

Supplementary Data

ESI-MSMS analyses. ESI-MSMS was performed on an Applied Biosystems API-4000 tandem triple quadrupole instrument operating in negative mode. Ion scanning was carried out with the Analyst software with the following settings: Dwell time, 50.0 msec; declustering potential (DP), -10.0 V; entrance potential (EP), -10.0 V; collision energy, -40.0 eV, collision cell exit potential (CXP), -15.0 V; Collision gas (CAD), 4; Curtain gas (Cur) 10, Ion source gas 1 (GS1), 30; ion source gas 2 (GS2), 25; ion spray voltage (IS), -4500 V; temperature (TEM), 250°C; interface heater (ihe) status is on. Multiple-reaction-monitoring mode for m/z 677→597 and 682→602 transitions. Resolution of Q1, unit; Resolution of Q2, unit.

The sample (5 μ l of the 100 μ l sample in 5 mM ammonium formate in methanol, see main text) was infused into the mass spectrometer with an Agilent LC system. After sample injection, acetonitrile was infused for 2 min at 80 μ L/min. Acetonitrile was then infused for 3 min at 400 μ L/min for washing. MSMS data was collected in the period 0.5-2 min after sample injection. The amount of product was calculated from the ratio of integrated ion peak intensities of product (IDS-P) to internal standard (IDS-IS), multiplied by the amount of added internal standard. Enzymatic activity was calculated from the amount of product from a DBS sample minus that from a blank control (2 mm filter paper punch without blood), which was divided by the incubation time and the volume of blood (1.6 μ L of blood in a 2 mm DBS punch).

Synthesis of IDS-S. Given below is a small scale (milligram amount) synthesis of

IDS-S. Optimization of a large scale synthesis to prepare multi-gram amounts of IDS-S is under development and will be reported elsewhere. The first step in the synthesis of IDS-S is to deacetylate commercial grade heparin and to fully sulfate the exposed amino groups. Dowex 50X8-200 resin (60 mL, wet volume) was put in a column ($4 \times 25\text{cm}$) in a cold room at 4°C and washed with 60 mL of 0.1 N NaOH, followed by 120 mL of H_2O , 120 mL of 0.1 N HCl, and 240 mL of H_2O . Heparin (sodium salt, 10 g, USB, cat. 16920) was dissolved in 60 mL of chilled (4°C) water and loaded on the cation exchange column. After the solution passed through the column, the column was washed with 120 mL of chilled (4°C) H_2O . The eluant during heparin loading and the water washing were combined and lyophilized to give Heparin free acid.

The free acid was dissolved in 48 mL of 70% hydrazine aqueous solution containing 1% (w/v) hydrazine sulfate. The solution was stirred in a round bottom flask with a water-filled reflux condenser for 8 hr with immersion of the round bottom flask in an oil bath at 96°C . The solvent was removed by rotary evaporation (water bath at 60°C , water aspirator). Then 5 mL of water was added, and solvent was removed again, and this was repeated once (to remove residual hydrazine).

The residue was dissolved in 10 mL of H_2O and dialyzed (dialysis tubing from SpectroPor, MW cutoff < 2,000 Da) against 10 liters of deionized H_2O (Milli-Q water, Millipore Inc.) for 12 h (water was changed once after 6 h). Dialysis was done with magnetic stirring at room temp. Extra room is left in the bag in the bag to allow for volume expansion during dialysis. The volume expansion can be considerable (the bag pressure is checked periodically to be sure it is not going to burst). The solution in the dialysis bag was collected and lyophilized to give a white fluffy powder.

The powder was dissolved in 160 mL of Milli-Q-grade water. Reagent grade Na₂CO₃ (6 g) and sulfur trioxide-pyridine complex (6 g, recently purchased bottle, Aldrich Cat. S7556) were added in 1 portion. The solution was stirred at 50°C (water bath) for 24 hr in a capped round bottom flask. The solution was dialyzed against 15 liters of Milli-Q-grade water at room temperature for ~9 hr and then against another 15 liters for ~9 hr. The solution was lyophilized to give a white powder. The yield of deacetylated, resultfated heparin is 6.5 g. The mass loss is presumably due to removal of salt present in the commercial heparin.

