

**A fucan sulfate with pentasaccharide repeating units from
the sea cucumber *Holothuria floridana* and its anticoagulant
activity**

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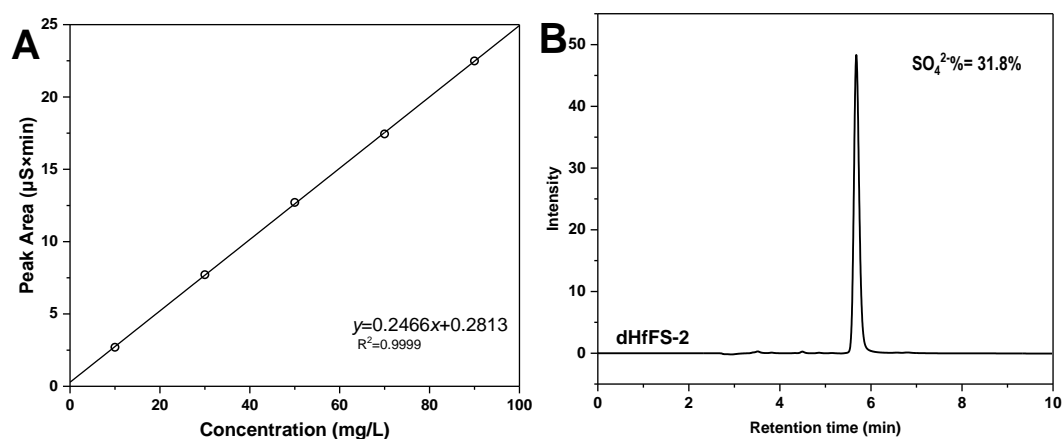


Figure S1. The calibration curve for sulfate standards (A) and sulfate elution profiles of dHfFS-2 obtained by ion chromatography (B).

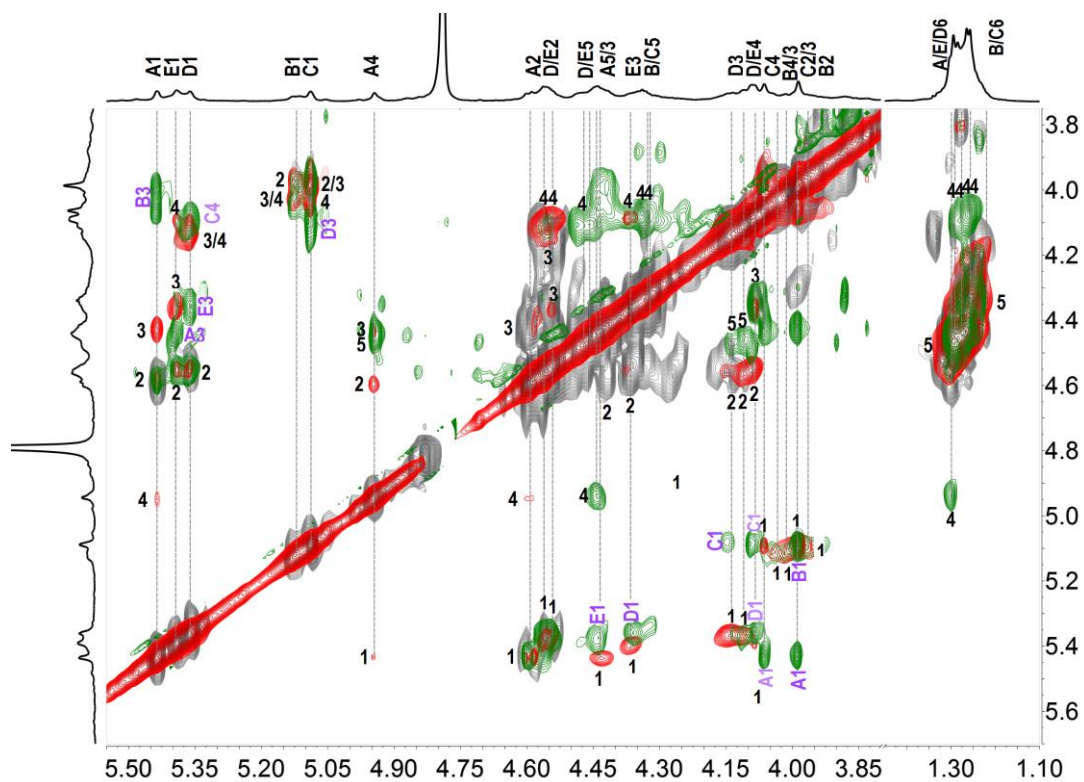


Figure S2. Overlapped spectra of ^1H - ^1H COSY (gray), TOCSY (red) and ROESY (green) of dHfFS-1.

