Supporting Information for:

Low-molecular weight sulfated marine polysaccharides: promising molecules to prevent neurodegeneration in mucopolysaccharidosis IIIA?

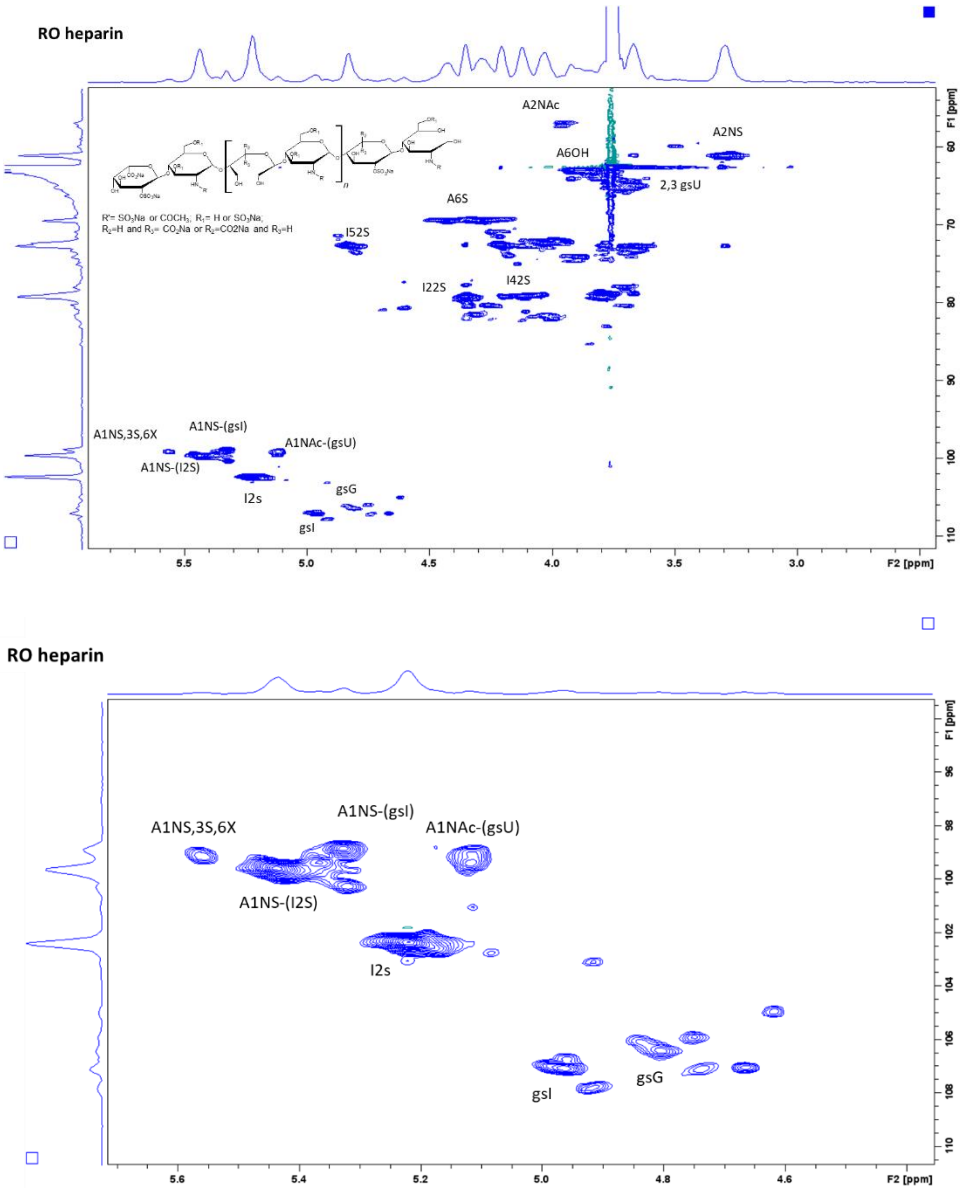


Fig. S1. HSQC of RO heparin. On top, signals of the ring region. On the bottom, signals of the anomeric region. ANS =N -sulfated-glucosamine, ANAc = N-acetyl-glucosamine, I = iduronic acid, G = glucuronic acid, gs= glycol-split, U= uronic acid.

