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Supplementary data

Precise structure and anticoagulant activity of fucosylated glycosaminoglycan from *Apostichopus japonicus*: analysis of its depolymerized fragments

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SUPPLEMENTAL FIGURES AND TABLES

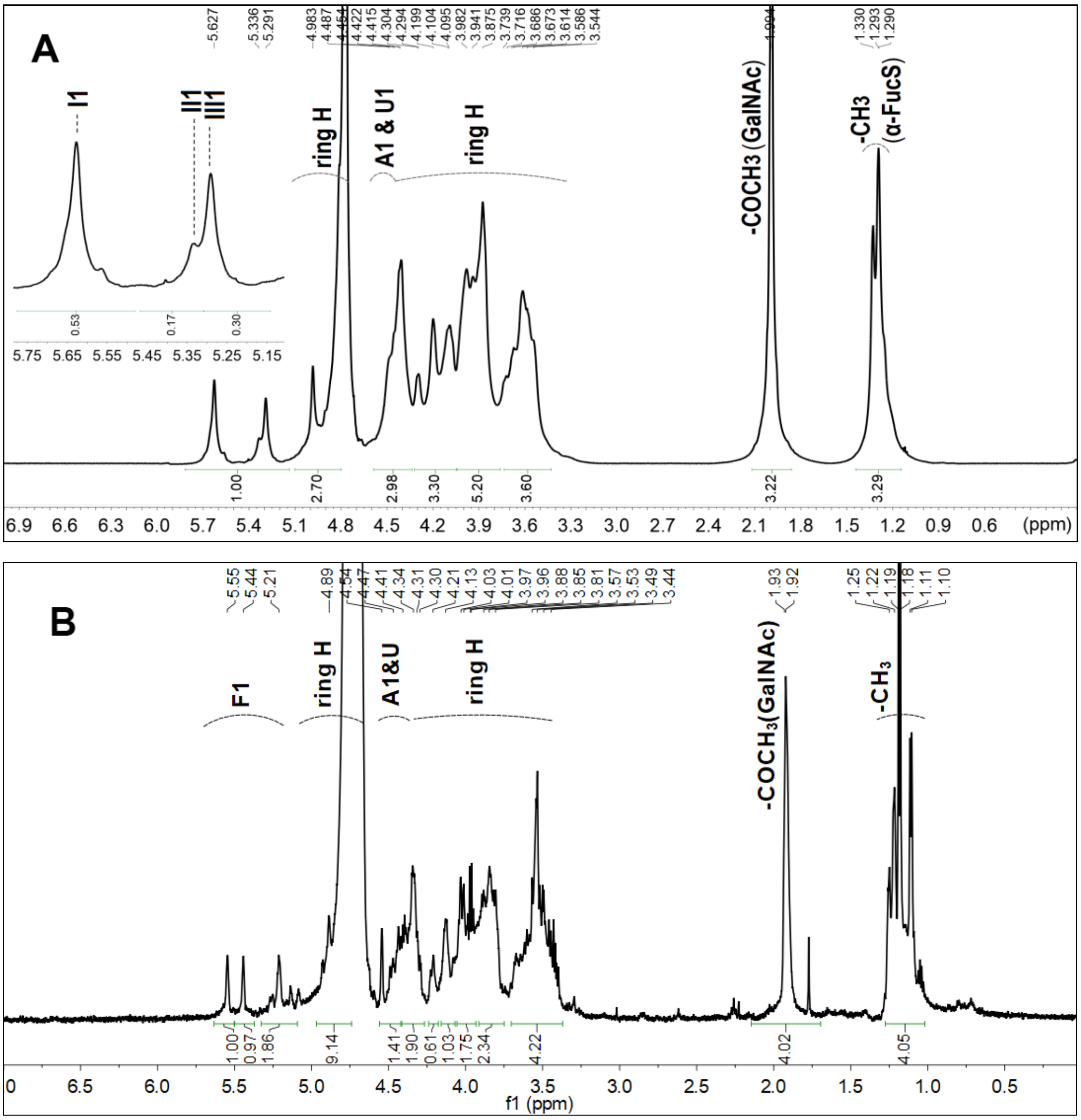


Figure S1. 1H-NMR spectra of AjFG (A) and dAjFG (B)

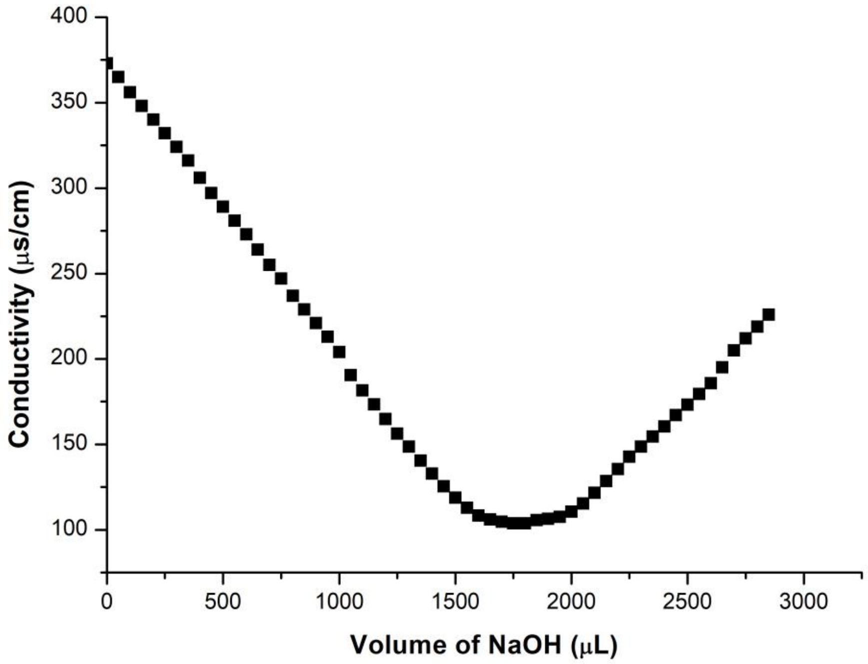
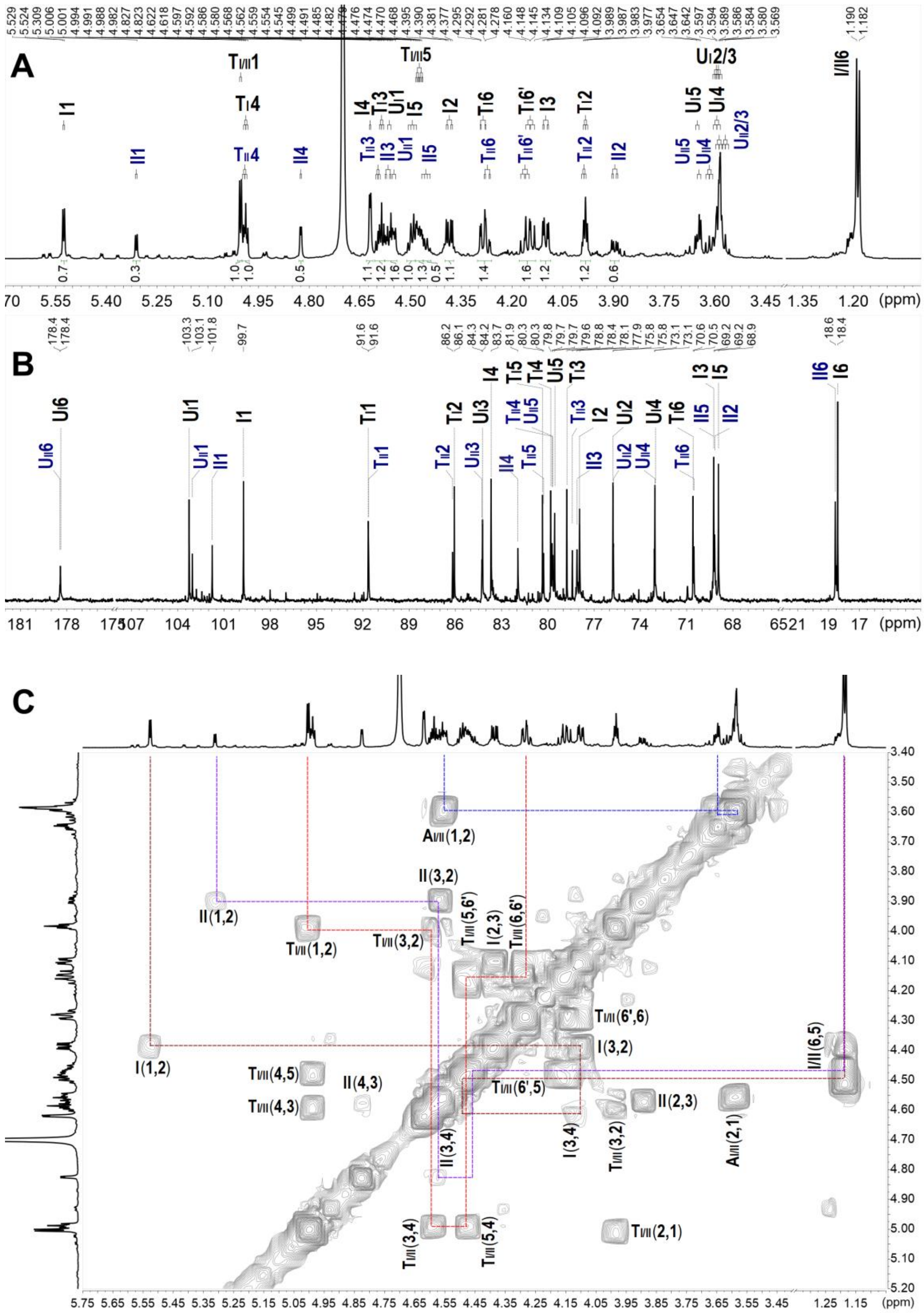


