

Supplementary Materials

Structural characterization and anticoagulant potential of *Colochirus quadrangularis* fucosylated glycosaminoglycan 5–12 oligomers with unusual branches

Xuedong Zhang ^{1,†}, Guangwei Yan ^{2,†}, Xinming Liu ^{1,†}, Jiewen Fu ¹, Xiang Shi ^{1,2}, Pei Cao ¹,
Yuqian Sun ³, Shengping Zhong ¹, Jiale Nong ¹, Peiqi Jiang ¹, Yonghong Liu ¹, Baoshun
Zhang ^{2,*}, Qingxia Yuan ^{1,*}, Longyan Zhao ^{1,*}

¹ Guangxi Key Laboratory of Marine Drugs, Institute of Marine Drugs, Guangxi University of
Chinese Medicine, Nanning 530200, China

² College of Pharmaceutical Sciences, Southwest University, Chongqing 400716, PR China.

³ Instrumentation and Service Center for Molecular Sciences, Westlake University, Hangzhou
310024, China

*Correspondence: zbs360@swu.edu.cn (B. Zhang); qingxiayuan@163.com (Q. Yuan);
zhaolongyan-dra@163.com (L. Zhao).

[†] These authors contributed equally to this work.

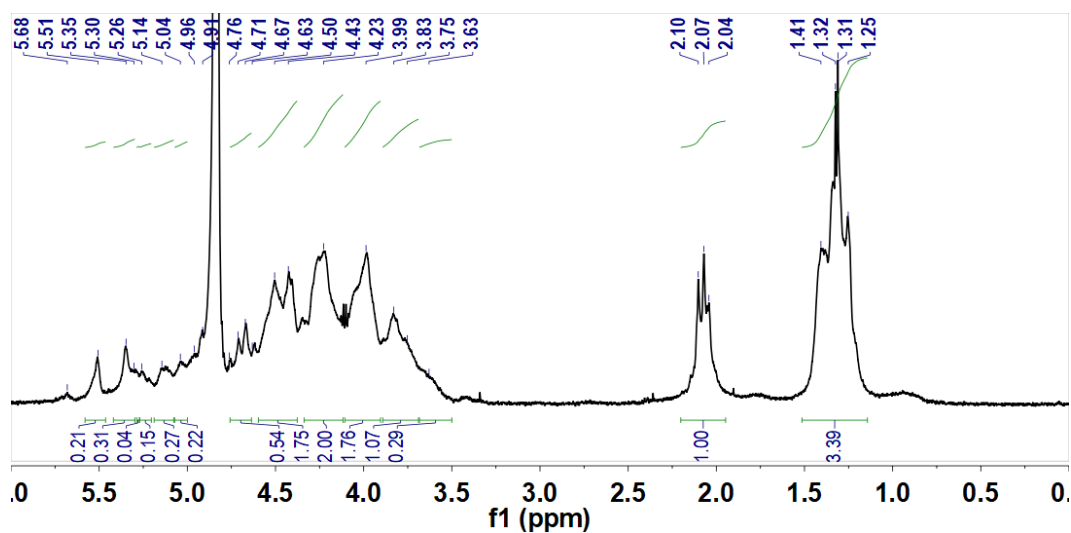


Figure S1. ^1H NMR spectrum of CqFG

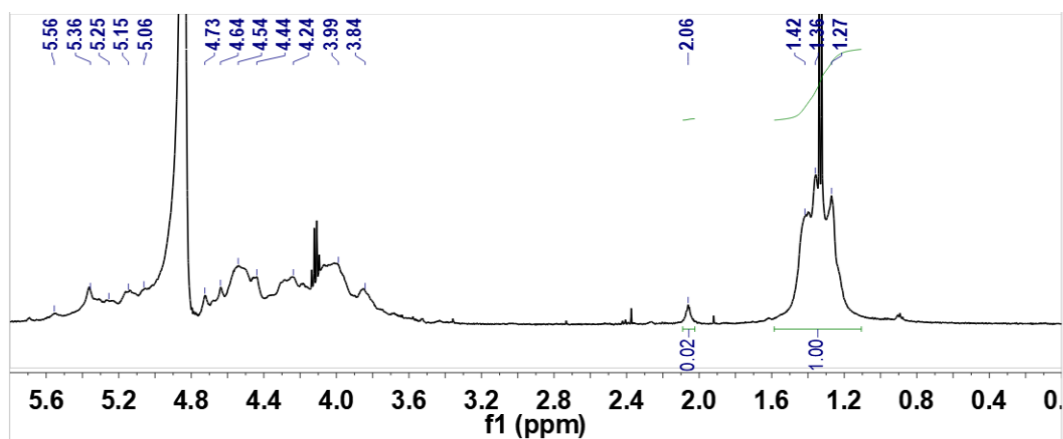


Figure S2. ^1H NMR spectrum of high-molecular-weight polysaccharide component isolated from dCqFG

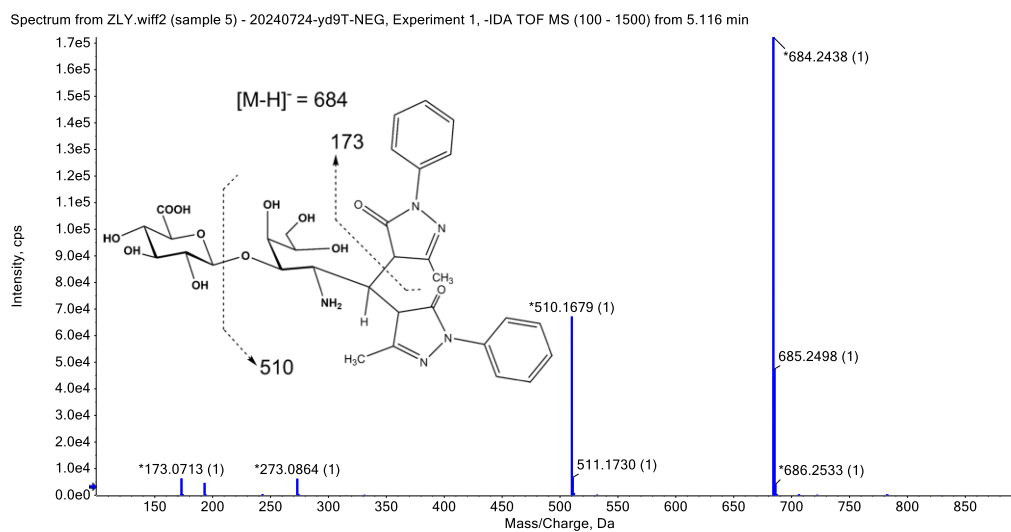


Figure S3. Negative ESI-MS spectrum of PMP-labeled acidolysis-resistant disaccharide from CqFG (peak marked x in Fig. 1E).

