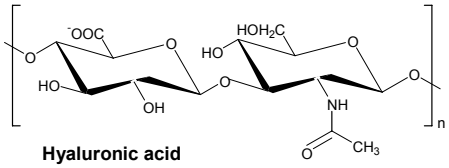
Heparansulfat



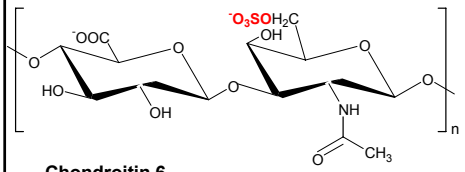
GlcA **GlcNAc**



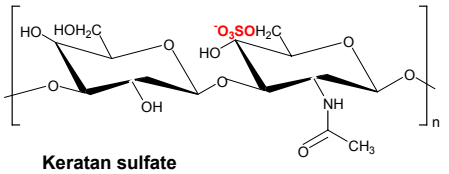
Chondroitin 4-sulfate



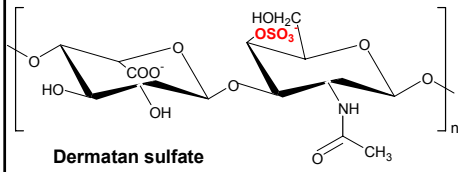
Hyaluronic acid



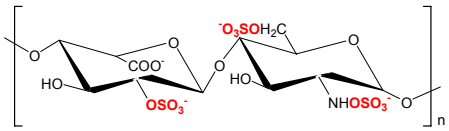
Chondroitin 6-sulfate



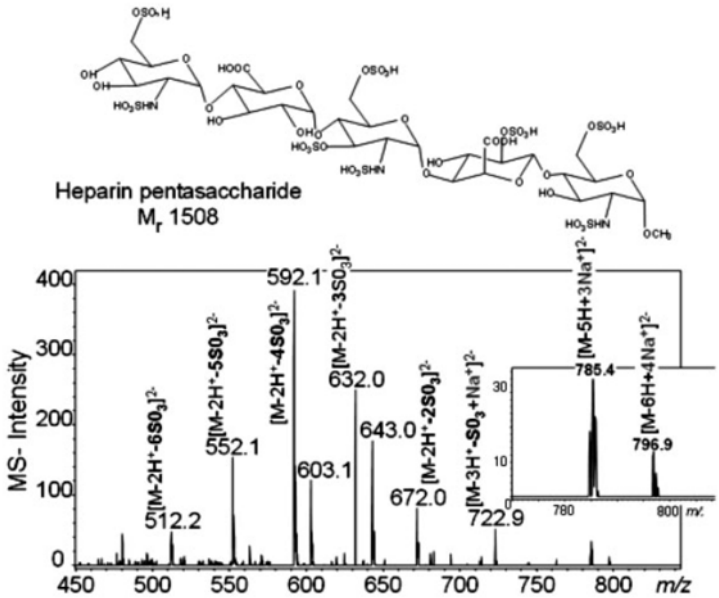
Keratan sulfate

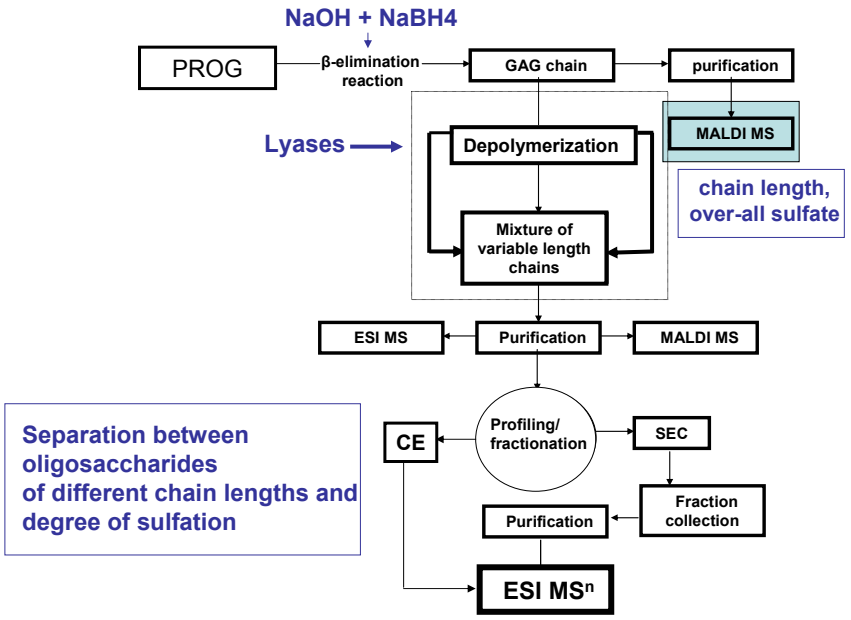


Dermatan sulfate