Figure S2. Conductometric titration curves of AjFG.



A
TI/II1
TI/II5
UI2/3
I/II6
I1
II1
TI4
TII4
II4
I4
TI3
UI1
I5
I2
TI6
TI6'
I3
TI2
TII3
II3
UII1
II5
TII6
TII6'
TII2
II2
UI5
UI4
UII5
UII4
UII2/3
(ppm)
B
UI6
UII6
UI1
UII1
II1
I1
TI1
TII1
TI2
TII2
UI3
UII3
I4
II4
T5
TII4
UII5
TII5
T4
UI5
TI3
TII3
I2
II3
UI2
UII2
UI4
UII4
TI6
TII6
I3
II5
I5
II2
II6
I6
(ppm)
C
AI/II(1,2)
II(3,2)
II(1,2)
TI/II(1,2)
TI/II(3,2)
TI/II(5,6')
I(2,3)
TI/II(6,6')
TI/II(6',6)
I(1,2)
I(3,2)
I/II(6,5)
TI/II(4,5)
II(4,3)
TI/II(6',5)
TI/II(4,3)
II(2,3)
II(3,4)
I(3,4)
TI/II(3,2)
AI/II(2,1)
TI/II(3,4)
TI/II(5,4)
TI/II(2,1)
(ppm)

