Science Advances
AAAS

Supplementary Materials for

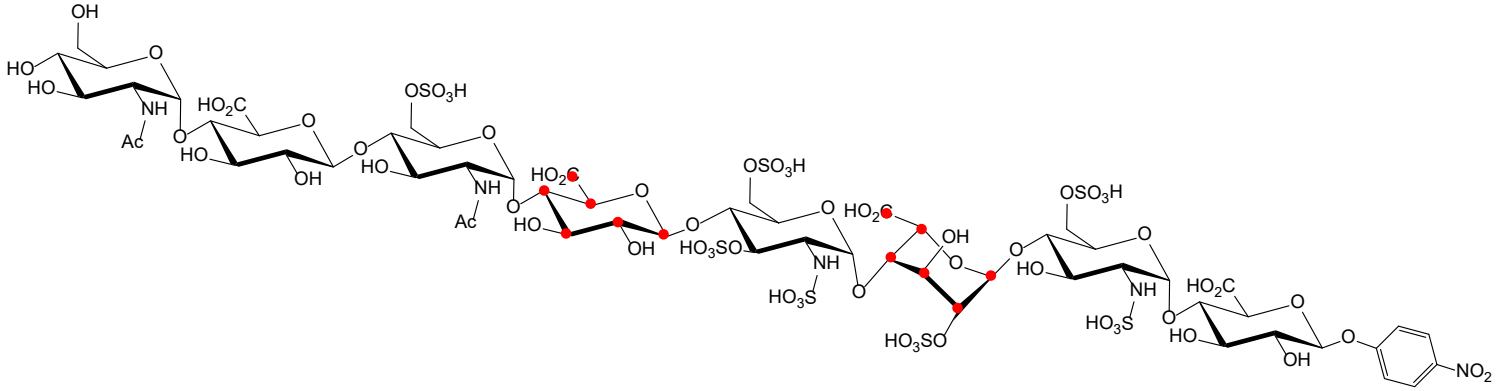
Increased 3-*O*-sulfated heparan sulfate in Alzheimer's disease brain is associated with genetic risk gene *HS3ST1*

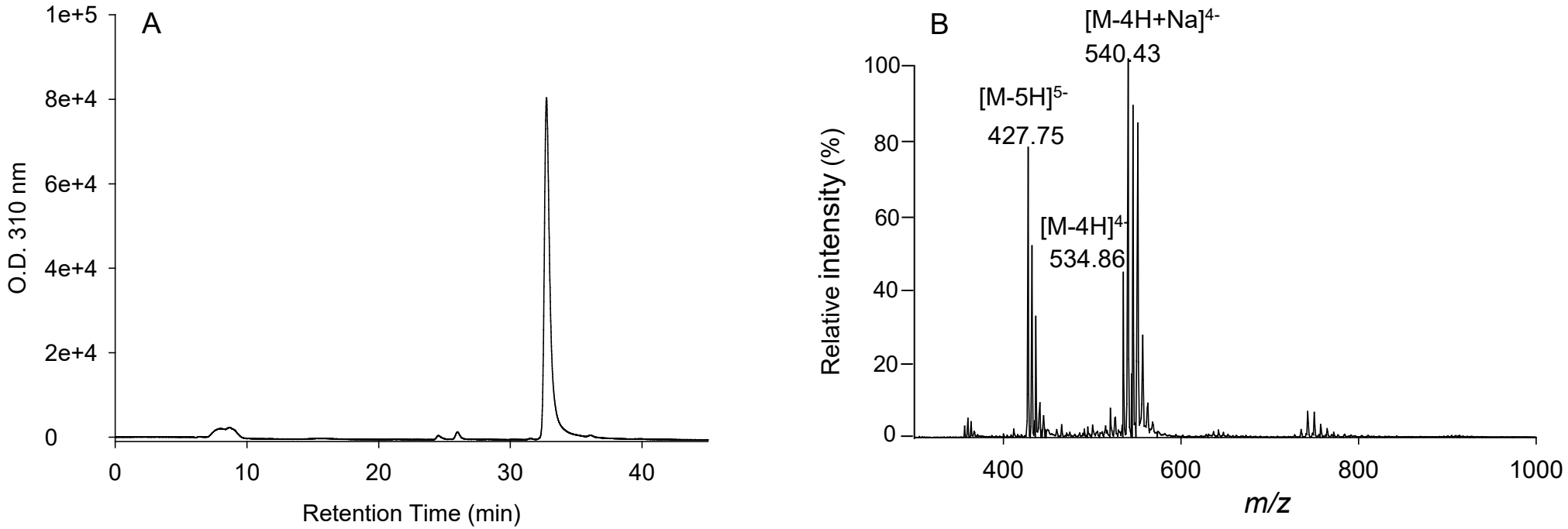
Zhangjie Wang *et al.*

Corresponding author: Jian Liu, jian_liu@unc.edu

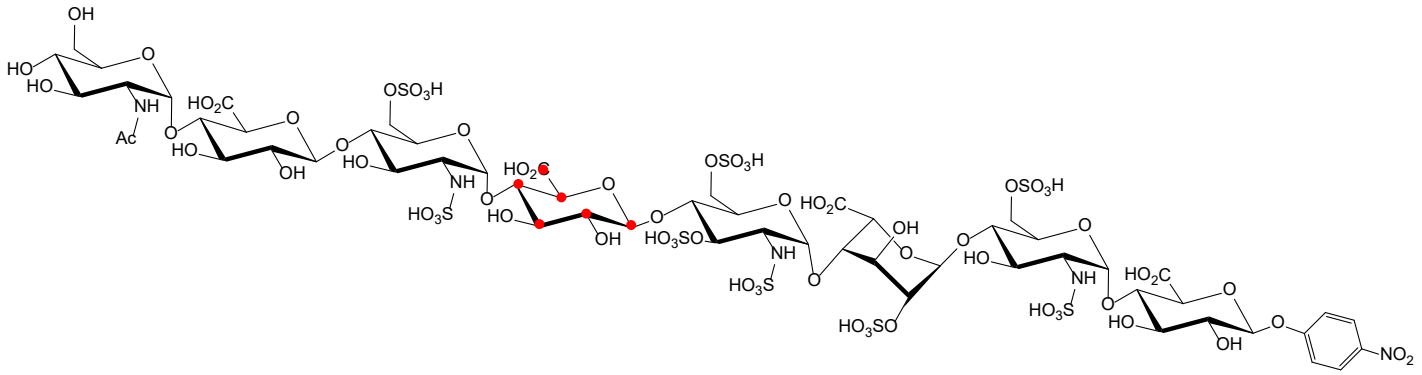
Sci. Adv. **9**, eadf6232 (2023)
DOI: 10.1126/sciadv.adf6232

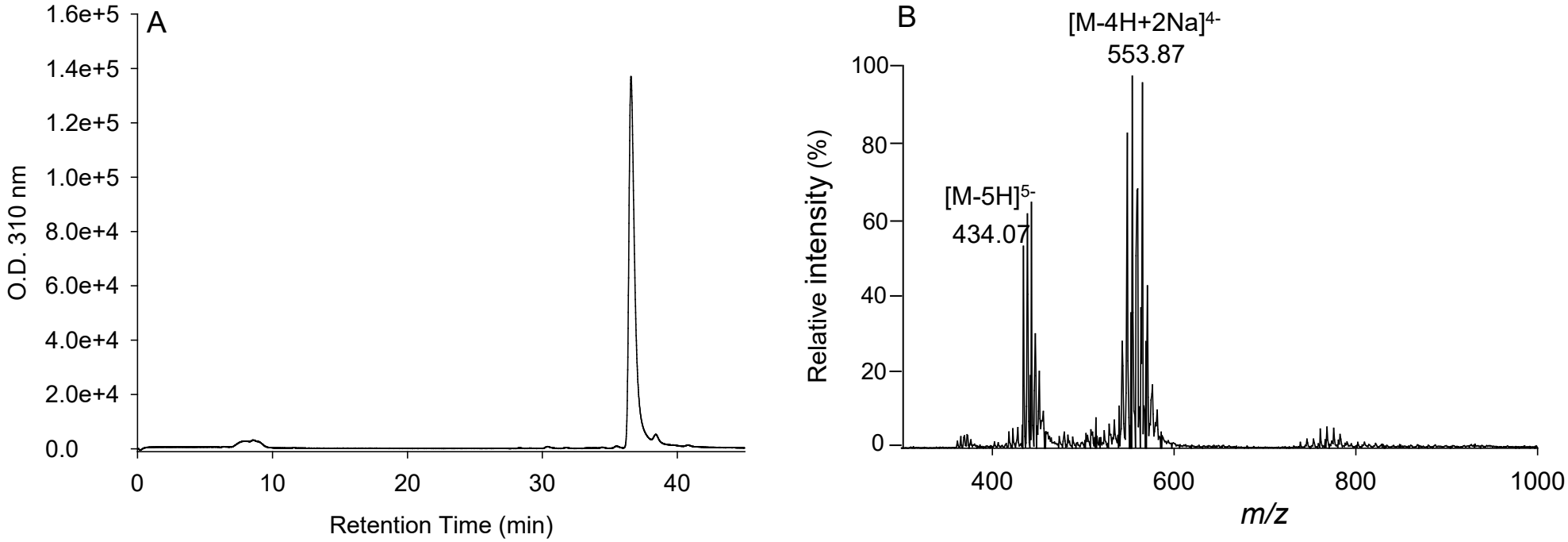
This PDF file includes:



