Electronic Supplementary Material (ESI) for Analytical Methods.

Supplements

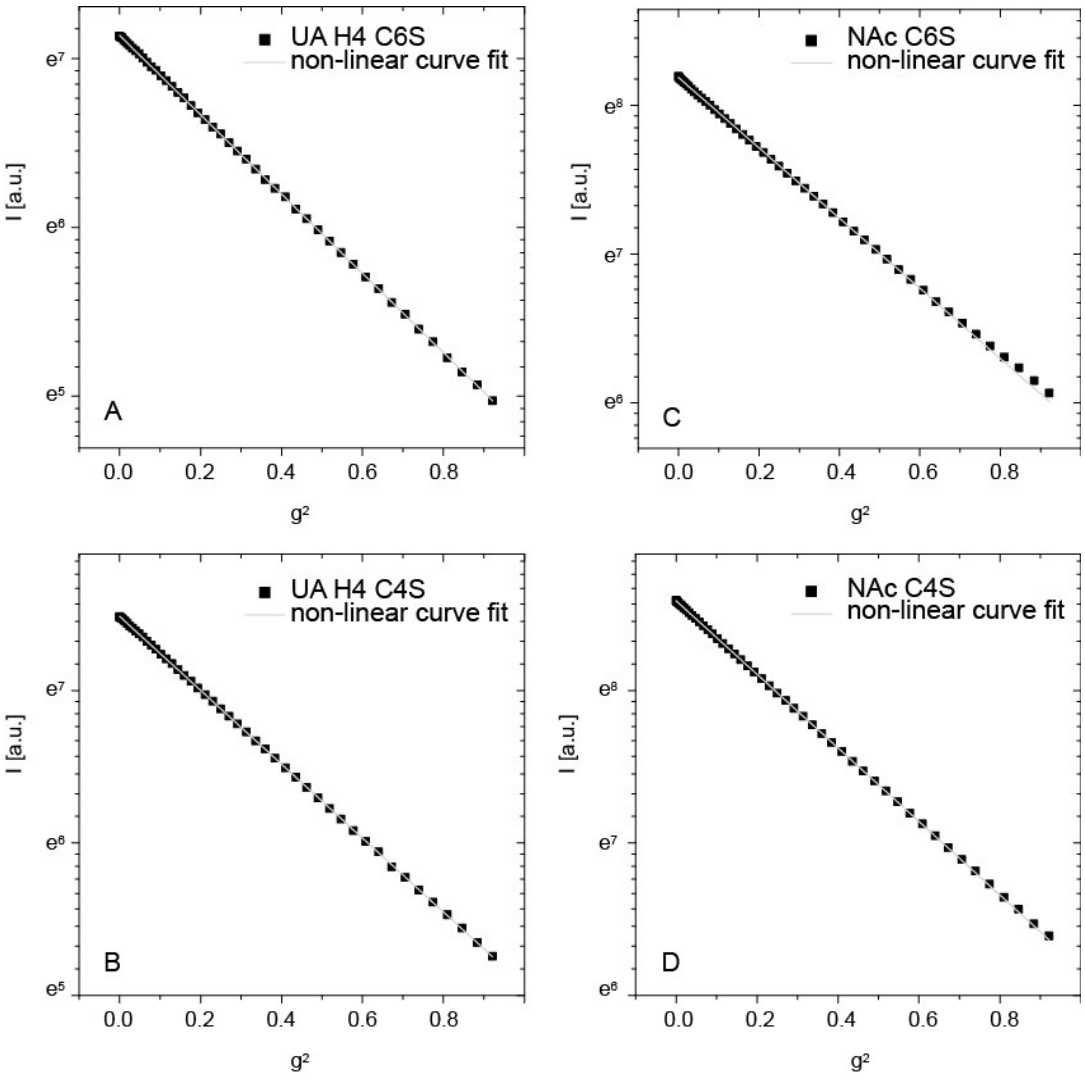

Figure S1
Non-linear curve fitting for the four observed resonances: (A) UA H4 C6S, (B) UA H4 C4S, (C) NAc C6S, and (D) NAc C4S. For details see Table S1.