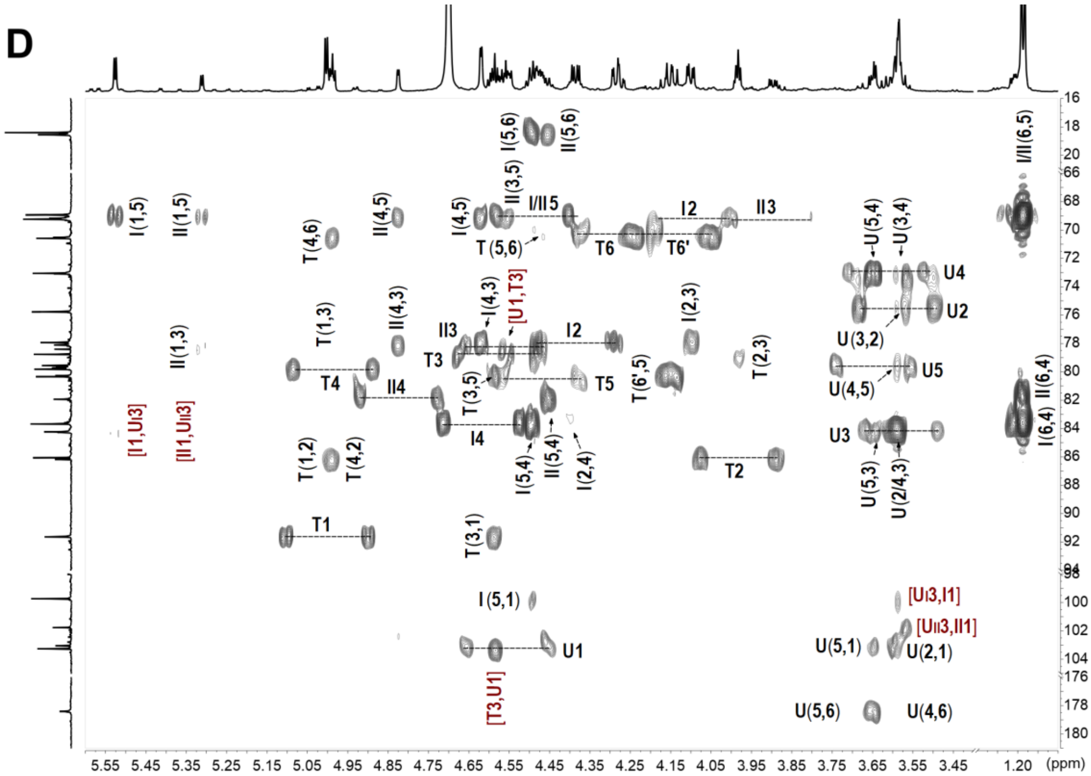
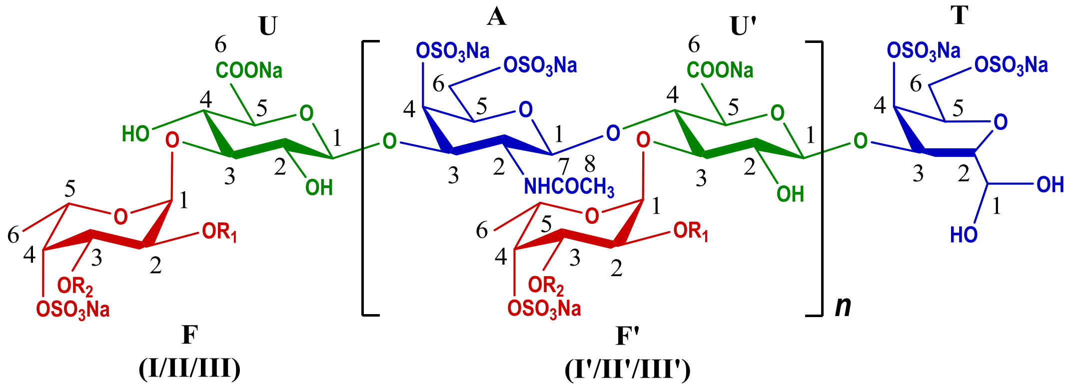
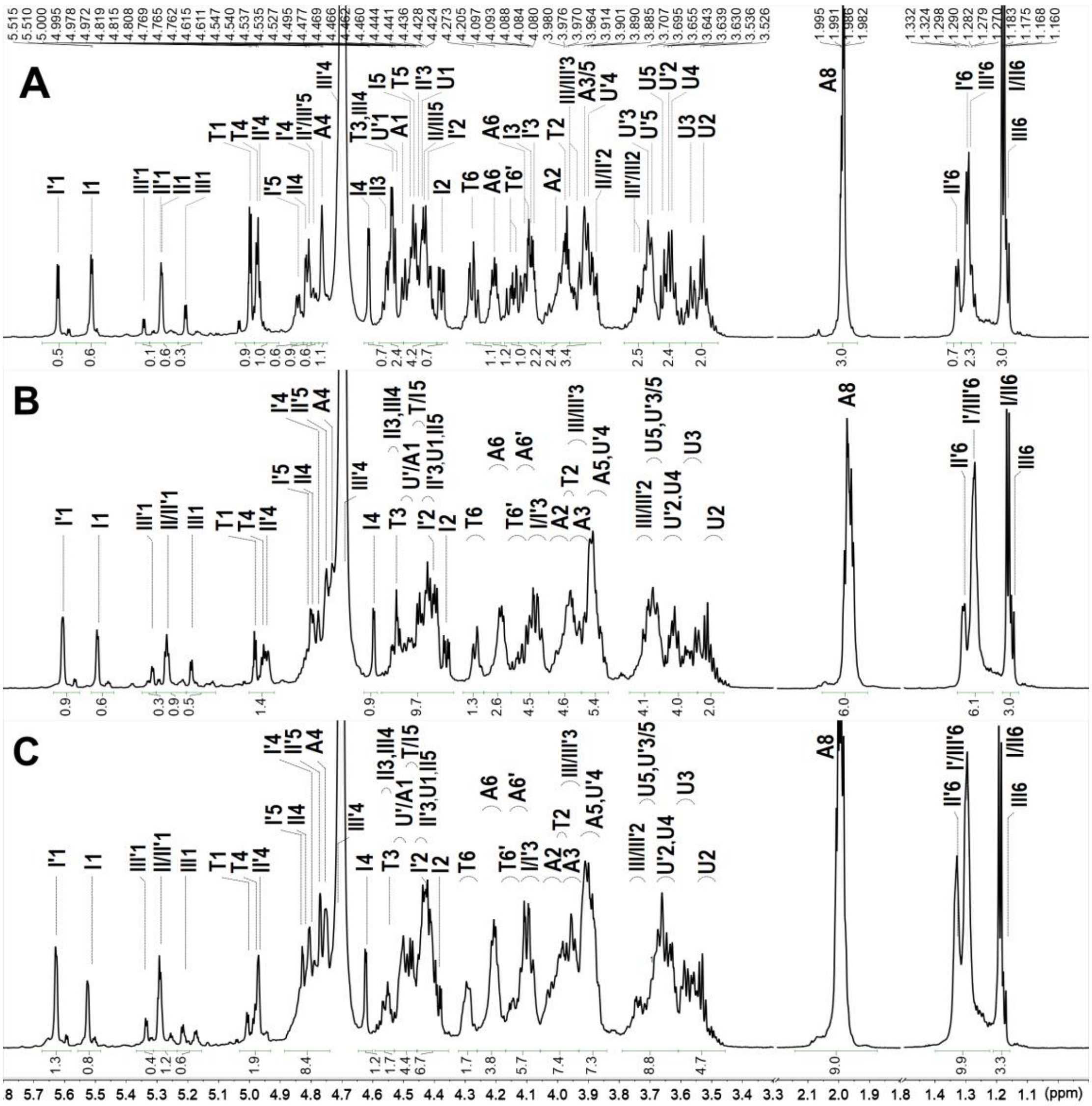


Figure S3. ^{1}H NMR (A),^{13}C NMR (B), ^{1}H-^{1}H COSY (C) and ^{1}H-^{13}C HMBC (D) of Fr-1. I, Fuc$_{2S4S}$; II, Fuc$_{3S4S}$; U, GlcA; U$_{I}$ & U$_{II}$, U substituted with I or II, respectively; T, anTal-diol; T$_{I}$ & T$_{II}$, T linked with U$_{I}$ or U$_{II}$, respectively.



R_1= -SO_3Na, R_2= -H in I & I'; R_1= -H, R_2= -SO_3Na in II & II'; R_1= R_2= -H in III & III';

Fr-2, n = 1; Fr-3, n = 2; Fr-4, n = 3.



