

**Using NMR to dissect the chemical space and *O*-sulfation effects within *O*- and *S*-glycoside analogues of heparan sulfate**

Maria C.Z. Meneghetti<sup>a#</sup>, Lucy Naughton<sup>b,c#</sup>, Conor O'Shea<sup>d,c#</sup>, Dindet S.-E. Koffi Teki<sup>e</sup>, Vincent Chagnault<sup>e</sup>, Helena B. Nader<sup>a</sup>, Timothy R. Rudd<sup>f,g</sup>, Edwin A. Yates<sup>g</sup>, José Kovensky<sup>e,\*</sup>, Gavin J. Miller<sup>d,c\*</sup> & Marcelo A. Lima<sup>b,c\*@</sup>

<sup>a</sup> Departamento de Bioquímica, Instituto de Farmacologia e Biologia Molecular, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Três de Maio, 100, São Paulo, SP 04044-020, Brazil

<sup>b</sup> School of Life Sciences, Keele University, Keele, Staffordshire, ST55BG, UK

<sup>c</sup> Centre for Glycosciences, Keele University, Keele, Staffordshire, ST55BG, UK

<sup>d</sup> Lennard-Jones Laboratories, School of Chemical and Physical Sciences, Keele University, Keele, Staffordshire, ST55BG, UK

<sup>e</sup> Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources (LG2A), UMR 7378 CNRS, Université de Picardie Jules Verne, 33 rue Saint Leu, F-80039 Amiens Cedex, France

<sup>f</sup> National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire, UK

<sup>g</sup> Department of Biochemistry and Systems Biology, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK

# These authors are joint first authors

\* Senior authors

@ Correspondence: Marcelo A. Lima, Centre for Glycosciences, Keele University, Keele, Staffordshire, ST55BG, UK, m.andrade.de.lima@keele.ac.uk

The current address for TRR is Analytical and Biological Sciences, Research & Development, Science, Research and Innovation, Medicines & Healthcare products Regulatory Agency, Blanche Lane, South Mimms, Hertfordshire, EN6 3QG, UK

Supplementary figures

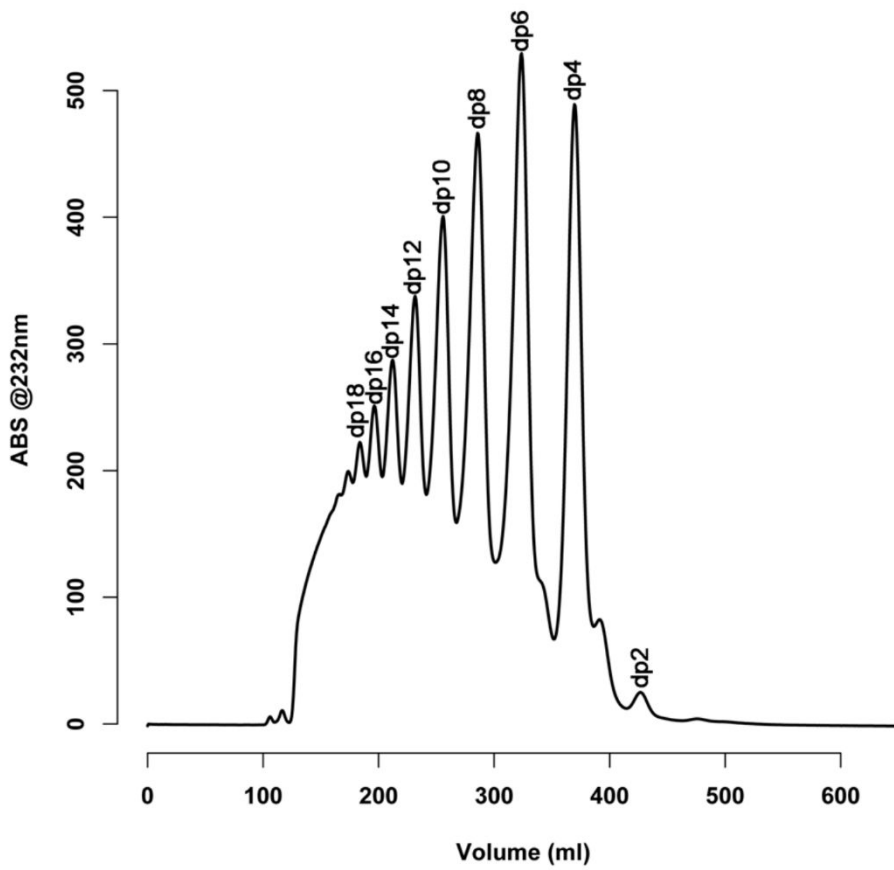


Figure S1. Biogel P10 oligosaccharide fractionation.