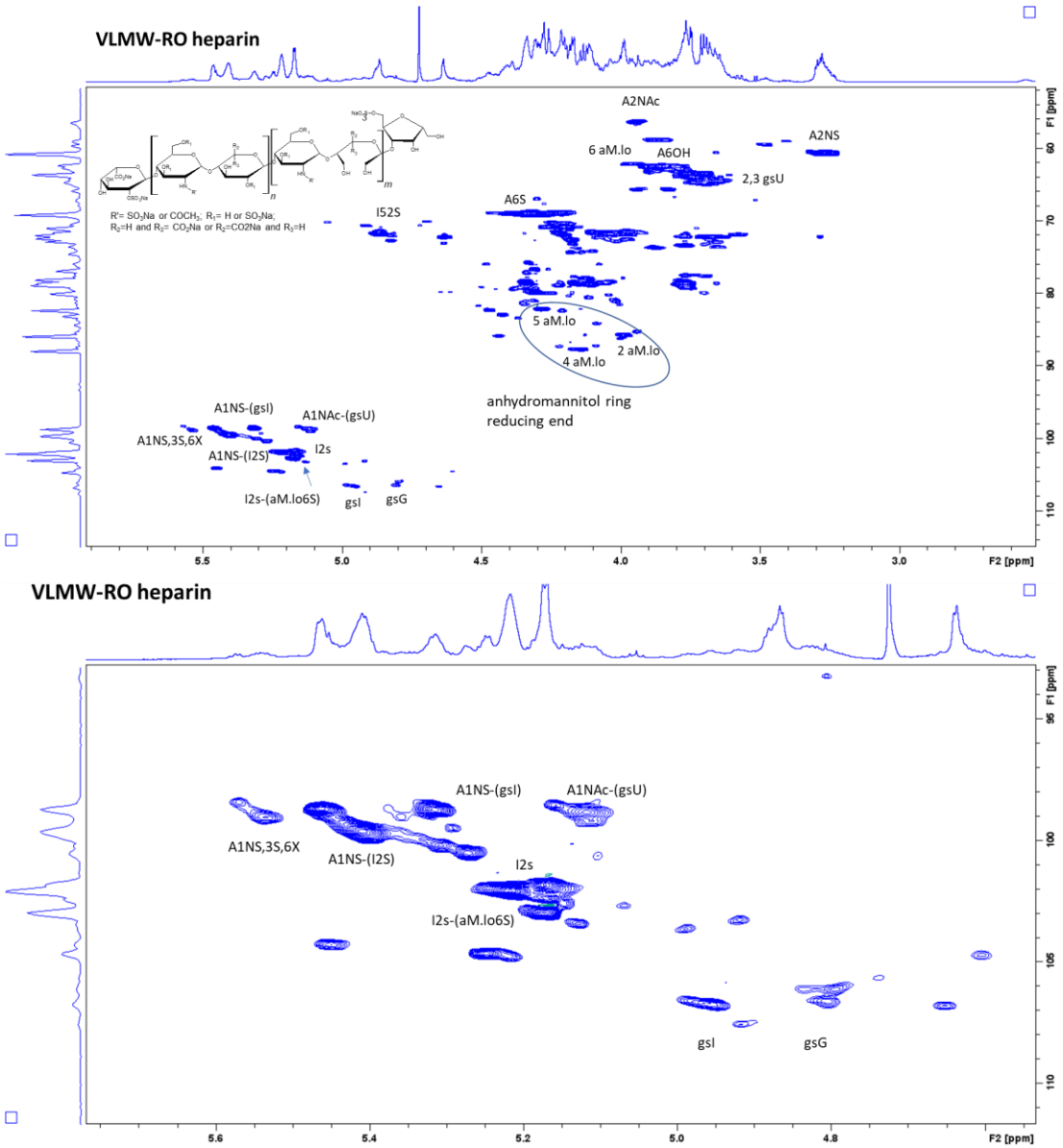
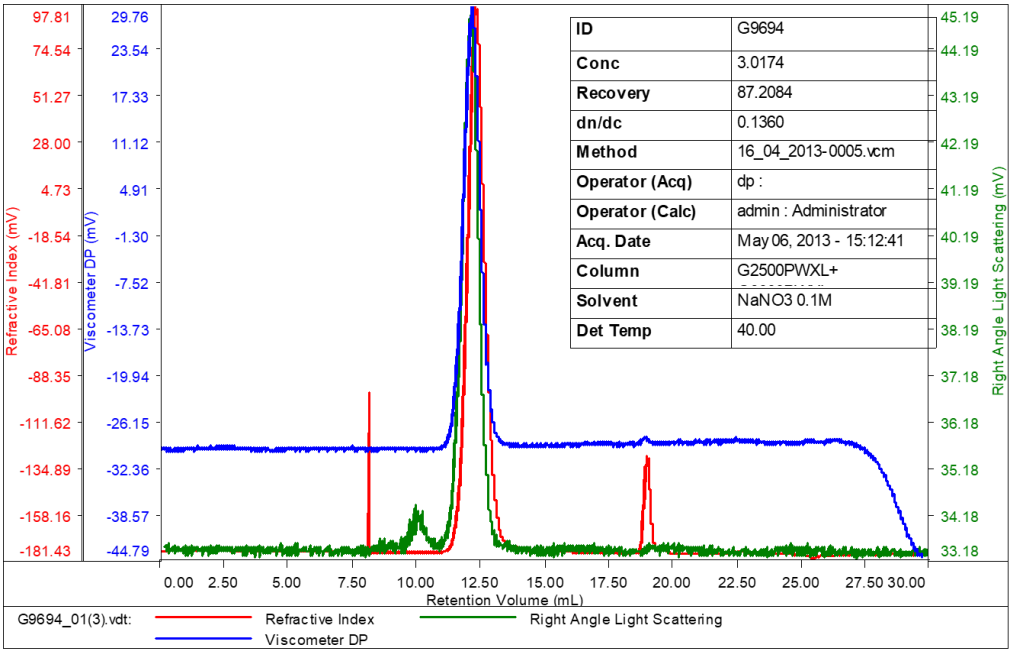


Fig. S2. HSQC of VLRO heparin. On top, signals of the ring region. On the bottom, signals of the anomeric region. ANS =N -sulfated-glucosamine, ANAc = N-acetyl-glucosamine, I = iduronic acid, G = glucuronic acid, gs= glycol-split, U= uronic acid, aM.ol= anhydromannithol.



Sample	Mn	Mw	Mp	MwMn	IV	Rh	MH - a	MH - k
G9694_01(3).vdt	9.885	16.591	15.164	1.678	0.1653	3.40	0.917	-4.648

Fig. S3. TDA analysis of RO heparin. Size-exclusion chromatography profile of RO heparin. Molecular weight parameters are indicated.

Fig. S4. TDA analysis of VLRO heparin. Size-exclusion chromatography profile of RO heparin. Molecular weight parameters are indicated. (pdf attached at the end of this file)

Table S1. Monosaccharide composition (wt%), sulfur and protein contents (wt%) of native diabolican and infernan EPS and their corresponding depolymerized LMW derivatives.

	Monosaccharide composition (wt%)						S	Proteins
	Gal	Glc	GalA	GlcA	GalNAc	GlcNAc	(wt%)	(wt%)
Diabolican	-	-	-	19.4	12.7	20.7	0	1.9
LMW diabolican	-	-	-	19.7	14.1	19.8	0	0.3
Infernan	13.8	17.5	6.4	9.8	-	-	2.9	15
LMW infernan	13.0	15.2	7.1	10.9	-	-	3.0	1.5

Gal: galactose, Glc: glucose, GalA: galacturonic acid, GlcA: glucuronic acid, GalNAc: N-acetyl-galactosamine, GlcNAc: N-acetyl-glucosamine. The protein content in the EPS samples was determined using bicinchoninic acid method (BCA-Kit, Sigma). LMW highly sulfated derivatives, A5_3 and A5_4, were not analyzed in term of their monosaccharide composition since sulfate groups prevent the correct derivatization and formation of the per-O-trimethylsilyl methyl glycosides. The residual protein part corresponds to proteins that can be co-eluted with the EPS at the same time.