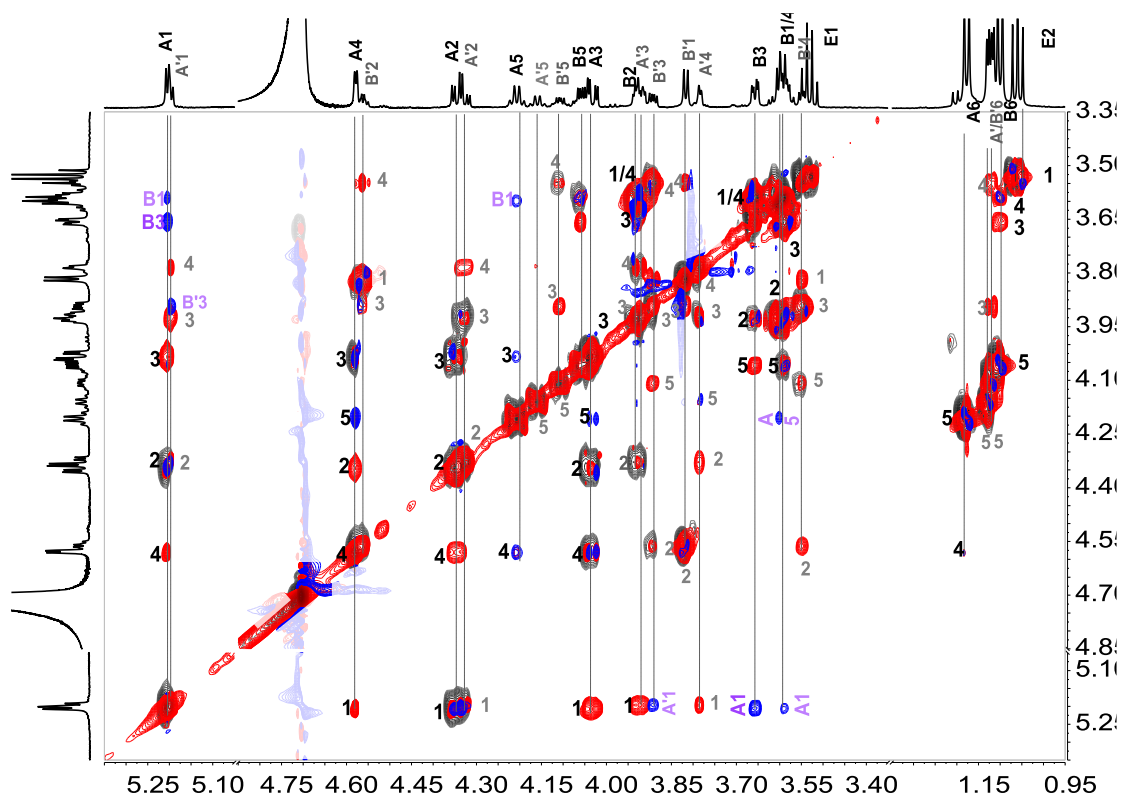
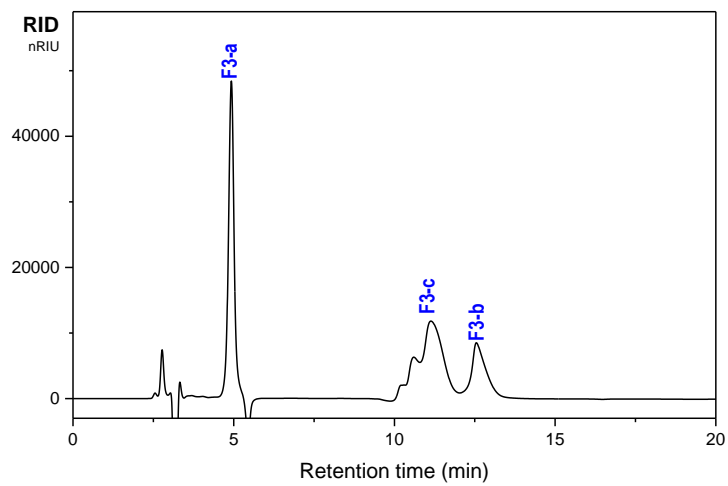


Figure S4. Overlapped spectra of ^1H - ^1H COSY (gray), TOCSY (red) and ROESY (blue) of F2.