Table S1

Results of the non-linear curve fitting for the four observed resonances: (A) UA H4 C6S, (B) UA H4 C4S, (C) NAc C6S, and (D) NAc C4S. Used function: $y = exp\ (-D \times (\gamma^2) \times (c^2) \times (\delta^2) \times (\Delta - \delta/3) \times x) \times I0$

Set parameters: $\gamma = 4.26 \times 10^7$ Hz/T; $c = 1.872$ T/m; $\delta = 0.004$ s; $\Delta = 0.05$ s.

A	**UA H4 C6S**	χ^2 reduced	577.657
		cor. R^2	0.99996
		value	standard error
	D	$\mathbf{4.744 \times 10^{-10}}$	$\mathbf{6.11 \times 10^{-13}}$
	I0	1256.61282	0.65058

C	**NAc C6S**	χ^2 reduced	131.34184
		cor. R^2	0.99989
		value	standard error
	D	$\mathbf{4.776 \times 10^{-10}}$	$\mathbf{1.03 \times 10^{-12}}$
	I0	3590.2808	3.10605

B	**UA H4 C4S**	χ^2 reduced	7.47607
		cor. R^2	0.99998
		value	standard error
	D	$\mathbf{4.897 \times 10^{-10}}$	$\mathbf{5.06 \times 10^{-13}}$
	I0	1783.38401	0.74459

D	**NAc C4S**	χ^2 reduced	33.87918
		cor. R^2	0.99999
		value	standard error
	D	$\mathbf{4.876 \times 10^{-10}}$	$\mathbf{3.56 \times 10^{-13}}$
	I0	5369.73407	1.58373

