

# Fragmentation of sulfated oligosaccharides and optical spectroscopy

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*Laboratoire des sciences analytiques – J. Lemoine*  
*Lyon University*

# Outline

## ■ I. Introduction

- What is at stake?
- The fragmentation of sulfated oligosaccharides by CID
- The experimental set up

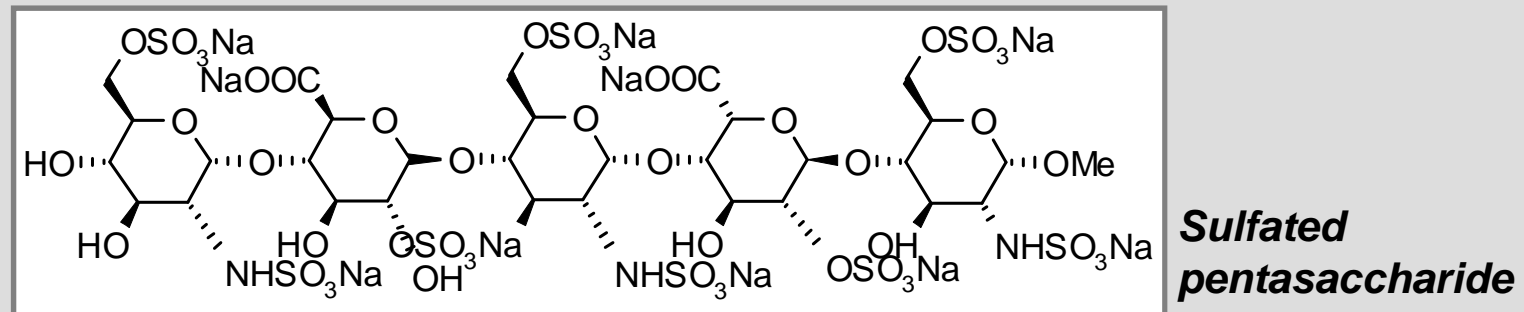
## ■ II. Results

- Gas phase optical spectroscopy
- CID/UVPD fragmentation for disaccharides and tetrasaccharide

## ■ III. Conclusion

# What is at stake ?

## Fondaparinux : Anticoagulant drug



### → Structurally more complex than conventional drug

- Sequence of sugar residues
- Localization of sulfate groups

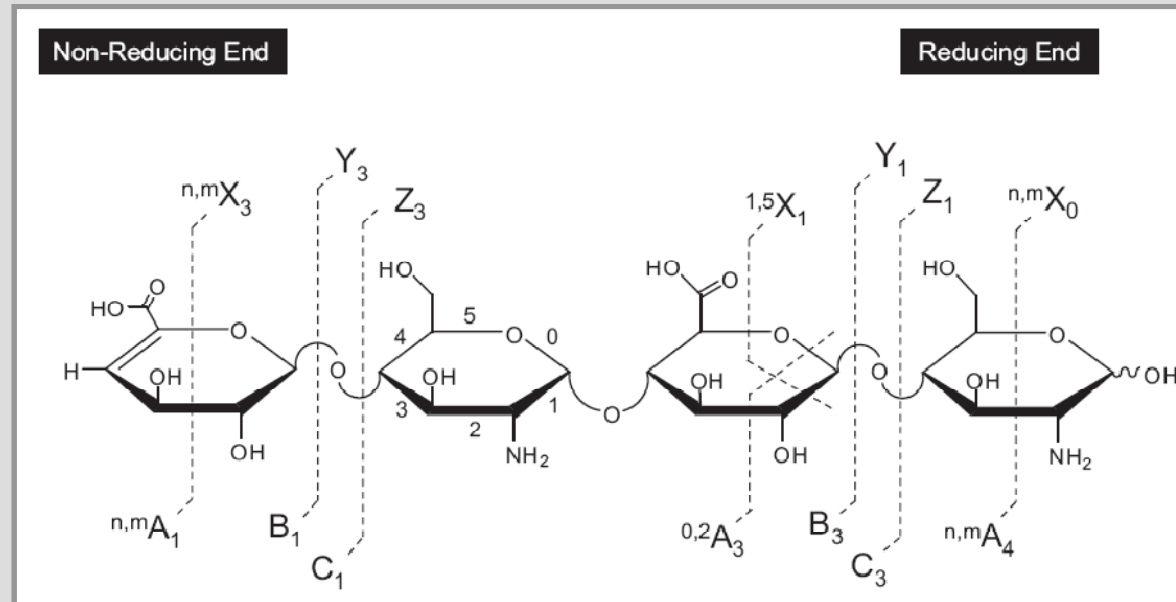
### → Tools for the structural identification

