Supporting Information

A New Approach to Glycan Targeting. Enzyme Inhibition by Oligosaccharide Metalloshielding.

John B. Mangrum, Brigitte J. Engelmann, Erica J. Peterson, John J. Ryan, Susan J. Berners-Price, and Nicholas P. Farrell*

Mass Spectral conditions and details of enzyme inhibition assay

Mass Spectrometry and Polynuclear Platinum Binding to Oligosaccharides.

Mass spectra were acquired on a Micromass-Waters Qtof-2 mass spectrometer (Milford, MA) equipped with a custom-built microspray source. Samples were introduced by flow injection at flow rates of 0.5-0.7 $\mu\text{L}/\text{min}$ using a syringe pump (Harvard Apparatus). Electrospray source conditions were kept constant with a source temperature of 120°C and spray voltage of 2.0 kV. In tandem mass spectrometry (MS/MS) experiments, collisional energy was varied from 10 eV to 30 eV. Heparan sulfate octasaccharide was purchased from V-Labs (Covington, LA) and reconstituted in 18 M Ω water to form a 375 μM stock solution. Platinum compounds were reconstituted in 18 M Ω water to a stock concentration of 500 μM . Samples were mixed at a 1:2 molar ratio in 18 M Ω water with a final concentration of 10mM NH_4OAc . Mixtures were incubated at 37°C for 20 minutes then dialyzed in a custom flow through micro dialysis chamber at 3 $\mu\text{L}/\text{min}$ using 13,000 molecular weight cut off (MWCO) hollow cellulose fibers from 107 SpectraPor, (Rancho Dominguez, CA) and recollected.

Figure S1. ESI-MS/MS with 10V of applied collisional energy for free octasaccharide DP8 and adducted with polynuclear platinum complexes.

Figure S2. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 @20 V

Figure S3. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 @30 V

Figure S4. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to AH44 (6+) @20 V

Figure S5. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to AH44 (6+) @30 V

Figure S6. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to TriplatinNC (8+) @20 V

Figure S7. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to TriplatinNC (8+) @ 30 V

Figure S1.

Figure S1. ESI-MS/MS with 10V of applied collisional energy. **I** DP8; (A) Free DP8, (B) $-(SO_3)^-$, (C) $(SO_3)^{-}_2$, (D) $(SO_3)^{-}_3$, (E) $(SO_3)^{-}_4$, (F) $(SO_3)^{-}_5$, (G) $(SO_3)^{-}_6$; **II** DP8-AH44; (A) DP8-AH44, (B) loss of $(SO_3)^-$, (C) $(SO_3)^{-}_2$; **III** DP8-TriplatinNC; (A) DP8-TriplatinNC (B) loss of $(SO_3)^-$

Figure S2

Figure S2. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 @20 V. (A) Free Octasaccharide, (B) loss of $(\text{SO}_3)^-$, (C) loss of $(\text{SO}_3)^{-}_2$, (D) loss of $(\text{SO}_3)^{-}_3$, (E) loss of $(\text{SO}_3)^{-}_4$, (F) loss of $(\text{SO}_3)^{-}_5$, (G) loss of $(\text{SO}_3)^{-}_6$, (H) loss of $(\text{SO}_3)^{-}_7$, (I) loss of $(\text{SO}_3)^{-}_8$

Figure S3

Figure S3. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 @30 V. (A) loss of $(\text{SO}_3)^-_7$ (B) $(\text{SO}_3)^-_8$, (C) $(\text{SO}_3)^-_9$, (D) loss of $(\text{SO}_3)^-_{10}$

Figure S4

Figure S4. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to AH44 (6+) @20 V. **(A)** AH44- Octasaccharide, **(B)** loss of $(\text{SO}_3)^-$, **(C)** $(\text{SO}_3)^{-}_2$, **(D)** $(\text{SO}_3)^{-}_3$

Figure S5

Figure S5. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to AH44 (6+) @30 V.
(A) loss of $(\text{SO}_3)^-_3$, (B) $(\text{SO}_3)^-_4$, (C) $(\text{SO}_3)^-_5$

Figure S6

Figure S6. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to TriplatinNC(8+) @20 V.
(A) TriplatinNC-Octasaccharide, (B) loss of $(\text{SO}_3)^-$, (C) loss of $(\text{SO}_3)^-_2$, (D) loss of $(\text{SO}_3)^-_3$

Figure S7

Figure S7. ESI-MS/MS Fragmentation pattern of free octasaccharide DP8 adducted to TriplatinNC (8+)@ 30 V. (A) TriplatinNC-Octasaccharide, (B) loss of $(\text{SO}_3)^-$, (C) $(\text{SO}_3)^{-}_2$, (D) $(\text{SO}_3)^{-}_3$,

Enzyme Inhibition Assay

Materials

Fondaparinux was manufactured by Gland Pharma Limited (India) for Dr. Reddy's Laboratories Limited (Bachepalli, India). The heparinase I was purchased from Sigma Aldrich (St. Louis, Mo, USA). 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-1) was purchased from Dojindo Laboratories (Kamimashiki-gun, Japan). The R9-Tat protein was purchased from AnaSpec (Fremont, California, USA).

Heparinase I Dose Response.

The assay adapted the method of ref. 21 to give a colorimetric read-out of enzyme cleavage efficiency using the tetrazolium salt WST-1.^[21] First, a standard curve was constructed using Fondaparinux, initially dissolved as an isotonic solution of NaCl and H₂O, over the range 0 μ M – 200 μ M in a 40 mM sodium acetate buffer (pH 5.0) in each 96-well plate. For the samples, 40 μ M fondaparinux was placed in the wells of a 96-well plate. The appropriate concentration (1:3 stoichiometry, 120 μ M) of each platinum drug dissolved in water or R9-Tat protein was then added to the wells. The plate was then incubated at 37 °C for appropriate time points. Noncovalent interaction is immediate – for comparison with cisplatin (to allow covalent binding) an incubation time of up to 23 h. can be used. BBR3464 gave consistent readings after 3h. For the standard curve and for assessment of inhibition, heparinase I (Sigma Aldrich, from *Flavibacterium heparinum*) in 20mM Tris-HCl, pH 7.5, 50mM NaCl, 4mM CaCl₂ and 0.01% BSA was added after the incubation period to each well so that the final concentration was 0.28 μ M. The plate was then incubated at 37 °C for 3 hours. A 1.69 mM solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-1) was then prepared in a 0.1 M NaOH solution. A 100 μ L aliquot of this WST-1 solution was added to each well to stop the assay. The plates were covered with Parafilm and developed at 60 °C for 60 minutes. The absorbance was measured at 584 nm using a μ Quant plate reader (Bio-Tek Instruments, Inc) and % inhibition calculated *versus* control.