Organic & Biomolecular Chemistry

Comparable stabilisation, structural changes and activities can be induced in FGF by a variety of HS and non-GAG analogues: Implications for sequence-activity relationships

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Supplementary

PCA data

Supplementary Table 1 Systematically modified heparins studied by ^{13}C NMR and analysed using principal component analysis.

		Position of Substitution		
Notation	**H-**	**I2 / R_1**	**A6 / R_2**	**A2 / R_3**
$I_{2OH}\ A^{6OH}_{NH2}$	**1**	H	H	H
$I_{2OH}\ A^{6OH}_{NS}$	**2**	H	H	SO_3^-
$I_{2OH}\ A^{6S}_{NS}$	**3**	H	SO_3^-	SO_3^-
$I_{2S}\ A^{6S}_{NH2}$	**4**	SO_3^-	SO_3^-	H
$I_{2S}\ A^{6S}_{NAc}$	**5**	SO_3^-	SO_3^-	$COCH_3$
$I_{2S}\ A^{6OH}_{NS}$	**6**	SO_3^-	H	SO_3^-
$I_{2OH}\ A^{6S}_{NH2}$	**7**	H	SO_3^-	H
$I_{2OH}\ A^{6S}_{NAc}$	**8**	H	SO_3^-	$COCH_3$
$I_{2OH}\ A^{6OH}_{NAc}$	**9**	H	H	$COCH_3$
$I_{2S}\ A^{6OH}_{NH2}$	**10**	SO_3^-	H	H
$I_{2S}\ ^{A6OH}_{NAc}$	**11**	SO_3^-	H	$COCH_3$
$I_{2S}\ A^{6S}_{NS}$	**12**	SO_3^-	SO_3^-	SO_3^-

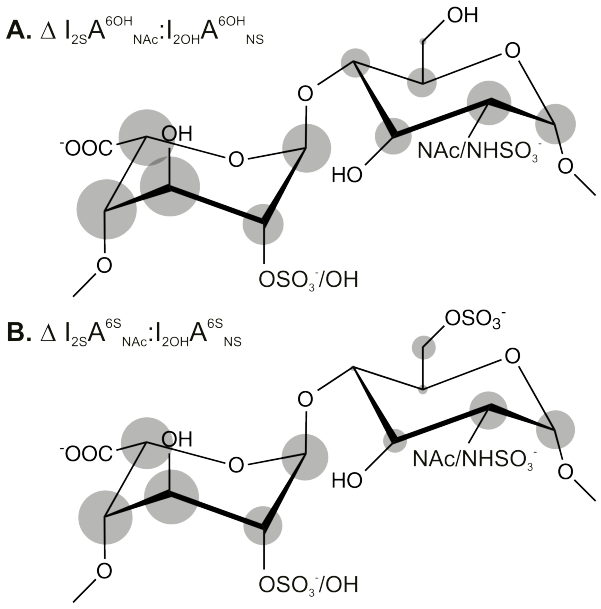
I-*N* refers to the N^{th} position of iduronate and A-*N* to the N^{th} position of glucosamine (aminosugar) residues respectively.

Supplementary Table 2 ^{13}C chemical Shift assignments (ppm) for 12 chemically modified heparins

	H1	***H2***	***H3***	***H4***	***H5***	***H6***	***H7***	***H8***	***H9***	***H10***	***H11***	***H12***
A1	95.8	98.2	98.1	93.7	96.6	100.0	97.9	97.1	97.1	97.2	96.8	99.5
A2	57.0	60.5	60.3	57.1	56.2	60.8	57.6	56.2	56.2	58.0	56.6	60.7
A3	71.8	72.5	72.4	70.9	73.0	72.4	73.9	72.5	72.3	73.5	72.9	72.5
A4	79.0	80.2	80.1	78.2	79.3	80.5	79.6	79.6	79.6	80.1	80.6	78.8
A5	74.3	73.5	71.5	72.0	72.3	73.8	72.7	71.8	73.7	74.2	74.2	72.0
A6	61.9	62.4	68.7	68.8	69.6	62.6	69.0	68.8	62.3	62.8	62.9	69.2
I1	104.5	104.3	104.6	101.3	102.2	102	104.8	104.6	104.3	101.9	102.3	102.1
I2	71.7	72.2	71.1	75.3	76.8	77.6	72.9	72.0	72.5	76.0	76.6	78.9
I3	71.8	71.5	70.4	65.4	67.3	70.7	72.4	71.4	72.2	67.0	67.1	72.1
I4	77.4	77.8	77.2	73.0	74.2	78.7	77.8	77.0	77.1	74.4	74.1	79.0
I5	71.8	72.2	71.2	69.8	70.8	71.4	72.8	71.9	72.6	70.1	70.6	72.3