Heparin





MS of GAGs

Ionization in positive and negative mode possible

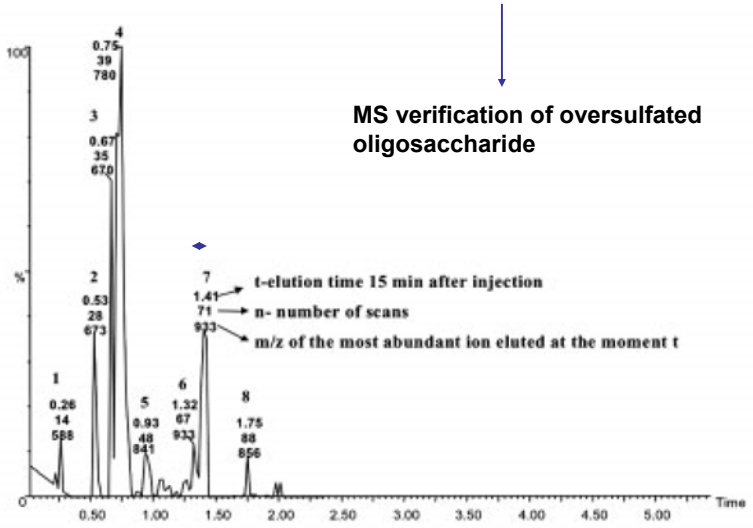
To distinguish between regularly-, under- and over-sulfated oligosaccharides

Challenges:

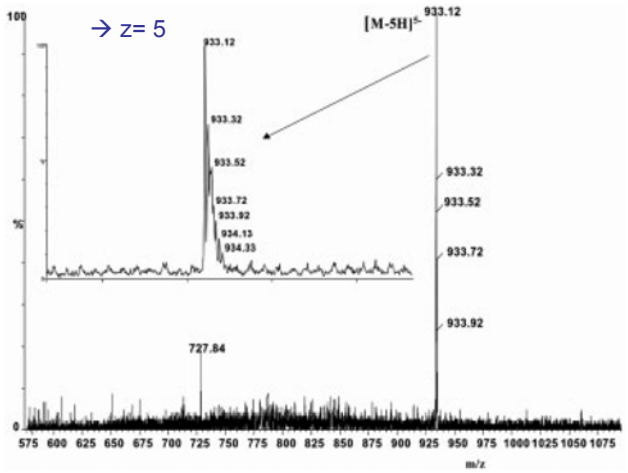
(-) Long GAG chains insufficiently ionized
(-) Very labile sulfate ester groups
(-) Over-sulfated ions difficult to detect in the presence of regularly sulfated ions