The total amount of sulfated proteoglycans (PGs) is considered as the recovered material from the first purification step and is expressed in cpm (radioactivity units). No visible difference was observed between untreated and treated cells regarding the total amount of proteoglycans (**Figure S1,** two-way ANOVA with Dunnett's *post-hoc*, 0 *vs* 20, 50 or 100 µg/ml), showing that treatment with different LMW glycans did not alter the biosynthesis of proteoglycans. Control and treated cells presented increased amount of PGs in the extracellular compartment compared to the intracellular space.

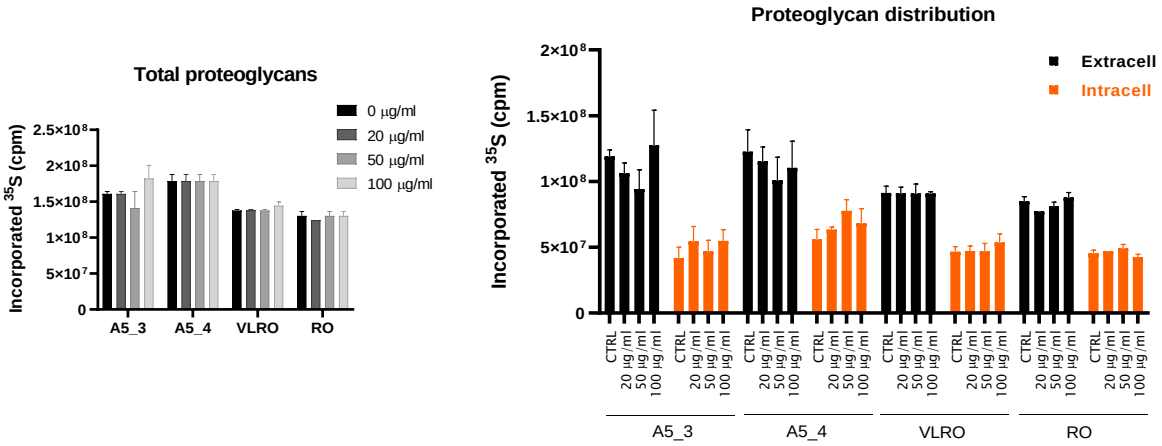


Fig. S5. Distribution of proteoglycans in untreated and treated MPSIIIA fibroblasts. Left graph: total incorporated radioactivity ($^{35}SO_4$) corresponding to total proteoglycans. Right graph: repartition of incorporated radioactivity (proteoglycans) between extracellular and intracellular compartments. For each compound, the average of two independent experiments is reported ± SEM (except RO 20 µg/ml for which just one experience is reported). As control, the average of 8 independent experiments ± SEM is reported.

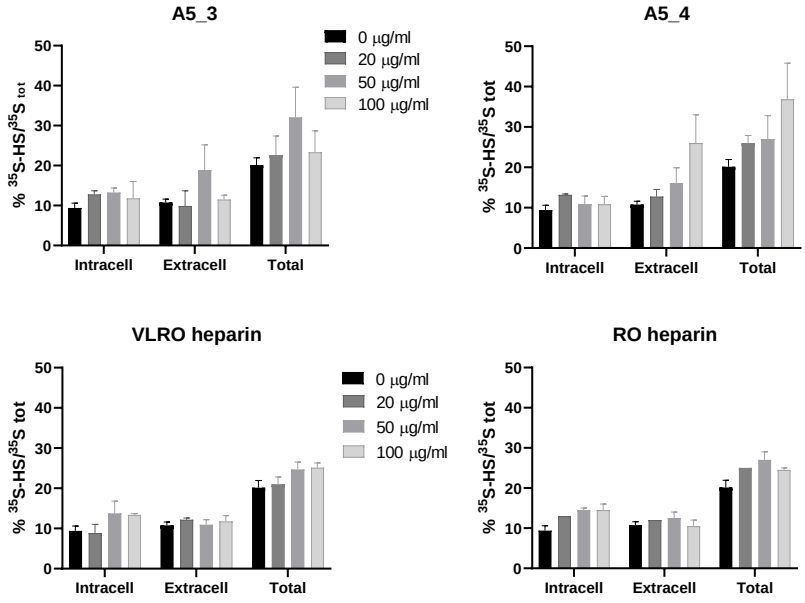


Fig. S6. Distribution of heparan sulfate in untreated and treated MPSIIIA fibroblasts. The graph reports the %HS on total incorporated radioactivity (PGs) in extracellular and intracellular compartments. Total PGs are considered as 100%. For each compound, the average of two independent experiments is reported ± SEM (except 20 μg/ml RO for which a single experiment is reported). For controls, the average of 8 independent experiments ± SEM is reported.

Table S2. Size distribution of HS in MPS-IIIA cells treated with 0-50-100 μg/ml HPSE inhibitors. HS was isolated from control and treated cells and analyzed by PAGE. Molecular weight (M) is expressed in kDa. When possible, the average of two independent analyses ± SD is reported. The range was calculated at half peak height.

		Intracellular		**Extracellular**	
	μg/ml	M_{peak}	M_{range}	M_{peak}	M_{range}
A5_3	0	smear	smear	43.1±12.1	32.1-56.8
	50	34.8 ±1.7	21.1-54.1	43.3±11.3	28.4-61.0
	100	36.2 ±2.7	22.5-55.2	46.2±12.2	30-62.2
A5_4	0	smear	smear	35.6 ±2.4	24.2-47.9
	50	34.2 ±6.2	23.7-50.6	41.1 ±4.3	23.7-50.2
	100	34.6 ±6.3	23.9-50.5	32.1 ±2.1	21.3-49.9
VLRO	0	smear	smear	39 ±2.6	27.3-51.1
	50	34 ± 2.6	15.2-50.9	37.9 ±2.9	26.5-51.0
	100	35 ± 2	17.9-51.4	36.3 ±7.3	27.0-50.4
RO	0	smear	smear	36.4±1.4	22.6-48.7
	50	32.30	20.9-42.9	37.5±2.4	24.7-49.3
	100	32.2±2.3	22.2-43.8	37.9±1.9	24.0-49.9