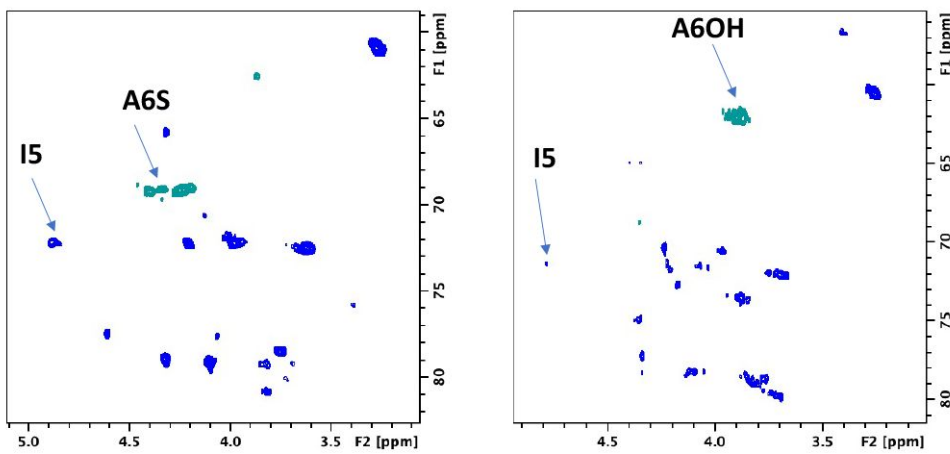
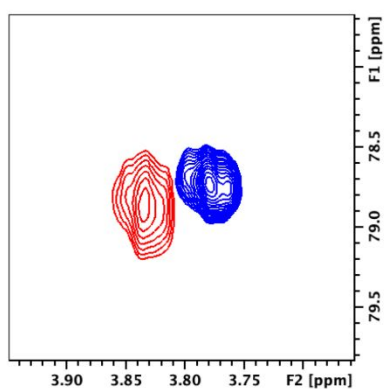
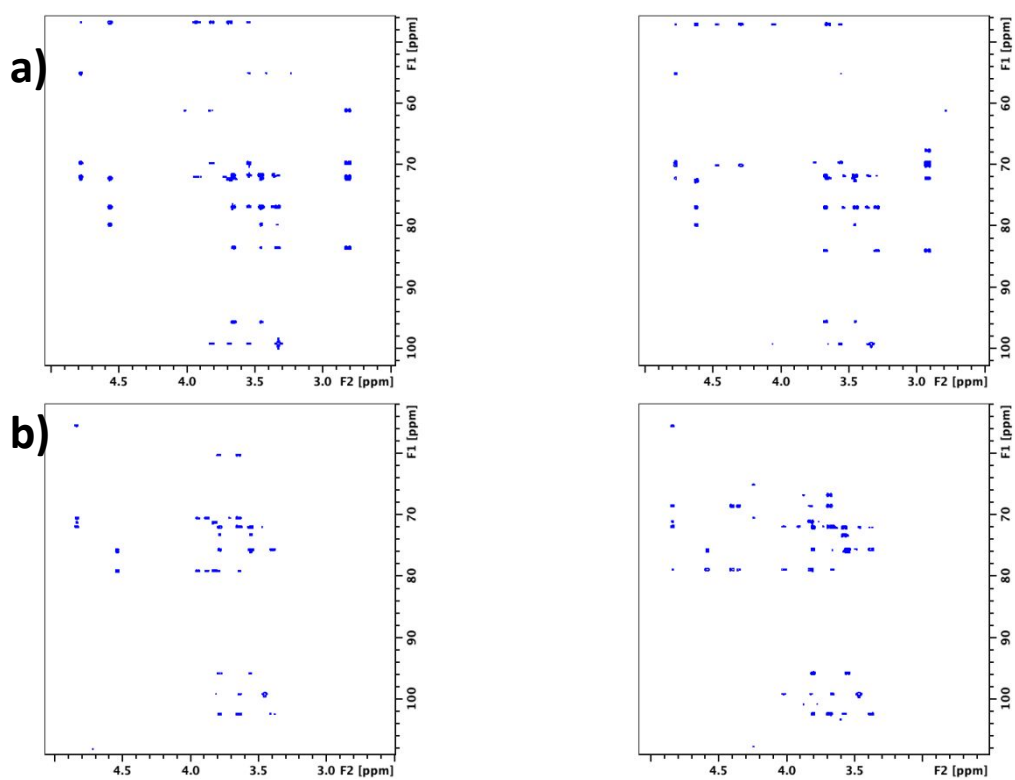


Figure S2. HSQC spectra for heparin and 6OH heparin oligosaccharide.



**Figure S3.** HSQC spectra showing the C4 region of 6S (blue) and 6OH (red) heparin derivatives.



**Figure S4. a)** J-resolved HMBC spectra for 6-OH and 6-OSO<sub>3</sub>H S-glycosides 1 (left) & 2 (right) **b)** J-resolved HMBC spectra for 6-OH and 6-OSO<sub>3</sub>H O-glycosides 3 (left) & 4(right).