**Tandem MS**

*And*

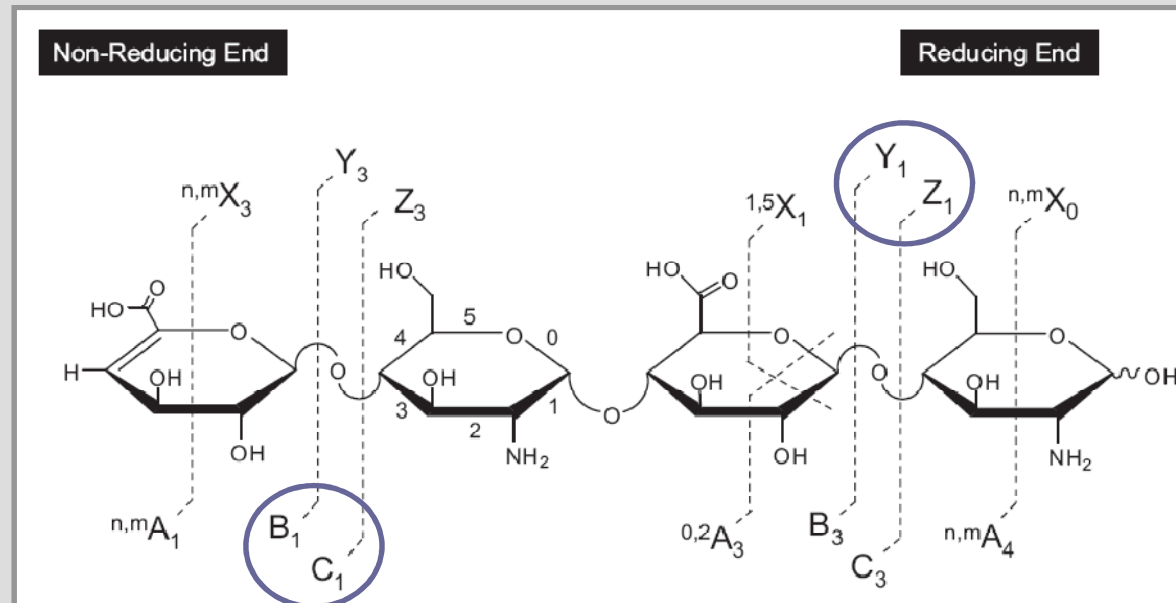
**NMR**

# Fragmentation in MS/MS



Domon, B.; Costello, C. E. *Glycoconjugate Journal*. **1988**, 5, 397-409.

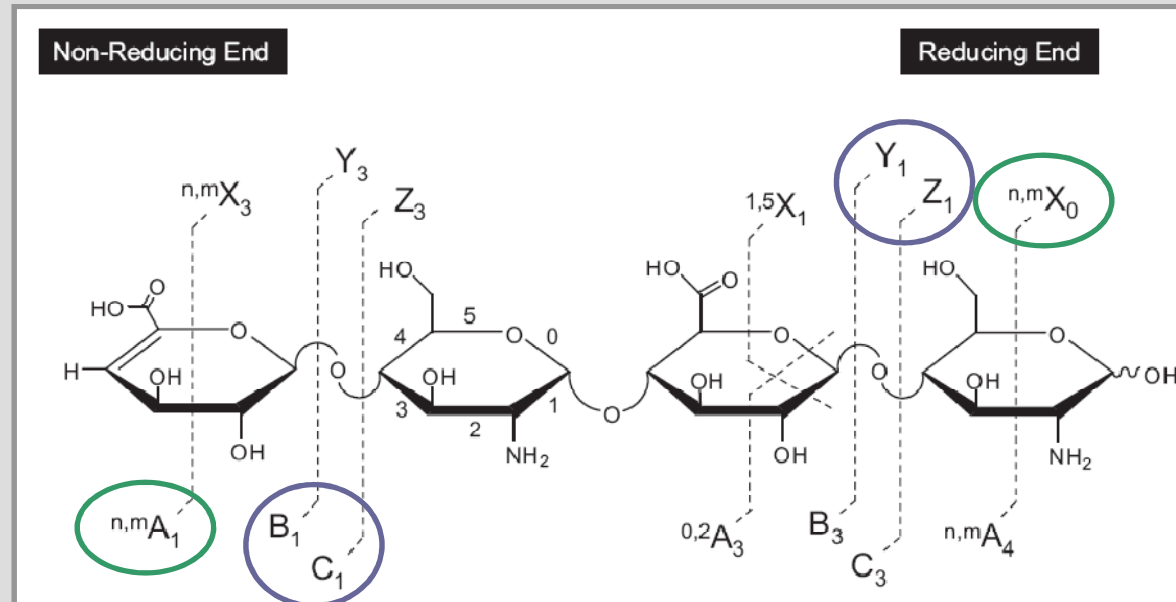
# Fragmentation in MS/MS



Domon, B.; Costello, C. E. *Glycoconjugate Journal*. **1988**, 5, 397-409.

➔ **Glycosidic fragments** ➔ *Identification of the sequence*

# Fragmentation in MS/MS



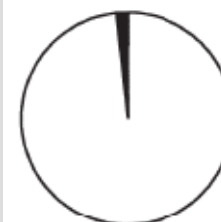
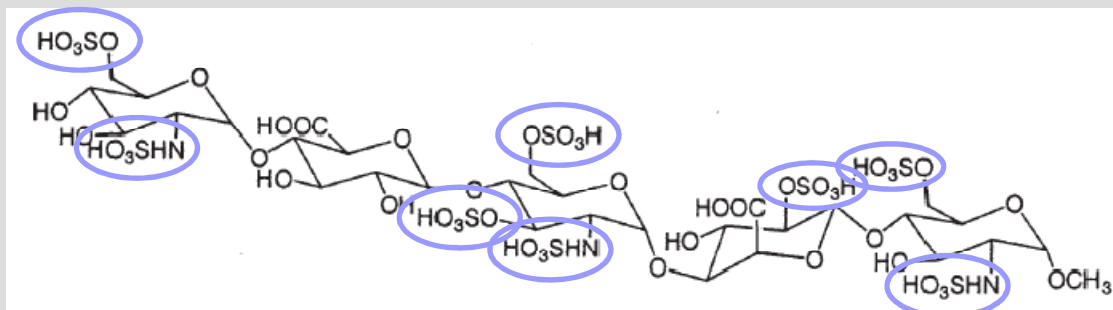
Domon, B.; Costello, C. E. *Glycoconjugate Journal*. **1988**, 5, 397-409.

➡ **Glycosidic fragments** → *Identification of the sequence*

➡ **Cross-ring fragments** → *Localization of sulfate groups*

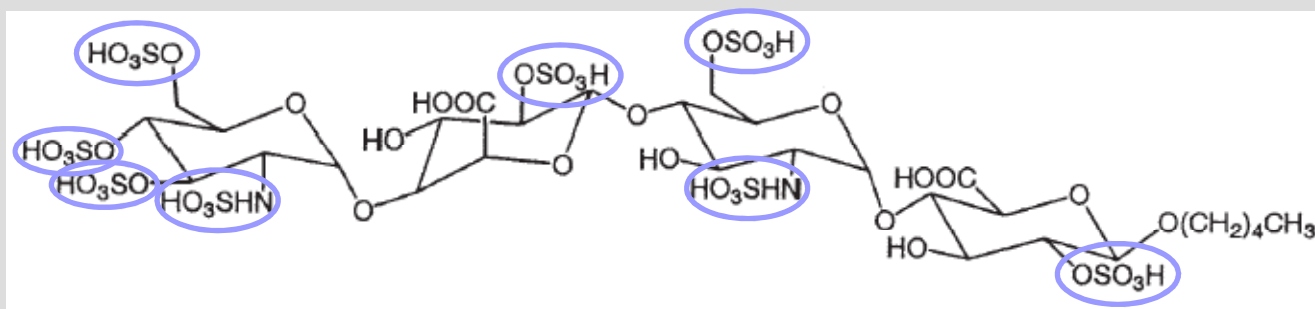
# CID

## Fragmentation dependent on the structure of oligosaccharide



- Glycosidic Cleavage
- SO<sub>3</sub> Loss
- H<sub>2</sub>SO<sub>4</sub> Loss
- Charge Loss