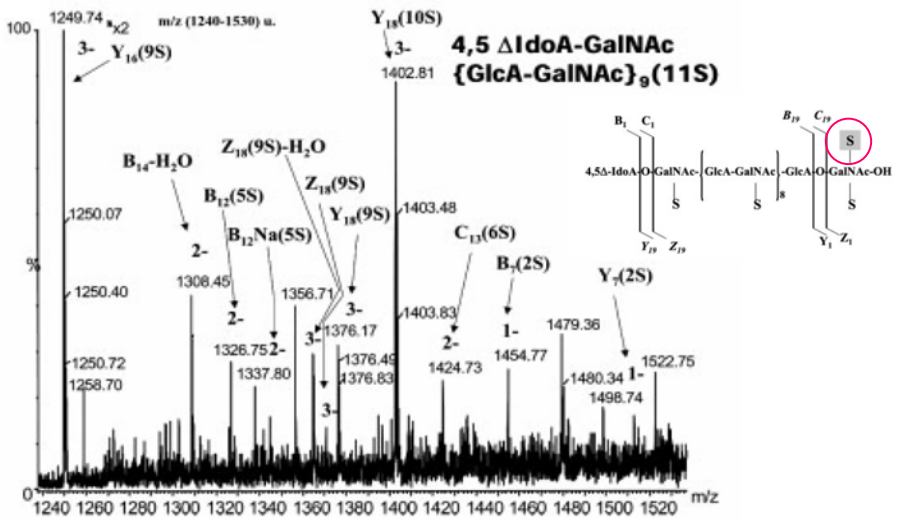
CZE separation of GAG derived oligosaccharides

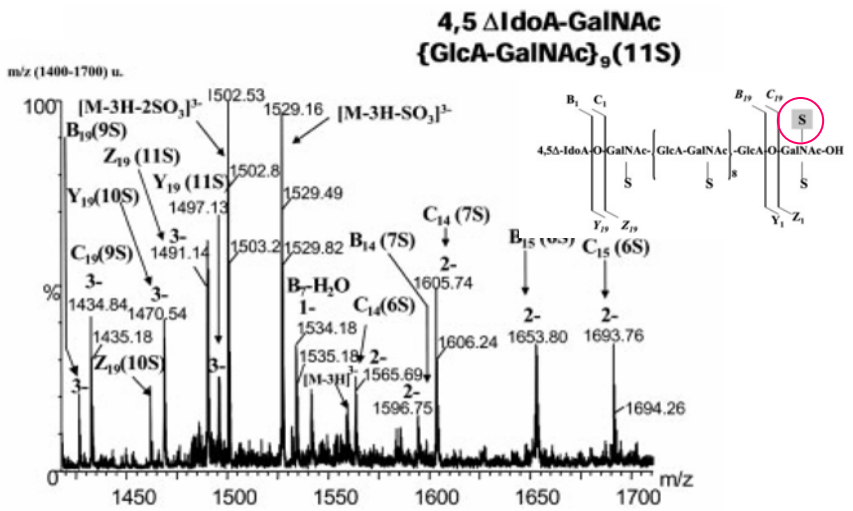
MS verification of oversulfated oligosaccharide



