Supporting Information for:

Heparin mapping using heparin lyases and the generation of a novel low molecular weight heparin

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Table S1 SAX-HPLC Peak Area Integration of Major Oligosaccharide Compositions in Heparins Digested by Heparin lyase 1, Heparin lyase 2 and Heparin lyase 3, respectively

	Hp1 (%)	Hp2 (%)	Hp3 (%)	Hp4 (%)	Hp5 (%)	Hp6 (%)	Hp7 (%)	Ave (%)	STDEVA (%)
Heparin lyase 1									
2a	0.87	0.86	0.75	0.78	0.77	0.81	0.79	0.80	0.05
2b	1.22	1.01	1.18	0.99	1.05	1.08	1.15	1.10	0.09
2c	4.44	4.65	4.51	4.55	3.99	4.11	3.98	4.32	0.30
2d	80.40	79.7	79.08	80.07	80.89	80.58	80.85	80.22	0.70
4a	0.32	0.40	0.43	0.40	0.39	0.44	0.41	0.40	0.04
4b	2.55	3.01	3.22	2.45	2.56	2.59	2.44	2.69	0.30
4c	8.96	9.01	9.54	9.44	9.11	9.03	8.97	9.15	0.20
4d	0.99	1.05	1.08	1.07	1.06	1.17	1.21	1.09	0.08
6a	0.29	0.31	0.21	0.25	0.18	0.19	0.20	0.23	0.05
Heparin lyase 2									
2a	0.93	0.89	0.97	0.98	0.95	1.10	1.02	0.98	0.07
2b	15.5	14.50	15.30	14.90	15.20	15.10	13.50	14.86	0.70
2c	9.10	9.00	10.10	8.90	8.10	7.90	7.40	8.64	0.90
2d	61.60	62.02	60.54	63.24	62.29	64.13	65.97	62.83	1.8
2e	3.51	3.31	3.49	3.41	3.11	2.99	2.89	3.24	0.30
2f	3.11	2.99	3.08	3.02	3.01	2.70	3.09	3.00	0.10
2g	1.44	1.69	1.72	1.71	1.70	2.10	1.79	1.74	0.20
2h	1.41	1.40	1.45	1.39	1.42	1.51	1.40	1.43	0.04
4e	0.89	1.80	0.91	0.15	1.81	0.11	0.51	0.88	0.70
4f	0.39	0.37	0.35	0.31	0.36	0.30	0.35	0.35	0.03
4g	1.92	1.87	1.91	1.83	1.87	1.85	1.91	1.88	0.03
4h	0.20	0.16	0.18	0.16	0.18	0.21	0.17	0.18	0.02
Heparin lyase 3									
2a	43.19	42.69	43.46	49.78	43.50	52.05	39.76	44.92	4.3
2b	4.66	4.28	3.85	4.06	5.70	4.30	4.75	4.51	0.60
2e	12.76	12.73	11.99	11.60	12.25	11.77	14.25	12.48	0.90
2f	11.61	11.2	12.1	13.92	12.75	13.91	15.20	12.96	1.5
4e	13.17	0.86	13.76	16.24	3.30	17.12	12.09	10.93	6.3
4i	14.62	29.1	14.85	4.40	22.50	0.85	13.95	14.32	9.7

Table S2 ^{1}H and ^{13}C chemical shifts for the tetrasaccharide standards [a]

	^{1}H/ppm	^{13}C/ppm		^{1}H/ppm	^{13}C/ppm
4a			**4f**		
A1	5.450	96.99	A1	5.074	100.9
A2	4.523	74.02	A2	3.733	70.39
A3	4.137	62.42	A3	4.153	66.83
A4	5.918	105.4	A4	5.724	107.6
B1	5.301	96.84	B1	5.324	96.80
B2	3.193	57.77	B2	3.863	53.39
B3	3.544	69.25	B3	3.723	69.19
B4	3.696	78.37	B4	3.773	78.15
B5	3.811	70.68	B5	3.943	69.03
B6a	3.727	60.06	B6a	4.373	65.87
B6b	3.823	60.06	B6b	4.103	65.87
C1	5.111	99.19	C1	4.573	100.5
C2	4.222	76.05	C2	3.353	73.55
C3	4.149	68.85	C3	3.593	76.71
C4	3.980	76.14	C4	3.703	76.07
C5	4.693	69.23	C5	3.693	77.03
D1	5.370	90.98	D1	5.364	91.12
D2	3.159	58.13	D2	3.353	57.27
D3	3.612	69.41	D3	4.463	75.71