[M-6H]<sup>6-</sup>



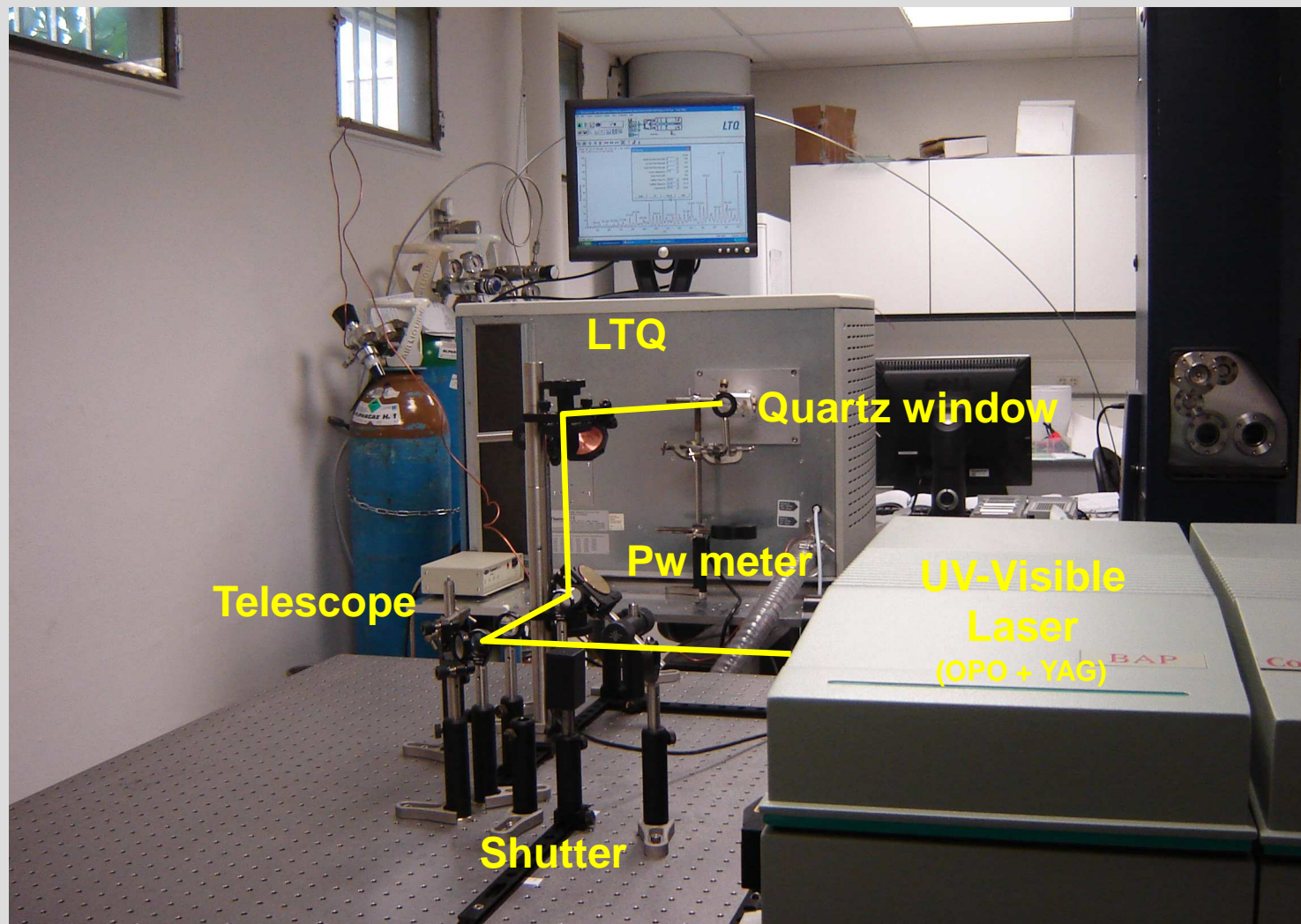
[M-6H]<sup>6-</sup>



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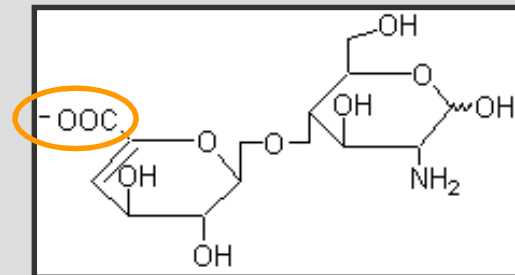
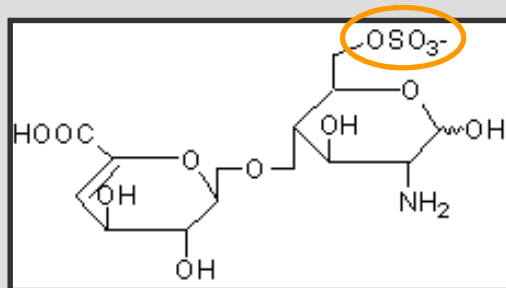
Naggar, E. F.; Costello, C. E.; Zaia, J. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1534-1544.