The next step is to degrade heparin with nitrous acid. A solution of pH 1.5 nitrous acid was prepared by mixing 50 mL of ice-cold 1 N HCl with 50 mL of ice-cold 1 M NaNO₂ (50mmol NaNO₂, 3.45g). After stirring for 1 min, the pH of the solution was tested on a pH meter. The pH is ~1.9-2.0, and a small amount (~5 mL) of 1 N HCl was used to adjust the pH to 1.5. The resulting nitrous acid solution was allowed to warm to 25°C (water bath).

Deacylated, resultfated heparin (1 g) was placed in an Erlenmeyer flask and dissolved in 1 mL of water. The freshly prepared nitrous acid solution was added in one portion with magnetic stirring, and the flask was not capped because N₂ gas was generated. The stirring was continued for 1 h at 25°C, and then the pH (~0.8 at this point) was adjusted to 4.0 (pH meter) by adding ~0.23 g of solid Na₂CO₃. The reaction mixture was stirred at room temperature for 12 hr. The pH was adjusted again to 6.0 (pH meter) by adding 1 M Na₂CO₃ aqueous solution. The volume of reaction mixture was reduced to 60 mL by rotary evaporation (45 °C water bath, water aspirator), and then 0.24 g of solid H₂N(CH₂)₇NHBz (1.04 mmol, synthesis described below) was added in one portion with magnetic stirring followed by 60 mL of reagent grade methanol to dissolve the amine. The mixture was stirred at room

temperature to allow Schiff base formation. After 12 h, 0.23 g of NaBH₃CN (3.66 mmol, Aldrich, Cat. 156159) was added in one portion, and stirring was continued at room temperature for 4 days.

Only a portion of the reaction mixture was submitted to purification; the remainder can be stored at -20 °C and processed when desired. Six mL out of 120 mL reaction mixture was transferred to a round bottom flask. The solvent was removed by rotary evaporation. Three mL of H₂O and 0.1 g of Na₂CO₃ were added. The solution was extracted with CH₂Cl₂ (2 x 3 mL). The aqueous layer was recovered followed by addition of 0.12 mL of acetic anhydride. The reaction was stirred at room temperature for 12 hr. The reaction mixture was adjusted to pH 5-6 by adding 1% trifluoroacetic acid in water and then submitted to HPLC.

The reaction mixture was purified by HPLC using a C18 reverse phase column (Vydac 218TP1022, 2.2 x 25 cm, 10 μ, 300 angstrom) at 6 ml/min using 5-20% solvent B in A over 120 min (solvent A is 0.01% CF₃COOH in water, solvent B is CH₃CN) with detection at 230 nm. The fraction at retention time 58.7 min was collected. The solvent was removed by lyophilization to give 1.2 mg of IDS-S, which was characterized by ESI-MS.

Synthesis of IDS-IS and IDS-P. A solution of pH 1.5 nitrous acid was prepared by mixing 50 mL of ice-cold 1 N HCl with 50 mL of ice-cold 1 M NaNO₂ (50mmol NaNO₂, 3.45g). After stirring for 1 min, the pH of the solution was tested on pH meter. The pH is close to 1.9~2.0. A small amount (~ 5mL) of 1 N HCl was used to adjust the pH to 1.5. The resulting nitrous acid solution was allowed to warm to 25°C (water bath).

Deacetylated and resultfated heparin (0.5 g) was put in an Erlenmeyer flask, and 50 mL of fresh prepared pH 1.5 HNO₂ solution was poured in one portion with

stirring. The reaction mixture was stirred at 25 °C (water bath) for 1 h. The pH was adjusted to 4.0 by adding Na₂CO₃ Powder. The reaction mixture was stirred at room temperature for 12 h. The pH was adjusted to 6.0 by adding 1 M Na₂CO₃ solution. The volume of solution was reduced to 30 mL by rotary evaporation. H₂N(CH₂)₇NHBz-d₅ (0.115g, 0.48 mmol) was added to the reaction mixture followed by the addition of 30 mL of methanol. The reaction mixture was stirred at room temperature for 12 h. NaBH₃CN (0.115 g, 1.83 mmol, Aldrich) was added, and the reaction mixture was stirred at room temperature for 4 days. The solvent was removed by rotary evaporation, and the residue was purified on HPLC as described above. The disulfated disaccharide conjugate fraction was collected and lyophilized to give 26 mg of product (yield 5.2%). ESI-MS (M+1) 764.4Da.