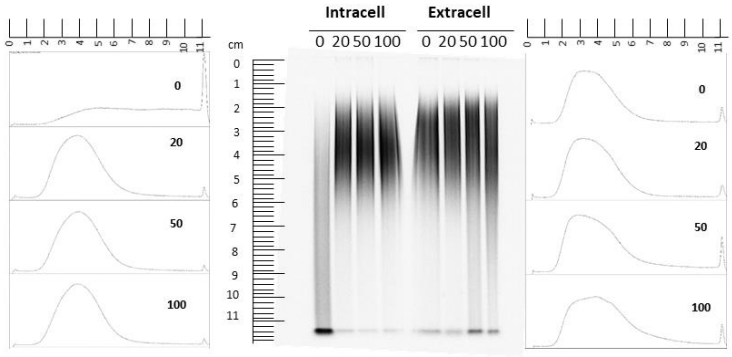


Fig. S7. Profiles of PAGE-NaCl of HS from MPS fibroblasts treated with A5_4. HS was isolated from control (0 µg/ml A5_4) and cells treated with 20-50-100 µg/ml A5_4, analyzed by PAGE-NaCl and detected by autoradiography 300,000 cpm of HS were loaded on the gel. The relative molecular weight is calculated by comparison with a standard curve. Profiles were obtained with the software ImageJ.

Table S3. Size distribution of HS in MPS-IIIA cells treated with 0-10-20 µg/ml A5_3. HS was isolated from control and treated cells and analyzed by PAGE and GFC. The molecular weight (M) is expressed in kDa. Results are from one experiment only. The range is calculated at half peak height.

	PAGE				**GFC**	
	Intracell		Extracell		intracell	
µg/ml	M_{peak}	M_{range}	M_{peak}	M_{range}	M_{peak}	M_{range}
0	9.2	5.3-12.3	48.3	18.4-79.1	6.8	3.1-15
10	43.9	10.8-60.7	50.3	21-64.8	20.4	6-84.7
20	43.4	20-62.2	49.3	20.7-80	38.5	12.8-98.8

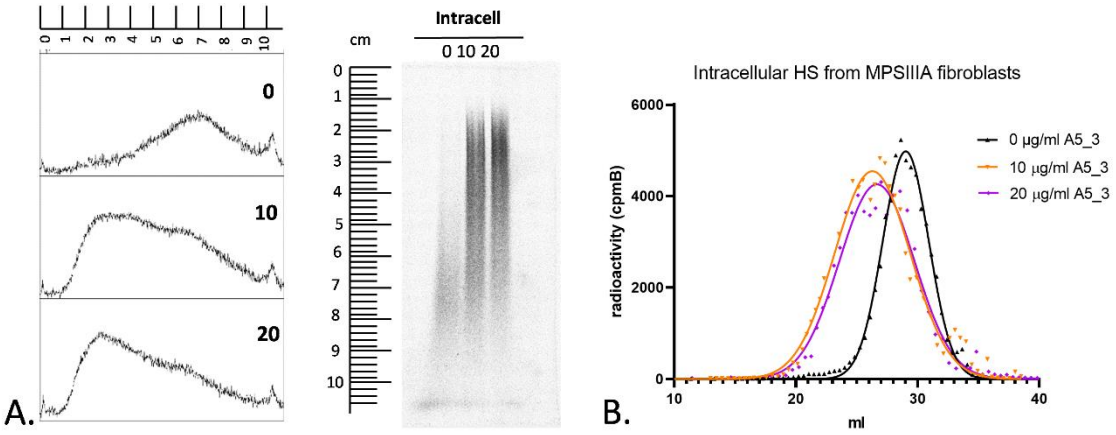


Fig. S8. Profiles of PAGE and gel filtration chromatography of HS from MPSIIIA fibroblasts treated with 10 µg/ml A5_3. HS was isolated from control (0 µg/ml A5_3) and cells treated with either 10 or 20 µg/ml A5_3, analyzed by PAGE and detected by autoradiography. 300,000 cpm of HS were loaded on the gel. Profiles were obtained with the software ImageJ. A) Profiles from PAGE. B) Superimposition of the GFC profiles of intracellular HS from control and treated cells. Treatment causes a shift towards higher molecular weights. The relative M was calculated by comparison with a standard curve. 75,000 cpm of HS were applied to the column.