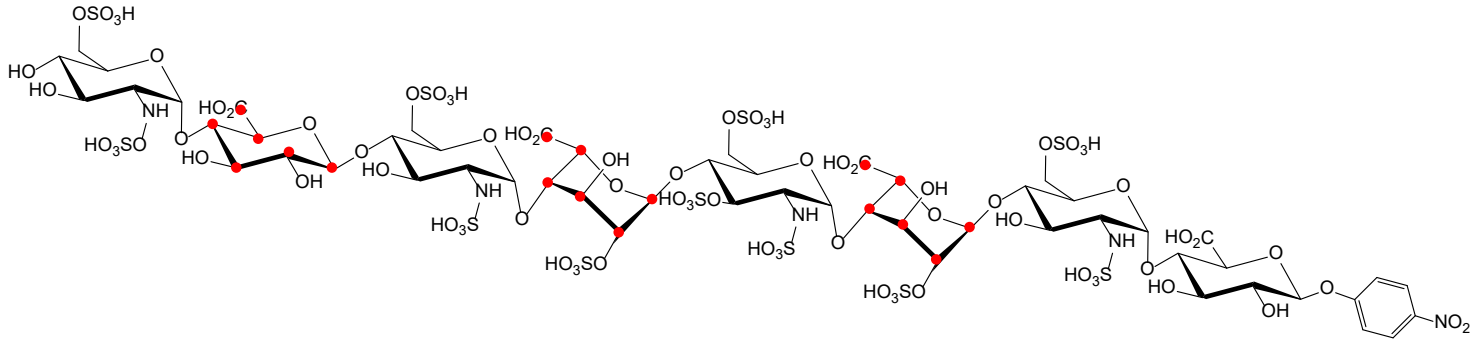


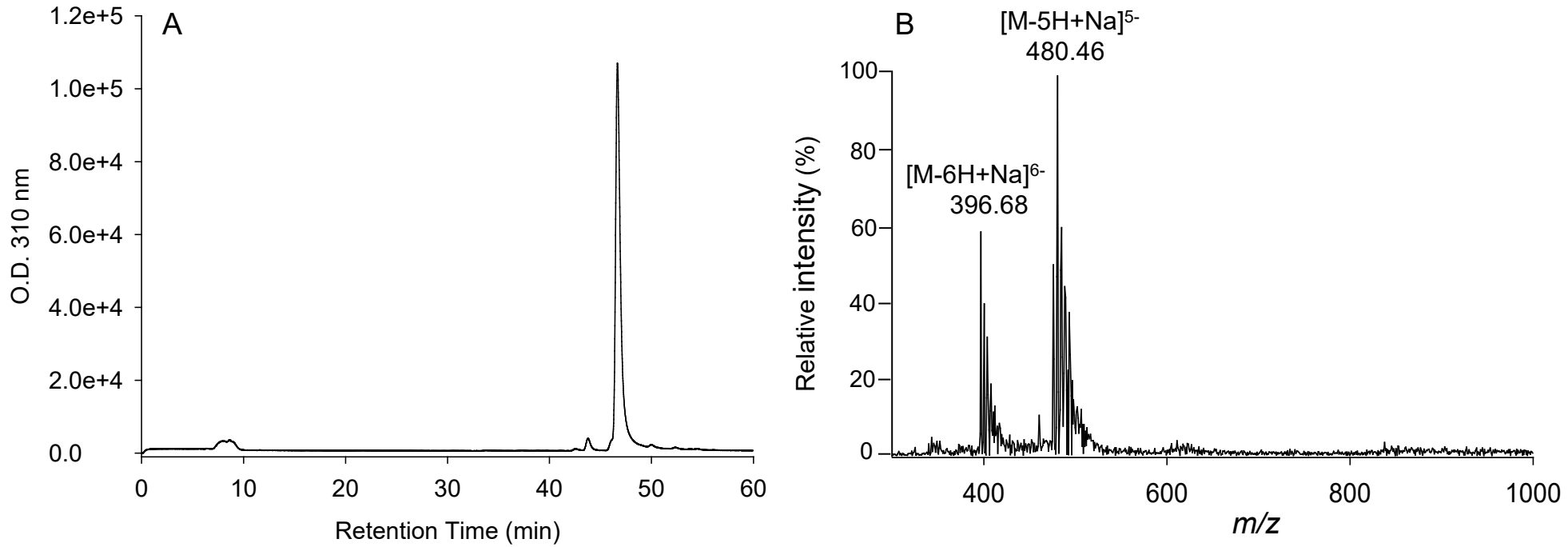
Suppl Fig S1. Purity and structure analysis of ^{13}C-labeled 8-mer-1 calibrant. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of ^{13}C-labeled 8-mer-1 calibrant. The molecular ions carrying 4 and 5 negative charges with Na adduct are indicated. The structure is shown above. Red circles represent ^{13}C-labeled sites.



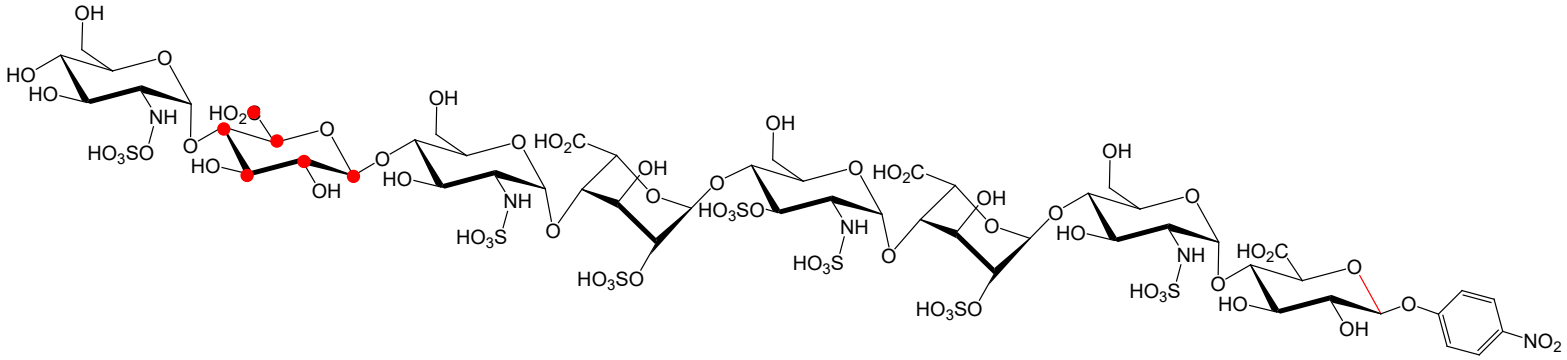


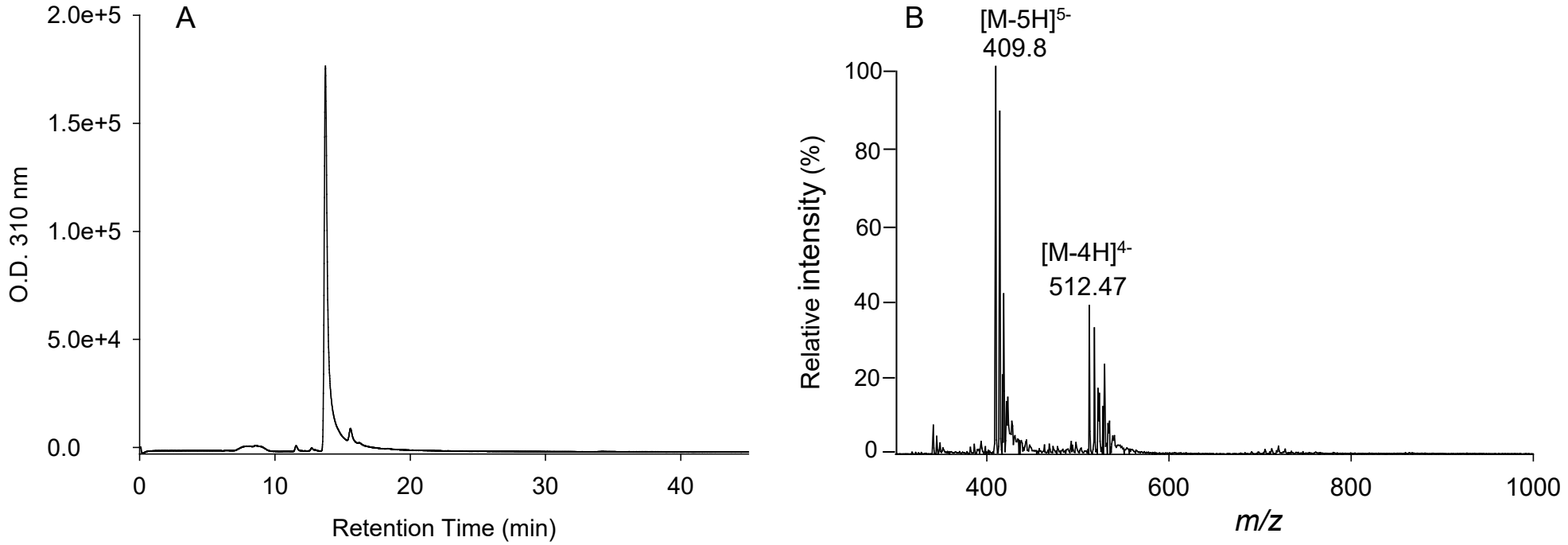
Suppl Fig S2. Purity and structure analysis of ^{13}C-labeled 8-mer-2 cailbrant. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of ^{13}C-labeled 8-mer-2 calibrant. The molecular ions carrying 4 and 5 negative charges with Na adduct are indicated. The structure is shown above. Red circles represent ^{13}C-labeled sites.