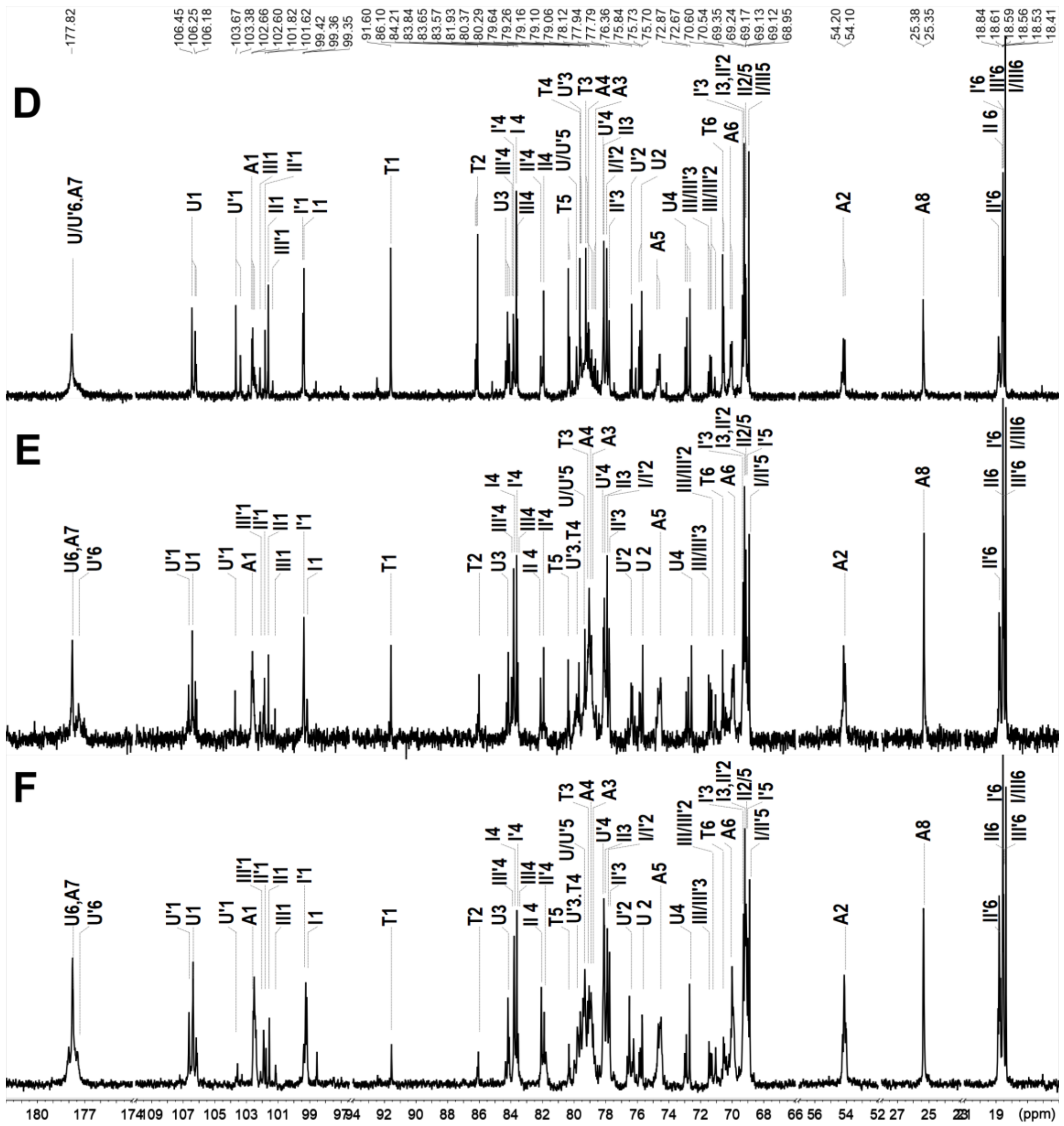
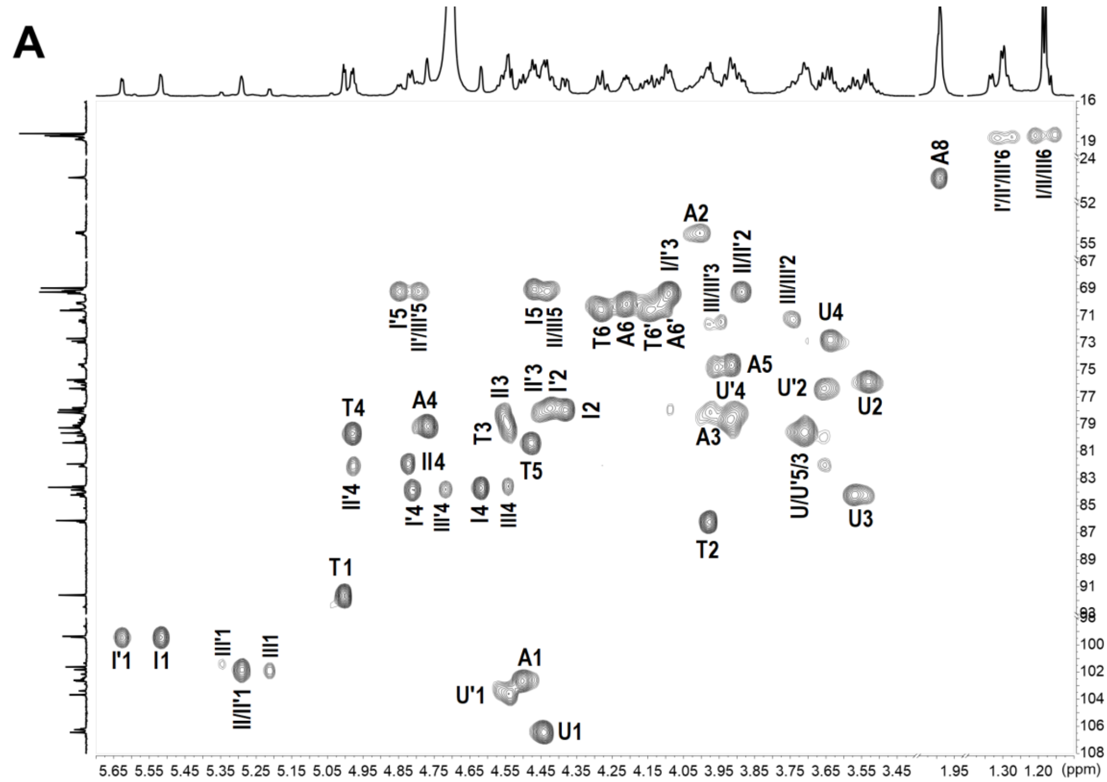
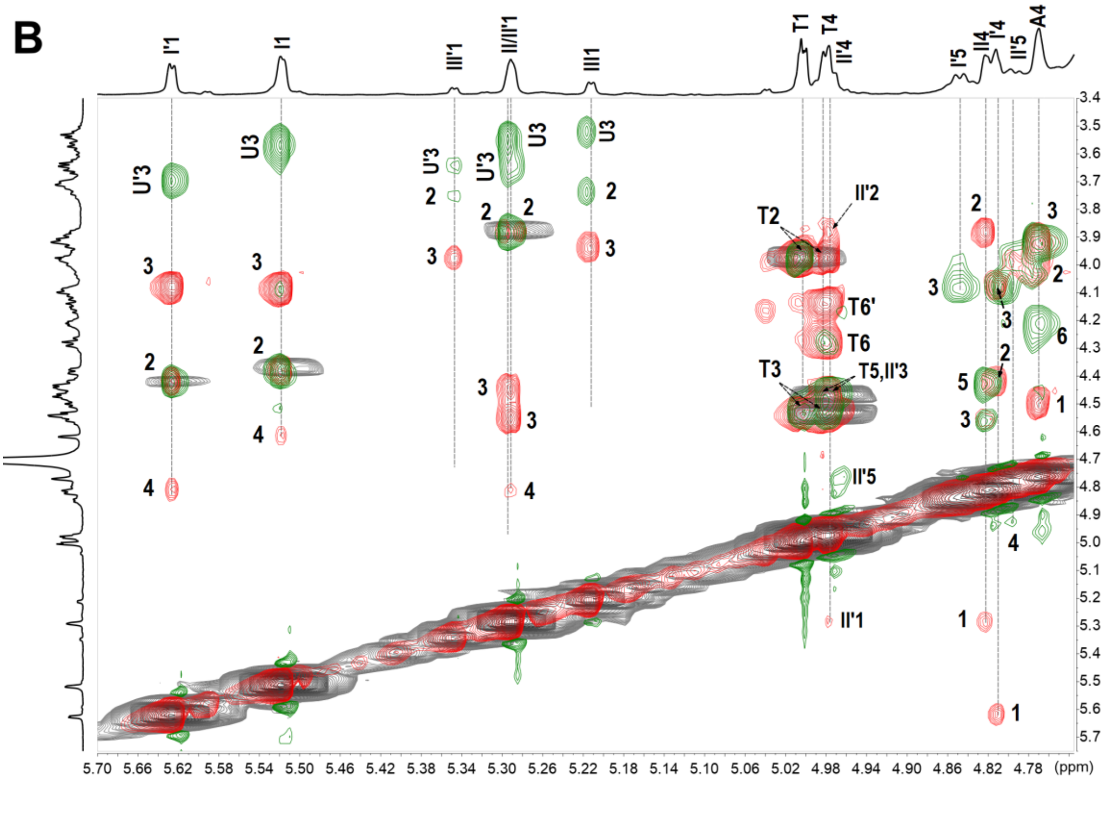


Figure S4. Structure formula, ^{1}H NMR (A,B,C), ^{13}C NMR (D,E,F) spectra of Fr-2 (A, D)**,** Fr-3 (B, E) and Fr-4 (C, F). Marked symbols are the same as shown in structure formula.