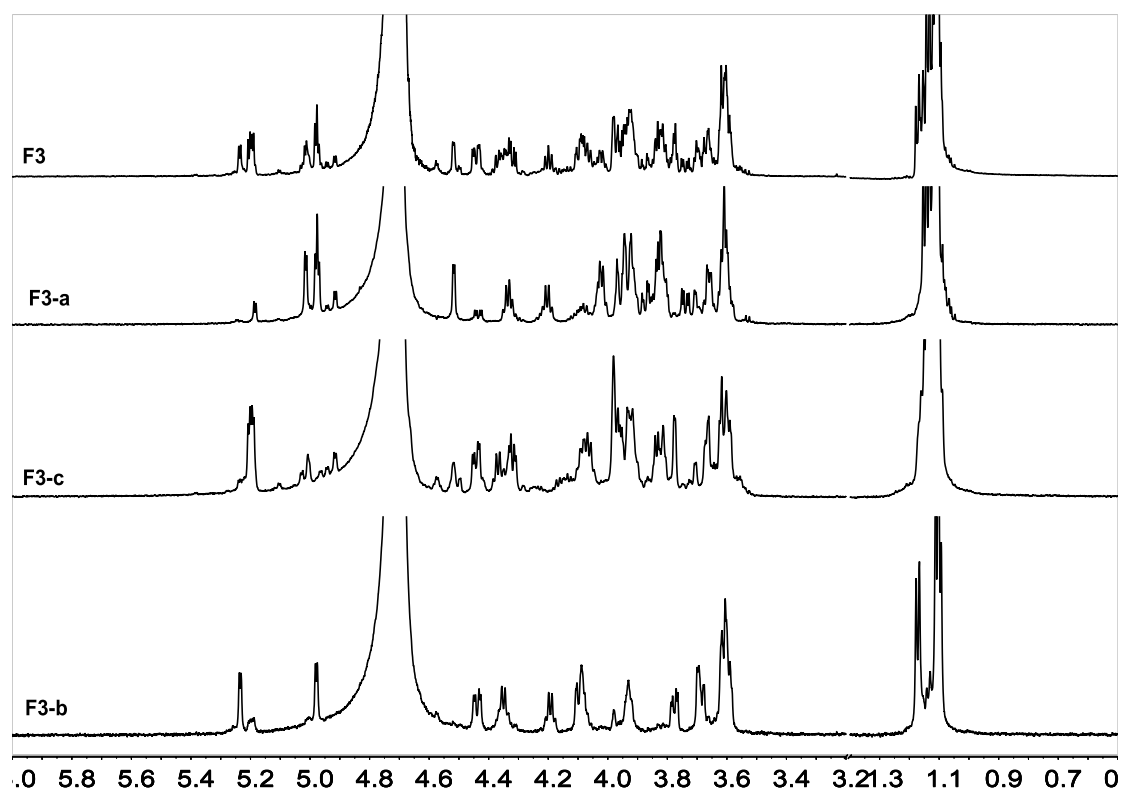


Figure S5. SAX chromatography of F3 and ^1H NMR spectra of F3, F3-a, F3-b and F3-c.