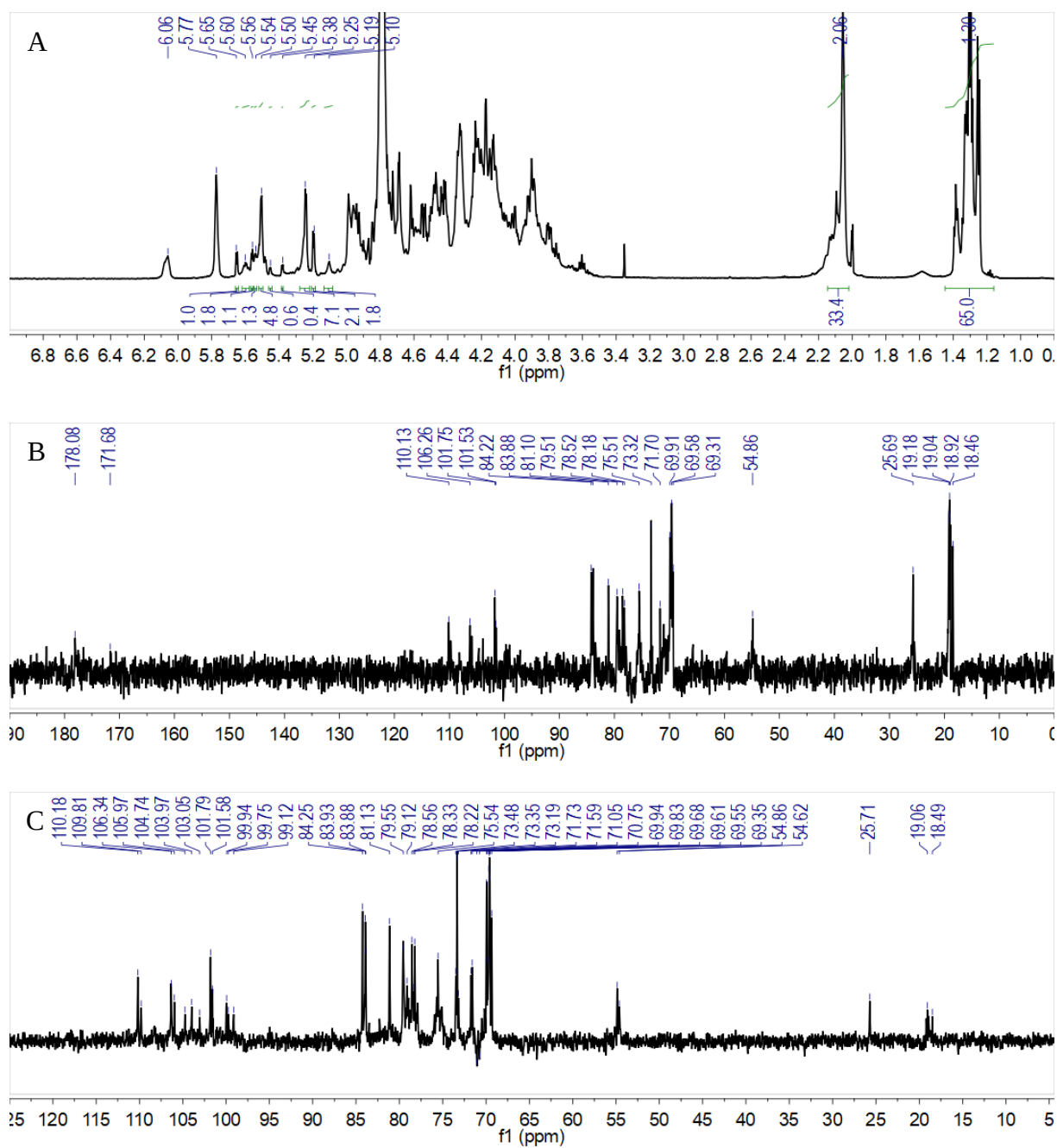


Figure S4. ^1H (A), ^{13}C (B), and DEPT-135 (C) NMR spectra of OF1.