The disaccharide conjugate (26 mg, 0.036 mmol) was dissolved in 5 mL of 0.06-0.07N HCl in anhydrous methanol. The latter was prepared by adding 5 mL of acetyl chloride (Aldrich, Cat. 114189) in one portion to 1 liter of anhydrous methanol (Aldrich, Cat. 322415), then leaving the mixture in a capped round bottom flask at room temperature for 24 h before use. The reaction mixture was stirred in a capped round bottom flask at room temperature for 24 hr. The solvent was removed in a vacuum centrifuge (Speed-Vac), and the residue was dissolved in 3 mL of 0.5 M aqueous NH₄OH. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed in a vacuum centrifuge, and the residue was dissolved in 2 mL of H₂O, followed by addition of Na₂CO₃ to adjust the pH to 9-10. One portion of acetic anhydride (20 µL) was added, and the reaction mixture was stirred at room temperature for 12 h. The solution was adjusted pH to 4-5 by addition of 5% CF₃COOH in water, and the mixture was submitted to purification by HPLC (Vydac 218TP1022 column, 6 mL/min, isocratic elution with 15% solvent B (0.08%

CF₃COOH in water), 85% solvent A (0.08% CF₃COOH in water), detection at 230 nm). The fraction at retention time 57 min was collected and lyophilized to give 12.3 mg of desulfated disaccharide IdoA-AnM-NAc(CH₂)₇NHBz-d₅ (Yield 57%). ESI-MS (M+1)⁺ 604.4.

Desulfated disaccharide IdoA-AnM-NAc(CH₂)₇NHBz-d₅ (5 mg, 8.28 μmol) was dissolved in 2 mL of anhydrous DMF (Aldrich) followed by addition of 4 beads of molecular sieves (4 angstrom). SO₃· Pyridine complex (26.5 mg, 165.6 μmol, Aldrich. Cat. S7556) was dissolved in 4 mL of anhydrous DMF and added to the reaction mixture in 4 portions (every 4 h). The reaction was stirred for an additional 12 h at room temperature. The reaction mixture was chilled in an ice-water bath, and 1 mL of water was added. The pH was adjust to 9 with 1 M Na₂CO₃. The solvent was removed in a Speedvac. The residue was dissolved in 1 mL of water and purified by HPLC (Vydac 218TP1022, 6 mL/min, 0-120 min using 14% CH₃CN in water with 0.08% CF₃COOH, detection at 230 nm). The fraction at retention time 52.5 min is the monosulfated product, and the fraction at retention time 81.3 min is the nonsulfated starting material. The product fraction was collected and lyophilized to give 1.8 mg of product (31.8% yield). ESI-MS (M+1) 684.4 Da.

IDS-P was made in the same way as for IDS-IS using non-deuterated benzoyl chloride.

Synthesis of H₂N(CH₂)₇NHBz and H₂N(CH₂)₇NHBz-d₅. H₂N(CH₂)₇NHBz was synthesized as follows. Heptamethylenediamine (4 g, 30.8 mmol, Aldrich Cat. D17408) was dissolved in 15 mL of reagent grade CH₂Cl₂ in a round bottom flask. Reagent grade Na₂CO₃ (0.5 g) was added in 1 portion followed by dropwise addition over ~ 2 hr with magnetic stirring of a solution of benzoyl chloride (0.6 ml, 5.17 mmol) in CH₂Cl₂ (30 mL). The flask was capped and the mixture stirred at room

temperature for ~12 hr. HPLC grade water (20 mL) was added, and the organic layer was separated and dried over anhydrous Na₂SO₄. Solvent was removed with a rotary evaporator, and the residue was purified by flash chromatography on silica gel (3.5 cm x 30 cm column, 40-63 µm, Silicycle, Inc.) using CHCl₃:CH₃OH, 1:4 with 5% triethylamine); R_f = 0.18, same solvent. The yield of product is 0.77 g (68 %). Electrospray ionization mass spectrometry gave 235 Da for (M+H⁺). ¹H-NMR (CDCl₃/(CH₃)₄Si) δ 7.7, d, 2H, Ar; 7.46-7.30, m, 3H Ar; 3.3, t, 2H, CH₂NHCO; 2.5, t, 2H, CH₂NH₂; 1.55, t, 2H, CH₂ 7 1.42, t, 2H, CH₂; 1.35, m, 6H CH₂.

