FIGURE S1. HSQC-NMR spectrum of GAGs from Aggrecan. Signals of the anomeric region are shown on the left while signals from the backbone are shown on the right. GalNAc: N-acetyl galactosamine, GlcNAc: N-acetyl-glucosamine, G: glucuronic acid, Gal: galactose, NeuAc: neuraminic acid, Xyl: xylose. CSs are constituted by GalNAc and G, while type II KS is constituted by Gal and GlcNAc units with NeuAc residues at the non-reducing end and GalNAc at the reducing end.
FIGURE S2. HSQC-NMR spectra of GAGs from cartilage: a) CS2, b) MO1, c) GRP1, d) FT3. Signals of the anomeric region are shown on the left while signals from the backbone are shown on the right. GalNAc: N-acetyl galactosamine, GlcNAc: N-acetyl glucosamine, G: glucuronic acid, Gal: galactose, NeuAc: neuraminic acid, Xyl: xylose, LR: linkage region. Signal from KS are indicated in bold.
FIGURE S3. 1D-NMR spectra of CS5-B (< 10kDa) before, a) and after, b) digestion with heparinases. Signals of HS are indicated and correspond to: a. N-acetyl (CH₃) GlcNAc (2.0 ppm); b. GlcNS, H-2 (3.2 ppm); c. GlcA, H-2 (3.4 ppm); e. IdoA, H-5 (4.9-5 ppm); f. GlcN (Ac or S) and IdoA2S H-1 (5.4 ppm). Arrows indicate the decrease of signals.
FIGURE S4. 1D-NMR spectra of GRP2-B before, a) and after, b) digestion with heparinases. No structural changes can be observed.

FIGURE S5. LC-MS profile of commercial porcine heparin digested with heparinases I, II, III mixture: structure assignment of the main peaks.
TABLE S1. Experimental data obtained by MS/MS fragmentation of the ion at m/z 774.148, attributed to \Delta U^{4,2,2}-LR. The selected ion was fragmentated with a collision energy of 50 eV and obtained ions are reported.

| Experimental m/z | z | Mass assignment | Theoretical m/z | Error (ppm) |
|------------------|---|----------------|----------------|-------------|
| 157.0135         | -1 | \Delta U (-H\text{$_2$}O) | 157.0132       | 1.9         |
| 161.0452         | -1 | Gal (-H\text{$_2$}O)     | 161.0445       | 4.3         |
| 175.0244         | -1 | \Delta U               | 175.0237       | 4.0         |
| 193.0347         | -1 | U                      | 193.0343       | 2.1         |
| 282.0294         | -1 | A$_{\text{NAG},65}$(-H\text{$_2$}O) | 282.0278       | 5.7         |
| 300.0392         | -1 | A$_{\text{NAG},68}$     | 300.0384       | 2.7         |
| 316.5431         | -2 | \Delta U^{3,1,1}        | 316.5418       | 4.1         |
| 337.0758         | -1 | \Delta U-Gal           | 337.0765       | 2.1         |
| 378.1050         | -2 | \Delta U^{2,0,1}       | 378.1031       | 5.0         |
| 396.1150         | -1 | U^{2,0,1}              | 396.1137       | 3.3         |
| 458.0606         | -2 | \Delta U^{2,1,1}       | 458.0599       | 1.5         |
| 536.1259         | -1 | \Delta U^{3,0,1}(-H\text{$_2$}O) | 536.1246       | 2.4         |
| 616.0816         | -1 | \Delta U^{3,1,1}(-H\text{$_2$}O) | 616.0814       | 0.3         |
| 631.1705         | -1 | \Delta U-Gal-Gal-Xyl   | 631.1716       | 1.7         |
| 649.1818         | -1 | U-Gal-Gal-Xyl          | 649.1822       | 0.6         |
TABLE S2. Experimental data obtained by MS/MS fragmentation of the ion at m/z 546.0, attributed to ΔU5,2,2. The selected ion was fragmentated with a collision energy of 50 eV and obtained ions are reported.

| Experimental m/z | z | Mass assignment | Theoretical m/z | Error (ppm) |
|------------------|---|----------------|----------------|-------------|
| 157.0144         | -1 | ΔU (-H₂O)       | 157.0132       | 7.6         |
| 175.0244         | -1 | ΔU              | 175.0237       | 4.0         |
| 282.0294         | -1 | ΔNAc,6S (-H₂O)  | 282.0278       | 5.7         |
| 300.0378         | -1 | ΔNAc,6S         | 300.0384       | 2.0         |
| 396.1150         | -1 | U2,0,1          | 396.1137       | 3.3         |
| 458.0606         | -2 | ΔU2,1,1         | 458.0599       | 1.5         |
| 536.1187         | -1 | ΔU3,0,1 (-H₂O)  | 536.1246       | 11          |
| 554.1377         | -1 | ΔU3,0,1         | 554.1363       | 2.5         |
FIGURE S6. LC-MS profiles of heparinases digestion of HS from osteochondromas. a) OC2-A >10 kDa, b) OC2-B <10 kDa, c) OC8-A >10 kDa, d) OC8-B <10 kDa.
FIGURE S7. LC-MS profiles of heparinases digestion of HS from osteochondromas. a) OC1-A >10 kDa, b) OC1-B <10 kDa, c) OC3-A >10 kDa, d) OC3-B <10 kDa.
FIGURE S8. LC-MS profiles of heparinases digestion of HS from osteochondromas. a) OC6-A >10 kDa, b) OC6-B <10 kDa, c) OC7-A >10 kDa, d) OC7-B <10 kDa.
FIGURE S9. LC-MS profiles of heparinases digestion of HS from chondrosarcomas. a) CS2-A (>10 kDa) (b) CS2-B (<10 kDa), c) CS4-A (>10 kDa), d) CS4-B (<10 kDa).
FIGURE S10. LC-MS profiles of heparinases digestion of HS from chondrosarcomas. a) CS5-A (>10 kDa) (b) CS5-B (<10 kDa), c) CS6-A (>10 kDa), d) CS6-B (<10 kDa).