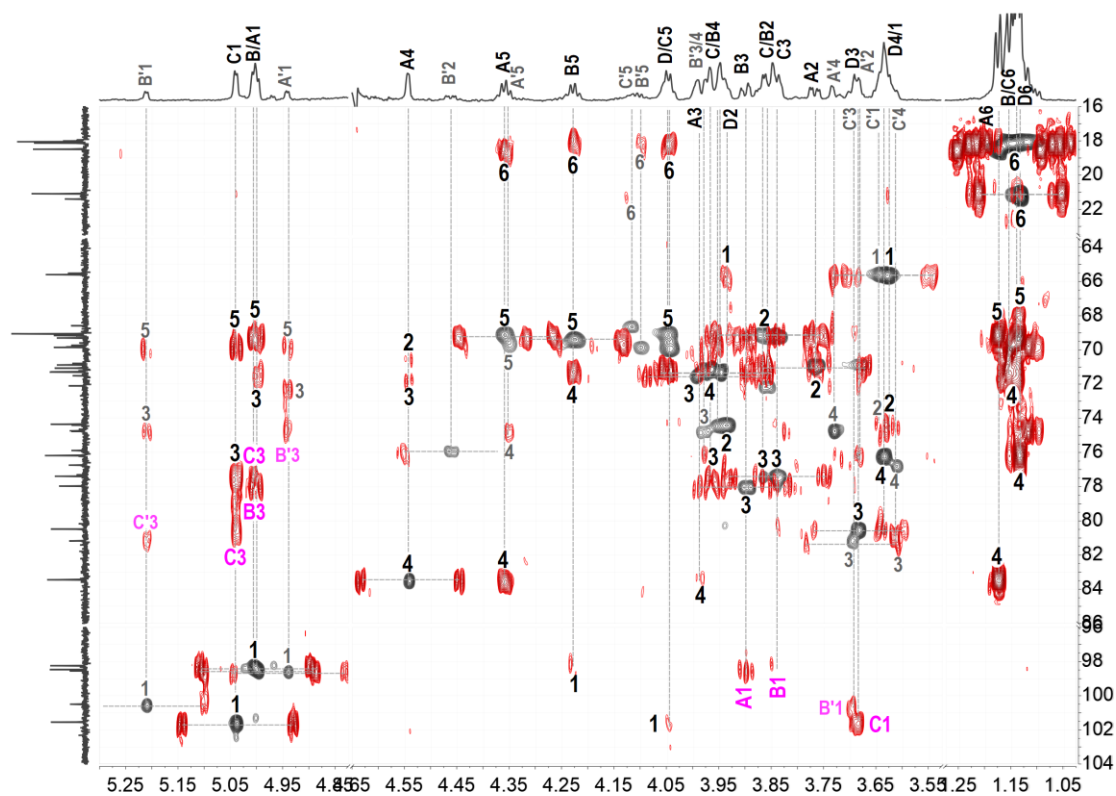


Figure S6. ^1H - ^{13}C HSQC (gray) and HMBC (red) spectra of F3-a.

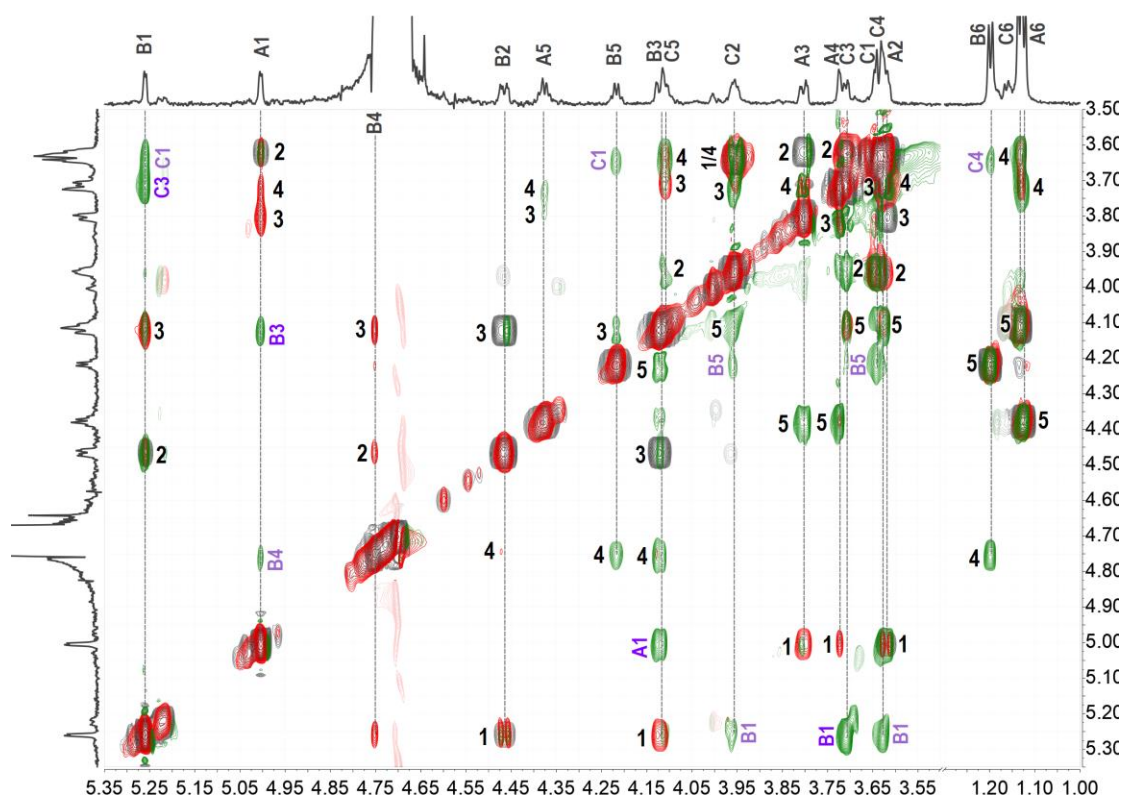
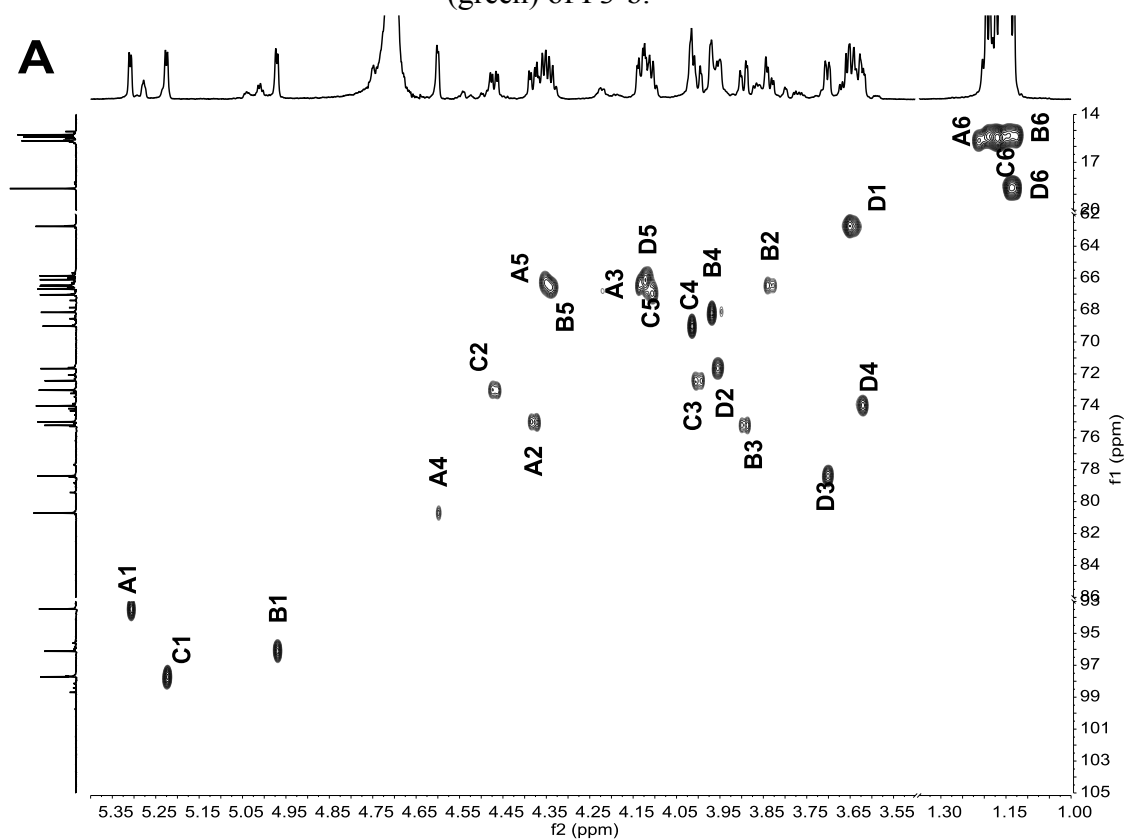