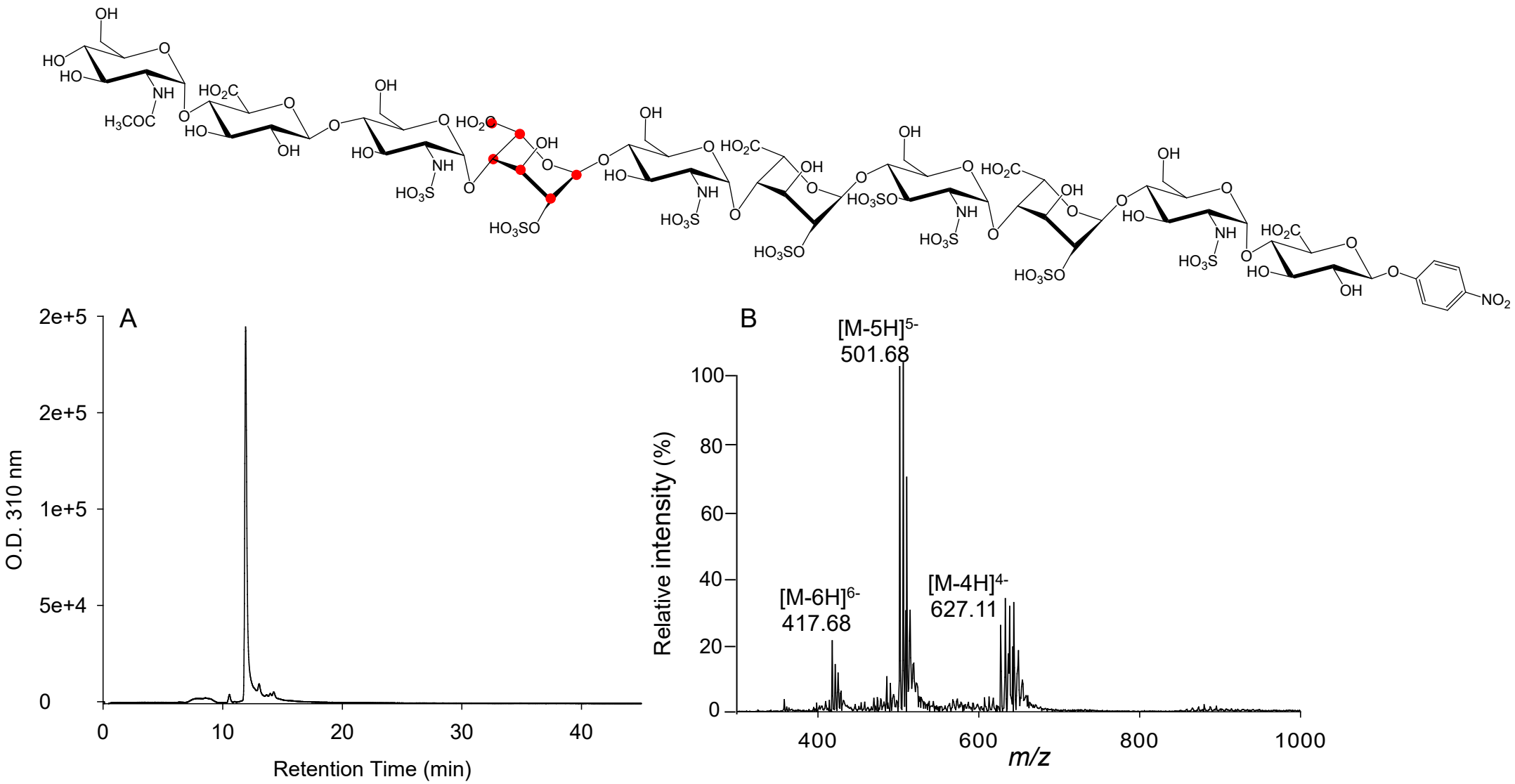


Suppl Fig S3. Purity and structure analysis of ^{13}C-labeled 8-mer-3 calibrant. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of ^{13}C-labeled 8-mer-3 calibrant. The molecular ions carrying 5 and 6 negative charges with Na adduct are indicated. The structure is shown above. Red circles represent ^{13}C-labeled sites.

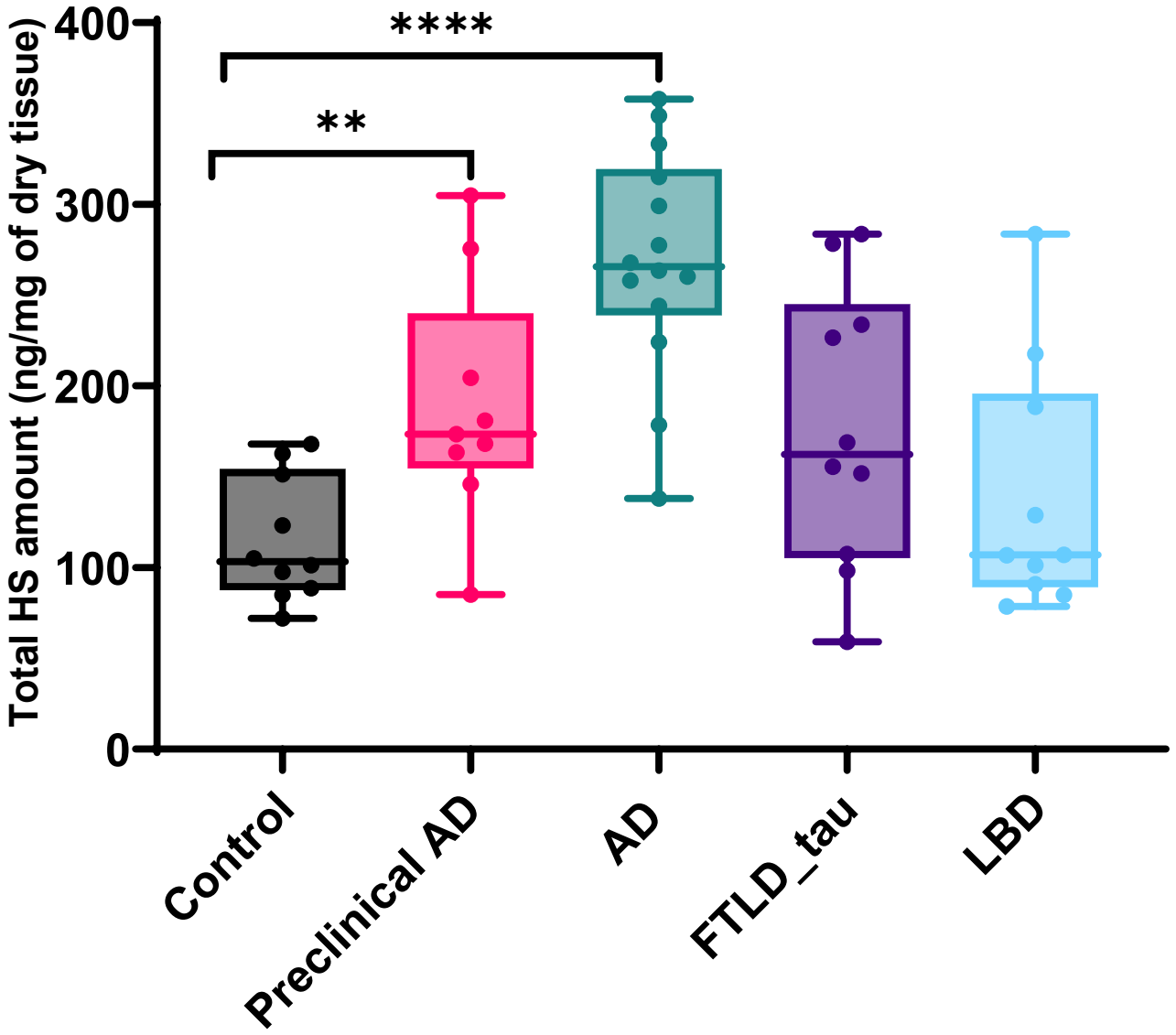




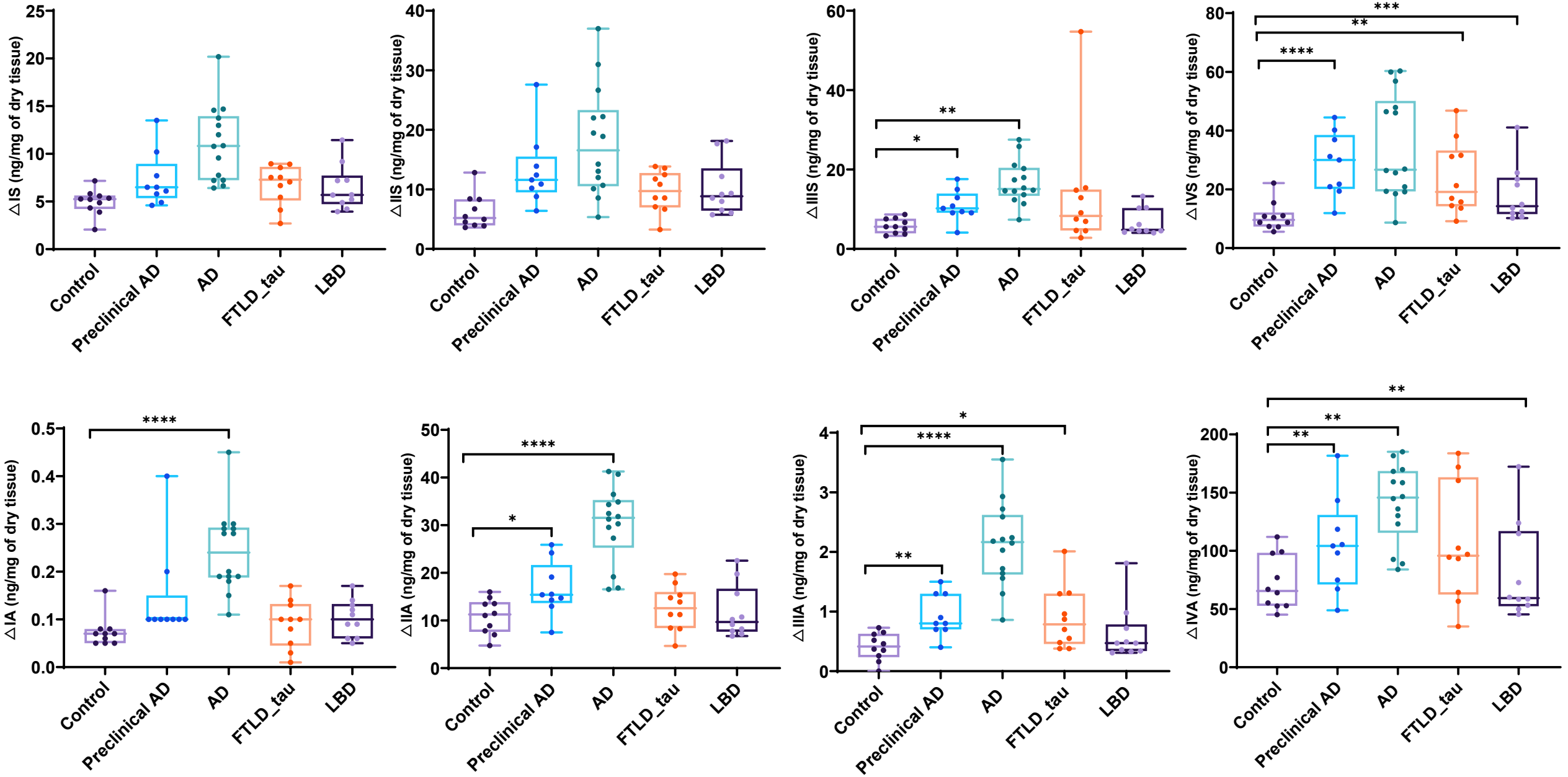
Suppl Fig S4. Purity and structure analysis of ^{13}C-labeled 8-mer-4 calibrant. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of ^{13}C-labeled 8-mer calibrant 4. The molecular ions carrying 4 and 5 negative charges are indicated. The structure is shown above. Red circles represent ^{13}C-labeled sites.