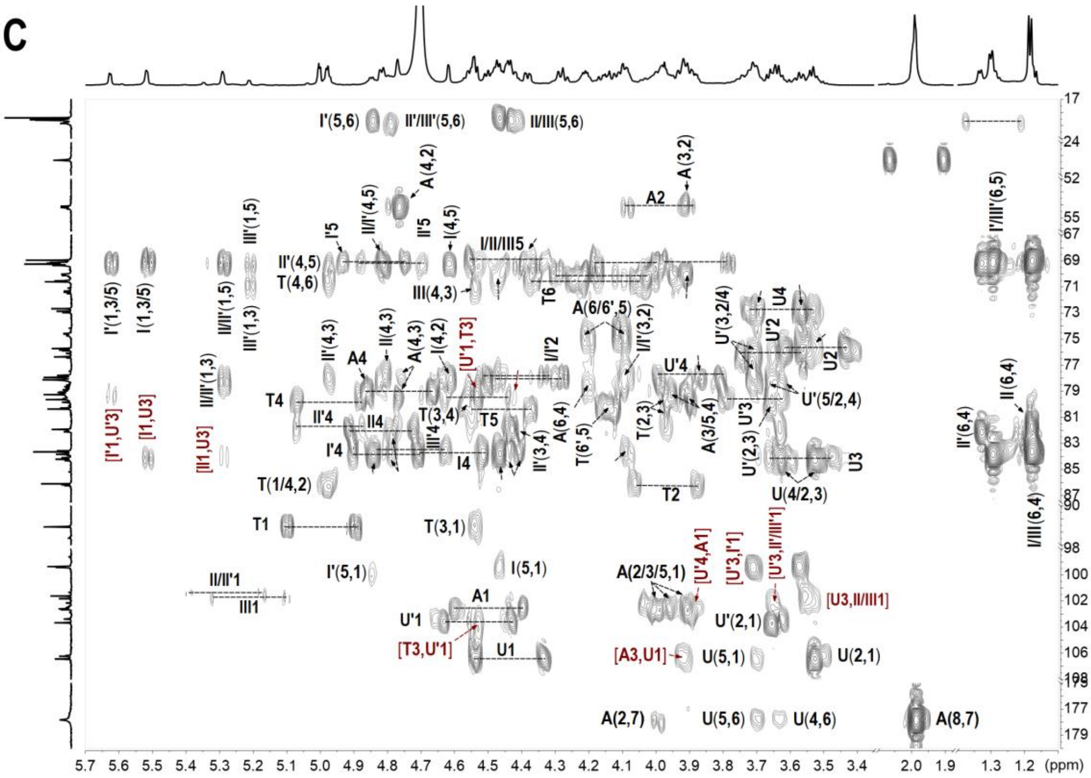
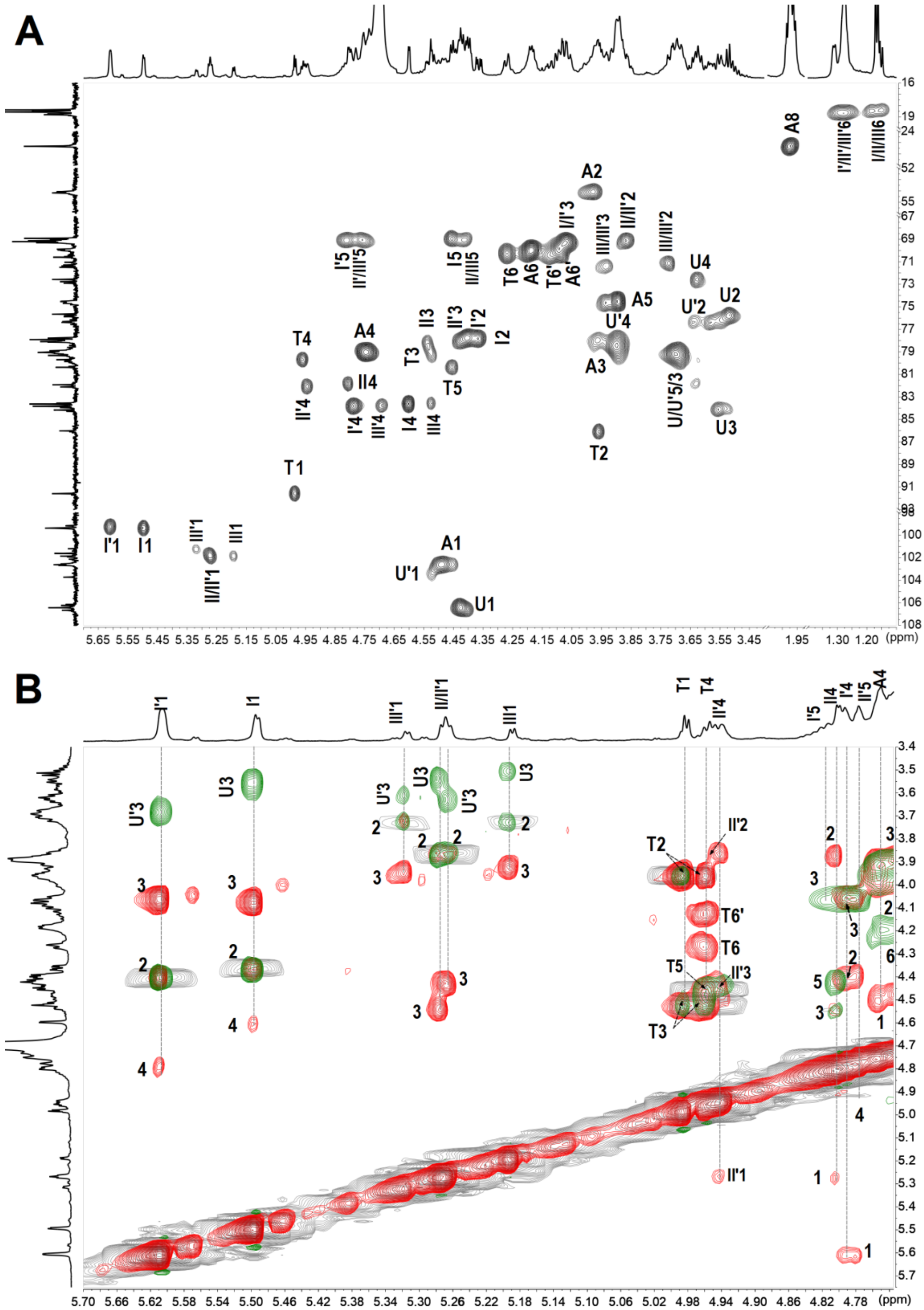


Figure S5. ^{1}H-^{13}C HSQC (A), superposition of ^{1}H-^{1}H COSY (black), TOCSY (red), and ROESY (green) (B), ^{1}H-^{13}C HMBC (C) spectra of Fr-2. Marked symbols are the same as in Figure S3.