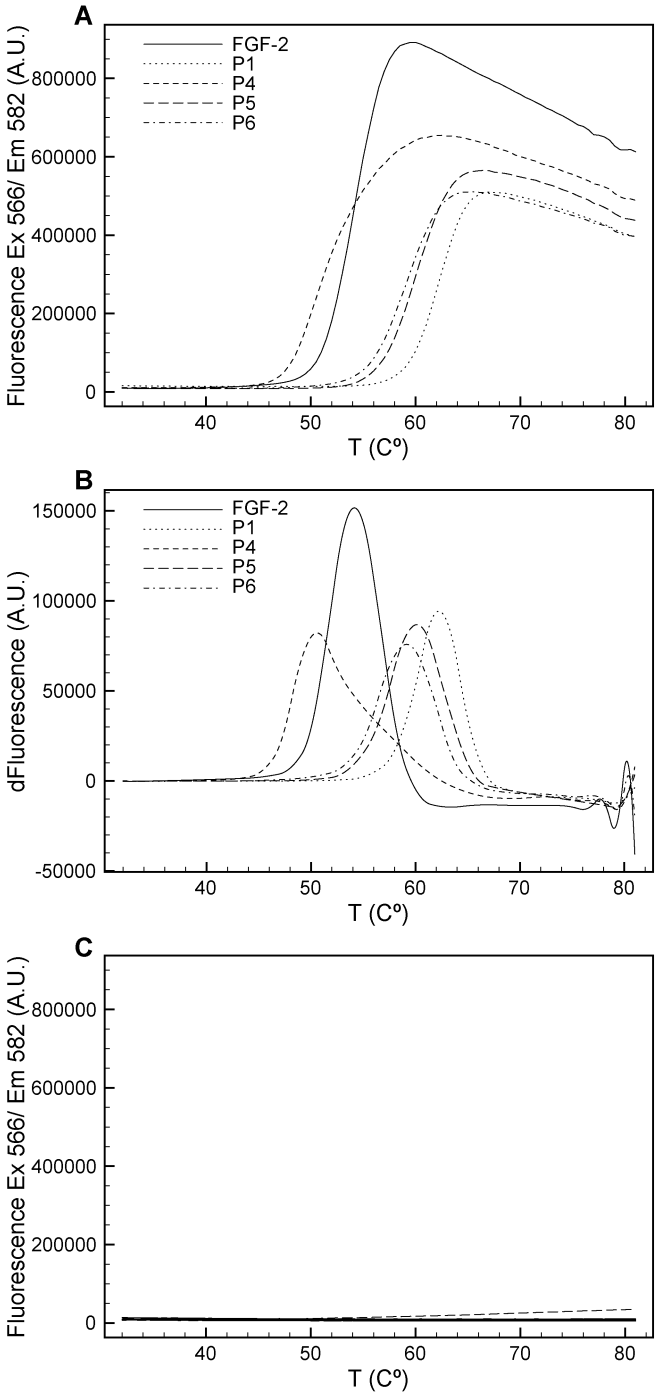
Supplementary Table 3 Loadings derived from the principal component analysis of ^{13}C chemical shift values