Figure S7. Overlapped spectra of ^1H - ^1H COSY (gray), TOCSY (red) and ROESY (green) of F3-b.



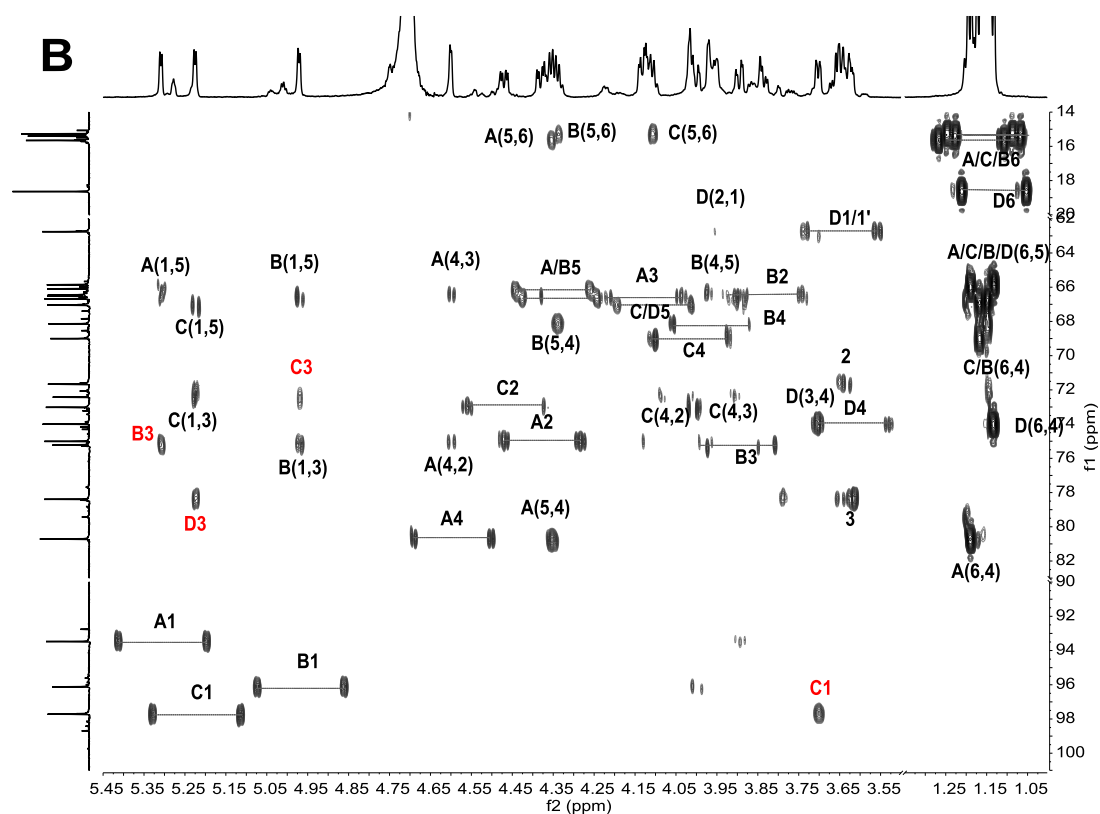


Figure S8. ^1H - ^{13}C HSQC and HMBC spectra of F4.