A
B
A8
I'/II'/III'6
I/II/III6
A2
I/I'3
II/II'2
III/III'3
III/III'2
I'5
II'/III'5
I5
II/III5
T6
A6
T6'
A6'
U4
U2
U'2
A5
U'4
A4
T4
T3
II3
II'3
I'2
I2
A3
T5
II4
U/U'5/3
II'4
I'4
III'4
I4
III4
U3
T2
T1
III'1
III1
I'1
I1
II/II'1
A1
U'1
U1
(ppm)
II'2
T6'
T6
II'3
II'1
I'5
A4

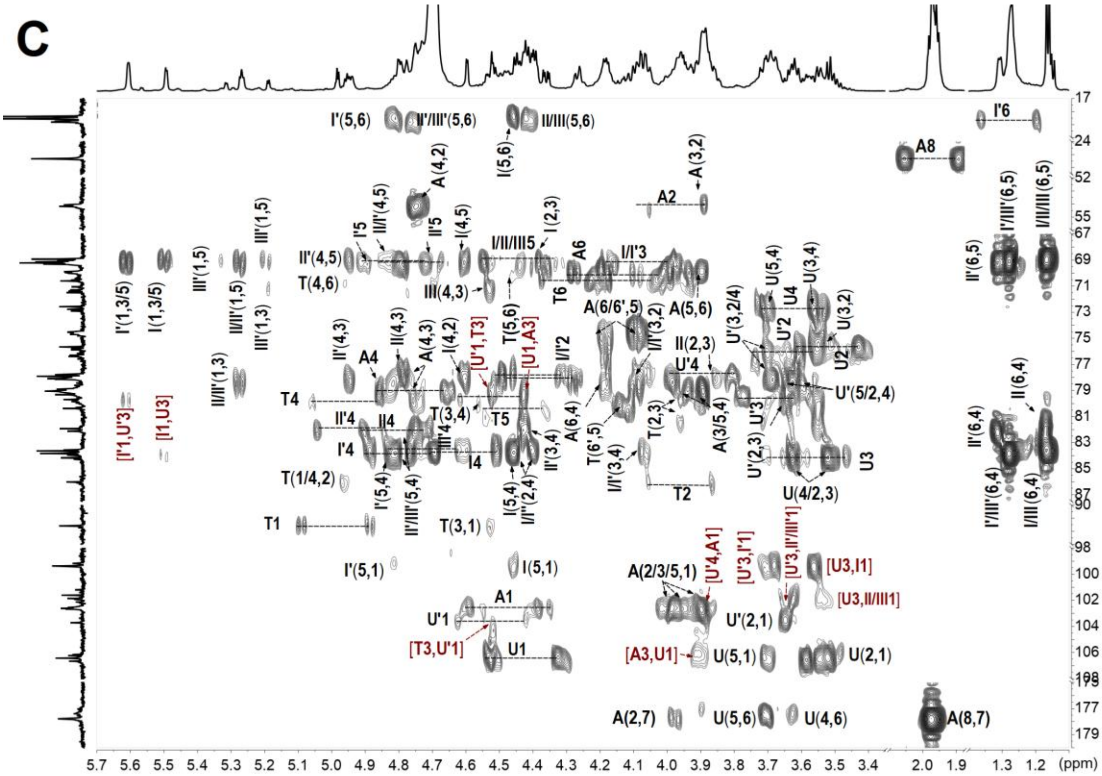


Figure S6. ^{1}H-^{13}C HSQC (A), superposition of ^{1}H-^{1}H COSY (black), TOCSY (red), and ROESY (green) (B), ^{1}H-^{13}C HMBC (C) spectra of Fr-3. Marked symbols are the same as in Figure S3.

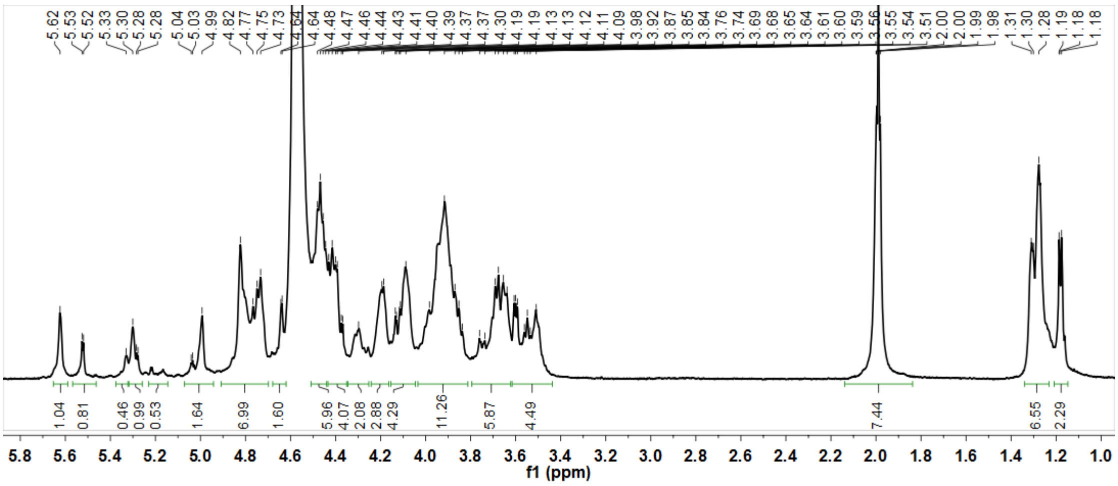


Figure S7. ^{1}H-NMR spectrum of fraction 5.