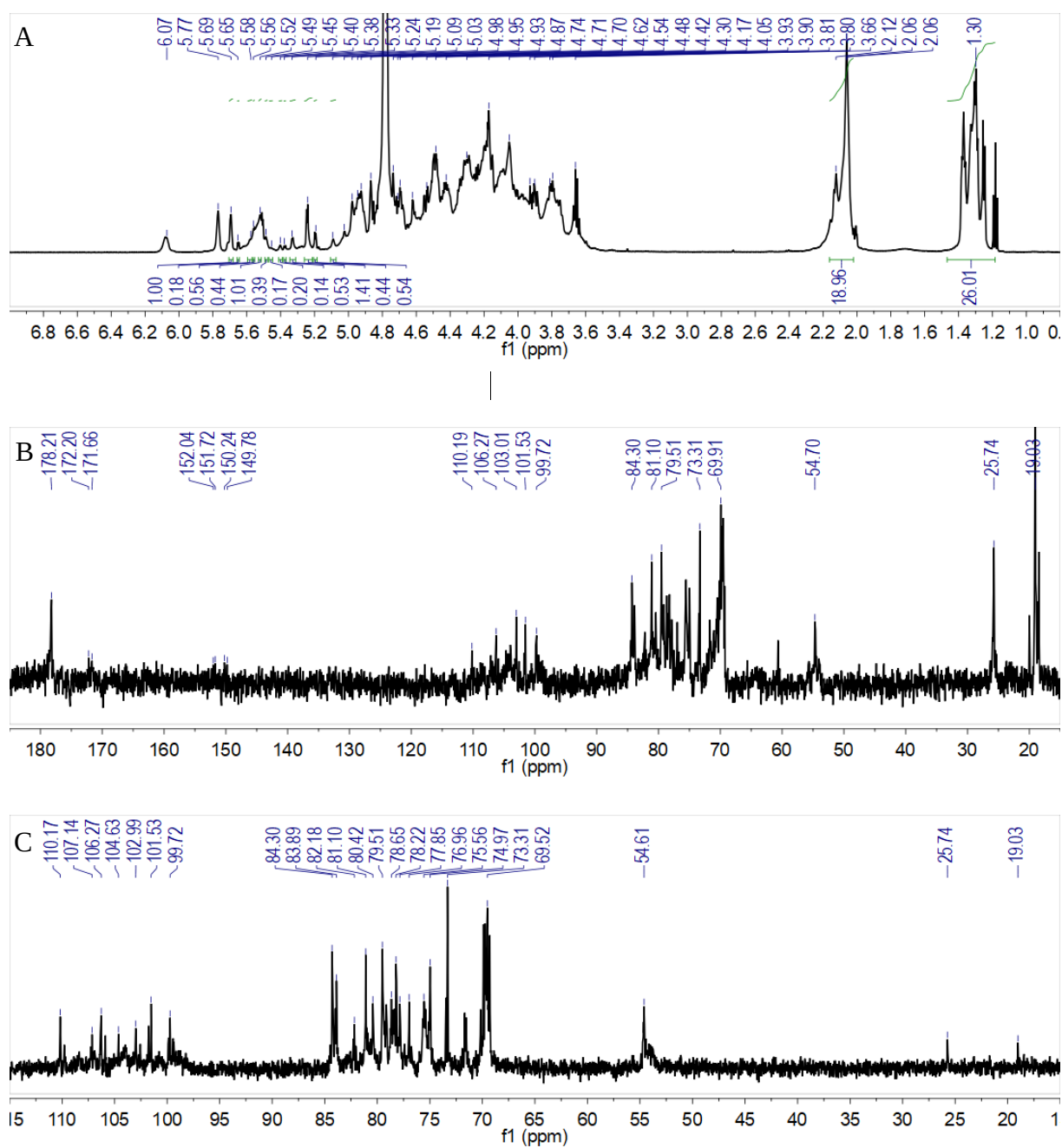


Figure S7. ^1H (A), ^{13}C (B), and DEPT-135 (C) NMR spectra of OF2.

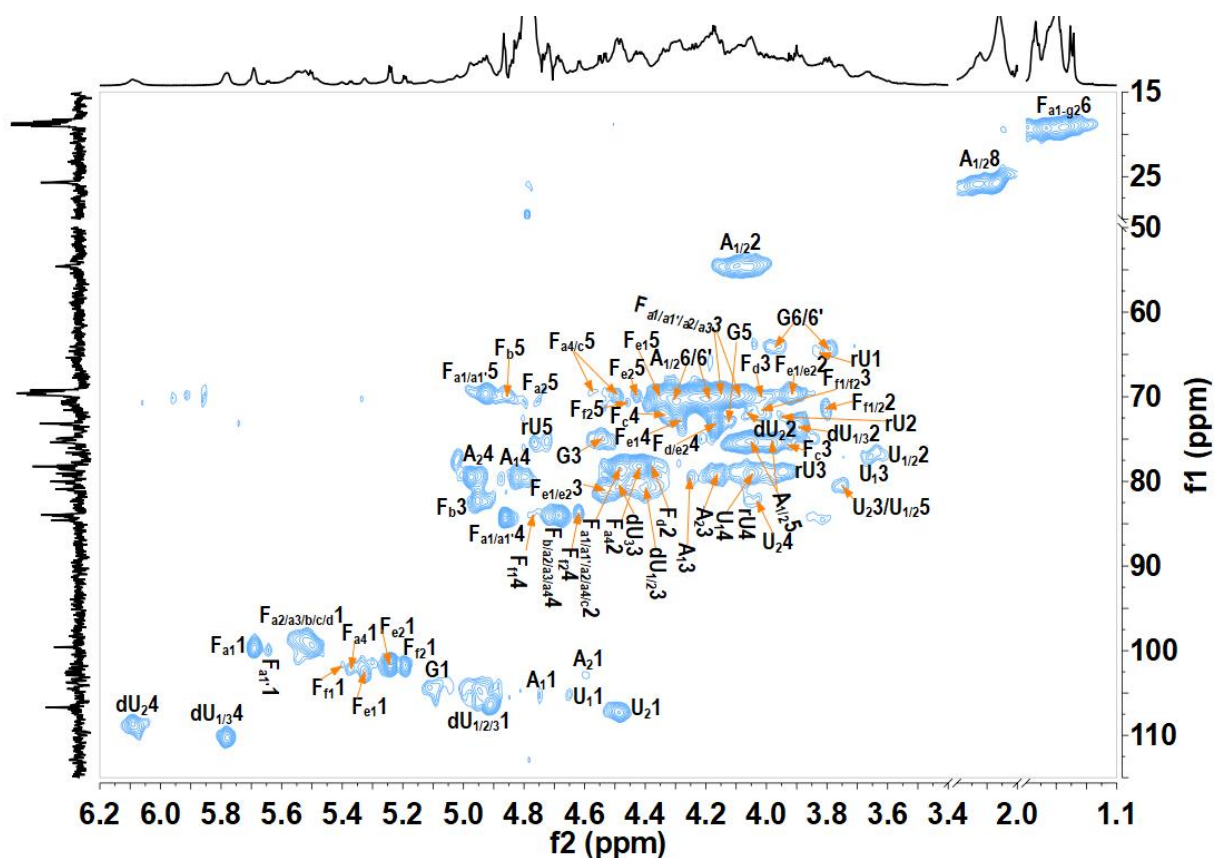
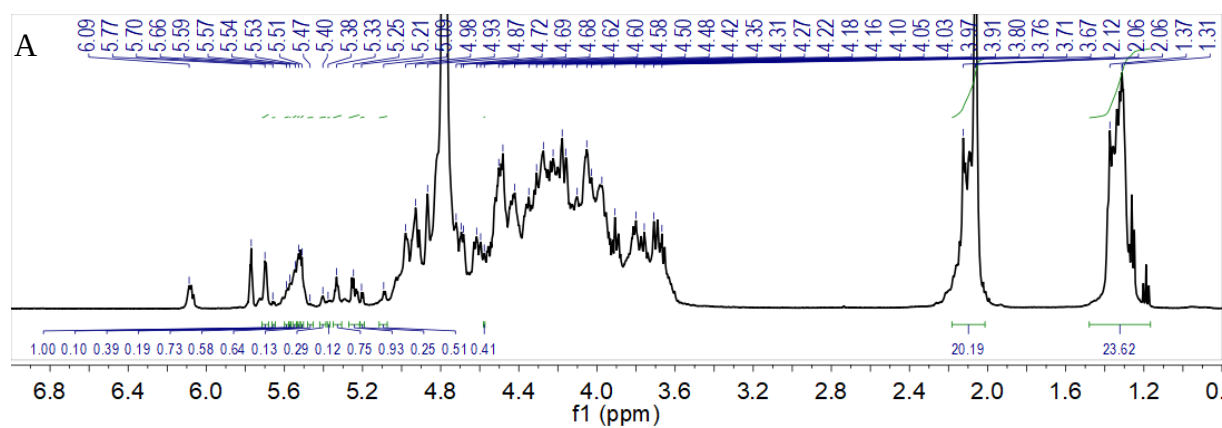