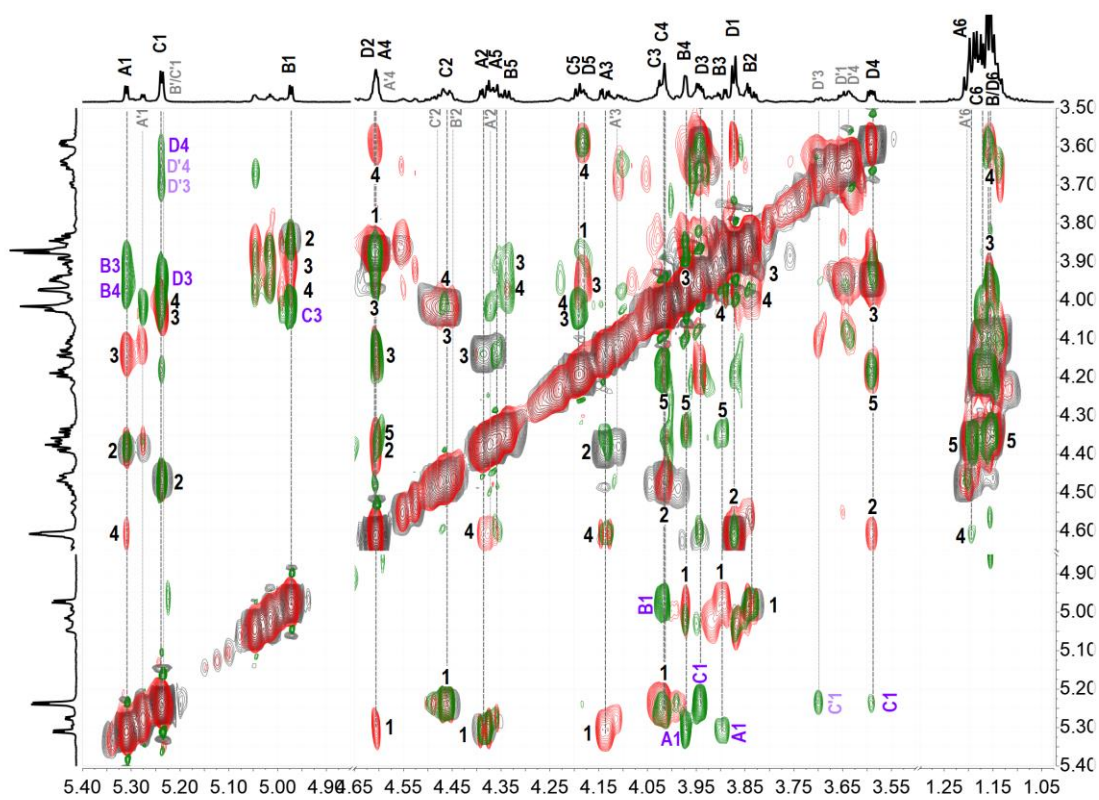


Figure S9. Overlapped spectra of ^1H - ^1H COSY (gray), TOCSY (red) and ROESY (green) of F5.

Table S1 ^1H and ^{13}C chemical shifts of the oligosaccharide F1

Fraction	Residue		Chemical shift (ppm)					
			1	2	3	4	5	6
F1-a	A: L-Fuc_{2S}-α(1-	H	5.183 ($J_{1,2}=4.02$)	4.342	3.918	3.793	4.079	1.125
		C	100.37	77.98	70.00	74.61	70.05	17.90
	B: -3)-L-Fuc-ol	H	3.627 ($J_{1,1'}=11.58$)	3.935	3.647	3.591	4.066	1.105
			3.597 ($J_{1,2}=4.74$)					
		C	65.53	74.36	80.89	76.81	68.56	21.40
F1-b	A': L-Fuc_{4S}-α(1-	H	5.006 ($J_{1,2}=4.20$)	3.747	3.884	4.515	4.156	1.171
		C	101.49	70.91	71.17	83.14	69.42	18.53
	B': -3)-L-Fuc-ol	H	3.623 ($J_{1,1'}=11.58$)	3.926	3.664	3.582	4.032	1.112
			3.597 ($J_{1,2}=4.74$)					
		C	65.63	74.26	80.33	76.26	68.82	21.28
F1-c	A'': L-F_{2S4S}-α(1-	H	5.209 ($J_{1,2}=4.08$)	4.350	4.032	4.584	4.211	1.184
		C	100.25	77.77	69.11	83.25	69.80	18.43
	B'': -3)-L-Fuc-ol	H	3.623 ($J_{1,1'}=11.58$)	3.926	3.664	3.582	4.032	1.112
			3.597 ($J_{1,2}=4.74$)					
		C	65.51	74.31	81.08	76.81	68.56	21.28
F1-d	rF: L-F_{2S4S}-ol	H	3.764 ($J_{1,2}=4.56$)	4.412	4.248	4.273	4.269	1.105
		C	62.79	82.33	73.70	84.14	69.80	21.49

