

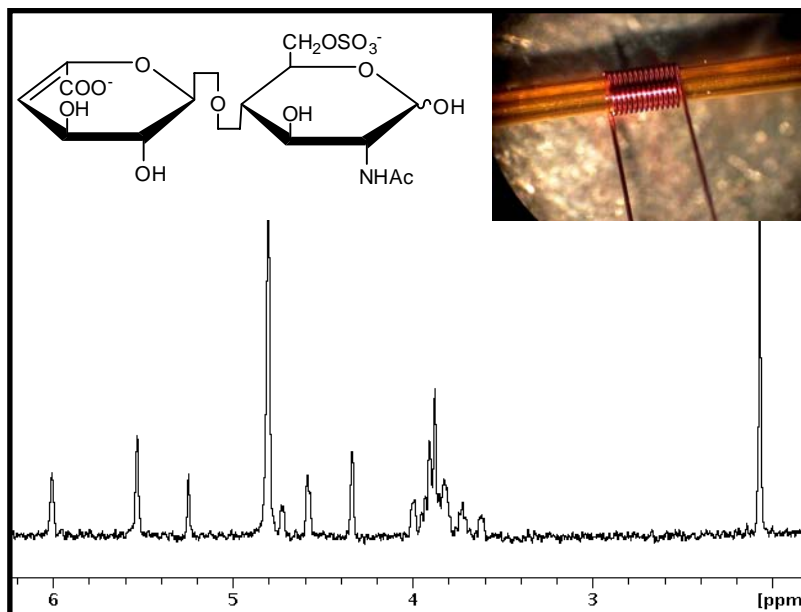
## Structure Elucidation of Heparin-Derived Oligosaccharides Using Microcoil NMR

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Heparin oligosaccharides are highly sulfated linear polysaccharides that display a wide range of biological activities through interaction with proteins, including growth factors and chemokines. Structure elucidation of heparin is complicated by its microheterogeneity arising from variations in sulfation patterns and stereochemistry. Although much progress has been made in the determination of the oligosaccharides responsible for protein binding, those that have been determined are by and large sequences that are in relatively high abundance, for example the pentasaccharide responsible for binding antithrombin III and inhibiting the coagulation cascade. To access the structures of rare heparin protein binding motifs, sensitive and specific analytical techniques are needed. Although, NMR spectroscopy is a powerful tool for molecular structure determination, it trails other analytical techniques in its sensitivity, requiring milligram quantities of material for analysis. Obtaining such quantities of pure oligosaccharides, especially the less abundant rare sequences, is a tedious and laborious process involving enzymatic digestion followed by several separation steps.

Microcoil NMR technology is a relatively inexpensive method of enhancing the mass sensitivity of NMR and allows characterization of mass-limited compounds using micrograms of material. NMR microcoil detection can improve sensitivity by lowering mass detection limits, which can be further enhanced by coupling to the online separation method capillary isotachopheresis (cITP). This technique utilizes electrophoretic separation with a discontinuous buffer system and can concentrate charged analytes by 2 to 3 orders of magnitude. Analysis with cITP-NMR requires only micrograms of material and facilitates the rapid identification of known heparin oligosaccharides; expediting the identification of unknown or novel structures. In addition, because NMR is a non-destructive analytical method, samples can be collected following cITP-NMR experiments for subsequent examination using mass spectrometry. We are developing a spectral database based on cITP-NMR spectra of heparin disaccharides to expedite the identification of specific substructures. The database is constructed by conducting experiments on single component standards of each disaccharide, which can be compared with spectral information obtained for heparin-derived oligosaccharide unknowns. Our goal is to extend this approach to the analysis of more complex mixtures and larger oligosaccharides.



The 600 MHz cITP-NMR spectrum of the heparin disaccharide shown above, measured for 1.5  $\mu\text{g}$  injected. The cITP-NMR experiment was performed with a homebuilt microcoil probe, similar to that shown here, with a detection volume of 25 nL.

In addition to on-line cITP-NMR experiments, we use a commercial Protasis/MRM microcoil probe to perform one- and two-dimensional NMR experiments for solutions containing small amounts (i.e. 10-20  $\mu\text{g}$ ) of heparin-derived oligosaccharides. Along with results of electrospray mass spectrometry experiments, the cITP and two-dimensional NMR results permit unambiguous structure elucidation, even for novel heparin oligosaccharides.