# Experimental set up

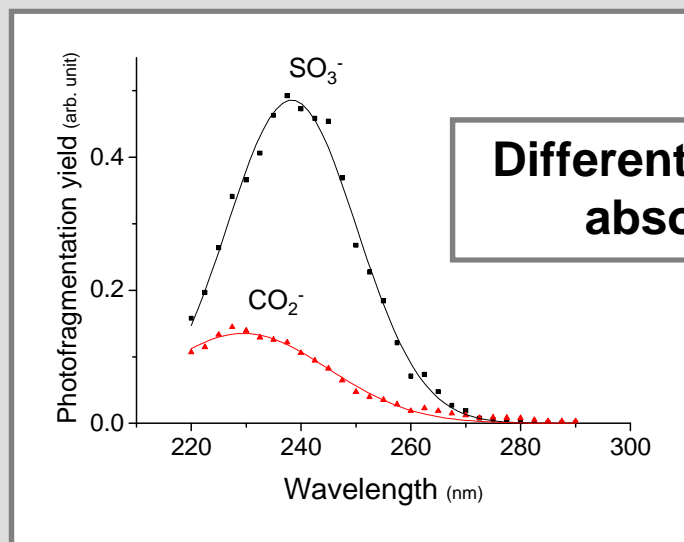


**UVPD** *UltraViolet PhotoDissociation*

# Optical spectroscopy

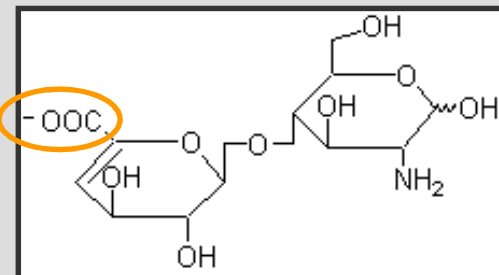
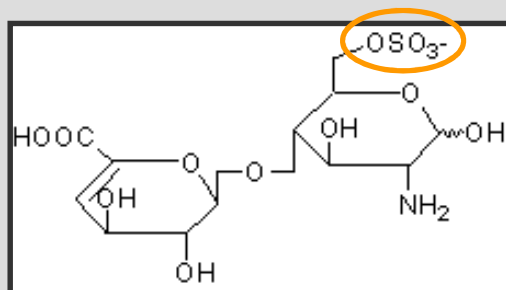


➔ **Photofragmentation yield =  $f(\lambda)$**



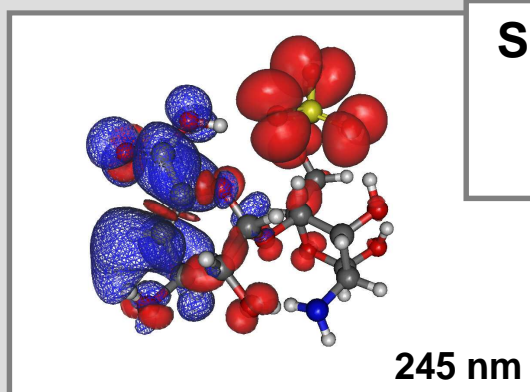
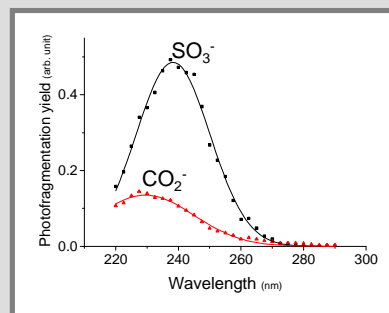
**Different maximum absorption**