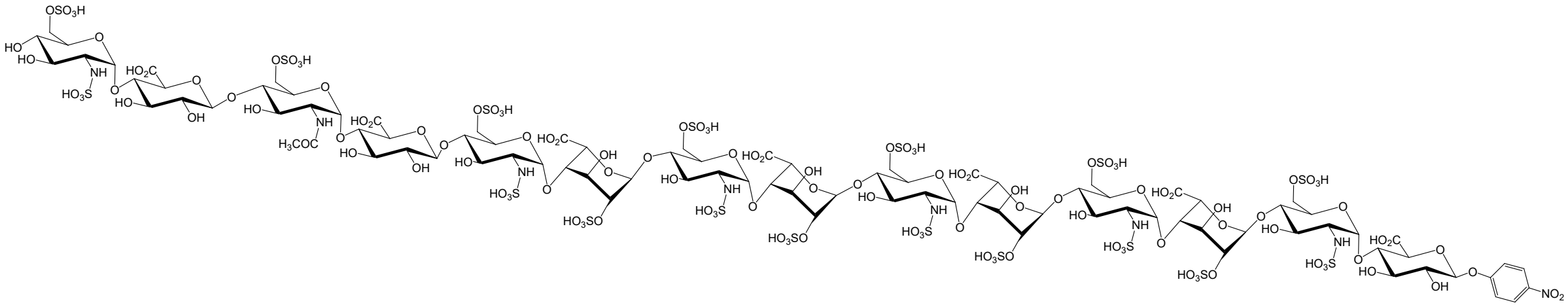
Suppl Fig S5. Purity and structure analysis of ^{13}C-labeled 10-mer-5 calibrant. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of ^{13}C-labeled 10-mer-5 calibrant. The molecular ions carrying 4, 5 and 6 negative charges with Na adduct are indicated. The structure is shown above. Red circles represent ^{13}C-labeled sites.

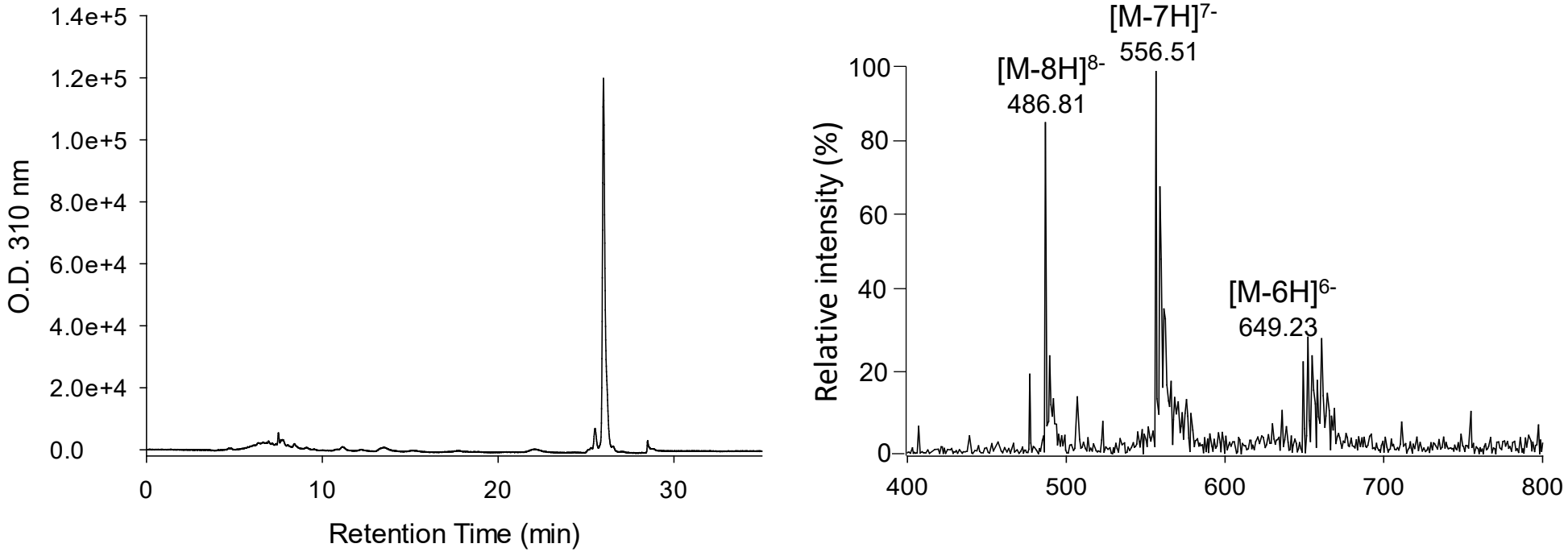


Suppl Fig S6. The amount of total HS from human brain tissue with different neurodegenerative diseases. The data are presented as mean ± S.D. p value was determined by two tailed unpaired t test, *p < 0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

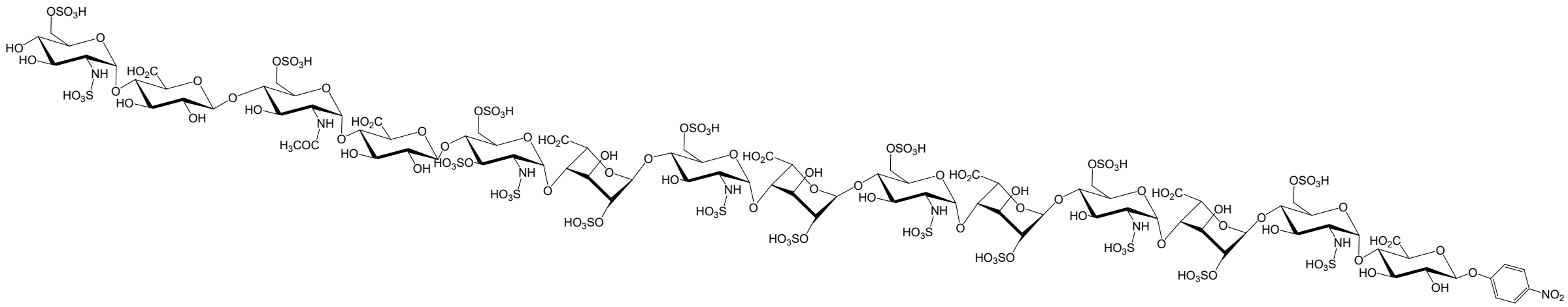


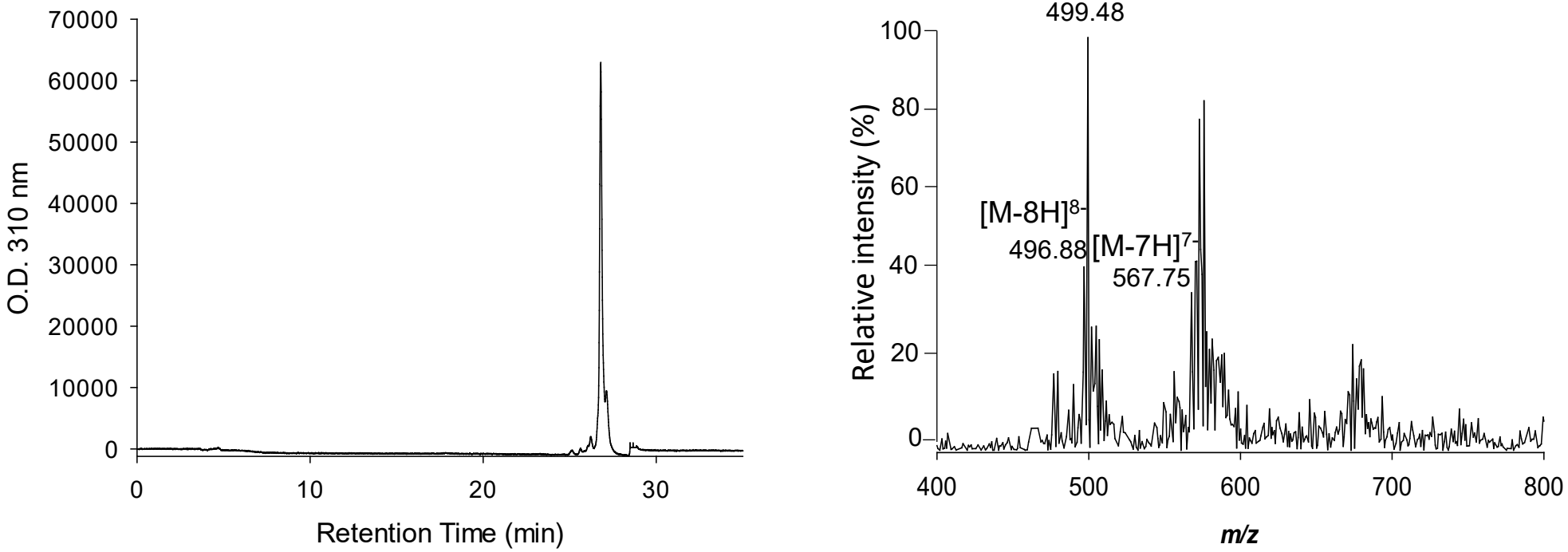
Suppl Fig S7. The amount of individual disaccharide from human brain tissue with different neurodegenerative diseases. The data are presented as mean ± S.D. p value was determined by two tailed unpaired t test, *p < 0.05, **p<0.01, ***p<0.001 and ****p<0.0001.