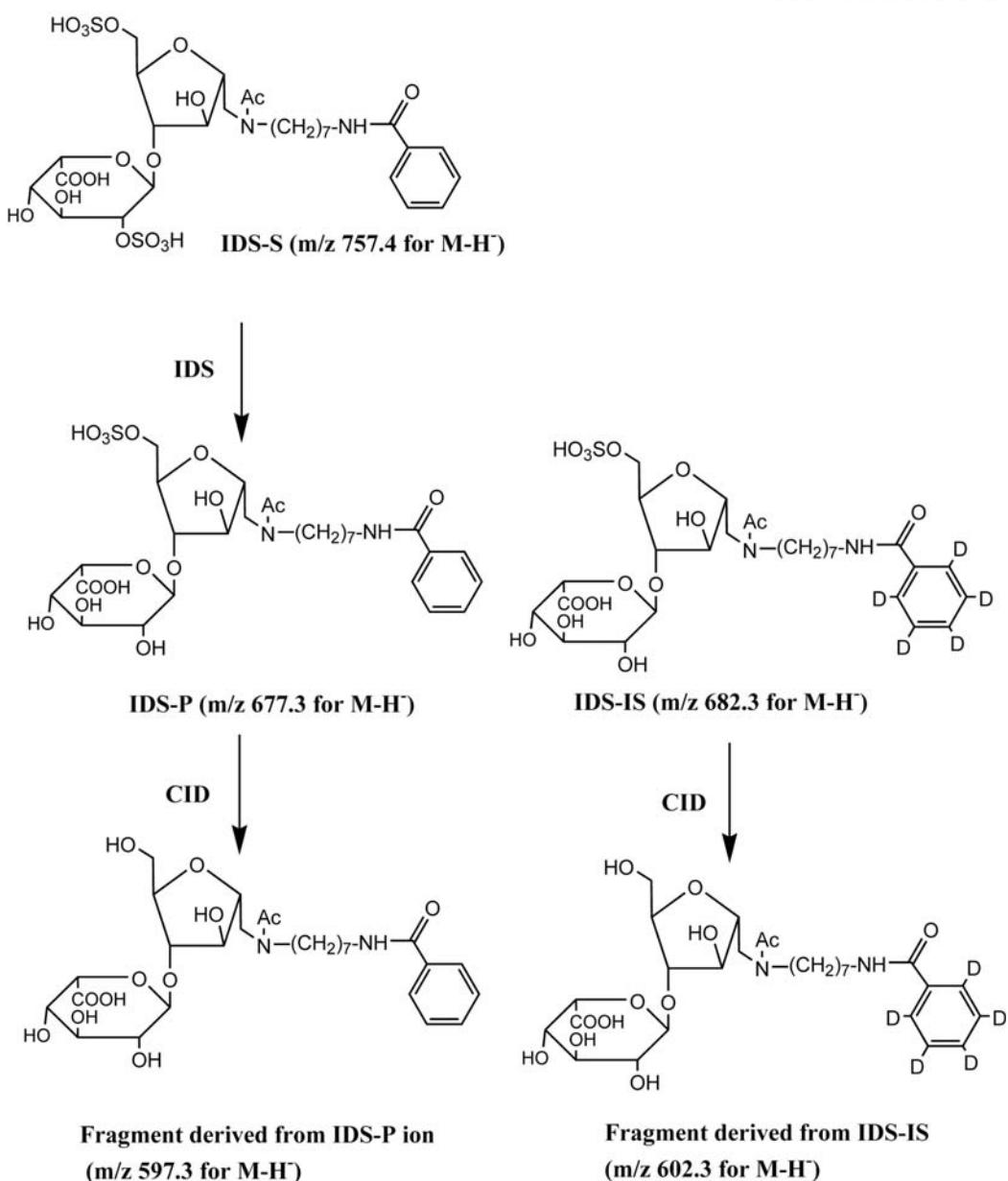
H₂N(CH₂)₇NHBz-d₅. was prepared as follows. H₂N(CH₂)₇NH₂ (1 g, Aldrich, Cat. D17408) was dissolved in 5 mL CH₂Cl₂ followed by addition of 0.5 g Na₂CO₃. Deuterated benzoyl chloride (0.2g, Aldrich Cat. 366048) was dissolved in 25 mL CH₂Cl₂, and the solution was slowly added dropwise to the diamine solution with stirring. The reaction mixture was stirred at room temperature for 12 h in a capped flask. Water (10 mL) was added, and the pH was adjusted to 9-10 using 1 N NaOH. The solution was extracted with CH₂Cl₂ (3 x 20 mL). The extracts were combined and concentrated by rotary evaporation. The residue was purified on silica gel chromatography with CHCl₃:CH₃OH (1:4) with 5% triethylamine, R_f = 0.18) to give 0.15 g product (yield 46%). ESI-MS (M+1)⁺ 240 Da.