# Optical spectroscopy

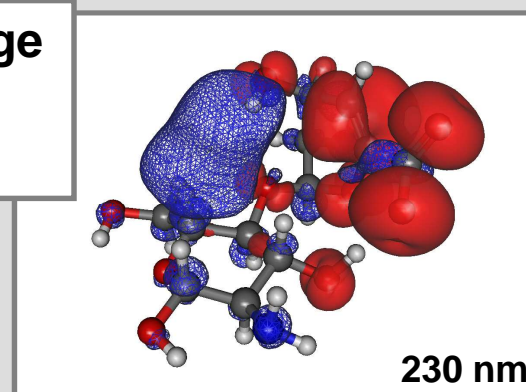


## ➔ Comparison with theory

TD-DFT using B3LYP and PBE0 functionals with the 6-311+G\* basis

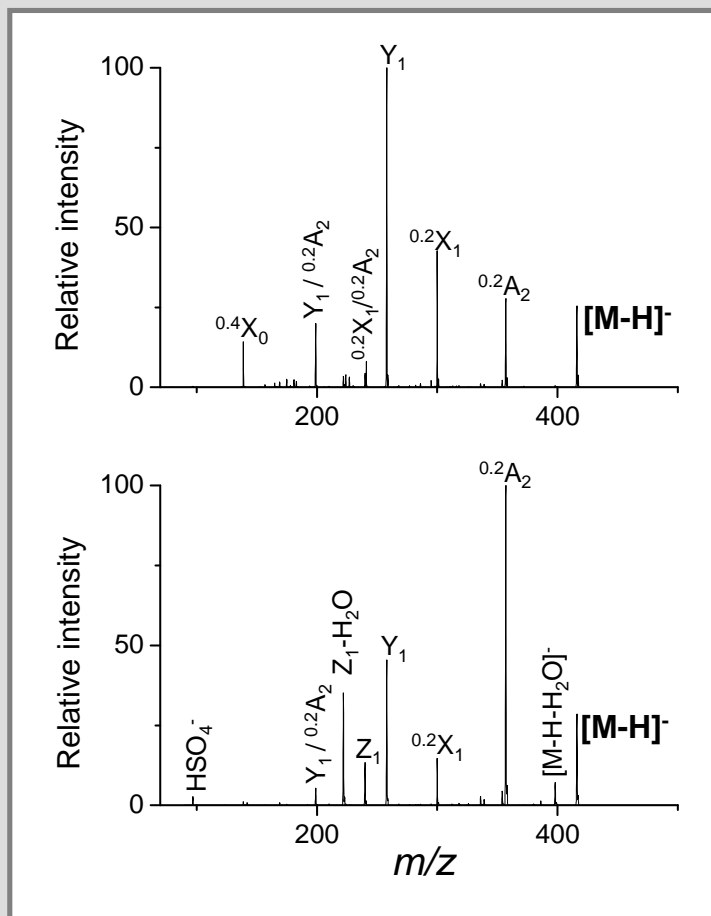


Specific charge transfer excitations



Differential electronic density between fundamental state and excited state

# Disaccharide fragmentation

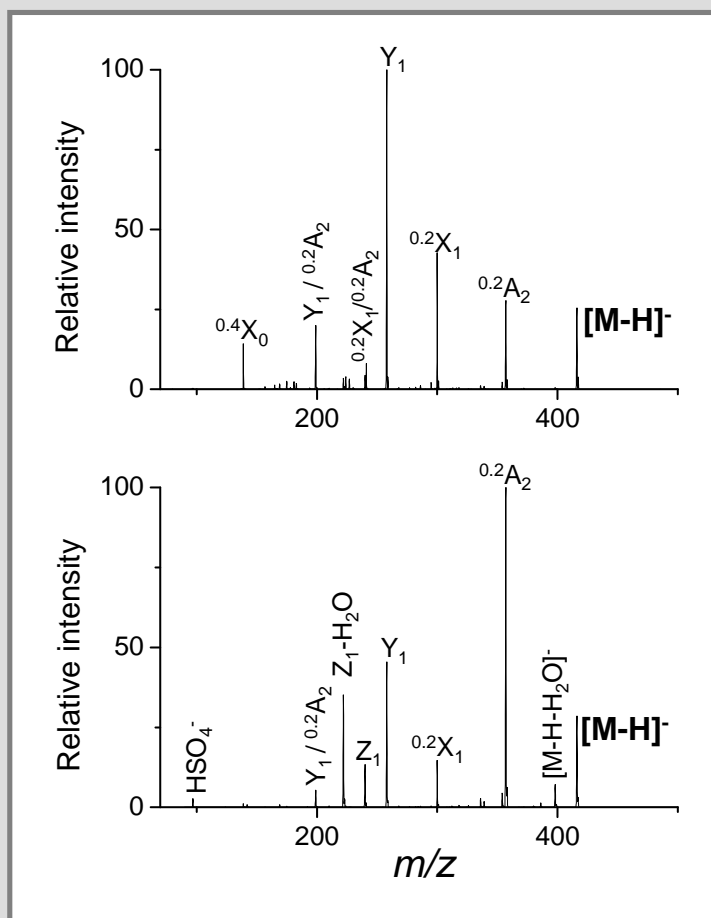


UVPD  
240 nm

CID

Rcaud, A.; Antoine, R.; Joly, L.; Mesplet, N.; Dugourd, P.; Lemoine, J. Wavelength-Tunable Ultraviolet Photodissociation (UVPD) of Heparin-Derived Disaccharides in a Linear Ion Trap. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1645-1651.

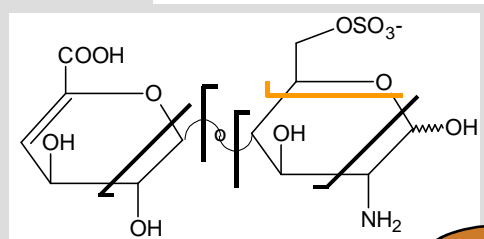
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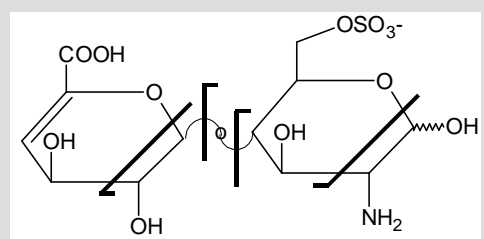
UVPD  
240 nm

CID

Additional cross-ring fragment

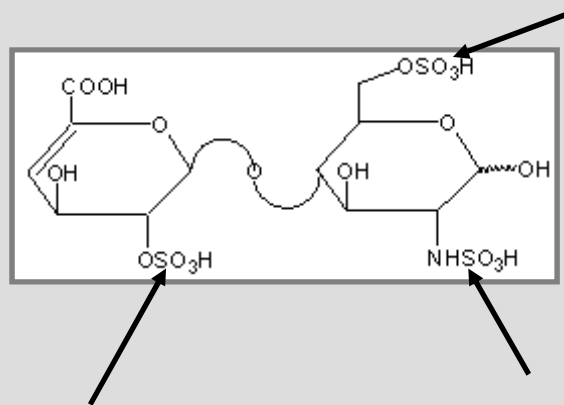


Localization of sulfate group

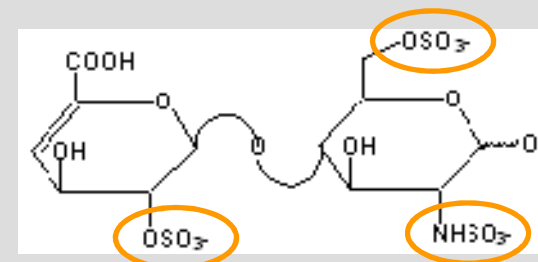
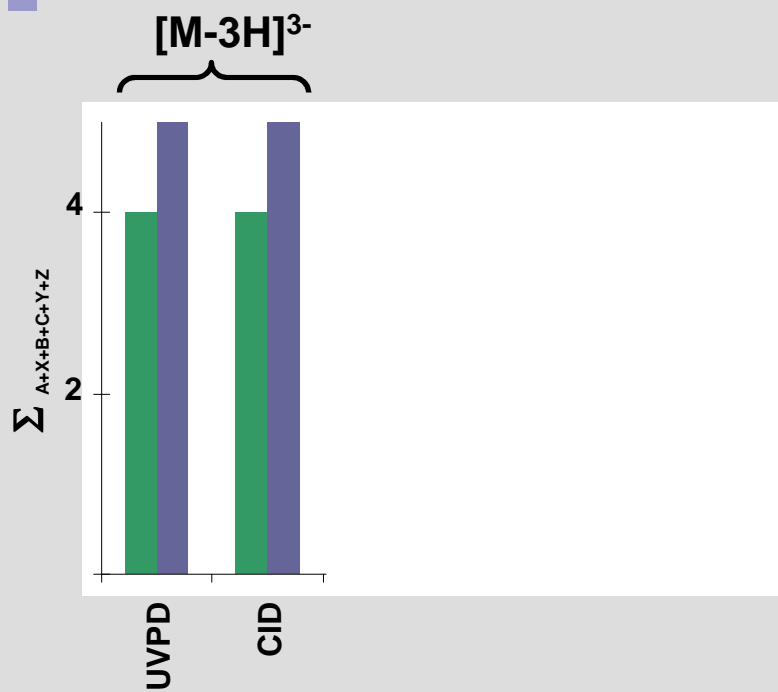