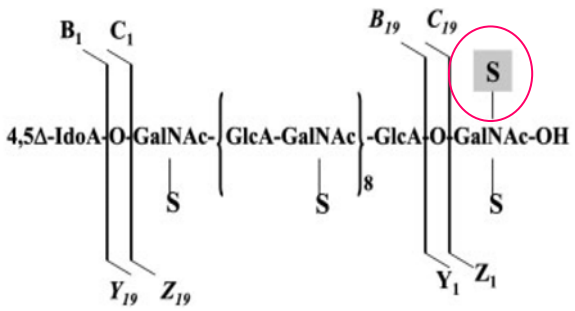
CZE-MS/MS of GAGs

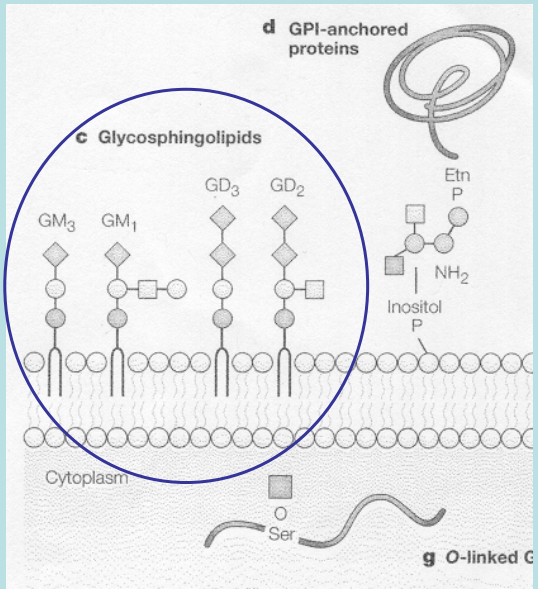



4,5 ΔIdoA-GalNAc
{GlcA-GalNAc}9(11S)
m/z (1240-1530) u.
1249.74
x2
3- Y16(9S)
Y18(10S)
3-
1402.81
B14-H2O
Z18(9S)-H2O
B12(5S)
Z18(9S)
Y18(9S)
B12Na(5S)
C13(6S)
B7(2S)
Y7(2S)
1250.07
1250.40
1250.72
1258.70
2-
1308.45
2-
1326.75
2-
1337.80
1356.71
3-
1376.17
3-
1376.49
1376.83
3-
1403.48
1403.83
2-
1424.73
1-
1454.77
1479.36
1480.34
1-
1498.74
1522.75
%
100
0
1240 1260 1280 1300 1320 1340 1360 1380 1400 1420 1440 1460 1480 1500 1520
m/z
4,5Δ-IdoA-O-GalNAc-{GlcA-GalNAc}s-GlcA-O-GalNAc-OH


4,5 ΔIdoA-GalNAc
{GlcA-GalNAc}9(11S)
m/z (1400-1700) u.
[M-3H-2SO3]3-
1502.53
1529.16
[M-3H-SO3]3-
B19(9S)
Z19 (11S)
Y19 (11S)
1502.8
1529.49
Y19(10S)
1497.13
C14 (7S)
3-
1491.14
1503.2
1529.82
B14 (7S)
C15 (6S)
C19(9S)
2-
1605.74
3-
1434.84
3-
1470.54
B7-H2O
1-
1534.18
C14(6S)
1435.18
1606.24
2-
1653.80
2-
1693.76
Z19(10S)
1535.18
2-
1565.69
[M-3H]3-
2-
1596.75
1694.26
%
100
0
1450 1500 1550 1600 1650 1700
m/z
4,5Δ-IdoA-O-GalNAc-{GlcA-GalNAc}s-GlcA-O-GalNAc-OH