	D4	3.627	77.38	D4	3.943	73.11
	D5	3.847	70.49	D5	3.943	71.23
	D6a	3.823	60.05	D6a	3.853	60.10
	D6b	3.823	60.05	D6b	3.763	60.10
4g				**4h**		
	A1	5.080	100.9	A1	5.074	101.0
	A2	3.729	70.49	A2	3.703	70.65
	A3	4.168	66.94	A3	4.193	67.21
	A4	5.728	107.7	A4	5.714	107.8
	B1	5.348	96.74	B1	5.544	97.32
	B2	3.859	53.40	B2	3.233	57.89
	B3	3.737	69.22	B3	3.603	69.49
	B4	3.786	77.94	B4	3.793	77.63
	B5	3.949	69.05	B5	3.941	68.80
	B6a	4.380	65.90	B6a	4.395	65.82
	B6b	4.103	65.90	B6b	4.094	65.82
	C1	4.551	100.9	C1	4.573	101.0
	C2	3.306	73.61	C2	3.328	72.84
	C3	3.615	76.67	C3	3.762	76.74
	C4	3.721	76.32	C4	3.695	76.97
	C5	3.696	77.09	C5	3.757	76.19
	D1	5.373	91.20	D1	5.364	91.20

	D2	3.363	56.95	D2	3.373	56.99
	D3	4.421	75.56	D3	4.405	75.58
	D4	3.940	73.32	D4	3.946	73.28
	D5	4.144	69.12	D5	4.141	69.16
	D6a	4.372	66.40	D6a	4.368	66.39
	D6b	4.217	66.40	D6b	4.221	66.39
4e				**4i**		
	A1	5.264	101.0	A1	5.254	101.0
	A2	3.893	69.33	A2	3.906	69.11
	A3	4.063	65.61	A3	4.053	65.69
	A4	5.844	107.19	A4	5.834	107.1
	B1	4.603	104.0	B1	4.603	104.0
	B2	3.651	70.18	B2	3.641	70.10
	B3	3.794	81.50	B3	3.792	81.56
	B4	4.026	68.11	B4	4.023	68.25
	B5	3.633	74.93	B5	3.630	74.91
	B6	3.732	61.03	B6	3.717	61.07
	C1	4.453	101.3	C1	4.453	101.4
	C2	3.598	69.75	C2	3.593	69.77
	C3	3.759	82.07	C3	3.763	82.02
	C4	4.107	68.43	C4	4.113	68.65
	C5	3.633	74.93	C5	3.630	74.91

C6	3.653	60.83	C6	3.670	60.88
D1	4.353	102.9	D1	4.373	102.7
D2	3.294	72.75	D2	3.273	72.69
D3	3.535	73.75	D3	3.543	73.69
D4	3.794	76.29	D4	3.792	76.27
D5ax	3.303	62.92	D5ax	3.315	62.90
D5eq	4.026	62.92	D5eq	4.029	62.90
CH_a	4.179	68.50	SerCH-α	3.729	54.95
CH_b	3.984	68.50	SerCH-βa	4.092	69.50
CH_c	3.533	49.16	SerCH-βb	3.876	69.50

[a] The chemical shifts listed above are observed from HMQC spectra, the protons of acetyl groups resonance at 2.03 ppm are not shown.