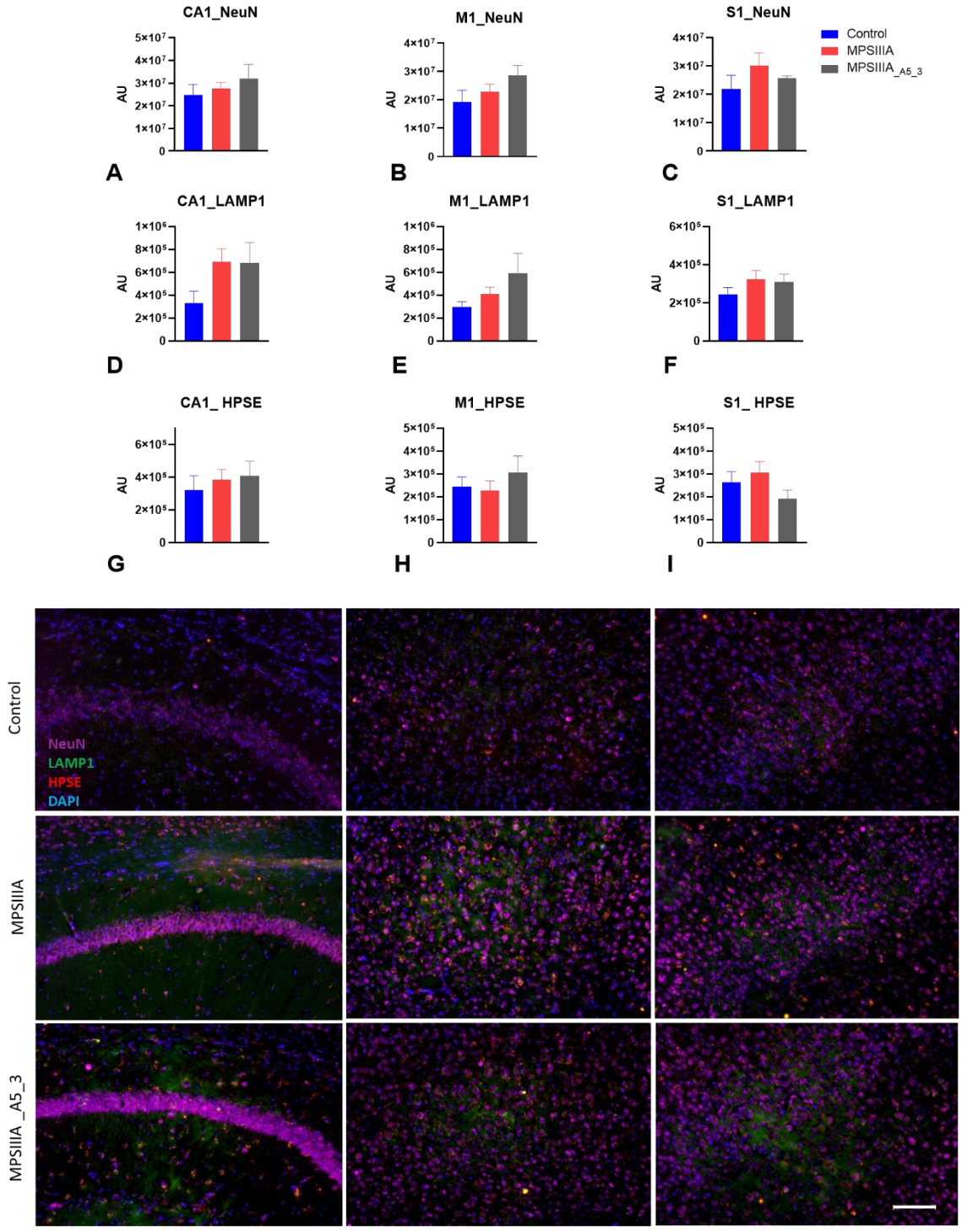


Fig. S9. Immunohistochemistry analysis of HPSE, LAMP1 and neuronal markers in the brain of 12-weeks-old mice. NeuN (graphs A-C), LAMP1 (graphs D-F) and HPSE (graphs G-I) were quantified in one region of the hippocampus (CA1, left panel) and in two regions of the cortex (M1 and S1, central and right panels, respectively). Representative images for each region are shown below. Nuclei were stained in blue with DAPI, LAMP1 in green, HPSE in red, NeuN in purple. Scale bar: 50µm. Data are expressed as mean ± SEM (n=6) and assessed by One-way ANOVA followed by Tukey's *post hoc* or Kruskal-Wallis. No significant differences were observed among groups.

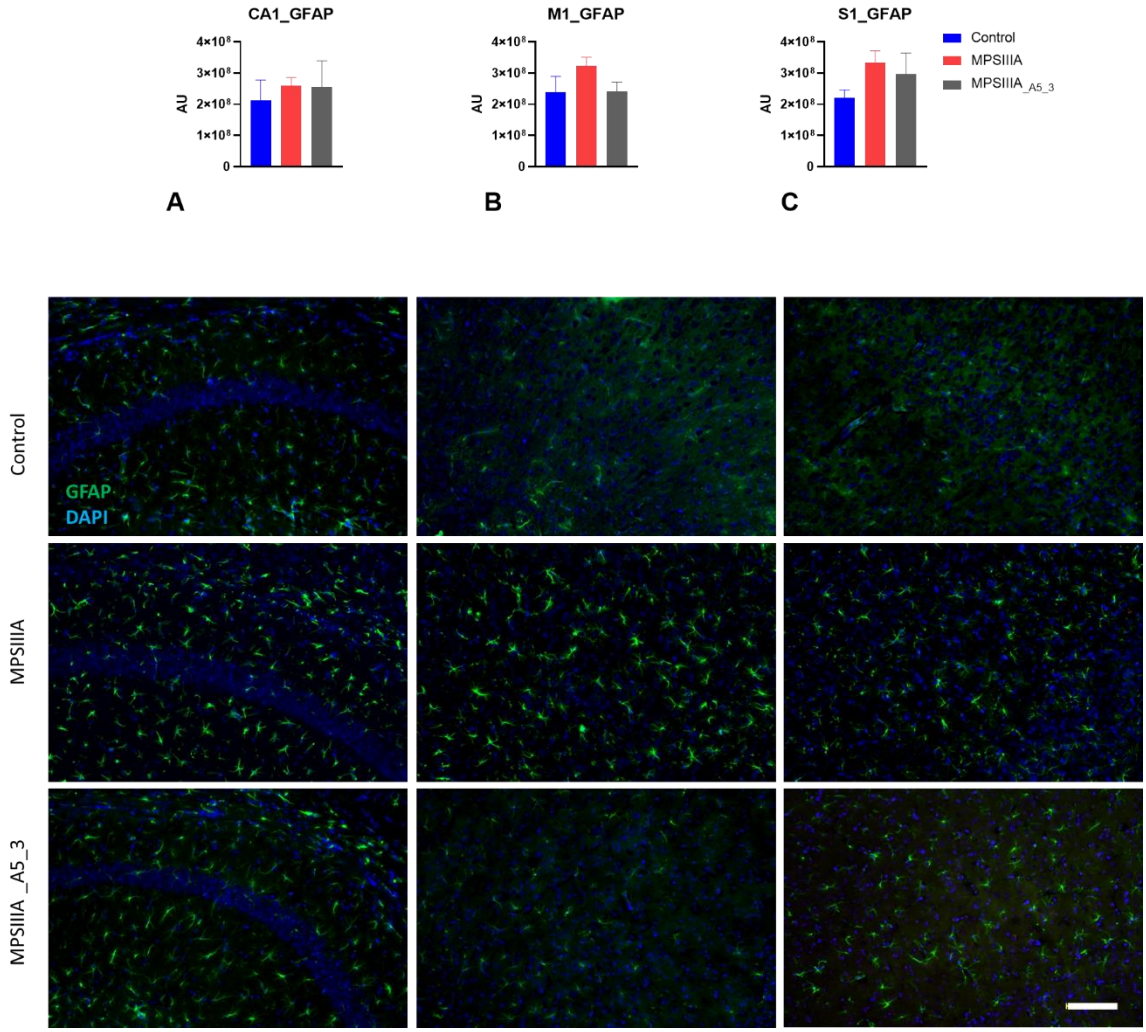


Fig. S10. Immunohistochemistry analysis of astrocyte markers in the brain of 12-weeks-old mice. GFAP was quantified in one region of the hippocampus (CA1, left panels and graph A) and in two regions of the cortex (M1, central panels and graph B and S1, right panels and graph C). Representative images for each region are shown below. Nuclei were stained in blue with DAPI and in green for GFAP. Scale bar: 50µm. Data are expressed as mean ± SEM (n=6) and assessed by One-way ANOVA followed by Tukey's *post hoc* or Kruskal-Wallis. No significant differences were observed among groups.