Figure S10. ^1H - ^{13}C HSQC spectrum of OF2.



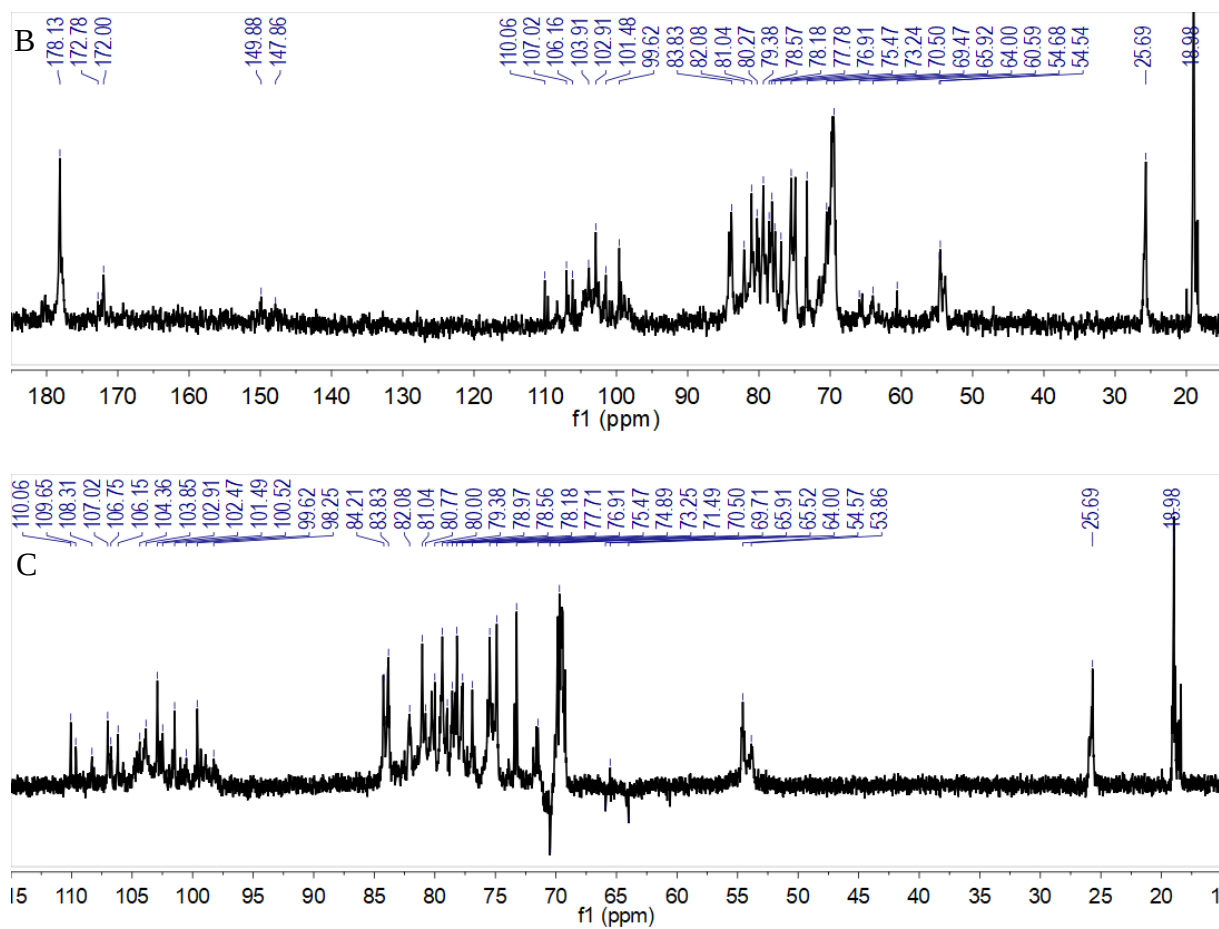


Figure S11. ^1H (A), ^{13}C (B), and DEPT-135 (C) NMR spectra of OF3.