Table S3 Molecular Weight and Activity Assay of Heparin and Low Molecular Weight Heparin

Sample	Number Average Molecular Weight, M_N ± SD (Da)[a]	Weight Average Molecular Weight, M_W ± SD (Da)[a]	Polydispersity Index, PDI	aPTT (s)[b]	IIa (relative activity)[b]	Xa (relative activity)[b]
Heparin	8300 ± 200	12000 ± 300	1.4	220	1.3	1.0
LMWH (commercial)	4800 ± 150	7200 ± 200	1.5	52	1.0	2.6
LMWH (novel)	5300 ± 200	7700 ± 100	1.4	60	1.0	1.4

a. Three independent determinations.
b. Value ±5%

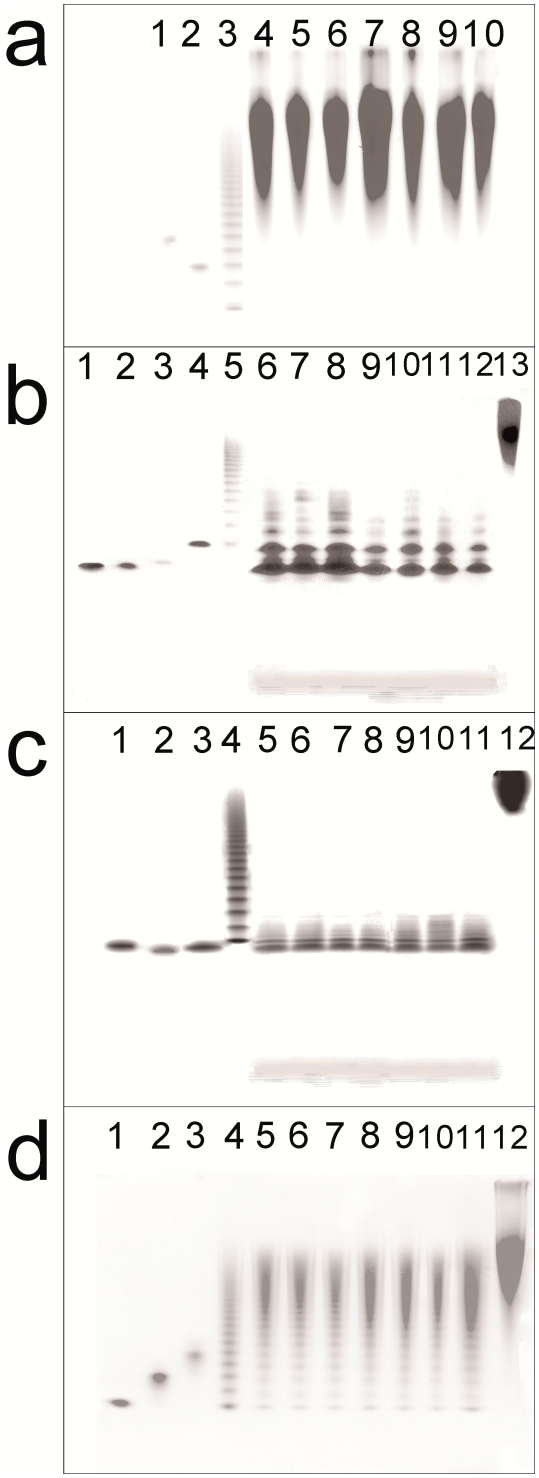

Figure S1 PAGE analysis of untreated heparins and heparin lyases treated heparins. Panel a is 15% PAGE gel analysis of the untreated seven pharmaceutical heparins, Panel b and panel c are 22% PAGE gels analysis of heparins exhaustively digested by heparin lyase 1 and heparin lyase 2, respectively. Panel d is 15% PAGE gel analysis of heparin lyase 3 treated heparins. Oligosaccharide standards, bovine heparin ladder and untreated heparin are used in these gels as controls. The faint bands at the bottoms of panel b and panel c are disaccharides stained by the mixture of alcian Blue and azure A.