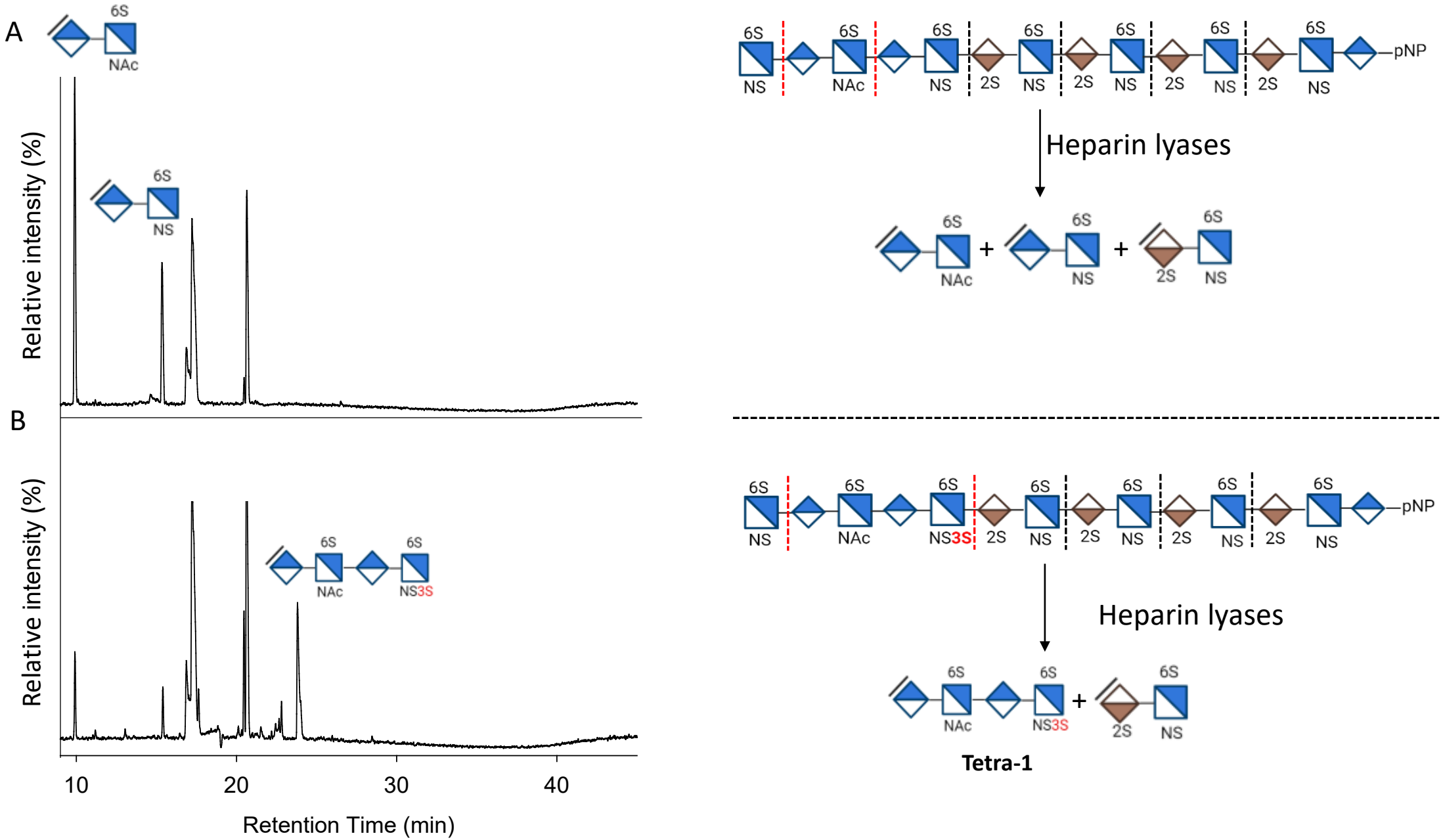


Suppl Fig S8. Purity and structure analysis of 14-mer-1. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of 14mer-1. The molecular ions carrying 8, 7 and 6 negative charges with Na adduct are indicated. The structure is shown above.

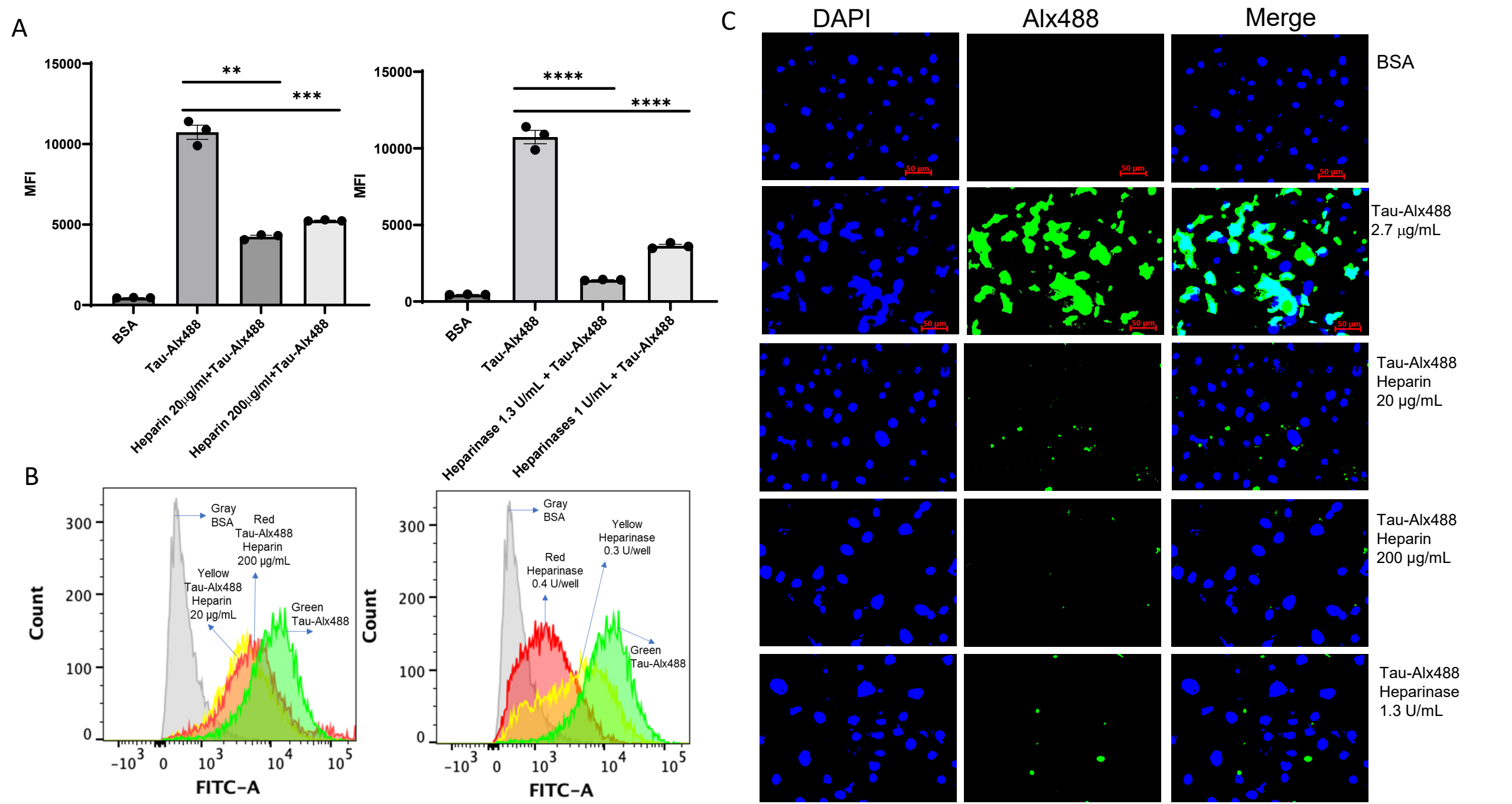




Suppl Fig S9. Purity and structure analysis of 14-mer-2. Panel A shows the SAX-HPLC chromatogram. Panel B shows the ESI-MS spectrum of 14mer-2. The molecular ions carrying 8 and 7 negative charges with Na adduct are indicated. The structure is shown above.

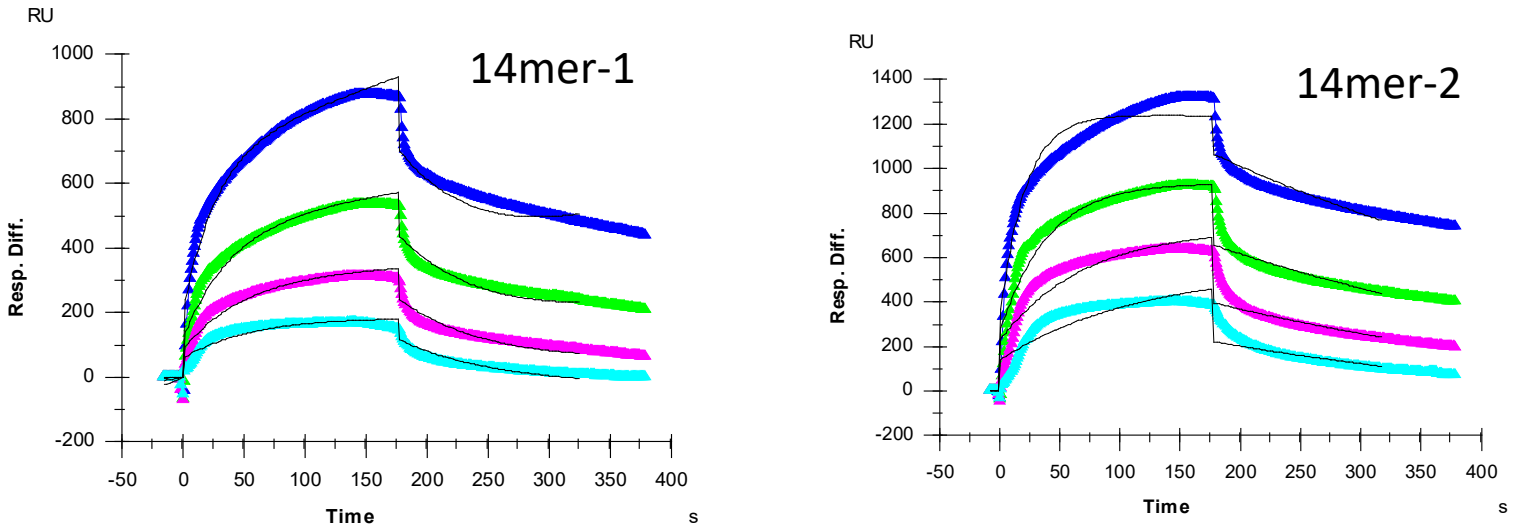


Suppl Fig S10. The LC-MS analysis of heparin lyases digest of 14-mer-1 and 14-mer-2. Panel A shows the LC-MS analysis of 14-mer-1. Panel B shows the LC-MS analysis of 14-mer-2.



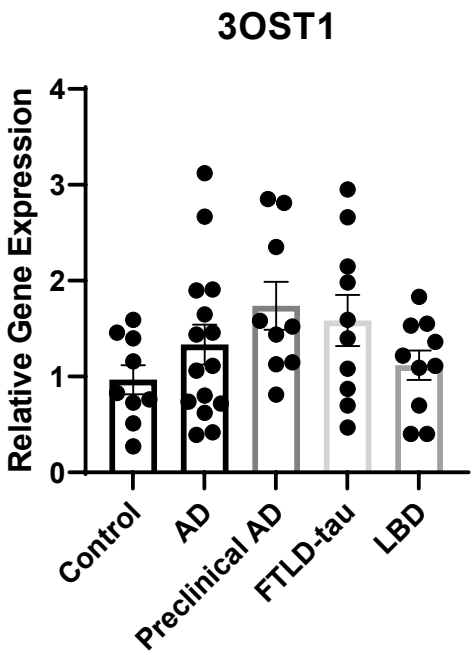
Suppl Fig S11. The inhibition of the cellular binding and cellular internalization of protein tau by heparin and heparinase. Legend is continued on

Suppl Fig S11. The inhibition of cellular internalization of protein tau by heparin and heparinase. A) shows the internalization of Alexa488 labeled tau (Tau-Alx488, 2.7μg/mL, 56 nM) to the cells in the absence or the presence of heparin (20 or 200 μg/mL, 1.67 and 16.7 μM, respectively) or heparinase (1.3 U/mL or 1 U/mL). B) shows overlayed five plots from flow cytometry analysis after the cells incubated with BSA (1.6 μg/mL, 24 nM, grey line), with Tau-Alx488 (2.7 μg/mL (56 nM), 0.3 mL/well, green line), Tau-Alx488 (2.7 μg/mL (56 nM), 0.3 mL/well) and heparin (20 μg/mL, 1.67 μM) or heparinase (1 U/mL) (yellow line) and Tau-Alx488 and heparin (200 μg/mL, 16.7 μM) or heparinase (1.3 U/mL) (red line). C) shows the microscopic images of the cells after incubation with BSA (1.6 μg/mL, 24 nM, grey line), or Tau-Alx488 (2.7 μg/mL (56 nM), 0.3 mL/well) with or without heparin (200 μg/mL, 16.7 μM) or heparinase (1.3 U/mL).