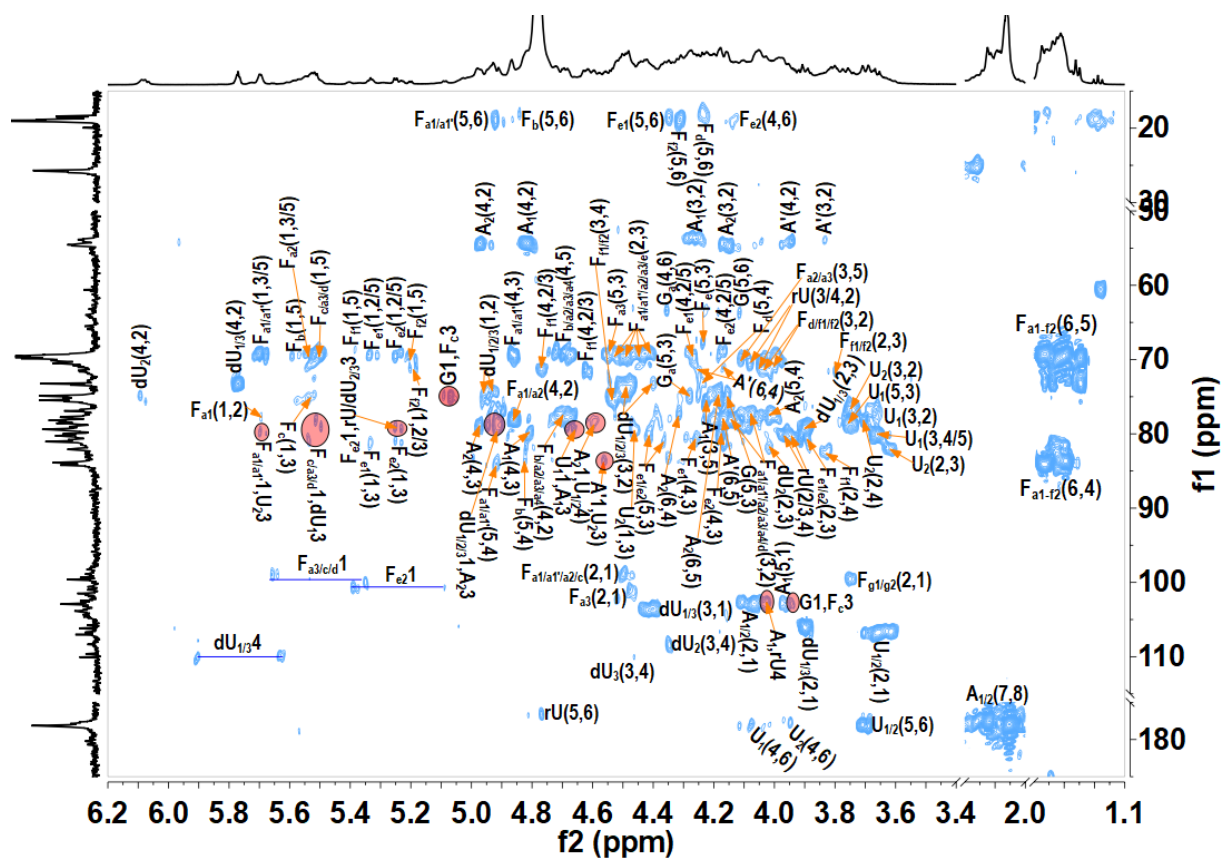
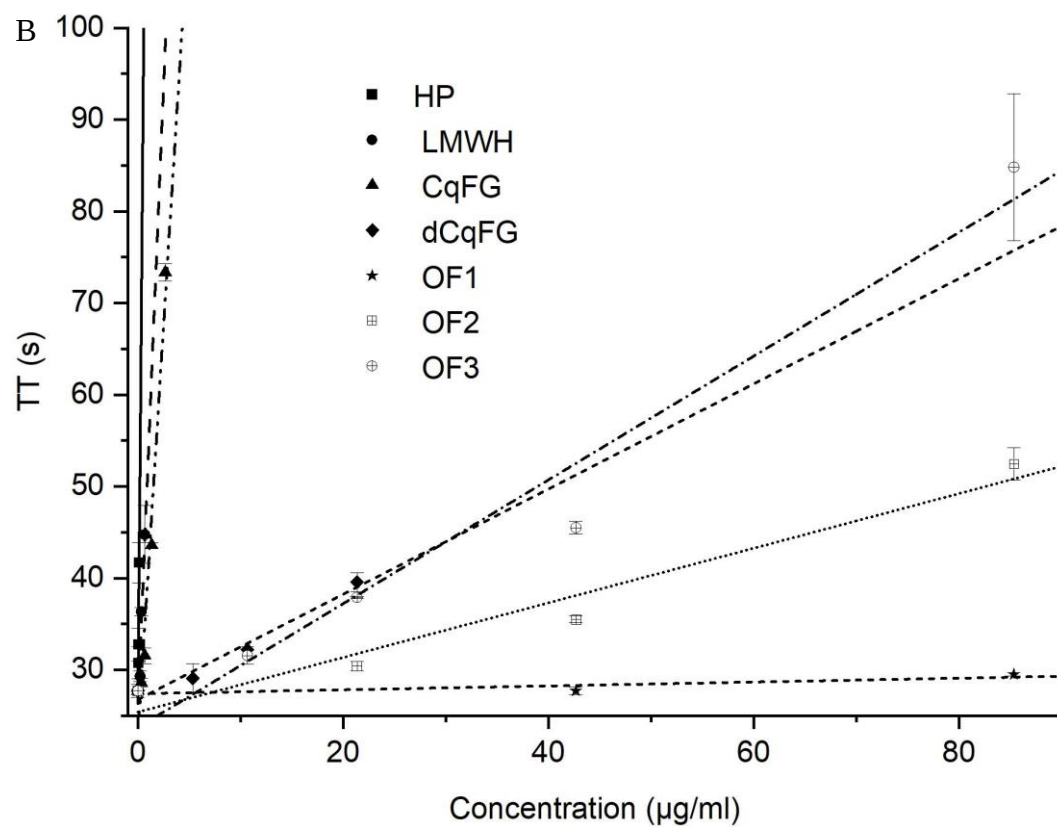
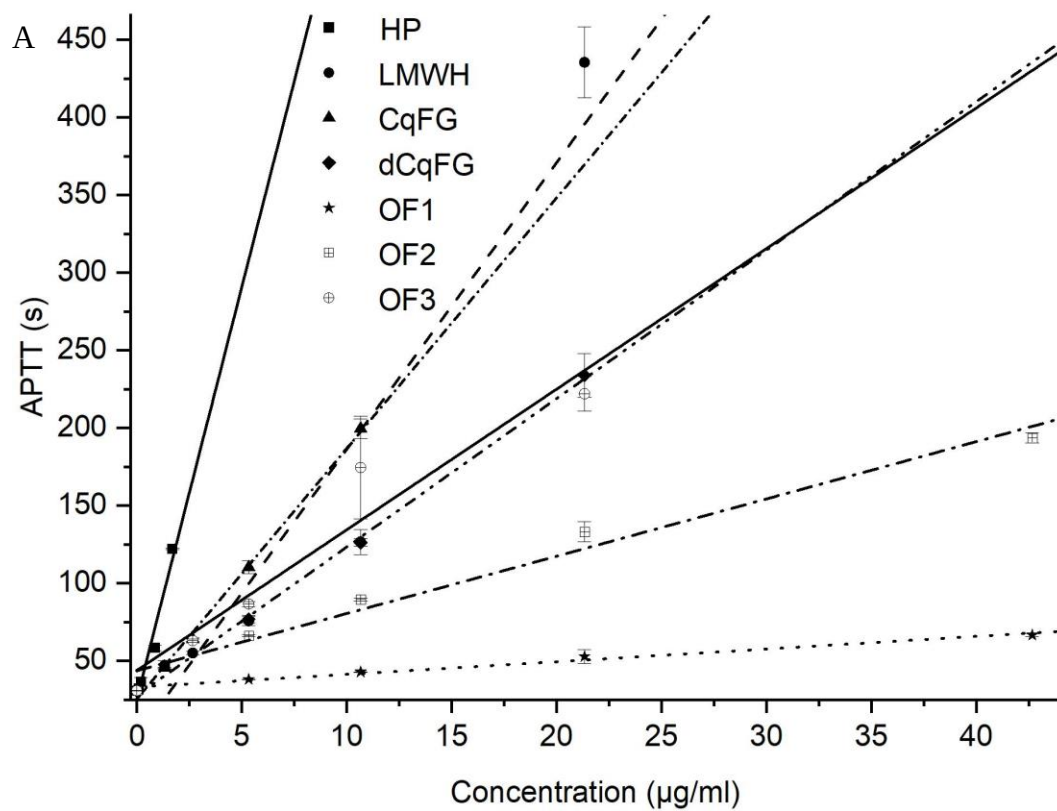