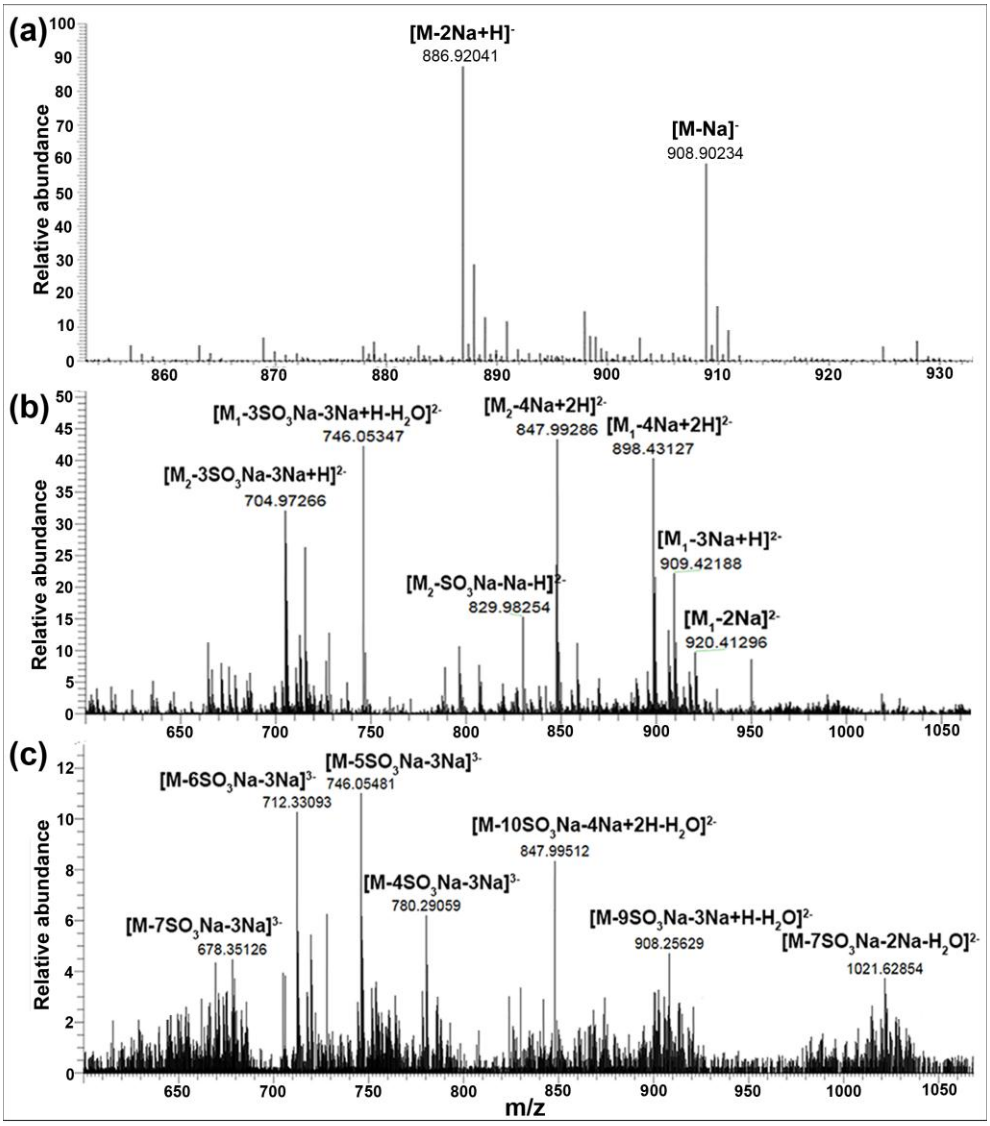


Figure S8. ESI-Q-TOF MS spectra of Fr-1 (A), Fr-2 (B), and Fr-3 (C).

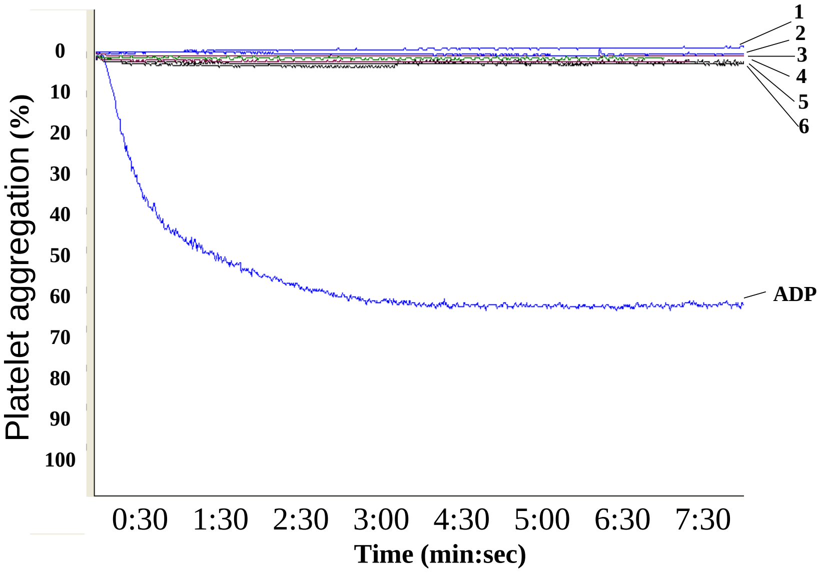
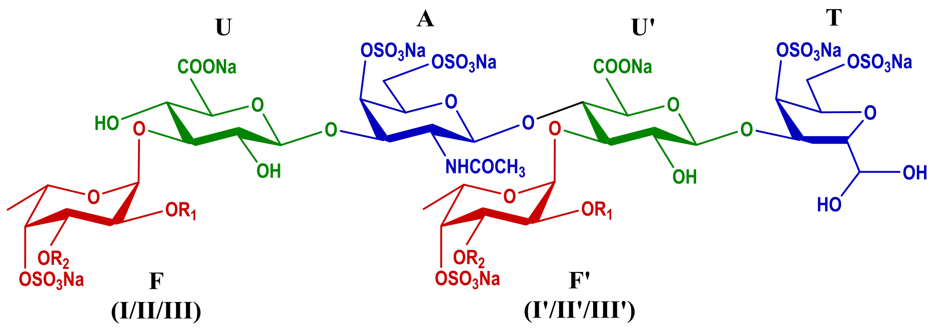


Figure S9. Effects of Fr-1–Fr-4 (1–4), AjFG (5), and dAjFG (6) (50 μg/mL) on platelet aggregation (n = 2).

Table S1. $^{1}H/^{13}C$ NMR chemical shift assignments of Fr-2. (δ, ppm; J, Hz)

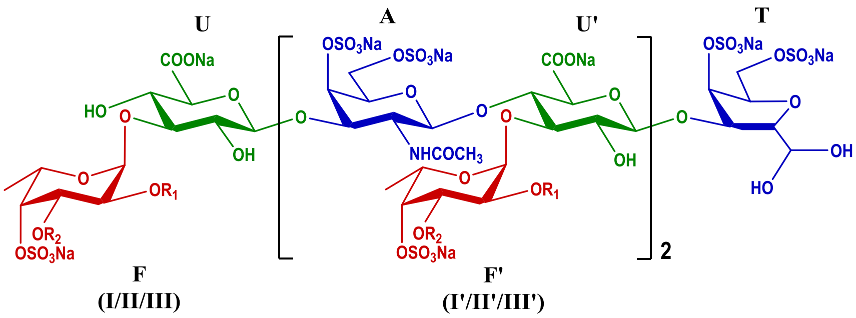


R_1= $-SO_3Na$, R_2= -H in I & I’; R_1= -H, R_2= $-SO_3Na$ in II & II’; R_1= R_2= -H in III & III’.