Position	***Component 1/I2*** (Proportion of variance 38.8%)	***Component 2/A2*** (Proportion of variance 22.3%)	***Component 3/A6*** (Proportion of variance 18.5%)
A1	0.047	0.867	0.043
A2	-0.162	0.863	0.013
A3	0.073	0.058	-0.030
A4	0.015	0.192	0.512
A5	-0.048	-0.064	0.943
A6	-0.058	-0.059	-0.993
I1	0.975	0.018	-0.008
I2	-0.859	0.275	-0.056
I3	0.771	0.603	0.008
I4	0.523	0.839	0.0160
I5	0.765	0.502	-0.058



A schematic of the effect of "transfering" an *O*-sulfate from iduronate to glucoamine (position-2) for 6-de-*O*-sulfated (A) and 6-*O*-sulfated (B) polysaccharides.

DSF data



Supplementary Figure 1. The principle of DSF data analysis in the presence of illustrative plant polysaccharides and visualisation of background signal of Sypro Orange. **A.** Melting curve profile of 10 μM FGF-2 in the presence of selected plant polysaccharides (**P1**, **P4**, **P5** and **P6**) obtained as described in the Methods section. **B.** The first derivatives of each melting curve from the panel **A**, where each maximum of the first derivative indicates the Tm (melting temperature) of the protein-polysaccharide complex. **C.** Thermal profile of all the plant polysaccharides used in this study incubated with Sypro Orange. The abscissa scale has been adjusted to that of panel **A** for comparison.

These data preclude the possibility that the fluorescence reading was affected by the interaction of the dye with the polysaccharides.

Supplementary Table 4. For each set of compounds (heparin derivarives (D1-D9) and chemically sulfated plant polysaccharides (P1-P11)), the melting temperatures of the polysaccaride-FGF complexes, recorded in triplicate, are presented. The calculation of normalised values (Tnorm) and relative complex stabilities was performed as described in Methods.

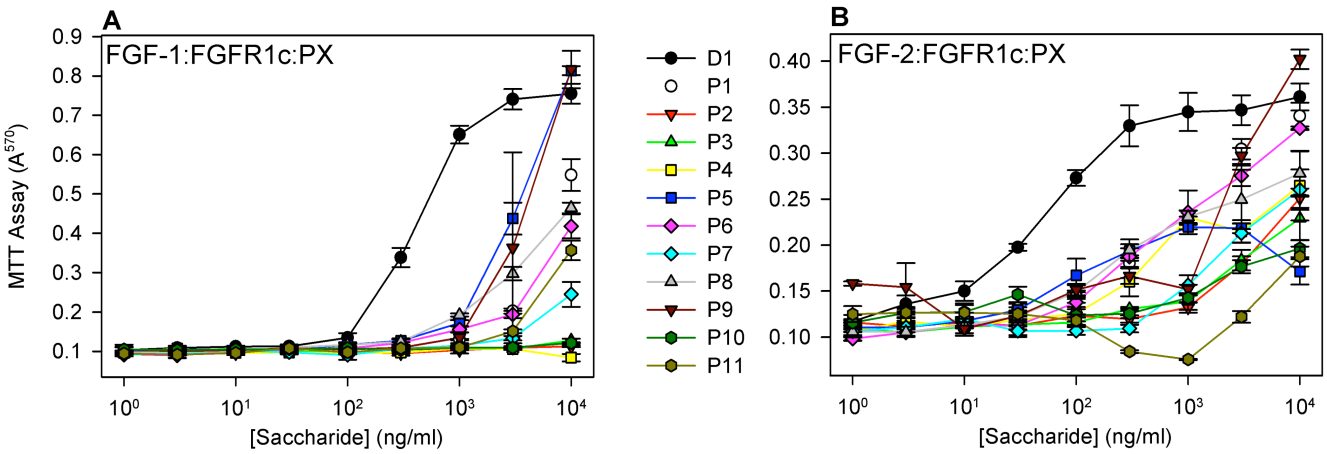
FGF1	*Tm1*	*Tm2*	*Tm3*	*Mean*	*SDEV*	*Tnorm*	*±*	*Relative complex stability*	*±*
PBS	48.60	48.41	48.51	48.51	0.10	0.00	0.16	0.00	0.01
HEP	62.30	62.30	62.18	62.26	0.07	13.75	0.14	1.00	0.01
D2	52.42	52.22	52.57	52.40	0.18	3.90	0.22	0.28	0.02
D3	48.58	48.73	48.75	48.68	0.09	0.18	0.15	0.01	0.01
D4	56.15	56.13	56.58	56.28	0.25	7.78	0.28	0.57	0.02
D5	49.10	48.82	48.83	48.92	0.16	0.41	0.20	0.03	0.01
D6	48.22	48.05	48.22	48.16	0.10	-0.34	0.16	-0.02	0.01
D7	50.95	50.87	51.20	51.01	0.17	2.50	0.21	0.18	0.02
D8	47.84	47.95	47.70	47.83	0.13	-0.68	0.17	-0.05	0.01
D9	61.39	61.36	61.76	61.51	0.22	13.00	0.25	0.95	0.02