Figure S12. ^1H - ^{13}C HMBC spectrum of OF3.



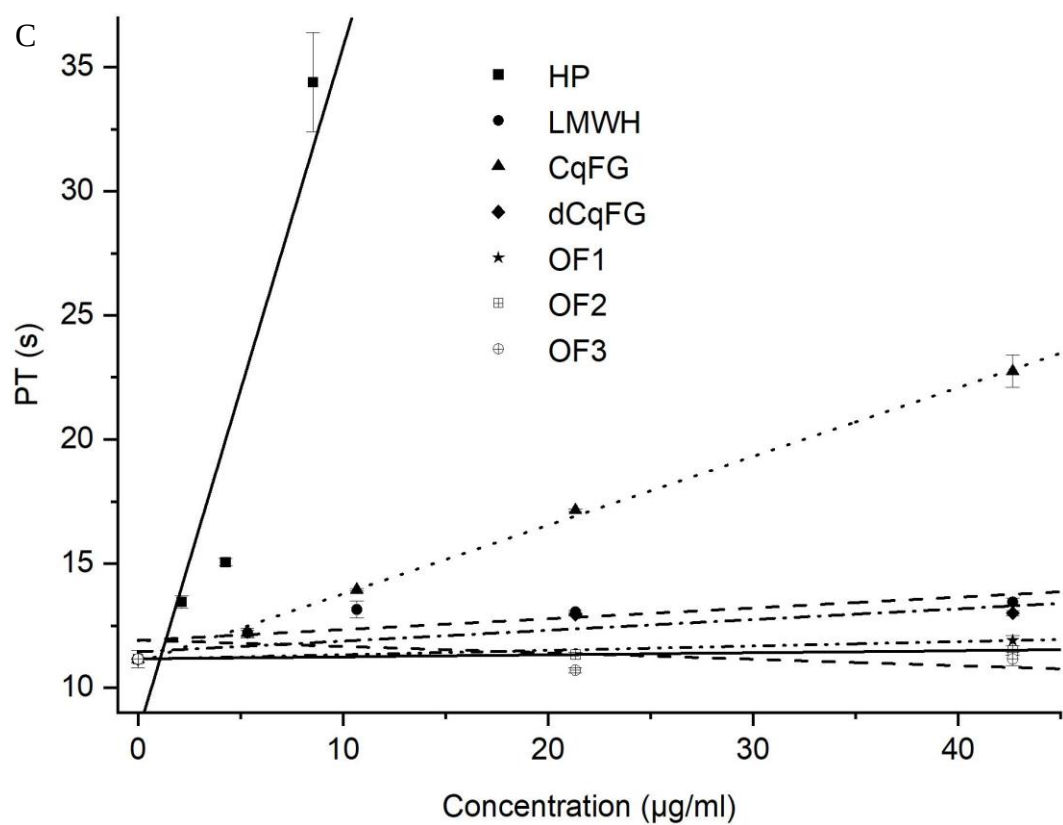


Figure S13. Effects of CqFG, dCqFG, and OF1–OF3 on APTT (A), TT (B) and PT (C) of human standard plasma.