Interaction	k_a (1/MS)*	k_d (1/S)	K_D (M)
tau-14-mer-1	9.70×10^4 ($\pm 2.78 \times 10^3$)	0.0133 ($\pm 3.31 \times 10^{-4}$)	1.37×10^{-7}
tau-14-mer-2	1.81×10^5 ($\pm 5.57 \times 10^3$)	2.6×10^{-3} ($\pm 1.88 \times 10^{-4}$)	1.44×10^{-8}

Suppl Fig S12. Determination of the binding affinity between tau and 14-mers. The SPR sensorgrams are shown on top. The data with (±) in parentheses are the standard deviations (SD) from global fitting of four injections tau at concentrations from 250, 125, 63 and 32 nM.



Suppl Fig S13. qPCR analysis of mRNA expression of 3-OST-1 from disease and patients. The experiment was done using the ViiA™ 7 Flex Real-Time PCR System (Thermo Fisher). The range of Ct of 3-OST-1, GAPDH, and CYC1 is 22.8 – 32.3, 16.3 – 23.9, 17.4 – 26.9. mRNA levels of 3-OST1 were quantified with the 2-ΔΔCt method and normalized to the internal control gene GAPDH and CYC1. The results are shown relative to the expression level of the control group.

Table S1. The quantity of ΔIS disaccharide (ΔUA2S-GlcNS6S) from neurodegenerative disease brains

The amount of ΔUA2S-GlcNS6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	5.23	4.6	9.57	5.44	5.7
2	5.81	4.9	7.75	2.7	4.89
3	4.32	6.5	13.75	8.56	4.22
4	5.27	5.8	7.2	4.1	5.68
5	2.06	13.5	6.41	6.67	5.21
6	3.89	6.1	10.78	7.54	3.94
7	5.58	7.7	14.57	7.05	7.23
8	5.35	10.2	7.24	7.52	7.19
9	7.17	6.5	10.86	8.91	11.44
10	4.87		12.99	8.96	9.19
11			20.18		
12			12		
13			14.69		
14			6.64		
Average±SD	4.96±1.28	7.31±2.69	11.05±3.82	6.75±1.98	6.47±2.24

The mass percentage of ΔUA2S-GlcNS6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	5.73	5.46	3.18	2.31	5.46
2	6.27	2.94	5.66	4.57	4.94
3	5.33	3.34	3.38	3.08	4.99
4	4.47	3.2	3.61	4.09	4.48
5	4.96	5.05	3.76	6.19	5.48
6	3.22	3.95	6.1	3.68	4.88
7	3.84	4.68	4.54	4.12	3.88
8	3.51	3.35	4.14	2.68	6.87
9	5.01	3.75	3.72	5.71	5.28
10	2.43		3.57	5.91	3.27
11			3.18		
12			4.96		
13			5.22		
14			2.46		
Average±SD	4.48±1.15	3.97±0.84	4.11±1.01	4.23±1.29	4.95±0.92

Table S2. The quantity of ΔIIS disaccharide (ΔUA-GlcNS6S) from neurodegenerative disease brains

The amount of ΔUA-GlcNS6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	3.98	6.4	10.67	8.58	9.16
2	5.43	8.8	10.1	3.25	8.53
3	4.52	11.6	13.04	13.84	6.07
4	3.87	10.2	5.35	6.67	7.99
5	3.6	27.6	8.55	7.07	6.52
6	4.97	12.4	18.9	11.75	5.74
7	6.73	13.9	26.66	12.48	12.16
8	8.31	17.1	11.98	13.56	9.32
9	12.83	10.9	19.46	10.85	17.68
10	8.41		22.01	8.56	18.14
11			37		
12			22.22		
13			30.99		
14			14.23		
Average±SD	6.27±2.75	13.21±5.83	17.94±8.79	9.66±3.25	10.13±4.28

The mass percentage of ΔUA-GlcNS6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	6.76	7.44	5.75	3.69	8.31
2	7.62	5.23	9.93	5.49	7.86
3	8.13	5.48	5.23	4.96	7.13
4	7.44	5.64	6.53	6.83	6.17
5	8.65	9.62	6.26	6.59	7.47
6	2.45	8.54	11.27	5.48	7.44
7	3.59	8.47	8.48	7.39	6.44
8	3.68	5.59	8.64	4.72	8.47
9	3.68	6.35	7.96	7	8.1
10	4.24		3.99	5.64	6.31
11			4.14		
12			4.7		
13			3.88		
14			3.29		
Average±SD	5.62±2.18	6.93±1.54	6.43±2.38	5.78±1.1	7.37±0.8

Table S3. The quantity of ΔIIIS disaccharide (ΔUA2S-GlcNS) from neurodegenerative disease bra

The amount of ΔUA2S-GlcNS in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	7.78	4.1	14.81	12.89	4.57
2	7.56	9.4	13.67	2.79	4.62
3	6.66	10.8	14.53	14.81	4.21
4	5.58	10.2	7.35	4.65	6.16
5	3.98	15	14.65	4.56	4.56
6	3.28	9.1	15.36	54.75	4.04
7	3.75	12.9	17.97	9.1	10.39
8	5.08	17.6	11.4	15.39	5.01
9	8.68	9.1	20.23	6.95	10.27
10	5.6		21.07	7.51	13.26
11			27.52		
12			17.4		
13			25.81		
14			12.38		
Average±SD	5.8±1.75	10.91±3.67	16.73±5.29	13.34±14.42	6.71±3.15

The mass percentage of ΔUA2S-GlcNS in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	4.71	4.82	3.87	5.48	4.34
2	4.21	5.63	7.07	4.71	4.62
3	5.04	5.39	5.23	5.33	4.97
4	5.34	5.68	6.75	4.67	4.86
5	5.71	5.62	6.1	4.23	4.86
6	4.78	6.06	8.32	19.53	5.05
7	4.99	7.85	6.56	5.33	5.57
8	5.41	5.77	7.24	5.46	4.78
9	5.32	5.21	6.94	4.45	4.74
10	4.69		5.53	4.95	4.7
11			5.6		
12			5.24		
13			5.33		
14			5.63		
Average±SD	5.02±0.42	5.78±0.8	6.1±1.08	6.41±4.39	4.85±0.3

Table S4. The quantity of ΔIVS disaccharide (ΔUA-GlcNS) from neurodegenerative disease brains

The amount of ΔUA-GlcNS in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	15.39	11.9	20.87	31.59	14.89
2	10.84	30	18.52	9.16	13.65
3	8.78	36.9	19.34	46.8	11.87
4	7.23	31.2	8.68	15.7	21.55
5	5.57	40.2	19.16	14.46	10.63
6	8.74	19.4	60.32	31.17	10.21
7	10.41	20.9	26.43	21.26	23.44
8	7.35	44.5	25.65	38.15	12.46
9	22.13	21.8	46.45	16.96	25.64
10	11.02		59.97	13.61	41.05
11			47.87		
12			46.04		
13			56.84		
14			26.92		
Average±SD	10.75±4.58	28.53±10.19	34.5±16.99	23.89±11.72	18.54±9.17

The mass percentage of ΔUA-GlcNS in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	12.16	13.84	19.43	13.81	14.03
2	11.76	17.7	10.22	15.47	13.97
3	7.37	16.94	11.31	16.84	14.03
4	12.53	17.05	15.67	15.92	16.08
5	11.41	14.35	16.89	13.44	12.71
6	9.46	13.83	16.04	12.8	13.78
7	7.16	12.81	17.69	13.1	12.18
8	7.13	14.5	15.72	13.22	11.27
9	6.88	12.64	15.01	10.91	11.68
10	6.56		7.79	8.97	14.55
11			7.59		
12			6.97		
13			6.3		
14			7.36		
Average±SD	9.24±2.36	14.85±1.79	12.43±4.49	13.45±2.21	13.43±1.39

Table S5. The quantity of ΔIA disaccharide (ΔUA2S-GlcNAc6S) from neurodegenerative disease bra

The amount of ΔUA2S-GlcNAc6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	0.05	0.1	0.19	0.1	0.11
2	0.08	0.1	0.18	0.01	0.09
3	0.08	0.1	0.3	0.14	0.06
4	0.06	0.1	0.11	0.05	0.09
5	0.05	0.4	0.15	0.08	0.06
6	0.07	0.1	0.2	0.1	0.05
7	0.07	0.1	0.29	0.1	0.13
8	0.05	0.2	0.19	0.17	0.12
9	0.16	0.1	0.29	0.13	0.17
10	0.07		0.3	0.03	0.14
11			0.28		
12			0.28		
13			0.45		
14			0.19		
Average±SD	0.07±0.03	0.14±0.1	0.24±0.08	0.09±0.05	0.1±0.04

The mass percentage of ΔUA2S-GlcNAc6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0.09	0.09	0.07	0.04	0.09
2	0.08	0.06	0.09	0.01	0.08
3	0.04	0.06	0.07	0.05	0.07
4	0.07	0.06	0.1	0.07	0.06
5	0.08	0.11	0.08	0.07	0.08
6	0.03	0.07	0.11	0.04	0.08
7	0.05	0.08	0.12	0.06	0.07
8	0.07	0.06	0.12	0.05	0.1
9	0.06	0.08	0.1	0.08	0.08
10	0.06		0.07	0.02	0.05
11			0.07		
12			0.11		
13			0.08		
14			0.06		
Average±SD	0.06±0.02	0.07±0.02	0.09±0.02	0.05±0.02	0.08±0.01

Table S6. The quantity of ΔIIA disaccharide (ΔUA-GlcNAc6S) from neurodegenerative disease brains