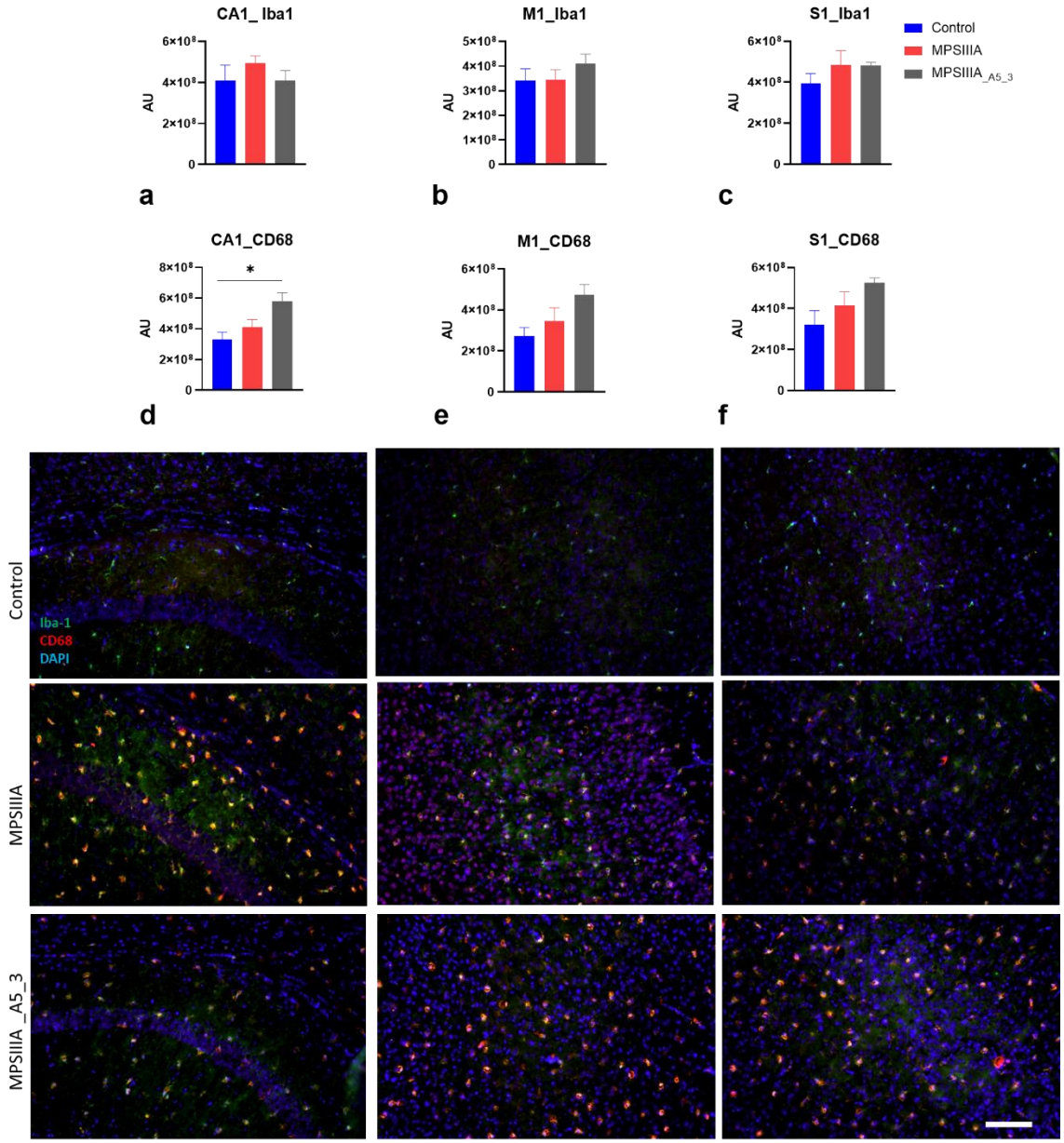


Fig. S11. Immunohistochemistry analysis of microglial activation markers in the brain of 12-weeks-old mice. Iba1 (graphs a-c) and CD68 (graphs d-f) were quantified in CA1 (left panel) and in two regions of the cortex (M1 and S1, central and right panels, respectively). Representative images for each region are shown below. Nuclei were stained in blue with DAPI, Iba1 in green, CD68 in red. Scale bar: 50µm. Data are expressed as mean ± SEM (n=6) and assessed by One-way ANOVA followed by Tukey's *post hoc* or Kruskal-Wallis. *Control *vs* MPSIIIA.

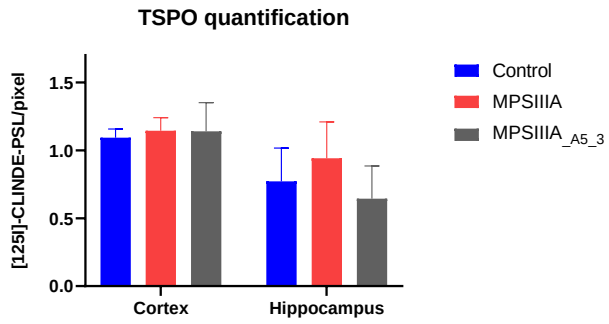


Fig. S12. TSPO level in the cortex and hippocampus of 12-week-old mice. Data are expressed as mean ± SD (n=6) and assessed by One-way ANOVA followed by Tukey's *post hoc* or Kruskal-Wallis**.** No significant differences were observed among groups.