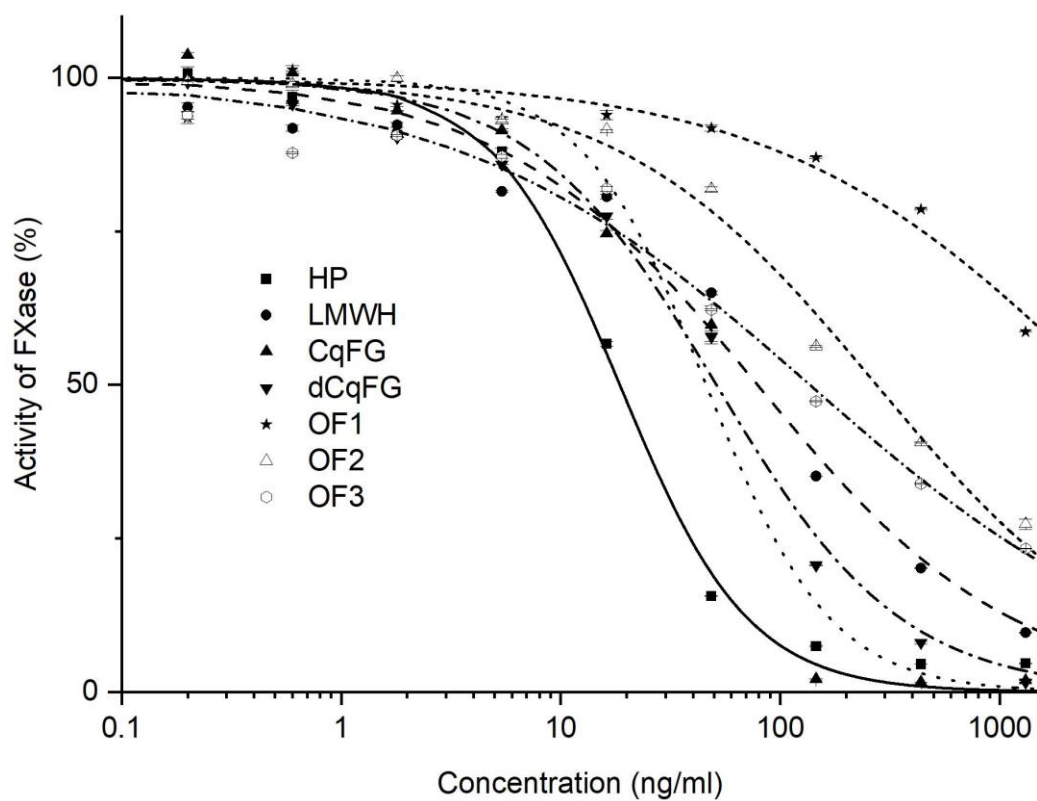


Figure S14. Effects of CqFG, dCqFG, and OF1–OF3 on intrinsic FXase

Table S1. $^1\text{H}/^{13}\text{C}$ NMR chemical shift assignments of **OF2** (δ , ppm).

Residues	H/C	Chemical shifts (δ , ppm) ^a								Connection patterns
		1	2	3	4	5	6	7	8	
F _{a1}	H	5.69	4.49	4.17	4.86	4.92	1.36			
α -L-FuC _{2S4S}	C	99.77	78.48	70.15	84.45	69.73	19.44			F _{a1} 1-U ₂ 3
F _{a1'}	H	5.65	4.51	4.13	4.87	4.96	1.33			
α -L-FuC _{2S4S}	C	100.06	78.40	70.15	84.45	69.56	19.44			F _{a1'} 1-U ₂ 3
F _b	H	5.57	4.59	4.95	4.70	4.86	1.36			
α -L-FuC _{2S3S4S}	C	98.55	78.57	82.31	84.12	69.98	19.10			F _b 1-dU ₂ 3
F _{a2}	H	5.56	4.50	4.12	4.65	4.75	1.37			
α -L-FuC _{2S4S}	C	99.01	78.56	69.98	84.75	69.98	19.10			F _{a2} 1-rU ₃
F _c	H	5.53	4.49	3.94	4.31	4.50	1.31			
α -L-FuC _{2S4S}	C	99.43	78.23	75.77	73.29	70.74	19.10			F _c 1-dU ₁ 3
F _{a3}	H	5.50	4.43	4.10	4.68	4.58	1.32			
α -L-FuC _{2S4S}	C	99.43	78.23	70.04	84.12	70.36	19.10			F _{a3} 1-dU _{1/2} 3
F _d	H	5.45	4.39	4.02	4.18	4.25	1.25			
α -L-FuC _{2S}	C	99.43	78.23	69.98	73.64	70.74	19.10			F _d 1-dU ₁ 3
F _{f1}	H	5.41	3.80	4.04	4.77	4.32	1.35			
α -L-FuC _{4S}	C	101.75	71.41	71.58	84.05	69.69	19.52			F _{f1} 1-U ₁ 3
F _{a4}	H	5.38	4.46	4.07	4.69	4.58	1.31			
α -L-FuC _{2S4S}	C	102.04	78.66	69.98	84.12	70.36	19.52			F _{a4} 1-dU _{1/2} 3
F _{e1}	H	5.33	3.89	4.50	4.28	4.37	1.30			
α -L-FuC _{3S}	C	102.55	69.73	81.38	73.22	70.15	19.39			F _{e1} 1-U ₁ 3
F _{e2}	H	5.24	3.91	4.55	4.18	4.43	1.31			
α -L-FuC _{3S}	C	101.75	69.73	81.38	73.31	69.98	19.39			F _{e2} 1-rU/dU _{2/3}
F _{f2}	H	5.20	3.80	4.00	4.62	4.46	1.30			
α -L-FuC _{4S}	C	101.92	71.41	71.41	83.74	70.23	19.52			F _{f2} 1-dU ₁ 3
G	H	5.09	3.93	4.54	4.36	4.18	3.80/3.98			
α -D-Gal _{3S4S}	C	104.69	69.81	75.16	80.67	71.85	64.60			G1-F ₃
dU ₁	H	4.96	3.88	4.35/4.41	5.78					
α -L- $\Delta^{4,5}$ GlcA	C	105.83	73.47	80.58	110.29	149.78	171.66			dU ₁ 1-A ₂ 3
dU ₂	H	4.94	4.01	4.20/4.32/4.42	6.07/6.09					
α -L- $\Delta^{4,5}$ GlcA	C	104.52	72.04	79.53	108.56	152.04	172.20			dU ₂ 1-A ₂ 3
dU ₃	H	4.93	3.91	4.23/4.48	5.77					
α -L- $\Delta^{4,5}$ GlcA	C	106.46	73.47	80.58	110.29	149.78	171.66			dU ₃ 1-A ₂ 3
A ₁	H	4.74	4.08	4.27	4.83/4.74	3.99	4.17/4.31		2.06	
β -D-GalNAC _{4S6S}	C	105.45	55.39	79.53	79.53	75.06	70.78	177.98	25.83	A ₁ 1-rU ₄
U ₁	H	4.65	3.61	3.66	4.03	3.78				
β -D-GlcA	C	105.20	76.63	77.43	78.94	80.46	178.21			U ₁ -A ₂ 3
A ₂	H	4.59	4.07	4.17	4.98/5.02	4.06	4.17/4.35		2.13	
β -D-GalNAC _{4S6S}	C	103.05	54.83	79.41	79.66/77.81	75.62	70.78	177.98	26.00	A ₂ -U _{1/2} 4
U ₂	H	4.49	3.65	3.75	4.03	3.75				
β -D-GlcA	C	107.26	76.18	80.58	82.35	80.46	178.21			U ₂ -A ₁ 3

rU	H	3.73/3.84	3.95	3.96	4.06	4.77	
β -D-GlcA-ol	C	65.68	72.04	79.07	82.18	75.07	177.23

^a Values in boldface and italic font indicate glycosylated and sulfated positions, respectively.