The amount of ΔUA-GlcNAc6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	13.44	7.5	34.33	11.26	9.16
2	16.01	13	34.88	4.66	8.2
3	14.82	14.7	40.71	17.92	6.73
4	11.5	14.3	16.82	8.43	10.2
5	11.06	25.9	31.26	8.27	7.79
6	4.73	15.4	32.45	13.89	7.18
7	7.01	19.1	29.55	15.35	15.65
8	7.82	24.2	16.54	19.73	10.72
9	13.53	15.5	30.29	11.24	19.76
10	8.89		31.82	14.77	22.56
11			41.27		
12			27.27		
13			36.47		
14			19.18		
Average±SD	10.88±3.49	16.62±5.35	30.2±7.64	12.55±4.43	11.8±5.3

The mass percentage of ΔUA-GlcNAc6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	6.84	8.94	9.47	4.76	8.72
2	7.87	7.76	10.9	7.87	8.19
3	7.66	7.46	7.7	6.45	7.96
4	8.34	7.92	10.1	8.41	8.1
5	9.05	9.69	9.21	7.68	8.25
6	8.26	10.34	12.66	6.93	8.92
7	10.58	11.67	10.36	8.94	8.4
8	12.04	7.96	10.26	7	10.33
9	10.94	8.89	10.76	7.21	9.12
10	13.04		12.82	9.74	8.03
11			14.29		
12			14.67		
13			12.2		
14			12.01		
Average±SD	9.46±1.96	8.96±1.31	11.24±1.89	7.5±1.32	8.6±0.69

Table S7. The quantity of ΔIIIA disaccharide (ΔUA2S-GlcNAc) from neurodegenerative disease brains

The amount of ΔUA2S-GlcNAc in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	0.65	0.4	2.15	0.7	0.33
2	0.37	0.8	1.3	0.38	0.31
3	0.73	0.7	1.72	0.96	0.36
4	0	0.7	0.86	0.38	0.47
5	0.16	0.9	1.64	0.48	0.49
6	0.26	1.3	2.21	0.55	0.34
7	0.35	1.5	1.56	1.3	1.81
8	0.52	1.3	2.72	2.01	0.47
9	0.62	0.8	2.03	0.87	0.98
10	0.46		2.18	1.31	0.72
11			3.55		
12			2.93		
13			2.24		
14			2.59		
Average±SD	0.41±0.22	0.93±0.34	2.12±0.67	0.89±0.49	0.63±0.44

The mass percentage of ΔUA2S-GlcNAc in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0.47	0.48	0.54	0.28	0.38
2	0.38	0.48	0.93	0.64	0.41
3	0.55	0.41	1.31	0.36	0.44
4	0.52	0.43	0.65	0.32	0.42
5	0.45	0.47	0.68	0.43	0.42
6	0.4	0.88	1.06	0.37	0.38
7	0.24	0.94	1.08	0.77	0.97
8	0.6	0.45	0.67	0.72	0.53
9	0	0.42	1.45	0.55	0.47
10	0.19		0.8	0.87	0.28
11			0.53		
12			0.62		
13			0.62		
14			0.63		
Average±SD	0.38±0.18	0.55±0.19	0.83±0.28	0.53±0.2	0.47±0.18

Table S8. The quantity of ΔIVA disaccharide (ΔUA-GlcNAc) from neurodegenerative disease brains

The amount of ΔUA-GlcNAc in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	112.01	48.9	146.64	160.33	60.1
2	99.23	98.2	136.04	35.07	58.66
3	76.94	118.5	158.51	171.96	49.91
4	66.96	104.2	84.03	56.62	72.78
5	52.59	143.2	168.22	64.36	53.3
6	45.19	74.9	181.71	102.37	45.39
7	52.85	67.3	130.22	96.98	114.76
8	64.01	181.7	144.83	183.77	58.41
9	98.02	105.4	159.29	94.51	123.9
10	55.21		185.04	93.29	172.34
11			92.59		
12			123.08		
13			169.74		
14			88.87		
Average±SD	72.30±22.08	104.7±38.07	140.63±32.31	105.93±47.90	80.96±39.80

The mass percentage of ΔUA-GlcNAc in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	61.6	57.42	54.46	68.45	55.65
2	59.78	58.47	50.83	59.24	57.6
3	62.99	58.57	64.1	61.74	58.71
4	58.22	57.7	53.2	58.13	56.71
5	56.62	51.74	53.24	59.99	58.62
6	68.86	51.66	31.87	48.77	57.54
7	65.56	41.21	46.56	57.33	60.87
8	62.5	59.65	47.34	64.99	54.56
9	63.74	60.71	49.81	60.82	56.91
10	61.99		54.76	61.48	60.55
11			55.74		
12			57.11		
13			60.93		
14			64.64		
Average±SD	62.19±3.35	55.24±5.81	53.19±7.95	60.09±4.89	57.77±1.89

Table S9. The quantity of Tetra-1 (ΔUA-GlcNAc6S-GlcA-GlcNS3S6S) from neurodegenerative disease brains

The amount of ΔUA-GlcNAc6S-GlcA-GlcNS3S6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	0.47	0.6	3.81	0.75	0.75
2	0.82	0.9	3.13	0.27	0.62
3	0.39	1.2	4.75	1.09	0.37
4	0.44	0.7	1.47	0.66	0.76
5	0.58	2.6	2.11	0.61	0.51
6	0.3	1.6	4.3	1.15	0.45
7	0.6	3.9	4.04	0.9	0.75
8	0.69	2.8	1.31	1.12	0.8
9	1.14	1.2	4.88	0.91	1.81
10	0.61		3.66	1.32	1.13
11			12.04		
12			5.36		
13			6.07		
14			2.48		
Average±SD	0.64±0.23	1.72±1.07	4.24±2.56	0.88±0.30	0.8±0.39

The mass percentage of ΔUA-GlcNAc6S-GlcA-GlcNS3S6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0.43	0.65	1.27	0.31	0.7
2	0.68	0.55	1.61	0.46	0.62
3	0.7	0.61	0.59	0.39	0.44
4	0.69	0.39	1.63	0.65	0.6
5	0.62	0.94	1.06	0.56	0.49
6	0.29	1.08	3.48	0.56	0.54
7	0.54	2.4	2.04	0.52	0.4
8	0.32	0.93	1.7	0.4	0.75
9	0.42	0.67	1.39	0.58	0.83
10	0.69		1.42	0.87	0.4
11			1.28		
12			1.71		
13			1.07		
14			0.81		
Average±SD	0.54±0.15	0.91±0.56	1.5±0.66	0.53±0.15	0.58±0.14

Table S10. The quantity of Tetra-2 (ΔUA-GlcNS6S-GlcA-GlcNS3S6S) from neurodegenerative disease brain

The amount of ΔUA-GlcNS6S-GlcA-GlcNS3S6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	0.44	0.1	0.91	0.2	0.68
2	0.58	0.4	0.57	0.02	0.54
3	0.36	1.2	0.84	0.97	0.16
4	0.2	0.9	0.37	0.25	0.72
5	0.49	2.1	0.55	0.07	0.57
6	0.1	1.1	1.94	0.66	0.09
7	0.33	2.5	1.58	1.01	0.66
8	0.72	1.7	0.77	0.45	0.62
9	1.11	0.6	1.36	1.56	2.14
10	0.42		2.15	0.96	0.93
11			5.22		
12			1.49		
13			2.98		
14			0.83		
Average±SD	0.48±0.27	1.18±0.75	1.54±1.24	0.6±0.47	0.71±0.53

The mass percentage of ΔUA-GlcNS6S-GlcA-GlcNS3S6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0.18	0.09	0.54	0.08	0.65
2	0.37	0.27	0.6	0.03	0.5
3	0.72	0.6	0.33	0.35	0.21
4	0.63	0.5	0.45	0.21	0.57
5	0.41	0.72	0.62	0.06	0.58
6	0.27	0.67	1.49	0.32	0.25
7	0.38	1.53	0.55	0.56	0.35
8	0.29	0.57	0.83	0.16	0.56
9	0.19	0.37	0.46	1	0.99
10	0.58		0.34	0.63	0.34
11			0.23		
12			0.3		
13			0.27		
14			0.21		
Average±SD	0.4±0.18	0.59±0.38	0.52±0.32	0.34±0.29	0.5±0.22

Table S11. The quantity of Tetra-3 (ΔUA-GlcNS6S-IdoA2S-GlcNS3S6S) from neurodegenerative disease brains

The amount of ΔUA-GlcNS6S-IdoA2S-GlcNS3S6S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	1.67	0	16.41	0	0.69
2	1.97	0.4	11.92	0.38	0.24
3	2.65	0.6	6.09	0	0.34
4	2.42	0.6	3.06	0	0.93
5	2.82	0.4	3.07	0.31	0.14
6	0	1	1.12	0.46	0.11
7	0.12	1.3	0.71	0	0
8	0.46	0.8	0.27	0.39	0.73
9	0.76	0.4	0	1.57	0.95
10	0.48		0.82	0	1.13
11			2.52		
12			1.33		
13			2.83		
14			1		
Average±SD	1.34±1.04	0.6±0.36	3.65±4.63	0.3±0.46	0.53±0.39

The mass percentage of ΔUA-GlcNS6S-IdoA2S-GlcNS3S6S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0	0	0.32	0	0.7
2	0.14	0.23	0.42	0.64	0.19
3	0.46	0.34	0.13	0	0.41
4	0.56	0.36	0	0	0.77
5	0.48	0.17	0.24	0.28	0.22
6	1.03	0.65	0.72	0.31	0.16
7	1.3	0.8	0.51	0	0
8	2.15	0.27	0.82	0.16	0.78
9	2.31	0.23	0.56	0.99	0.46
10	3.32		6.13	0	0.43
11			4.88		
12			2.19		
13			2.22		
14			1.18		
Average±SD	1.18±1.03	0.34±0.23	1.45±1.8	0.24±0.32	0.41±0.26

Table S12. The quantity of Tetra-4 (ΔUA-GlcNS-IdoA2S-GlcNS3S) from neurodegenerative disease brains

The amount of ΔUA-GlcNS-IdoA2S-GlcNS3S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	1.09	0.4	6.32	1.8	0.73
2	1.93	0.7	5.05	0.41	0.69
3	2.34	1.1	2.65	1.05	0.38
4	1.09	1.6	1.59	0.59	1.22
5	1.44	2.9	3.19	0.34	0.85
6	0.49	2.8	2.75	1.8	0.77
7	0.6	11	3.67	2.77	1.2
8	0.79	2	0.9	1.06	0.77
9	1.46	0.8	2.92	0.79	2.45
10	1.17		2.13	1.2	2.42
11			20.01		
12			3		
13			7.06		
14			2.61		
Average±SD	1.24±0.55	2.59±3.09	4.56±4.59	1.18±0.72	1.15±0.68