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# Influence of Charge state on fragmentation

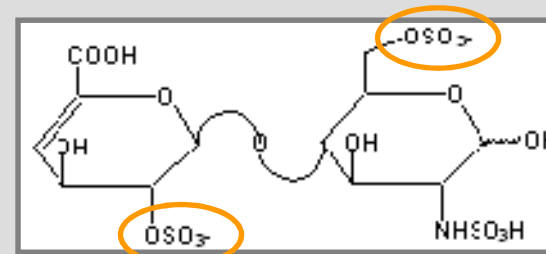
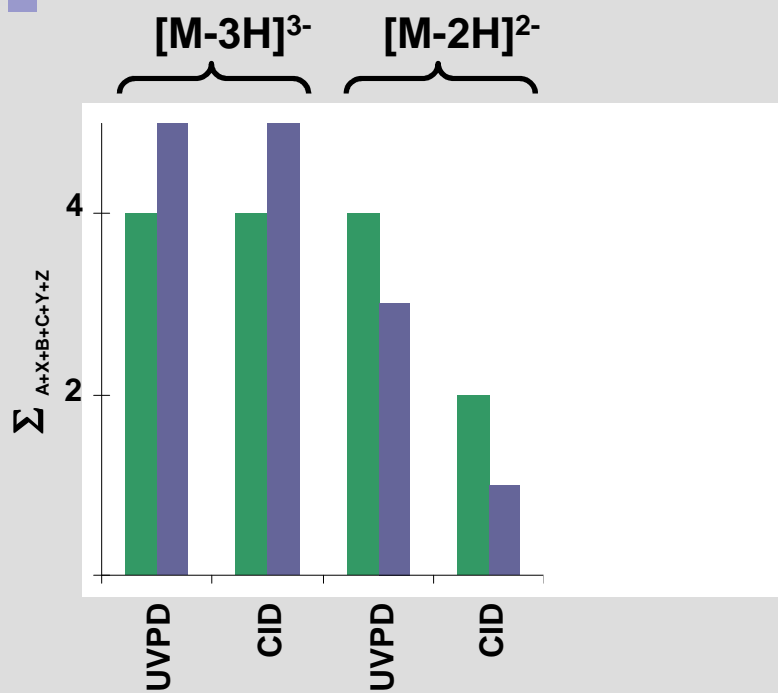




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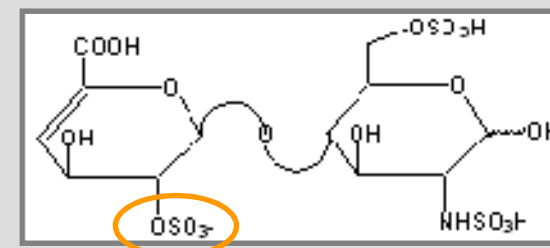
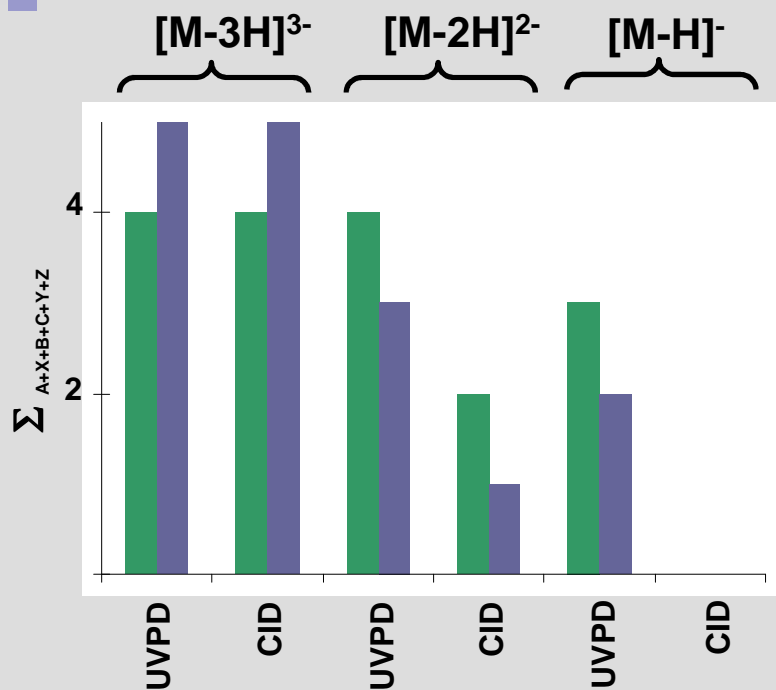
- Cross-ring fragments
- Glycosidic fragments