MS verification of oversulfated deca-saccharide



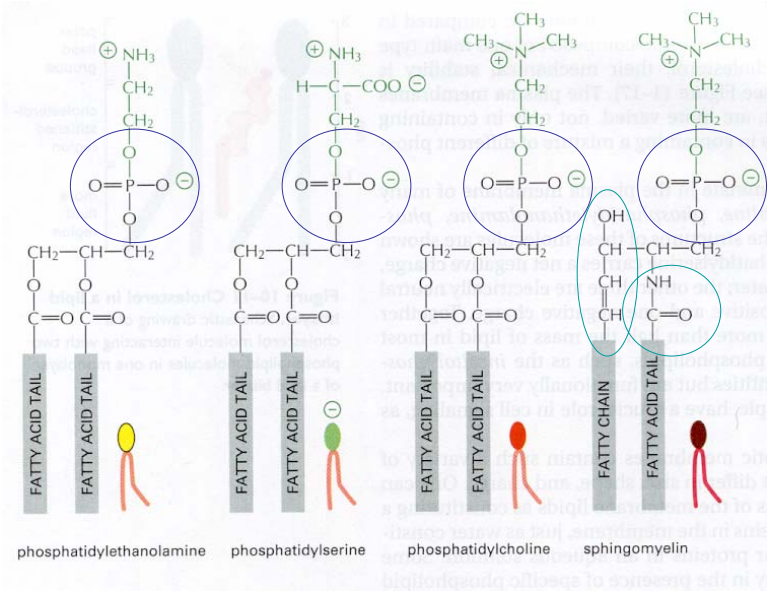


Glycolipide

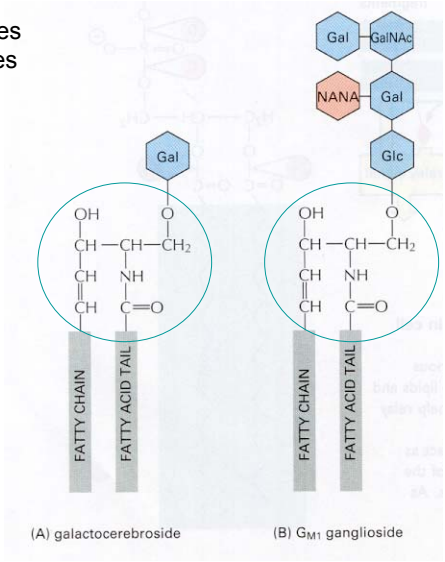
Glycosphingolipide

Ganglioside

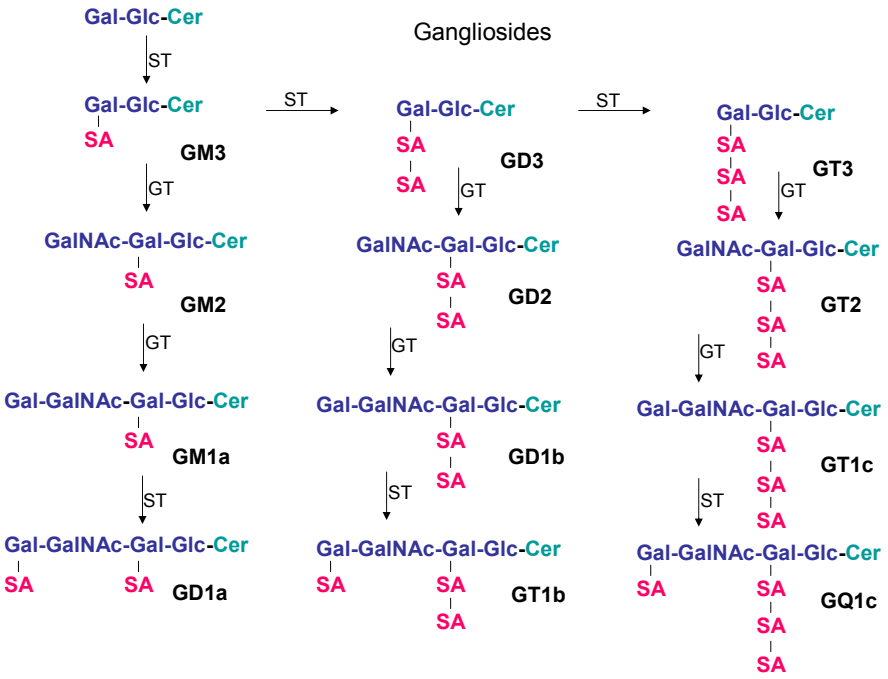
Phosphatidyl-Ester



Cerebrosides
Gangliosides



Gangliosides