FGF2	*Tm1*	*Tm2*	*Tm3*	*Mean*	*SDEV*	*Tnorm*	*±*	*Relative complex stability*	*±*
PBS	54.41	54.26	54.25	54.31	0.09	0.00	0.17	0.00	0.01
HEP	75.02	74.92	74.80	74.91	0.11	20.61	0.18	1.00	0.01
D2	58.79	59.02	59.41	59.07	0.31	4.76	0.34	0.23	0.02
D3	53.78	53.82	53.77	53.79	0.03	-0.51	0.14	-0.02	0.01
D4	66.02	66.46	65.95	66.14	0.28	11.84	0.31	0.57	0.02
D5	54.58	54.51	55.23	54.77	0.40	0.47	0.42	0.02	0.02
D6	54.19	54.26	54.14	54.20	0.06	-0.11	0.15	-0.01	0.01
D7	57.62	57.44	57.80	57.62	0.18	3.32	0.23	0.16	0.01
D8	54.46	54.37	54.14	54.32	0.17	0.02	0.22	0.00	0.01
D9	73.97	74.14	73.91	74.00	0.12	19.70	0.18	0.96	0.01

FGF1	*Tm1*	*Tm2*	*Tm3*	*Mean*	*SDEV*	*Tnorm*	*±*	*Relative complex stability*	*±*
PBS	48.00	47.85	47.88	47.91	0.08	0.00	0.22	0.00	0.02
HEP	62.04	62.31	62.40	62.25	0.19	14.34	0.28	1.00	0.02
P1	62.99	62.65	62.89	62.85	0.18	14.94	0.27	1.04	0.02
P2	45.11	45.13	45.33	45.19	0.12	-2.72	0.24	-0.19	0.02
P3	47.66	47.75	47.83	47.75	0.09	-0.16	0.22	-0.01	0.02
P4	54.30	53.17	53.61	53.69	0.57	5.78	0.60	0.40	0.04
P5	62.77	62.60	62.63	62.67	0.09	14.76	0.23	1.03	0.02
P6	59.66	60.00	60.25	59.97	0.30	12.06	0.36	0.84	0.03
P7	59.86	59.66	60.42	59.98	0.39	12.07	0.44	0.84	0.03
P8	61.01	60.71	60.84	60.85	0.15	12.94	0.25	0.90	0.02
P9	63.43	63.73	63.75	63.64	0.18	15.73	0.27	1.10	0.02
P10	47.88	47.85	47.61	47.78	0.15	-0.13	0.25	-0.01	0.02
P11	60.15	60.18	60.32	60.22	0.09	12.31	0.23	0.86	0.02