Supplementary Data Table 1. IDS Activities for Individual DBS

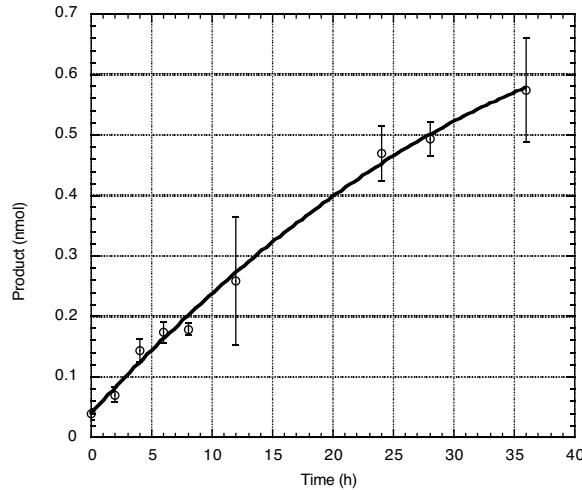
MPS-II patient	IDS activity ($\mu\text{mol hr}^{-1} (\text{L blood})^{-1}$)				
	Normal newborns				
1.714	24.5	26.1	51.5	21.1	50.8
1.010	14.8	24.5	29.0	37.2	49.7
0.635	22.2	21.4	42.0	38.4	59.0
0.306	10.4	19.5	36.5	42.9	56.1
0.995	15.3	26.1	40.1	39.0	83.4
0.357	12.2	40.8	30.4	57.0	
1.042	21.9	38.7	34.2	26.2	
1.917	21.2	35.4	45.8	60.3	
1.792	25.0	41.5	29.7	57.5	
0.109	18.9	58.3	41.4	59.2	
0.460	26.2	28.7	28.3	34.2	
0.043	28.7	28.9	38.3	55.0	
0.132	27.3	46.2	27.2	62.6	

The control value, obtained from an identical assay in which DBS was replaced with a 2 mm filter paper punch without blood, (see main text) was subtracted from all values.