Table S2. ESI-MS data assignments of fucooligosaccharides

Sample	Actual Peak	Charge	Negative Ion		Predicted Peak
			Structure	Formula	
F1	391.0932	1-	[M-Na] ⁻	C ₁₂ H ₂₃ O ₁₂ S ⁻	391.0916
F2	493.0325	1-	[M-Na] ⁻	C ₁₂ H ₂₂ NaO ₁₅ S ₂ ⁻	493.0303
	471.0502	1-	[M-2Na+H] ⁻	C ₁₂ H ₂₃ O ₁₅ S ₂ ⁻	471.0484
F3-a	683.2080	1-	[M-Na] ⁻	C ₂₄ H ₄₃ O ₂₀ S ⁻	683.2074
F3-b	639.0886	1-	[M-Na] ⁻	C ₁₈ H ₃₂ NaO ₁₉ S ₂ ⁻	639.0882
	537.1501	1-	[M-SO ₃ -2Na+H] ⁻	C ₁₈ H ₃₃ O ₁₆ S ⁻	537.1495
F4	887.0847	1-	[M-Na] ⁻	C ₂₄ H ₄₁ Na ₂ O ₂₆ S ₃ ⁻	887.0849
	865.1022	1-	[M-2Na+H] ⁻	C ₂₄ H ₄₂ NaO ₂₆ S ₃ ⁻	865.1030
	785.1461	1-	[M-SO ₃ -2Na+H] ⁻	C ₂₄ H ₄₂ NaO ₂₃ S ₂ ⁻	785.1461
	763.1637	1-	[M-SO ₃ -3Na+2H] ⁻	C ₂₄ H ₄₃ O ₂₃ S ₂ ⁻	763.1462
F5	1011.1595	1-	[M-H] ⁻	C ₂₄ H ₃₉ Na ₄ O ₂₉ S ₄ ⁻	1011.0056
	989.0233	1-	[M-Na] ⁻	C ₂₄ H ₄₀ Na ₃ O ₂₉ S ₄ ⁻	989.0237
	909.2215	1-	[M-SO ₃ -Na] ⁻	C ₂₄ H ₄₀ Na ₃ O ₂₆ S ₃ ⁻	909.0669
	887.0843	1-	[M-SO ₃ -2Na+H] ⁻	C ₂₄ H ₄₁ Na ₂ O ₂₆ S ₃ ⁻	887.0849
	865.1022	1-	[M-SO ₃ -3Na+2H] ⁻	C ₂₄ H ₄₂ NaO ₂₉ S ₃ ⁻	865.1030

Table S3. ^1H and ^{13}C chemical shifts of the fucooligosaccharide **F3-a** and **F3-b**

Fraction	Residue		chemical shift (ppm)					
			1	2	3	4	5	6
F3-a-I	A: L-Fuc_{4S}-α(1-	H	4.999 ($J_{1,2}=3.60$)	3.766	3.983	4.543	4.360	1.174
		C	98.48	70.88	71.30	83.44	69.06	18.48
	B: -3)-L-Fuc-α(1-	H	4.005 ($J_{1,2}=4.16$)	3.842	3.899	3.948	4.228	1.139
		C	98.25	69.16	77.96	71.28	69.30	18.12
	C: -3)-L-Fuc-α(1-	H	5.039 ($J_{1,2}=3.36$)	3.854	3.836	3.966	4.045	1.152
		C	101.54	69.06	77.39	71.06	69.85	18.12
	D: -3)-L-Fuc-ol	H	3.630	3.938	3.683	3.636	4.048	1.133
		C	65.60	74.34	80.48	76.18	69.07	21.09
	A': L-Fuc-α(1-	H	4.941 ($J_{1,2}=3.60$)	3.680	3.858	3.732	4.352	1.121
		C	98.55	70.79	72.10	74.73	69.73	18.00
F3-a-II	B': -3)-L-Fuc_{2S}-α(1-	H	5.210 ($J_{1,2}=3.68$)	4.462	3.983	3.993	4.101	1.165
		C	100.05	75.89	74.76	71.51	69.82	18.05
	C': -3)-L-Fuc-ol	H	3.643 ($J_{1,2}=3.36$)	3.950	3.696	3.613	4.109	1.148
		C	65.52	74.40	81.17	76.74	68.58	21.40
	A: L-Fuc-α(1-	H	5.003 ($J_{1,2}=4.24$)	3.622	3.803	3.723	4.376	1.125
		C	100.66	71.05	72.44	74.72	70.05	18.12
F3-b	B: -3)-L-Fuc_{2S4S}-α(1-	H	5.260 ($J_{1,2}=3.68$)	4.465	4.122	4.750	4.217	1.198
		C	100.47	75.84	75.08	81.91	69.70	18.41
	C: -3)-L-Fuc-ol	H	3.363	3.956	3.709	3.626	4.110	1.133
		C	100.89	74.33	81.32	76.86	68.69	21.39