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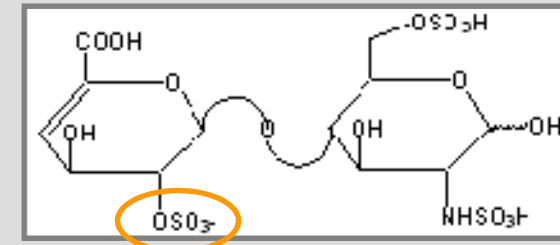
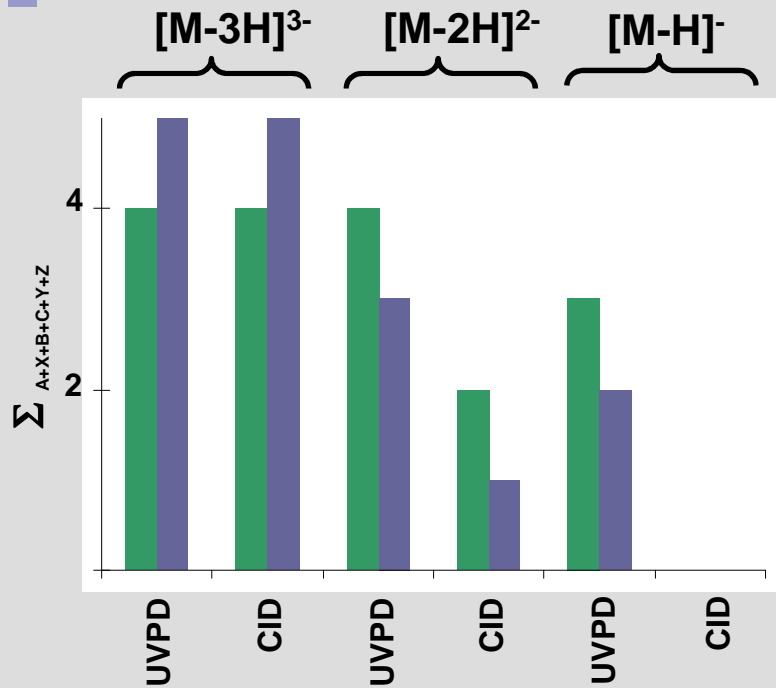


- Cross-ring fragments
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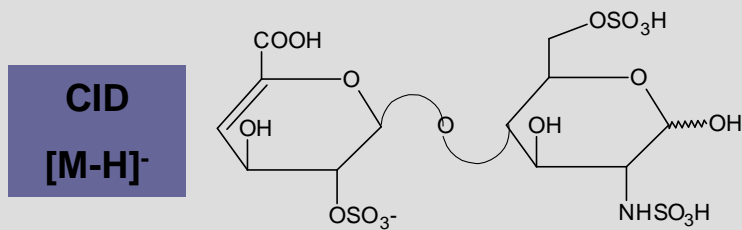
**Less structural information for low charge state**

# Influence of Charge state on fragmentation

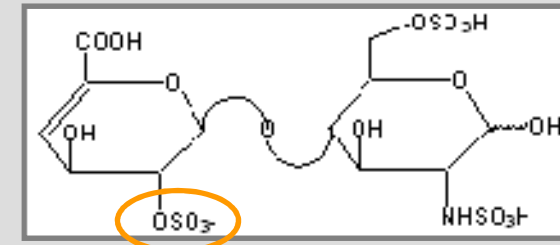
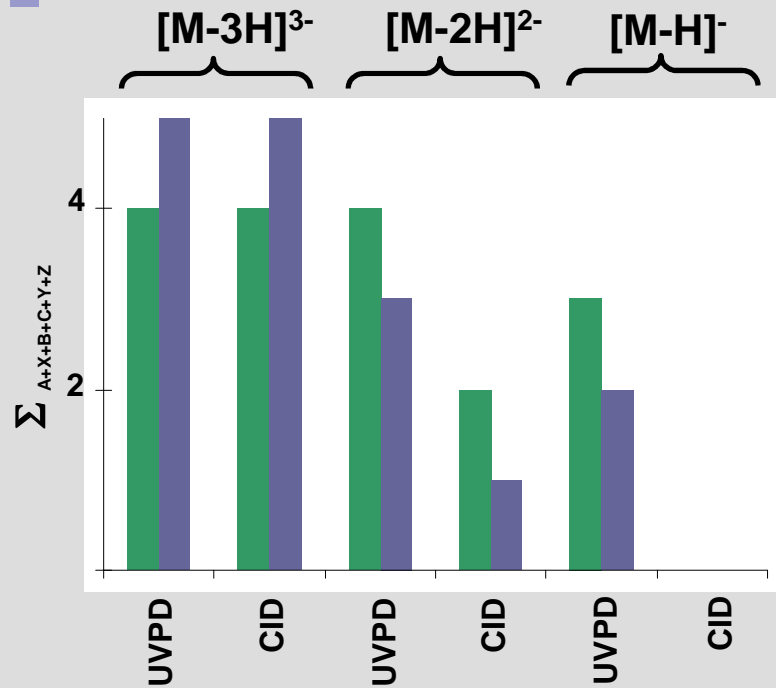


- Cross-ring fragments
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Less structural information for low charge state