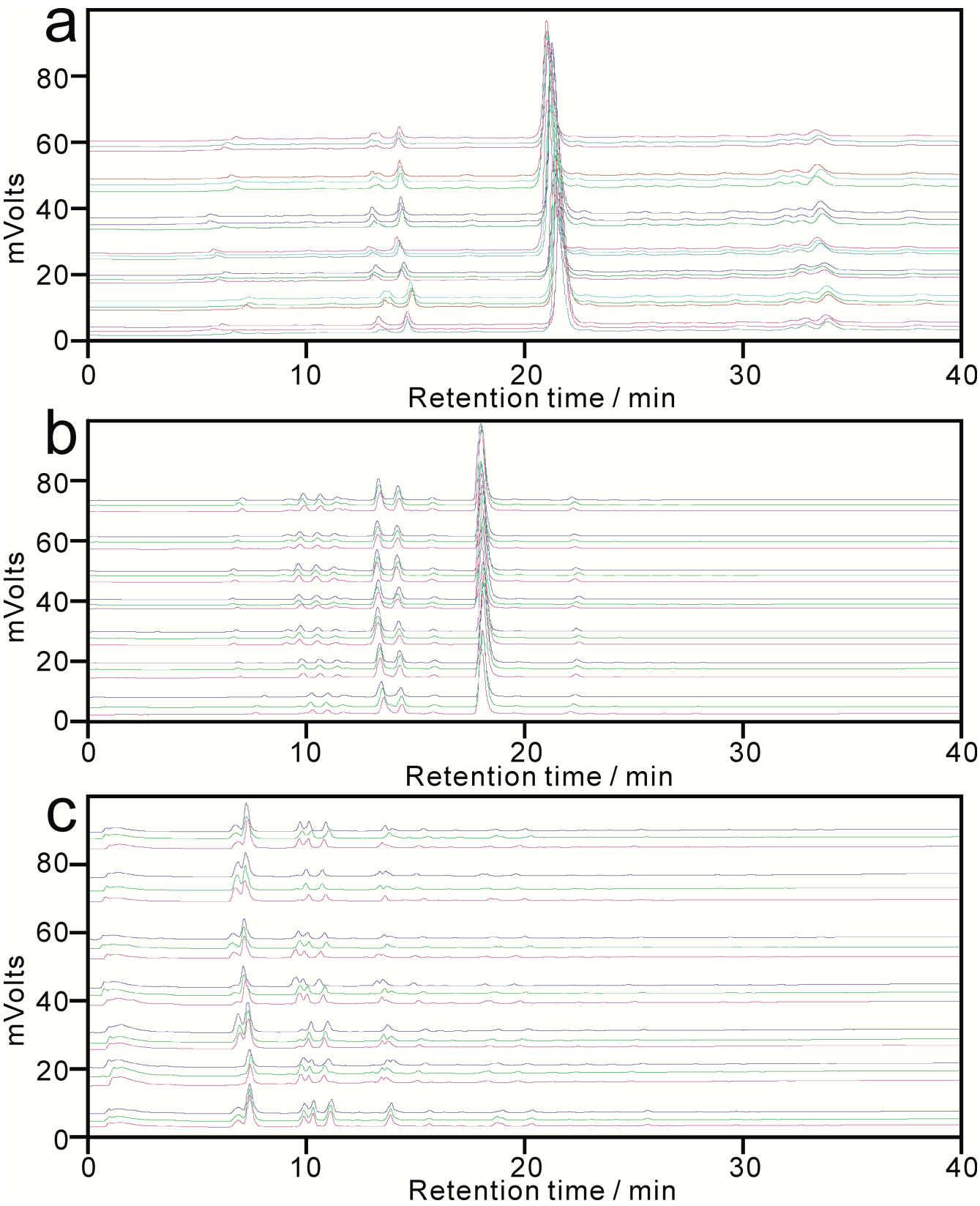

Figure S2 Chromatogram of heparins treated by heparin lyases. Panels a, b and c are SAX-HPLC analysis of the seven pharmaceutical heparins excessively digested by heparin lyase 1, heparin lyase 2 and heparin lyase 3, respectively. Each sample is analyzed in triplicate.