NMR acquisition parameters for F1 and F2 on 600 MHz NMR spectrometer

¹H NMR: Number of scans (NS), 16; Spectral width in hertz (SWH), 12019.2 Hz; Acquisition time (AQ), 2.7263; Receiver gain (RG), 30.1; Temperature (TE), 295.0 K; Relaxation delay (D1), 1.0 sec; Pulse width (P1), 11.5 µsec; Size of real spectrum (SI), 65536.

¹³C NMR: NS, 300; SWH, 36231.9 Hz; AQ, 0.9044; RG, 190.7; TE, 295.0 K; D1, 2.0 sec; P1, 10.00 µsec; SI, 32768.

¹H-¹H COSY: NS, 4; SWH, 4807.7, 4803.1 Hz; AQ, 0.2130; RG, 190.7; TE, 295.0 K; D1, 2.0 sec; P1, 11.5 µsec; SI, 1024.

¹H-¹H TOCSY: NS, 8; SWH, 4807.7, 4803.1 Hz; AQ, 0.2130; RG, 190.7; TE, 295.0 K; D1, 2.0 sec; P1, 11.5 µsec; SI, 1024.

¹H-¹H ROESY: NS, 8; SWH, 4807.7, 4803.1 Hz; AQ, 0.2130; RG, 190.7; TE, 295.0 K; D1, 2.0 sec; P1, 11.5 µsec; SI, 1024.

¹H-¹³C HSQC: NS, 2; SWH, 4807.7, 22624.4 Hz; AQ, 0.1065; RG, 190.7; TE, 295.0 K; D1, 1.5 sec; P1, 11.5 µsec.

¹H-¹³C HMBC: NS, 10; SWH, 4807.7, 25641.0 Hz; AQ, 0.4160; RG, 190.7; TE, 295.0 K; D1, 1.5 sec; P1, 11.5 µsec.

NMR acquisition parameters for dHfFS-1, F3-a, F3-b, F4 and F5 on 800 MHz NMR spectrometer

¹H NMR: Number of scans (NS), 4; Spectral width in hertz (SWH), 16025.6 Hz; Acquisition time (AQ), 2.0447; Receiver gain (RG), 5.7; Temperature (TE), 298.0 K; Relaxation delay (D1), 2.0 sec; Pulse width (P1), 7.1 µsec; Size of real spectrum (SI), 131072.

¹³C NMR: NS, 2000; SWH, 48076.9 Hz; AQ, 0.6816; RG, 2050; TE, 298.0 K; D1, 2.0 sec; P1, 12.00 µsec; SI, 32768.

¹H-¹H COSY: NS, 8; SWH, 8012.8, 8012.8 Hz; AQ, 0.1278; RG, 654.4; TE, 298.0 K; D1, 1.0 sec; P1, 7.38 µsec; SI, 1024.

¹H-¹H TOCSY: NS, 8; SWH, 8081.9, 8077.5 Hz; AQ, 0.1267; RG, 1440; TE,

298.0 K; D1, 1.5 sec; P1, 7.38 μ sec; SI, 1024.

^1H - ^1H ROESY: NS, 8; SWH, 8012.8, 8012.8 Hz; AQ, 0.1278; RG, 757.8; TE,
298.0 K; D1, 1.5 sec; P1, 7.38 μ sec; SI, 1024.

^1H - ^{13}C HSQC: NS, 8; SWH, 8012.8, 20161.3 Hz; AQ, 0.1278; RG, 2050; TE,
298.0 K; D1, 1.5 sec; P1, 7.38 μ sec.

^1H - ^{13}C HMBC: NS, 16; SWH, 8012.8, 34246.6 Hz; AQ, 0.2556; RG, 2050; TE,
298.0 K; D1, 1.5 sec; P1, 7.38 μ sec.