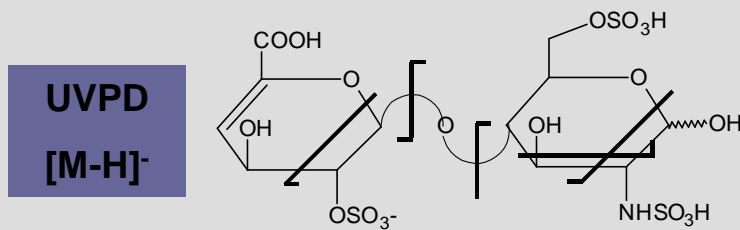


# Influence of Charge state on fragmentation



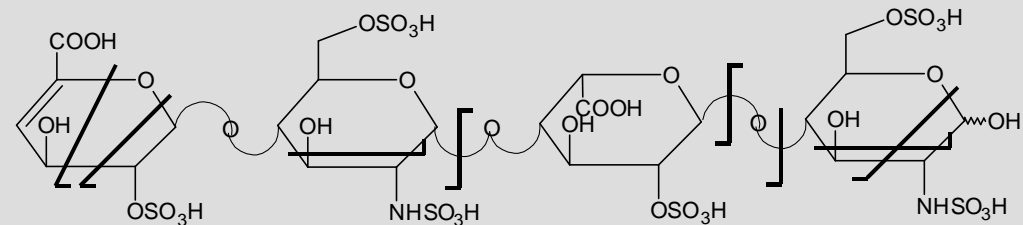
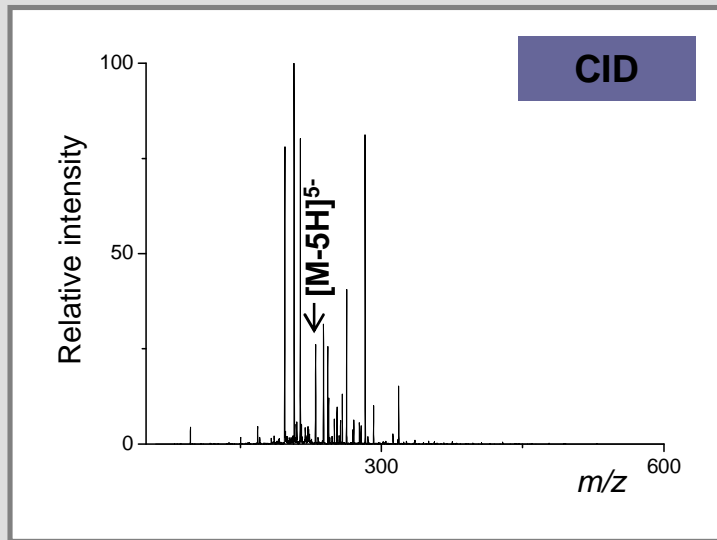
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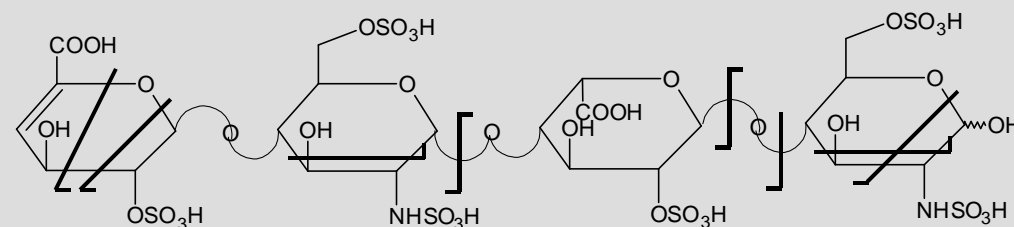
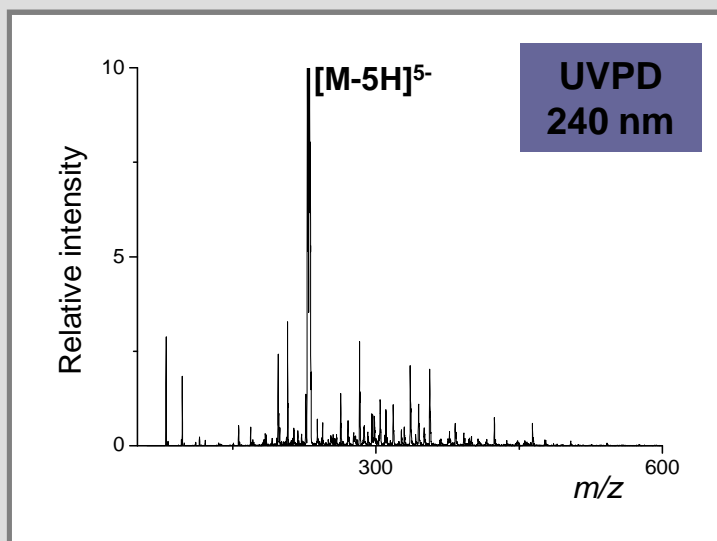
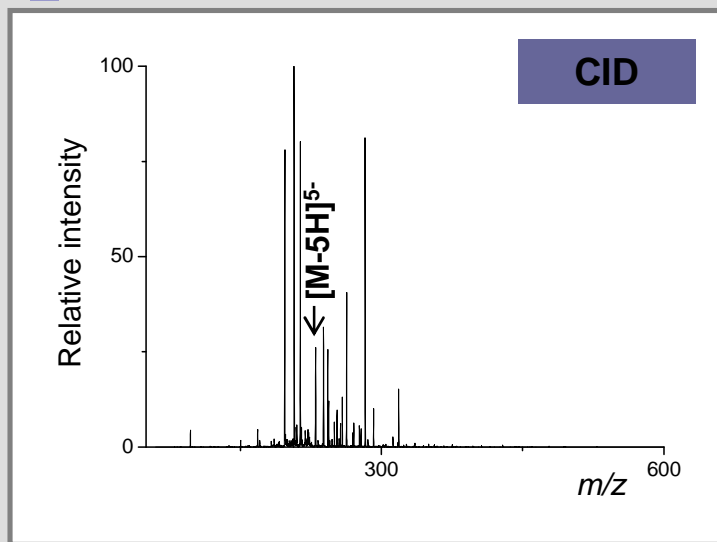


UVPD > CID

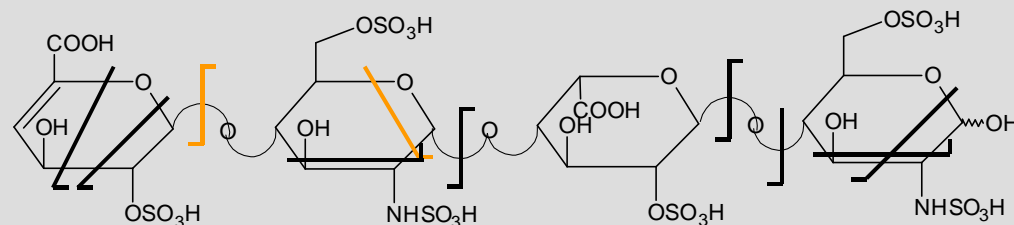
# Tetrasaccharide fragmentation



# Tetrasaccharide fragmentation



**Additional glycosidic and  
cross-ring fragments**



**More structural  
information in UVPD**

# Conclusion

- Optical spectroscopy
  - Acidic disaccharides absorb in near UV → excitation
  - Sensitive to the nature of acidic group
  
- Fragmentation
  - More structural information in UVPD

# Conclusion

- Optical spectroscopy
  - Acidic disaccharides absorb in near UV → excitation
  - Sensitive to the nature of acidic group
- Fragmentation
  - More structural information in UVPD

## Future works

- To improve the structural information : CID<sup>n</sup>, UVPD<sup>n</sup>
- To compare laser excitation with EDD
- To combine MS with LC

# Acknowledgment



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