Supplemental Figure 1

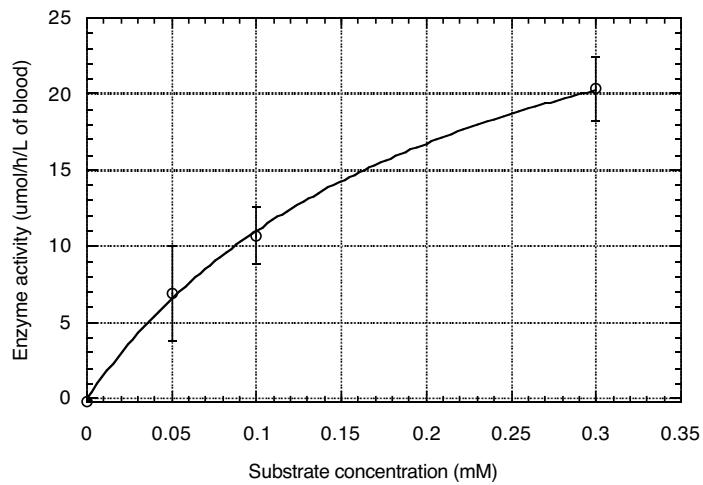


Supplementary Data Figure 2



Amount of IDS-generated product measured in DBS as a function of the incubation time. Reactions were carried out at 37 °C using 0.1 mM IDS-S and the conditions given in the maintext. Error bars are shown for duplicate analyses.

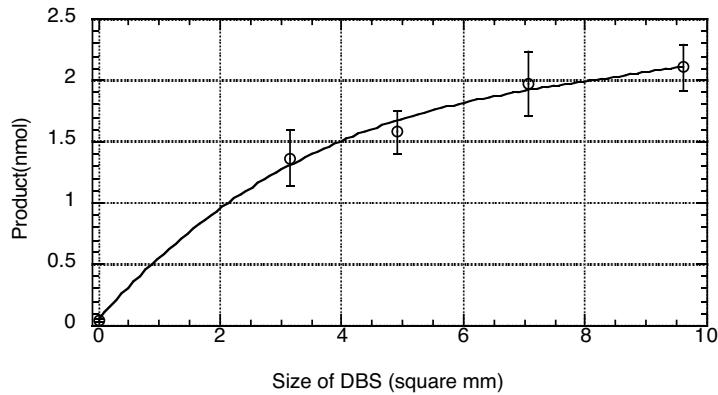
Supplementary Data Figure 3



Activity of IDS measured in DBS as a function of the concentration of IDS-S.

Reactions were carried out at 37 °C for 20 h using the standard assay given in the main text. Error bars are shown for duplicate analyses.

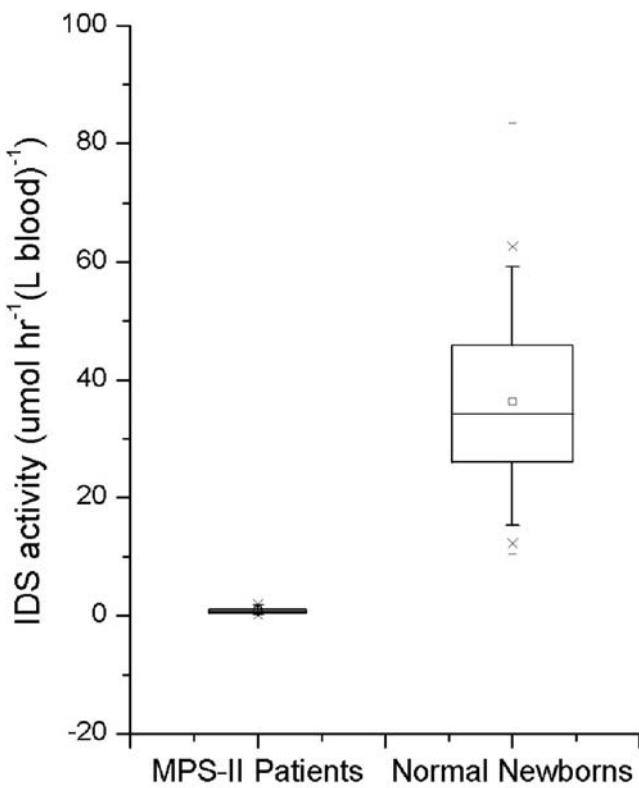
Supplementary Data Figure 4



Activity of IDS measured in DBS as a function of the size of the DBS punch.

Reactions were carried out at 37 °C for 20 h using the standard assay given in the main text. Error bars are shown for duplicate analysis.

Supplementary Data Figure 5



Shown is the same data as Figure 1 of the main text but plotted according to: square, average value; horizontal line in rectangle, median value; rectangle, 25-75 percentile; horizontal line with vertical line, 5-95 percentile; X, 1-99 percentile; horizontal lines, minimum and maximum values.