FGF2	*Tm1*	*Tm2*	*Tm3*	*Mean*	*SDEV*	*Tnorm*	*±*	*Relative complex stability*	*±*
PBS	54.72	54.70	54.48	54.64	0.13	0.00	0.23	0.00	0.01
HEP	77.75	77.55	77.79	77.70	0.13	23.06	0.23	1.00	0.01
P1	72.10	72.03	72.12	72.08	0.05	17.45	0.19	0.76	0.01
P2	52.28	52.14	52.16	52.19	0.07	-2.44	0.20	-0.11	0.01
P3	54.65	54.28	54.31	54.41	0.21	-0.22	0.28	-0.01	0.01
P4	55.29	55.07	55.02	55.13	0.14	0.49	0.23	0.02	0.01
P5	69.65	69.68	69.48	69.60	0.11	14.97	0.22	0.65	0.01
P6	64.19	63.55	63.52	63.75	0.38	9.12	0.42	0.40	0.02
P7	69.88	69.67	69.51	69.69	0.19	15.05	0.26	0.65	0.01
P8	59.01	58.51	58.48	58.66	0.30	4.03	0.35	0.17	0.02
P9	69.80	70.03	69.67	69.84	0.18	15.20	0.26	0.66	0.01
P10	54.80	54.15	54.58	54.51	0.33	-0.13	0.38	-0.01	0.02
P11	71.85	71.46	71.01	71.44	0.42	16.81	0.46	0.73	0.02

BaF3 Cell assay data



Supplementary Figure 2 The ability of heparin chemical derivates (DX) and sulfated plant polysaccharides (PX) to support signalling through FGFR1c/FGF-1 and -2 in a BaF3 cell assay.

Categories of activity were defined as:

FGF-1: None- P2, P4, P10; **low**- P7; **Medium**- P1, P6,P8,P11 and **Strong**- P5 and P9.

FGF-2: Low- P10, P11; **Medium**- P2,P4, P5, P7, P8, P9; **Strong**- P1 and P6.

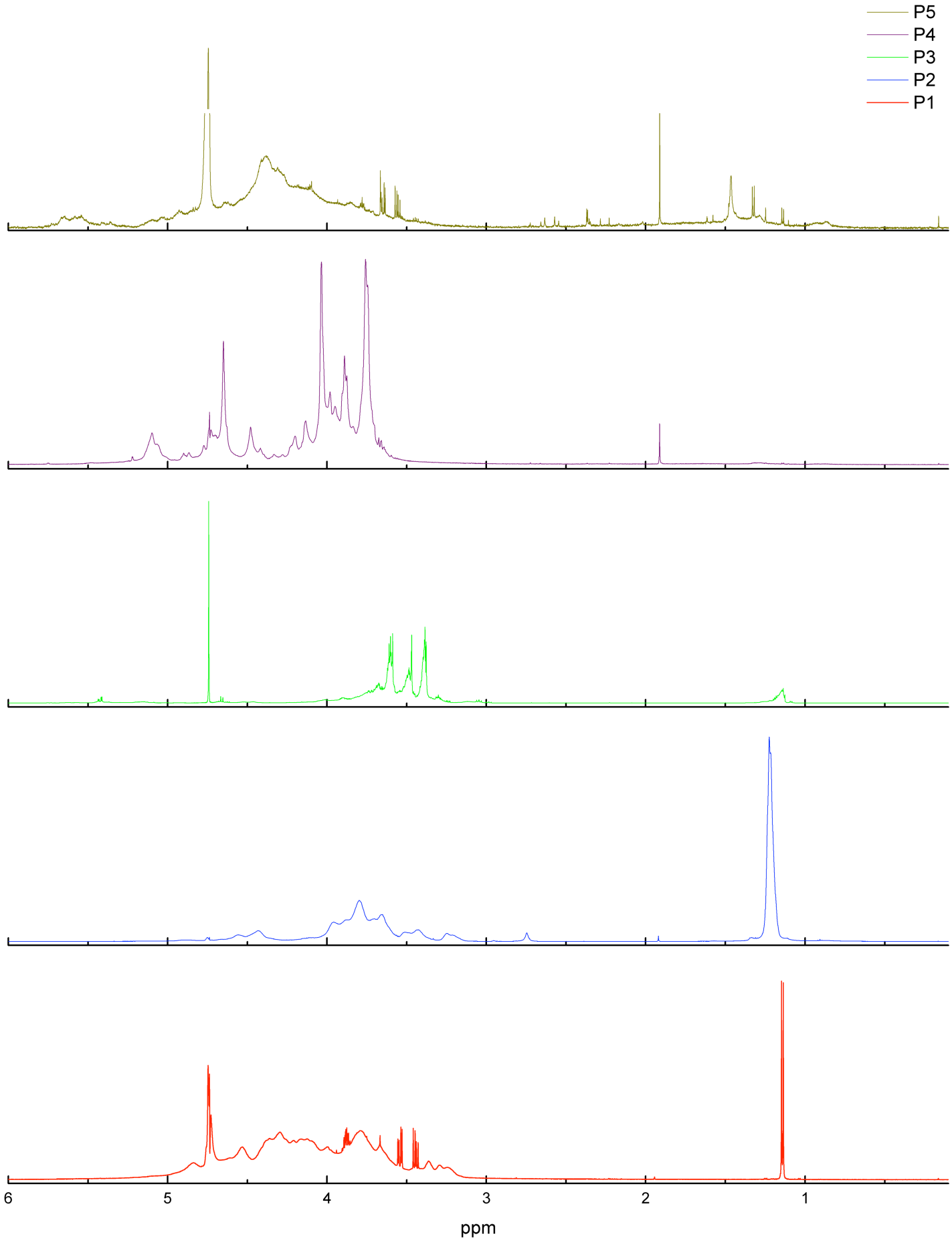
Supplementary Table 5 ***Compound key***. **A.** The eight modified heparin derivatives and the predominant substitution patterns of the repeating disaccharide. *I indicates iduronate, A glucosamine (aminosugar) while the sub- and superscripts indicate the presence of hydroxyl (nOH) or O-sulfate at position-n (nS) and N-acetyl (NAc) or N-sulfate (NS).* ***B.*** The starting materials from which each of the eleven chemically sulfated plant polysaccharides were prepared.