Table S2. ¹H and ¹³C chemical shift assignments of **OF3** (δ , ppm).

Residues	H/C	Chemical shifts (δ , ppm)								Connection patterns
		1	2	3	4	5	6	7	8	
F _{a1}	H	5.70	<i>4.49</i>	4.15	<i>4.87</i>	4.92	1.38			
α -L-FuC _{2S4S}	C	99.47	<i>78.10</i>	69.60	<i>84.08</i>	69.10	18.68			F _{a1} 1-U ₂ 3
F _{a1'}	H	5.66	<i>4.51</i>	4.13	<i>4.87</i>	4.93	1.35			
α -L-FuC _{2S4S}	C	100.06	<i>78.40</i>	69.60	<i>84.08</i>	69.10	18.68			F _{a1'} 1-U ₂ 3
F _b	H	5.59	<i>4.58</i>	<i>4.93</i>	<i>4.76</i>	4.84	1.37			
α -L-FuC _{2S3S4S}	C	98.21	<i>78.57</i>	<i>81.85</i>	<i>84.03</i>	69.35	18.68			F _b 1-dU ₂ 3
F _{a2}	H	5.57	<i>4.48</i>	<i>4.12</i>	<i>4.67</i>	4.79	1.35			
α -L-FuC _{2S4S}	C	100.06	<i>78.56</i>	69.56	<i>83.61</i>	69.35	18.68			F _{a2} 1-rU ₃
F _c	H	5.53	4.45	3.94	<i>4.31</i>	4.50	1.31			
α -L-FuC _{2S}	C	98.59	<i>77.76</i>	75.77	<i>73.29</i>	70.74	19.10			F _c 1-dU ₁ 3
F _{a3}	H	5.51	4.43	4.10	<i>4.68</i>	4.60	1.35			
α -L-FuC _{2S4S}	C	99.81	<i>77.85</i>	69.56	<i>83.70</i>	69.35	18.68			F _{a3} 1-dU _{1/2} 3
F _d	H	5.48	4.40	<i>4.02</i>	<i>4.18</i>	4.25	1.30			
α -L-FuC _{2S}	C	99.85	<i>78.02</i>	69.43	<i>73.05</i>	69.81	19.10			F _d 1-dU ₁ 3
F _{f1}	H	5.40	3.82	4.05	<i>4.77</i>	4.49	1.35			
α -L-FuC _{4S}	C	101.45	71.20	71.54	<i>83.61</i>	69.81	18.68			F _{f1} 1-U ₁ 3
F _{a4}	H	5.38	4.45	4.07	<i>4.70</i>	4.59	1.31			
α -L-FuC _{2S4S}	C	102.04	<i>78.66</i>	69.98	<i>84.12</i>	70.36	18.68			F _{a4} 1-dU _{1/2} 3
F _{e1}	H	5.33	3.89	<i>4.50</i>	4.28	4.35	1.32			
α -L-FuC _{3S}	C	102.17	69.14	<i>81.00</i>	73.05	69.90	18.68			F _{e1} 1-U ₁ 3
F _{e2}	H	5.25	3.91	<i>4.55</i>	4.17	4.40	1.31			
α -L-FuC _{3S}	C	101.37	69.14	<i>81.00</i>	73.05	69.90	18.68			F _{e2} 1-rU/dU _{2/3}
F _{f2}	H	5.21	3.80	4.01	<i>4.62</i>	4.32	1.31			
α -L-FuC _{4S}	C	101.45	71.20	71.54	<i>83.61</i>	69.73	18.68			F _{f2} 1-dU ₁ 3
G	H	5.09	3.93	<i>4.52</i>	<i>4.34</i>	4.16	3.83/3.96			
α -D-Gal _{3S4S}	C	104.15	69.01	<i>75.07</i>	<i>80.67</i>	72.17	63.96			G1-F _c 3
dU ₁	H	4.95	3.90	4.35/4.41	5.78					
α -L- $\Delta^{4,5}$ GlcA	C	106.04	73.22	80.67	109.78	147.86	172.00			dU ₁ 1-A ₂ 3
dU ₂	H	4.94	4.02	4.26/4.32/4.42	6.08					
α -L- $\Delta^{4,5}$ GlcA	C	104.19	72.65	81.00	108.23	149.88	172.78			dU ₂ 1-A ₂ 3
dU ₃	H	4.91	3.91	4.23/4.49	5.77					
α -L- $\Delta^{4,5}$ GlcA	C	106.04	73.22	80.67	109.78	147.86	172.00			dU ₃ 1-A ₂ 3
A ₁	H	4.78	4.04	4.26	<i>4.81</i>	3.99	<i>4.17/4.31</i>		2.06	
β -D-GalNAc _{4S6S}	C	103.85	54.04	79.83	<i>79.28</i>	74.95	<i>70.78</i>	177.98	25.83	A ₁ 1-rU ₄
U ₁	H	4.62	3.59	3.68	4.03	3.76				

β -D-GlcA	C	105.20	76.59	79.87	78.77	79.95	178.21			U ₁ 1-A ₃ 3
A ₂	H	4.60	4.07	4.18	<i>4.98/5.02</i>	4.02	<i>4.17/4.35</i>		2.13	
β -D-GalNAc _{4S6S}	C	102.63	54.12	79.41	<i>79.20/77.09</i>	74.95	<i>70.78</i>	177.98	26.00	A ₂ 1-U _{1/2} 4
A'	H	4.58	4.15	3.84	3.95	3.75	<i>4.17/4.26</i>			A'-U ₂ ?
β -GlcNAc _{6S}	C	103.14	54.37	75.41	<i>70.88</i>	77.40	<i>69.73</i>			
U ₂	H	4.48	3.63	3.76/3.84	3.99	3.76				
β -D-GlcA	C	106.71	76.71	79.95/83.82	78.77	79.95	178.21			U ₂ 1-A ₁ 3
rU	H	3.76/3.81	3.91	3.94	4.06	4.77				
β -D-GlcA-ol	C	65.01	73.31	78.61	80.25	75.20	177.23			

^a Values in boldface and italic font indicate glycosylated and sulfated positions, respectively.