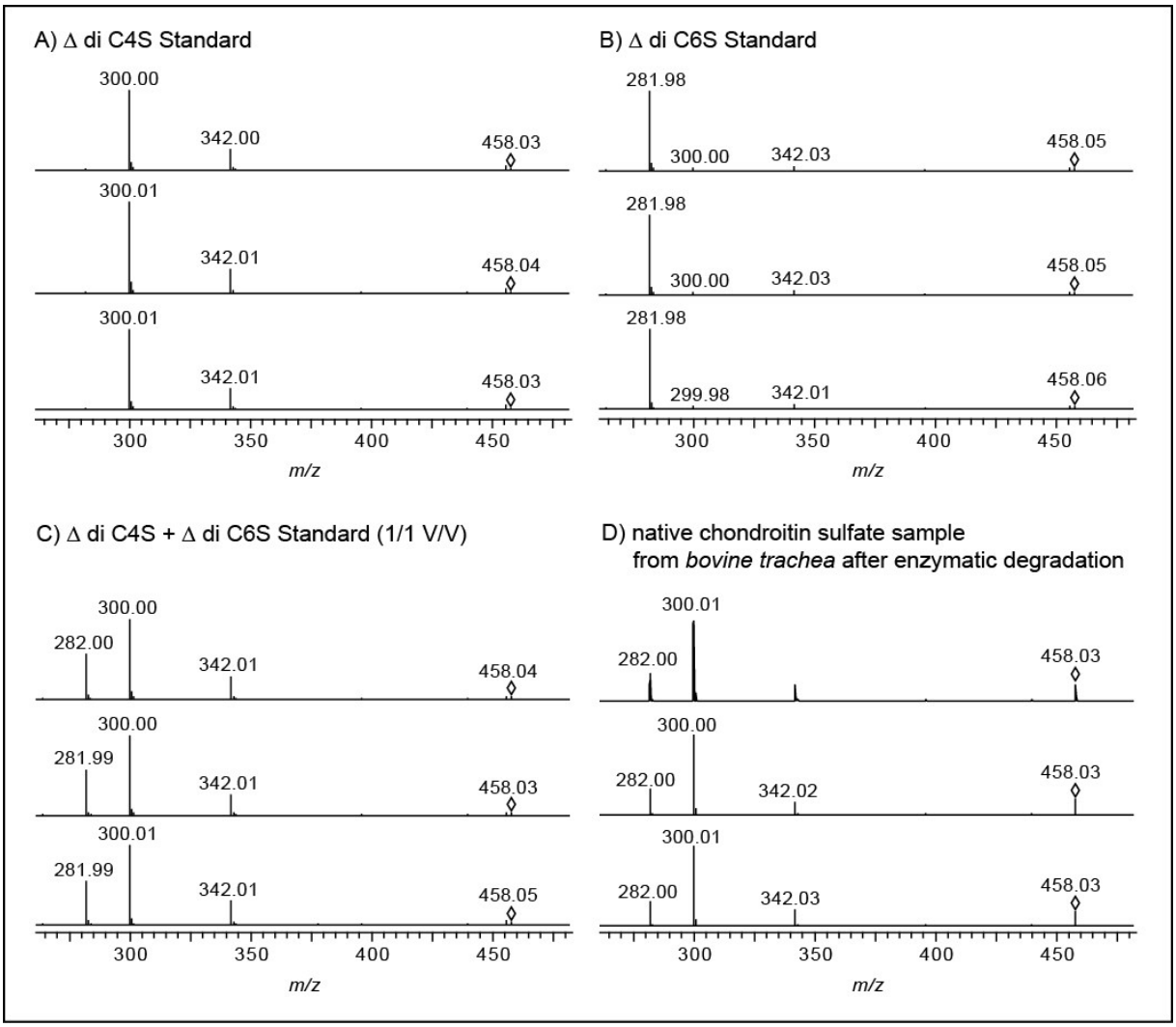

Figure S2
MS^2 spectra of *m/z* 456.10 in the negative ion mode of A) Δ di C4S standard, B) Δ di C6S standard, C) a mixture of both standard disaccharides, and D) the native chondroitin sulfate from *bovine trachea* subsequent to enzymatic digestion. For each sample three spectra are shown which were used for the evaluation of the individual parameters and normalizations. Parent ions are indicated by diamonds.

Table S2
Contribution of the two ions *m/z* 282 and *m/z* 300 as percentage of the total ion count (C300, C282). All values were determined three times for the two standard (A and B), a mixture (C) of both standards (1/1, V/V), and for the digested chondroitin sulfate sample from bovine trachea (D). Mean values and standard deviations are also listed.

sample		C300	C282
A) Δ di C4S standard	spectrum 1	70.85	1.18
	spectrum 2	70.25	1.15
	spectrum 2	71.75	1.23
	mean	**70.95**	**1.19**
	standard deviation	**0.75**	**0.04**
B) Δ di C6S standard	spectrum 1	3.47	81.24
	spectrum 2	3.35	81.98
	spectrum 2	2.95	82.56
	mean	**3.26**	**81.93**
	standard deviation	**0.27**	**0.66**
C) mixture of both standard 1: 1 (v:v)	spectrum 1	49.24	28.40
	spectrum 2	50.31	28.21
	spectrum 2	48.40	26.34
	mean	**49.32**	**27.65**
	standard deviation	**0.96**	**1.14**
D) after enzymatic degradation	spectrum 1	55.92	18.77
	spectrum 2	57.78	18.74
	spectrum 2	57.84	18.03
	mean	**57.18**	**18.51**
	standard deviation	**1.09**	**0.42**

With the contributions of the two ions C300 and C282 from A) and B) a system of equations for the relative quantification can be derived according to[18]:

$$C300 = (70.95 \pm 0.75)A + (3.26 \pm 0.27)B,\ \ C282 = (1.19 \pm 0.04)A + (81.93 \pm 0.66)B$$

The normalization of the ionization efficiency is achieved through application of these equations on a mixture with a known ratio of the two isomers (C):

$$(49.32 \pm 0.96) = (70.95 \pm 0.75)A + (3.26 \pm 0.27)B,$$
$$(27.65 \pm 1.14) = (1.19 \pm 0.04)A + (81.93 \pm 0.66)B$$

Table S3
Relative quantification of the two isomers by means of MS^2 according to[18]:

Contributions	A and B	Norm 1	A or B * Norm 1	calc. %
m/z 300	A = (0.7961 ± 0.0105)	(0.7005 ± 0.0080)	(0.5577 ± 0.017)	(64 ± 4)%
m/z 282	B = (0.2144 ± 0.0033)	(1.5263 ± 0.0144)	(0.3272 ± 0.0064)	(37 ± 2)%