A. Heparin derivatives		***B. Plant polysaccharides***	
Sample	***Compound***	***Sample***	***Starting material***
D1	$I_{2S}\,A^{6S}_{NS}$ (heparin)	**P1**	Tylose
D2	$I_{2S}A^{6S}_{NAc}$	**P2**	Ethyl Cellulose
D3	$I_{2OH}\,A^{6S}_{NS}$	**P3**	Hydroxypropylmethyl Cellulose
D4	$I_{2S}\,A^{6OH}_{NS}$	**P4**	Alginic Acid
D5	$I_{2OH}\,A^{6S}_{NAc}$	**P5**	Xanthan Gum
D6	$I_{2S}\,A^{6OH}_{NAc}$	**P6**	Locust Bean Gum
D7	$I_{2OH}\,A^{6OH}_{NS}$	**P7**	Gum Arabic
D8	$I_{2OH}\,A^{6OH}_{NAc}$	**P8**	Pectin
D9	$I_{2S3S}A_{6S3SNS}$	**P9**	ι-Carrageenan
		P10	Hydroxyethyl Cellulose
		P11	Glycogen Type II

Supplementary Table 6 Degree of sulfation of sulfate polysaccharides.

Polysaccharide	Precursor	D.O.S.[#] (per disaccharide)
P1	Tylose	1.0
P2	Ethyl Cellulose	1.5
P3	Hydroxypropylmethyl cellulose	2.5
P4	Alginic acid	1.6
P5	Xanthan gum	1.6
P6	Locust bean gum	0.9
P7	Gum arabic	0.8
P8	Pectin	1.3
P9	i-Carrageenan	2.1
P10	Hydroxyethyl cellulose	2.3
P11	Glycogen (II)	1.9