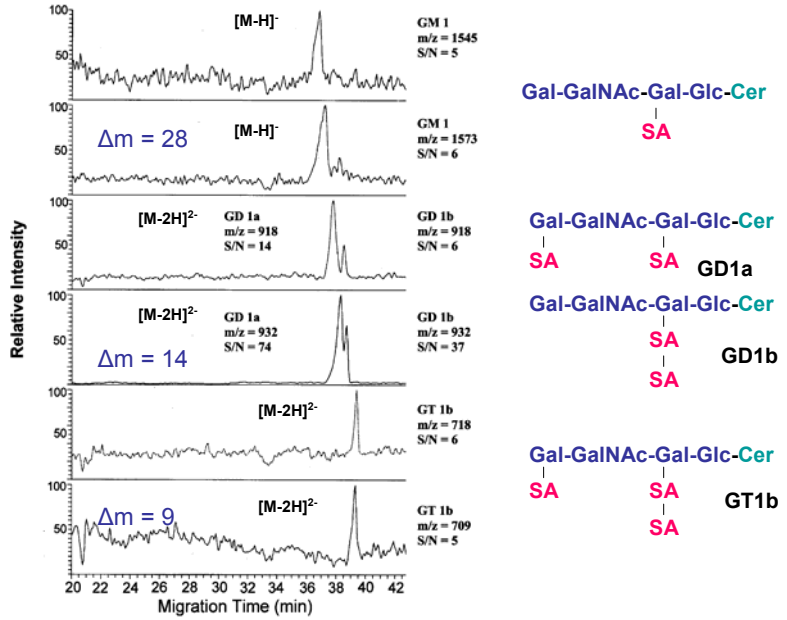


Figure 6.3

MS of Gangliosides

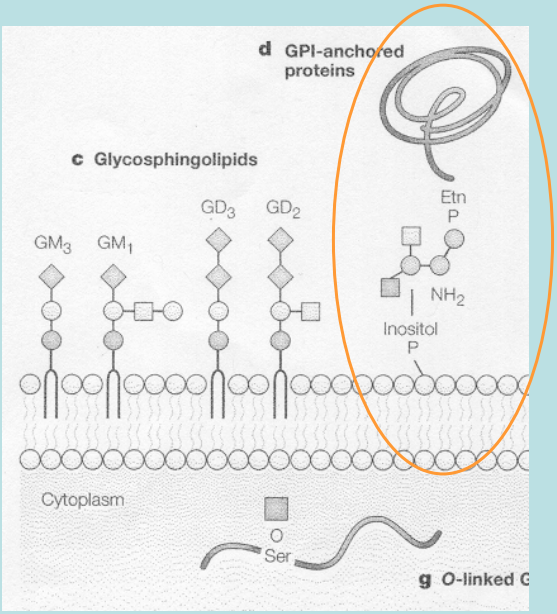
Ionization in negative mode

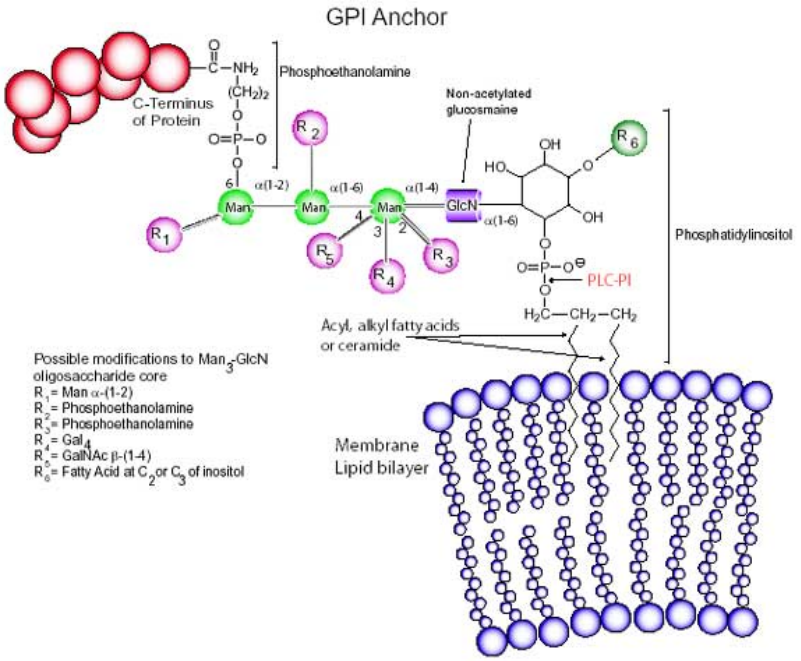
To distinguish between number of sialic acids and positions of attachment

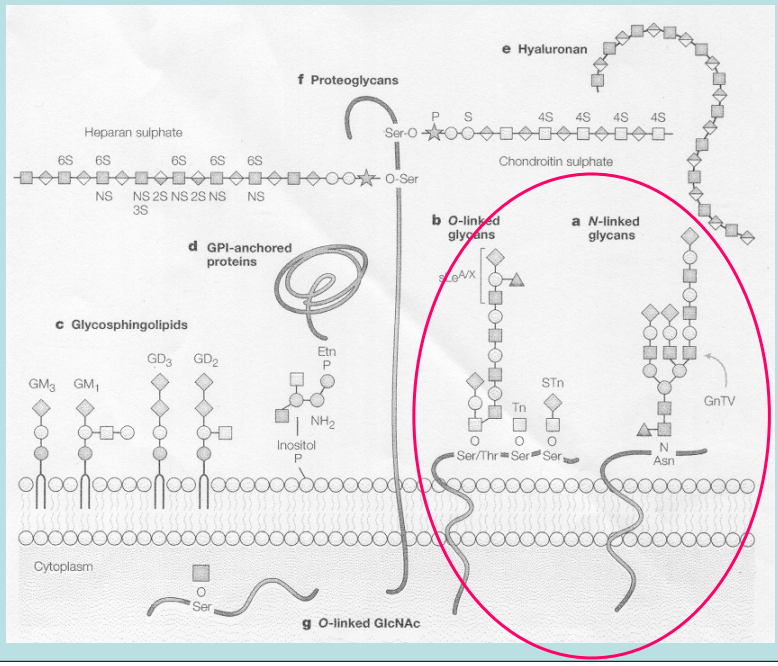
Challenges:

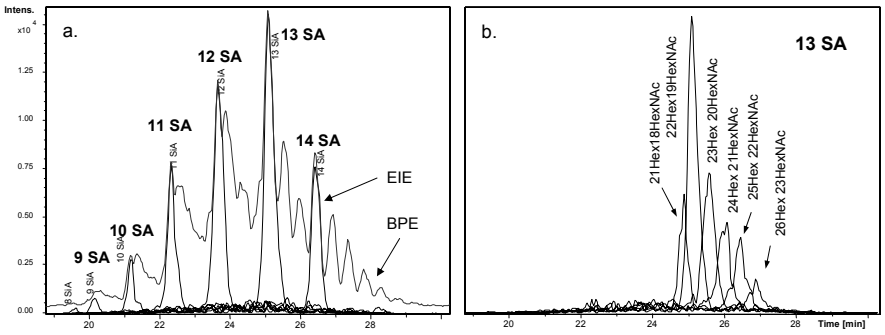
(-) positional isomers are isobaric

(-) difficult to distinguish by MS^n


d GPI-anchored proteins
c Glycosphingolipids
GM3
GM1
GD3
GD2
Etn
P
NH2
Inositol
P
Cytoplasm
O
Ser
g O-linked G


GPI Anchor
C-Terminus of Protein
Phosphoethanolamine
Non-acetylated glucosmaine
Phosphatidylinositol
PLC-PI
Acyl, alkyl fatty acids or ceramide
Possible modifications to Man3-GlcN oligosaccharide core
R1 = Man α-(1-2)
R2 = Phosphoethanolamine
R3 = Phosphoethanolamine
R4 = Gal4
R5 = GalNAc β-(1-4)
R6 = Fatty Acid at C2 or C3 of inositol
Membrane Lipid bilayer





a. Sialoforms

14 SiA. EIE 1776.2; 1887.2; 2012.9; 2156.7 (M: **30178.6**)
13 SiA: EIE 1869.0; 1993.6; 2135.8; 1759.1 (M: **29888.2**)
12 SiA: EIE 1720.5; 1827.9; 1949.7; 2089.0 (M: **29231.4**)
11 SiA: EIE 1682.0; 1786.9; 1906.0; 2042.0 (M: **28574.1**)
10 SiA: EIE 1643.2; 1704.9; 1818.5; 1948.2 (M: **27918.1**)
9 SiA: EIE 1604.5; 1704.9; 1818.5; 1948.2 (M: **27627.8**)
8 SiA: EIE 1662.6; 1773.6; 1900.2 (M: **26588.6**)

b. HexHexNAc isoforms

21Hex18HexNAc: EIE 1737.7; 1846.2; 1969.2; 2109.8 (M: **29522.5**)
22Hex19HexNAc: EIE 1869.0; 1993.6; 2135.8; 1759.1 (M: **29888.2**)
23Hex20HexNAc: EIE 1780.6; 1891.9; 2017.8; 2161.9 (M: **30252.6**)
24Hex21HexNAc: EIE 1802.1; 1914.6; 2042.2; 2188.1 (M: **30618.7**)
25Hex22HexNAc: EIE 1823.5; 1937.5; 2066.7; 2214.2 (M: **30983.9**)
26Hex23HexNAc: EIE 1845.1; 1960.4; 2091.0; 2240.3 (M: **31349.7**)