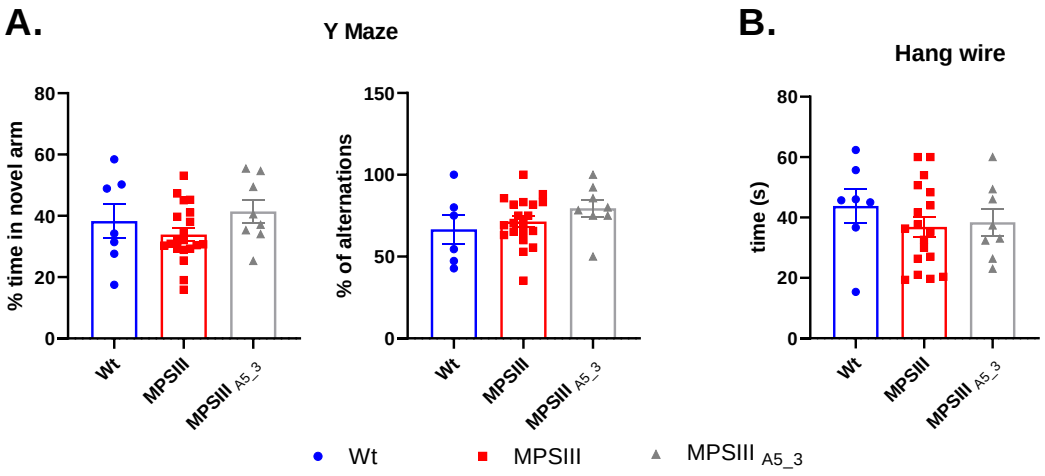


Fig. S13. Y maze and Hang Wire tests. Both male and female mice were subjected to different behavioral tests at 11 weeks of age. A) Results from the Y maze test. B) Results from the Hang Wire test. Data are expressed as mean ± SEM.

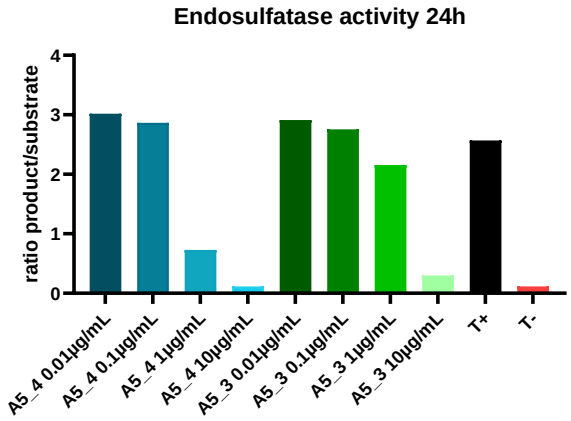


Fig. S14. Inhibition of SULFs by compounds A5_3 and A5_4. Heparin was digested with Hsulf-2, in presence of increasing concentrations (0.1-10 µg/mL, from dark to light) of A5_4 (blue) and A5_3 (green). After 24h incubation, heparin was exhaustively depolymerized and disaccharide content was analyzed by RPIP-HPLC. Results are expressed as the ratio of Hsulf-2 product/substrate disaccharides and compared to negative (T-, untreated heparin) and positive (T+, Hsulf-2 treated heparin in absence of inhibitor) controls.

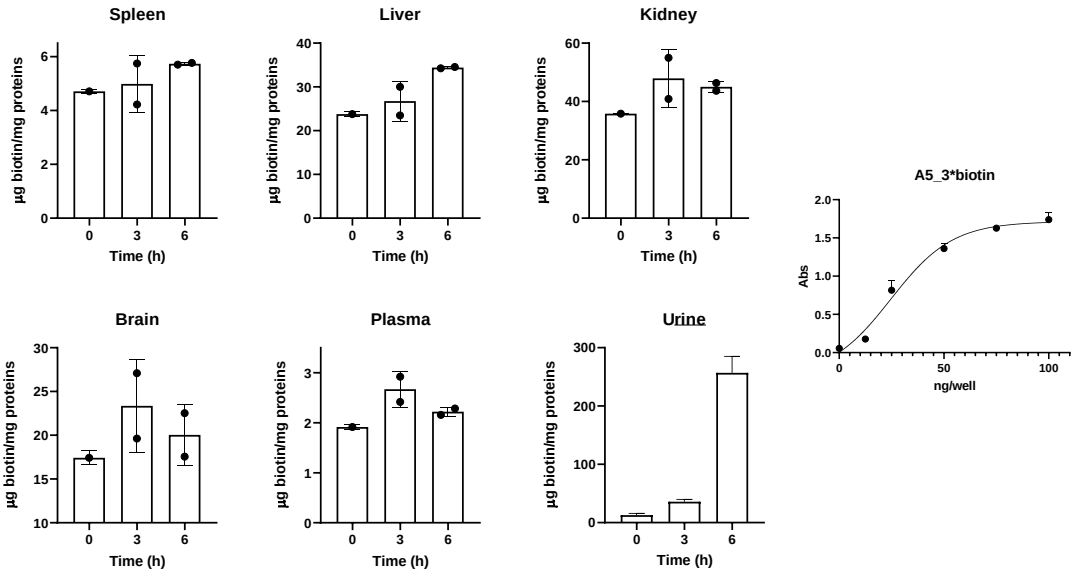


Fig. S15. Biodistribution of A5_3 in MPSIIIA mice. Mice were sacrificed after 0, 3 or 6h following an IP injection of 20 µg/g biotinylated A5_3. Detection of biotin was performed by ELISA. Results were normalized on the protein content of each organ. Quantification of proteins was performed on the extract using the Bradford method (n=2).