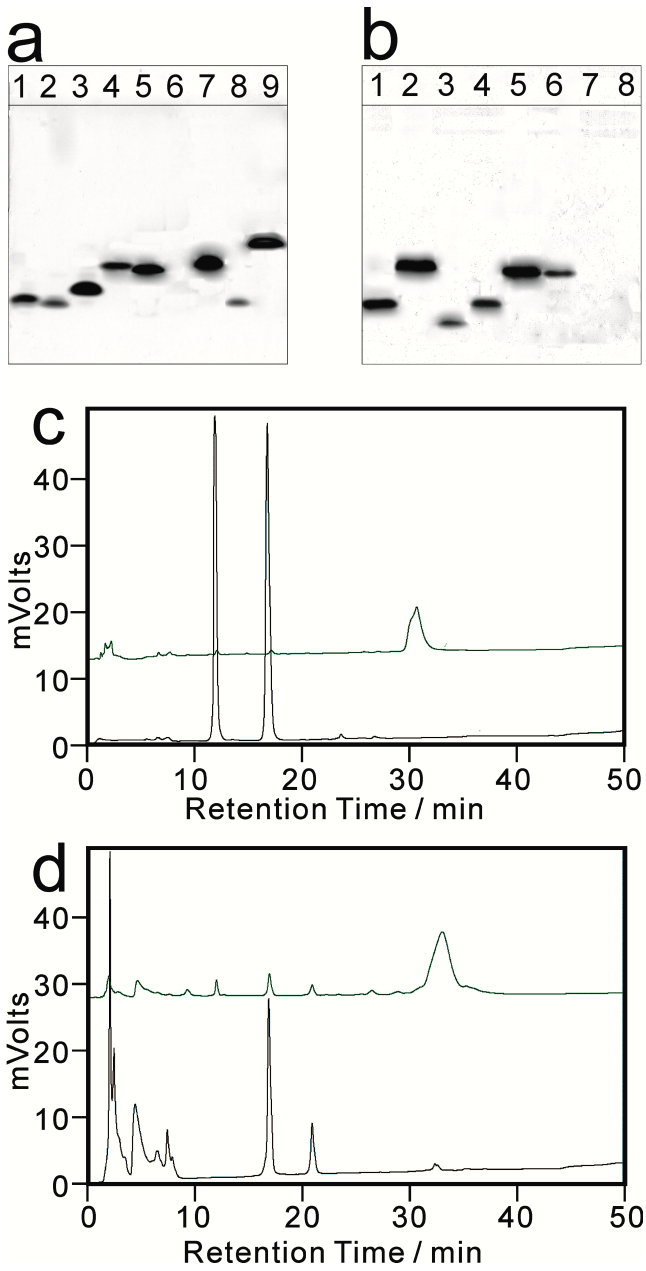

Figure S3 PAGE and SAX-HPLC analysis of oligosaccharides treated by heparin lyase 3. Panel a, lane 1 through lane 5 are untreated standards, lane 1 is **4c**, lane 2 is **4g**, lane 3 is **4d**, lane 4 is **6b** and lane 5 is **6a**. Lanes 6 through 9 are oligosaccharides incubated with excess heparin lyase 3. Lane 6 is **4c**, lane 7 is **6b**, lane 8 is **6a** and lane 9 is **8a**. Panel b, lane 1 is standard **4c**, lane 2 is standard **6c**, lane 3 is heparin lyase 1 treated **6c**, lane 4 is heparin lyase 3 treated **6c**, lane 5 is standard **6d**, lanes 6 through 8 are **6d** treated by heparin lyase 1, heparin lyase 2 and heparin lyase 3, respectively. Lane 7 and lane 8 are **6d** digested to disaccharides which are not able to be stained either by Alcian Blue or silver nitrate. Panel c is SAX-HPLC analysis of **6b** (upper) and **4c** (lower) treated by heparin lyase 3. Panel d is SAX-HPLC analysis of **8a** (upper) and **6a** (lower) treated by heparin lyase 3.