The mass percentage of ΔUA-GlcNS-IdoA2S-GlcNS3S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0.84	0.53	0.8	0.73	0.74
2	0.66	0.43	1.39	0.69	0.73
3	0.79	0.57	0.41	0.38	0.45
4	0.92	0.89	0.98	0.52	0.98
5	1.17	1.19	0.61	0.31	0.8
6	0.67	1.8	5.53	0.97	0.91
7	1.27	6.74*	1.15	1.56	0.66
8	1.9	0.67	2.01	0.38	0.72
9	1.04	0.48	1.46	0.51	1.13
10	1.7		2.36	0.79	0.9
11			2.07		
12			0.96		
13			1.15		
14			1.23		
Average±SD	1.1±0.4	0.82±0.44	1.58±1.22	0.68±0.35	0.8±0.18

Table S13. The quantity of Tetra-5 (ΔUA2S-GlcNS-IdoA2S-GlcNS3S) from neurodegenerative disease brains

The amount of ΔUA2S-GlcNS-IdoA2S-GlcNS3S in different neurodegenerative diseases (ng/mg)

Group.Name/No.	Control (ng/mg)	Preclinic AD (ng/mg)	AD (ng/mg)	FTLD_tau (ng/mg)	LBD (ng/mg)
1	0.49	0.2	1.12	0.16	0.21
2	0.73	0.4	0.96	0.12	0.24
3	0.5	0.4	1.31	0.21	0.19
4	0.46	0.3	1.01	0.22	0.25
5	0.45	0.8	1.27	0.15	0.22
6	0.09	0.7	1.11	0.44	0.22
7	0.18	1.3	0.69	0.54	0.37
8	0.19	0.7	0.39	0.2	0.26
9	0.39	0.4	1.06	0.31	0.5
10	0.39		4.52	0.22	0.55
11			4.97		
12			1.05		
13			1.69		
14			0.66		
Average±SD	0.39±0.18	0.58±0.32	1.56±1.34	0.26±0.13	0.3±0.13

The mass percentage of ΔUA2S-GlcNS-IdoA2S-GlcNS3S in different neurodegenerative diseases (%)

Group.Name/No.	Control	Preclinic AD	AD	FTLD_tau	LBD
1	0.16	0.25	0.29	0.07	0.22
2	0.19	0.25	0.35	0.2	0.28
3	0.19	0.22	0.2	0.08	0.22
4	0.28	0.19	0.35	0.2	0.21
5	0.39	0.33	1.23	0.14	0.2
6	0.3	0.46	1.35	0.26	0.25
7	0.48	0.82	0.37	0.3	0.21
8	0.41	0.24	0.49	0.07	0.28
9	0.44	0.21	0.38	0.2	0.23
10	0.53		0.42	0.14	0.2
11			0.4		
12			0.47		
13			0.73		
14			0.49		
Average±SD	0.34±0.12	0.33±0.19	0.54±0.33	0.17±0.08	0.23±0.03

Supplementary Table S14. The mass percentage of HS disaccharides and 3-*O*-sulfation tetrasaccharides in the brain samples

Di/tetra-saccharides	Mass percentage (%)															
	A1	A2	A3	A4	B1	B2	B3	B4	F1	F2	F3	F4	G1	G2	G3	G4
△UA-GlcNAc	52.47	51.08	52.82	55.90	55.77	50.18	47.89	52.26	51.89	52.17	56.44	52.32	52.23	55.63	51.61	51.48
△UA2S-GlcNAc	1.01	0.99	1.25	1.25	1.13	1.21	1.04	1.05	1.17	1.20	0.82	1.16	1.25	1.14	1.13	1.03
△UA-GlcNAc6S	8.32	8.23	7.60	7.78	8.47	8.52	9.87	8.87	7.63	7.40	7.70	7.88	7.37	7.68	7.49	7.24
△UA2S-GlcNAc6S	0.07	0.09	0.07	0.06	0.09	0.07	0.06	0.08	0.07	0.07	0.06	0.08	0.07	0.05	0.08	0.07
△UA-GlcNS	15.82	16.46	15.73	13.85	14.23	15.05	15.52	15.24	15.40	15.52	13.70	15.51	14.53	15.00	14.65	15.16
△UA2S-GlcNS	9.40	9.95	9.76	8.68	8.20	9.30	9.29	9.03	10.03	9.86	8.14	9.62	10.15	8.61	9.90	9.79
△UA-GlcNS6S	7.71	7.41	7.60	7.72	8.51	9.30	9.87	8.63	7.77	7.59	7.89	7.71	7.79	7.42	8.53	9.24
△UA2S-GlcNS6S	2.77	2.62	2.95	2.44	2.43	3.48	3.12	2.98	3.37	3.26	2.40	3.23	4.45	2.59	4.43	4.07
△UA-GlcNAc6S-GlcA-GlcNS3S6S	0.54	0.63	0.59	0.68	0.09	0.14	0.12	0.08	0.62	0.67	0.69	0.66	0.63	0.62	0.64	0.62
△UA-GlcNS6S-GlcA-GlcNS3S6S	0.54	0.63	0.39	0.34	0.18	0.85	0.81	0.40	0.82	0.87	0.38	0.66	0.70	0.31	0.72	0.48
△UA-GlcNS6S-IdoA2S-GlcNS3S6S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
△UA-GlcNS-IdoA2S-GlcNS3S	0.68	1.36	0.79	0.68	0.50	1.14	1.73	0.65	0.82	0.80	1.33	0.58	0.35	0.47	0.48	0.41
△UA2S-GlcNS-IdoA2S-GlcNS3S	0.61	0.45	0.52	0.62	0.50	0.64	0.46	0.48	0.48	0.53	0.38	0.58	0.49	0.47	0.40	0.48

Note: A group: HS3-OST-1 WT; B group: HS3-OST-1 KO; F group: HS3-OST-3a1 and 3b1 WT; G group: HS3-OST-3a1 and 3b1 DKO

Supplementary Table S15. The mass percentage of HS disaccharides and 3-*O*-sulfation tetrasaccharides in the liver samples

Di-/tetra-saccharides	Mass percentage (%)														
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	F1	F2	F3
△UA-GlcNAc	49.21	46.97	45.90	48.28	44.55	45.36	44.17	49.34	53.33	46.36	49.23	45.93	47.06	44.39	44.06
△UA2S-GlcNAc	0.73	0.65	0.53	0.63	0.51	0.47	0.56	0.62	0.71	0.61	0.61	0.69	0.68	0.70	0.48
△UA-GlcNAc6S	12.73	13.64	14.32	13.56	15.85	16.55	16.88	13.86	11.17	12.93	13.55	13.98	13.87	14.03	15.43
△UA2S-GlcNAc6S	0.06	0.07	0.07	0.00	0.06	0.08	0.06	0.09	0.05	0.07	0.08	0.08	0.09	0.05	0.06
△UA-GlcNS	13.96	15.44	16.06	16.82	16.53	16.47	16.75	14.92	14.42	16.61	15.39	16.44	16.77	17.85	16.75
△UA2S-GlcNS	3.72	3.32	3.46	3.16	4.38	3.83	4.20	4.16	3.50	3.20	3.29	3.69	3.49	3.42	2.94
△UA-GlcNS6S	7.38	7.72	8.46	7.41	10.68	10.15	11.04	10.32	6.50	7.35	7.50	7.83	7.57	7.94	9.60
△UA2S-GlcNS6S	4.90	5.34	4.46	4.25	7.05	6.71	5.96	6.38	3.96	4.08	4.82	4.99	4.60	5.08	4.92
△UA-GlcNAc6S-GlcA-GlcNS3S6S	0.11	0.14	0.13	0.18	0.17	0.08	0.13	0.13	0.10	0.14	0.15	0.15	0.00	0.00	0.00
△UA-GlcNS6S-GlcA-GlcNS3S6S	0.79	1.08	0.87	0.81	0.00	0.00	0.06	0.09	0.86	0.95	0.69	1.00	0.94	0.80	0.66
△UA-GlcNS6S-IdoA2S-GlcNS3S6S	4.34	3.25	3.86	2.80	0.00	0.00	0.00	0.00	3.61	5.38	2.99	2.76	2.98	3.62	2.88
△UA-GlcNS-IdoA2S-GlcNS3S	1.75	2.02	1.60	1.72	0.06	0.08	0.06	0.09	1.42	1.97	1.61	2.23	1.70	1.66	1.92
△UA2S-GlcNS-IdoA2S-GlcNS3S	0.34	0.36	0.33	0.36	0.11	0.08	0.13	0.13	0.41	0.34	0.23	0.31	0.34	0.35	0.30

Note: A group: HS3-OST-3a1 and 3b1 WT; B group: HS3-OST-3a1 and 3b1 DKO; C group: HS3-OST-1 WT; F group: HS3-OST-1 KO

Supplementary Table S16. HS analysis from MLE cells and HCT-116[a]

Abbreviation	Di/tetra-saccharides	Amount (ng/×10^6 cells)	
		MLEC	HCT-116
△IVA	△UA-GlcNAc	156.9	40.1
△IIIA	△UA2S-GlcNAc	9.4	0.5
△IIA	△UA-GlcNAc6S	12.7	8.9
△IA	△UA2S-GlcNAc6S	1.6	0.1
△IVS	△UA-GlcNS	60.7	34.8
△IIIS	△UA2S-GlcNS	14.0	3.0
△IIS	△UA-GlcNS6S	11.2	6.9
△IS	△UA2S-GlcNS6S	5.2	3.7
Tetra-1	△UA-GlcNAc6S-GlcA-GlcNS3S6S	0.7	2.6
Tetra-2	△UA-GlcNS6S-GlcA-GlcNS3S6S	0.0	1.2
Tetra-3	△UA-GlcNS6S-IdoA2S-GlcNS3S6S	0.0	0.0
Tetra-4	△UA-GlcNS-IdoA2S-GlcNS3S	0.1	1.5
Tetra-5	△UA2S-GlcNS-IdoA2S-GlcNS3S	0.1	1.6
Total HS amount		272.4	104.7

a. The data for HCT-116 taken from the publication by Ferreira and colleagues[26]. The data for HCT-116 was presented in ng/mg of protein in the paper. We converted to ng/×10^6 cells provided that 0.9 mg of protein was from 10^6 × cells.