[#] Degree of sulfation


P5
P4
P3
P2
P1
6
5
4
3
2
1
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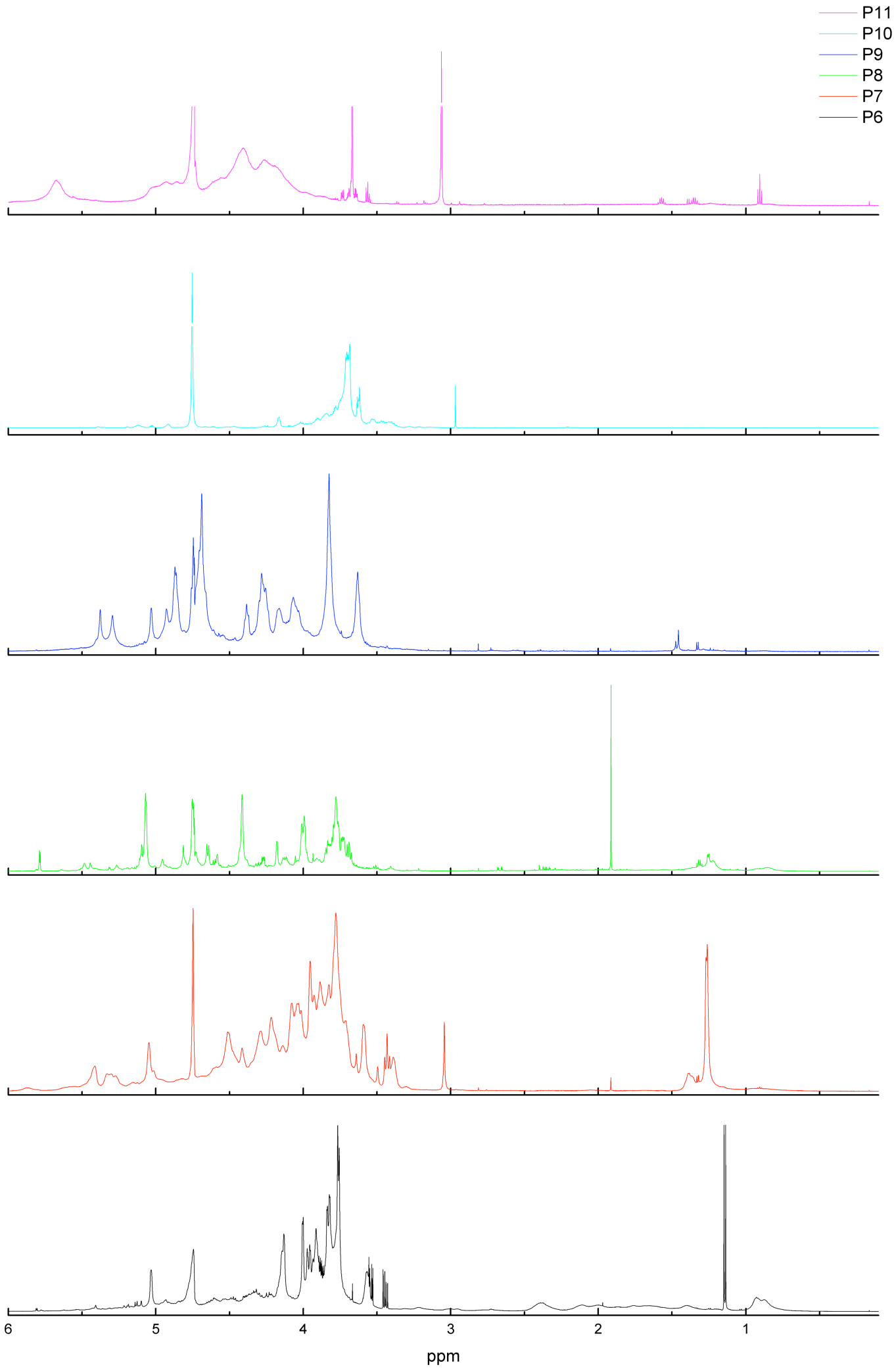
P11
P10
P9
P8
P7
P6
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4
3
2
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ppm

Supplementary Figure 7 ^{1}H NMR spectra of the chemically sulfated polysaccharides.