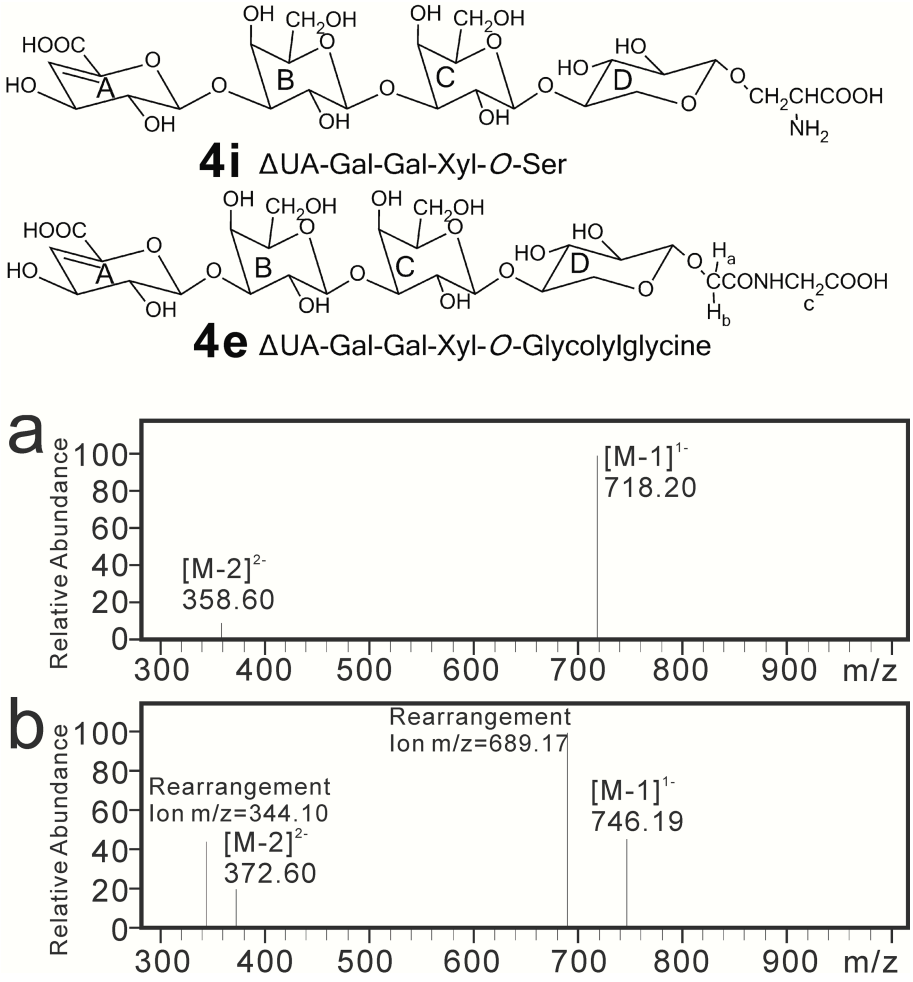

Figure S4 Structures and Mass Spectra of **4i** and **4e**. The structures of **4i** and **4e** with labels in the schematics shown here are corresponding to the explanation in the body of the paper and **Table S1** in the supporting information section. Panel a and panel b are the mass spectra for **4i** and **4e**, respectively.