		δ	**Couplings**		*δ*			*δ*	**Couplings**		*δ*
I	H-1	5.512	$J_{(1,2)}$ = 4.00	C-1	99.36	**U**	H-1	4.442	$J_{(1,2)}$ = 8.64	C-1	106.45 106.25
	H-2	**4.377**	$J_{(2,3)}$ = 10.56	C-2	**77.94**		H-2	3.526	$J_{(2,3)}$ = 8.96	C-2	75.72
	H-3	4.088	$J_{(3,4)}$ = 3.20	C-3	69.24		H-3	3.569	$J_{(3,4)}$ = 9.20	C-3	84.21
	H-4	**4.613**	--	C-4	83.65		H-4	3.629	$J_{(4,5)}$ = 8.32	C-4	72.66 72.89
	H-5	4.470	$J_{(5,6)}$ = 6.56	C-5	68.95		H-5	3.660		C-5	79.85
	H-6	1.178		C-6	18.41					C-6	177.82
II	H-1	5.285	$J_{(1,2)}$ = 4.16	C-1	101.62	**A**	H-1	4.500	$J_{(1,2)}$ = 8.24	C-1	102.66 102.60
	H-2	3.878	--	C-2	69.17		H-2	4.003	--	C-2	54.20
	H-3	**4.553**	--	C-3	**78.12**		H-3	3.974 3.918	--	C-3	78.83 78.64
	H-4	**4.817**	--	C-4	81.93		H-4	**4.765**	--	C-4	**79.03**
	H-5	4.433	$J_{(5,6)}$ = 6.56	C-5	69.12		H-5	3.956 3.915		C-5	74.75 74.56
	H-6	1.178		C-6	18.60		H-6	**4.205**	$J_{(5,6)}$ = 3.20	C-6	70.06
							H-6’	**4.105** **4.095**	$J_{(6,6')}$ =11.52	C-7	177.82
							H-8	1.986 1.982		C-8	25.38 25.35
III	H-1	5.207	$J_{(1,2)}$ = 4.08	C-1	102.15						
	H-2	3.742	--	C-2	71.32						
	H-3	3.945	--	C-3	71.50						
	H-4	**4.540**	--	C-4	**83.57**						
	H-5	4.433	$J_{(5,6)}$ = 6.62	C-5	68.95						
	H-6	1.163		C-6	18.41						
I’	H-1	5.621	$J_{(1,2)}$ = 4.00	C-1	99.42	**U’**	H-1	4.533	$J_{(1,2)}$ = 7.52	C-1	103.67 103.38
	H-2	**4.420**	--	C-2	**77.94**		H-2	3.639	--	C-2	76.36
	H-3	4.082	$J_{(3,4)}$ = 2.80	C-3	69.85		H-3	3.707	--	C-3	79.61
	H-4	**4.807**		C-4	**83.85**		H-4	3.918	--	C-4	**78.14**
	H-5	4.843	$J_{(5,6)}$ = 6.40	C-5	69.17		H-5	3.693		C-5	79.85

	H-6	1.295		C-6	18.56						C-6	177.82
II’	H-1	5.286	--	C-1	101.82		**T**	H-1	**4.998**	$J_{(1,2)}$ = 4.26	C-1	91.61
	H-2	3.880	--	C-2	69.24			H-2	3.978	--	C-2	86.10
	H-3	**4.451**	--	C-3	**77.78**			H-3	4.536	--	C-3	79.26
	H-4	**4.967**	--	C-4	**82.11**			H-4	**4.975**	$J_{(4,5)}$ = 4.88	C-4	79.64
	H-5	4.794	$J_{(5,6)}$ = 6.40	C-5	69.12			H-5	4.476	--	C-5	80.37
	H-6	1.328		C-6	18.85			H-6	4.277 4.265	$J_{(5,6)}$ = 2.32	C-6	70.60 70.53
								H-6’	4.153 4.134	$J_{(6,6')}$ =11.44		
III’	H-1	**5.342**	$J_{(1,2)}$ = 4.16	C-1	101.33							
	H-2	3.742	--	C-2	71.08							
	H-3	3.976	--	C-3	71.40							
	H-4	**4.718**		C-4	**83.93**							
	H-5	4.784	$J_{(5,6)}$ = 6.48	C-5	69.12							
	H-6	1.275		C-6	18.53							

Table S2. $^{1}H/^{13}C$ NMR chemical shift assignments of Fr-3. (δ, ppm; J, Hz)



R_1= -SO_3Na, R_2= -H in I & I’; R_1= -H, R_2= -SO_3Na in II & II’; R_1= R_2= -H in III & III’.

		δ		*δ*				*δ*		*δ*
I	H-1	5.492	C-1	99.17		**U**	H-1	4.426	C-1	106.43 106.23
	H-2	**4.361**	C-2	**77.91**			H-2	3.518	C-2	75.66
	H-3	4.073	C-3	69.22			H-3	3.555	C-3	84.17
	H-4	**4.596**	C-4	83.83			H-4	3.627	C-4	72.57 72.77
	H-5	4.456	C-5	68.92			H-5	3.630	C-5	79.32
	H-6	1.163	C-6	18.38					C-6	177.79
II	H-1	5.271	C-1	101.61		**A**	H-1	4.491	C-1	102.62 102.54
	H-2	3.866	C-2	69.13			H-2	3.978	C-2	54.18 54.07
	H-3	**4.535**	C-3	**78.09**			H-3	3.963	C-3	78.90
	H-4	**4.796**	C-4	82.12			H-4	**4.731**	C-4	**79.05**
	H-5	4.413	C-5	69.13			H-5	3.933 3.895	C-5	74.69 74.53
	H-6	1.163	C-6	18.53			H-6	**4.181**	C-6	69.88
							H-6’	**4.102**	C-7	177.79
							H-8	1.973	C-8	25.34 25.32
III	H-1	5.188	C-1	101.19						
	H-2	3.723	C-2	71.29						
	H-3	3.934	C-3	71.49						
	H-4	**4.525**	C-4	**83.56**						
	H-5	4.413	C-5	69.00						
	H-6	1.148	C-6	18.38						
I’	H-1	5.605	C-1	99.36		**U’**	H-1	4.518	C-1	103.72 106.66
	H-2	**4.403**	C-2	**77.91**			H-2	3.639 3.581	C-2	76.39 76.31
	H-3	4.073	C-3	69.30			H-3	3.699	C-3	79.85
	H-4	**4.776**	C-4	**83.63**			H-4	3.899	C-4	**78.14**

	H-5	4.817	C-5	69.57			H-5	3.685	C-5	79.32
	H-6	1.275	C-6	18.56					C-6	177.40
II’	H-1	5.265	C-1	101.86		**T**	H-1	**4.980**	C-1	91.59
	H-2	3.866	C-2	69.22			H-2	3.959	C-2	86.02
	H-3	**4.406**	C-3	**77.78**			H-3	4.523	C-3	79.13
	H-4	**4.943**	C-4	**81.92**			H-4	**4.956**	C-4	79.69
	H-5	4.751	C-5	68.92			H-5	4.455	C-5	80.38
	H-6	1.308	C-6	18.80			H-6	4.266	C-6	70.60
							H-6’	4.130		
III’	H-1	**5.314**	C-1	102.13						
	H-2	3.723	C-2	71.05						
	H-3	3.934	C-3	71.37						
	H-4	**4.690**	C-4	**83.96**						
	H-5	4.762	C-5	69.00						
	H-6	1.267	C-6	18.50						