Results Report

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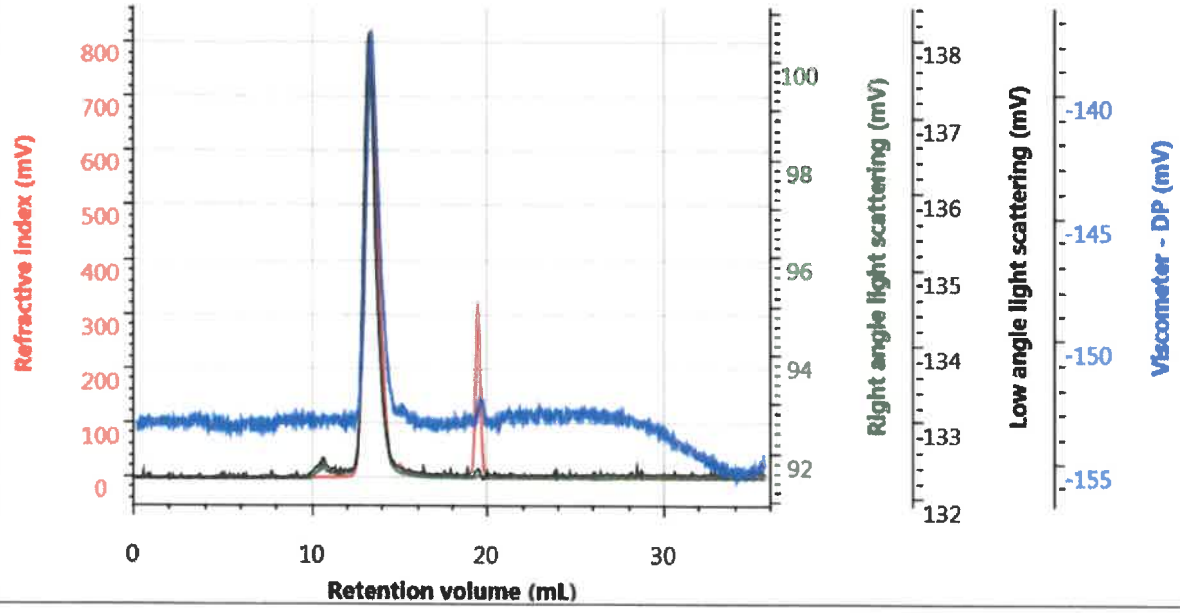
Chromatogram



Sample Info

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Concentration (mg/mL)	6.5
Acquisition date	29/03/2018 11:21:33
Injection volume (µL)	100
Sample dn/dc	0.13
Analysis date	03/04/2018 10:51:11
OMNISEC file name	8c522a9a-9d13-493d-962e-da5c7c82204b
Notes	N/C
Autosampler temperature (°C)	20
Column oven temperature (°C)	40
Column set	G2500PWXL + G3000PWXL
Detector temperature (°C)	40
Flow rate (mL/min)	0.600000023841858
Pump Pressure (MPa)	3.268

Results Report

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Results by sample and peak.

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	Peak 1
RV (mL)	13.34
Mn (g/mol)	2,819
Mw (g/mol)	3,365
Mz (g/mol)	4,450
Mw/Mn	1.194
IVw (dL/g)	0.04743
Rh(η)w (nm)	1.337
M-H a	0.6215
M-H log K (dL/g)	-3.509
RI peak (mV·mL)	743.4
UV peak (mV·mL)	N/C
RALS peak (mV·mL)	7.716
LALS peak (mV·mL)	4.843
DP peak (mV·mL)	14.04
Frac. of sample (%)	100
Recovery (%)	81.33

Results Report

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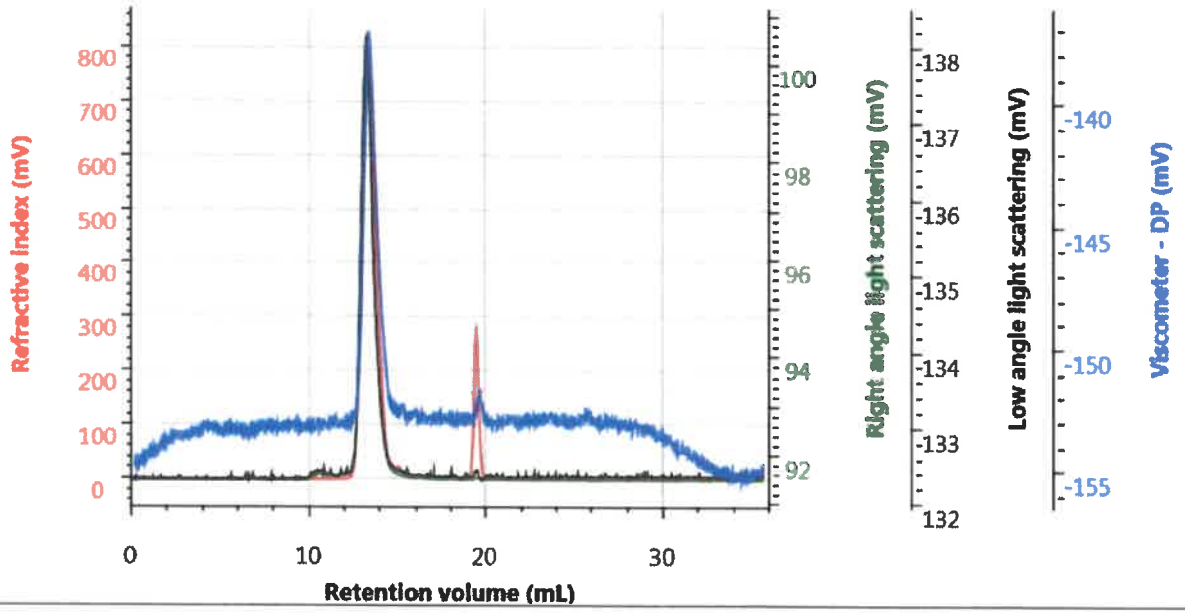
Chromatogram



Sample Info

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Sample dn/dc	0.13
Analysis date	03/04/2018 10:50:50
OMNISEC file name	f0fd0ba4-e2a6-415a-9743-1f5481018572
Notes	N/C
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Column oven temperature (°C)	40
Column set	G2500PWXL + G3000PWXL
Detector temperature (°C)	40
Flow rate (mL/min)	0.600000023841858
Pump Pressure (MPa)	3.268

Results by sample and peak.

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Mn (g/mol)	2,799
Mw (g/mol)	3,342
Mz (g/mol)	4,401
Mw/Mn	1.194
IVw (dL/g)	0.04712
Rh(η)w (nm)	1.331
M-H a	0.5788
M-H log K (dL/g)	-3.357
RI peak (mV·mL)	752.7
UV peak (mV·mL)	N/C
RALS peak (mV·mL)	7.764
LALS peak (mV·mL)	4.869
DP peak (mV·mL)	14.28
Frac. of sample (%)	100
Recovery (%)	82.35