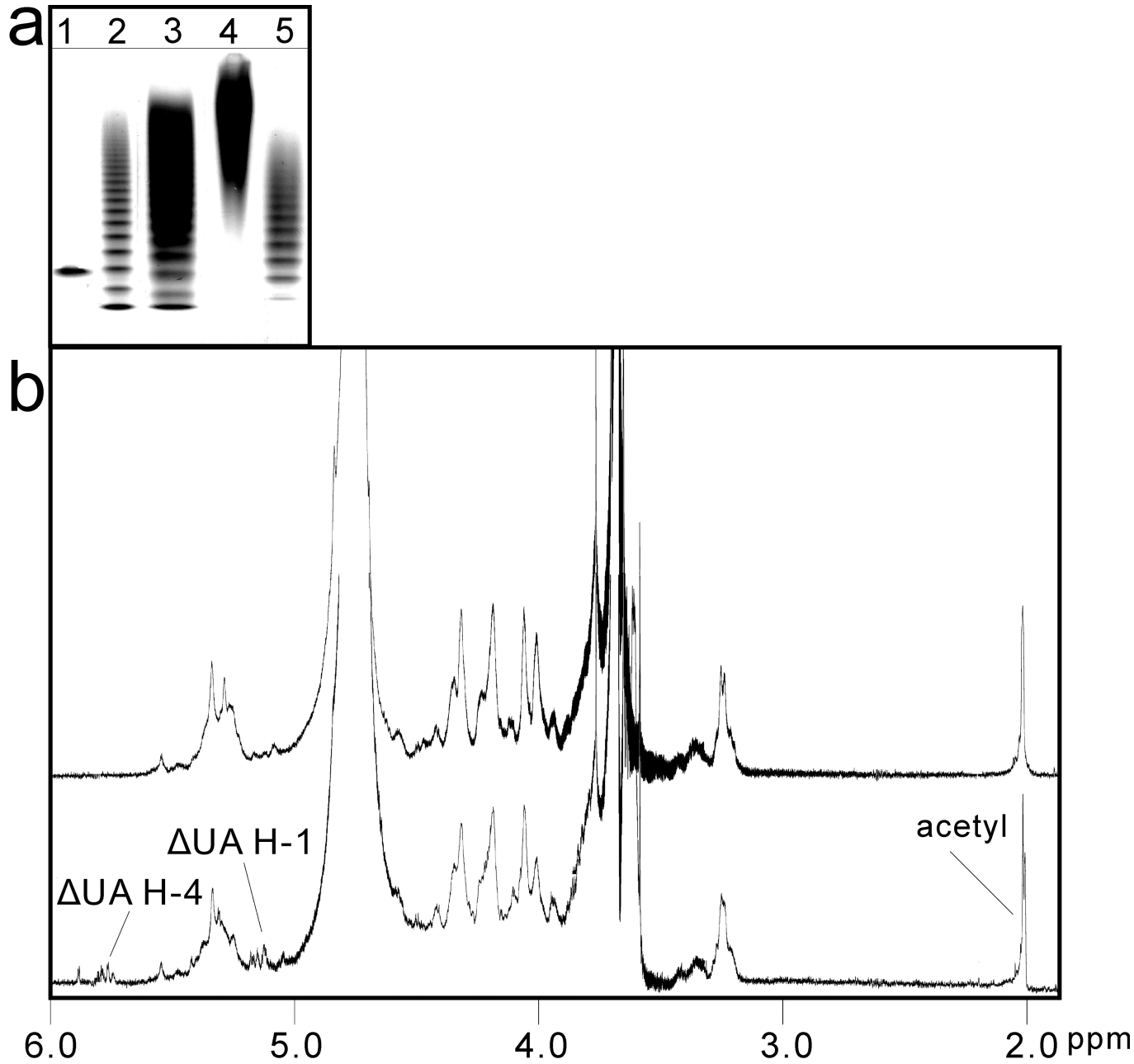

Figure S5 PAGE analysis and 1D NMR spectra of the new low molecular weight heparin and the heparin control. Panel a is 15% PAGE gel analysis of the new LMWH (lane 3), lane 1 is heparin dp10, lane 2 is bovine heparin ladder, lane 4 is untreated heparin and lane 5 is a commercial LMWH. Panel b shows the 1D NMR spectra of heparin treated with deactivated heparin lyase 3 (upper) and the new LMWH (lower). ΔUA H-1 and ΔUA H-4 stand for the signals of H-1 and H-4 in the unsaturated uronic acid residues at the non-reducing terminal of heparin derived oligosaccharides.