Supplementary Material for

Purification and characterisation of heparin-like sulfated polysaccharides with potent anti-SARS-CoV2 activity from snail mucus of *Achatina fulica*

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Table S1. ^{13}C NMR chemical shifts for acharan sulfate from snail mucus (AS-GAG). Carbons labeled as I and G refer to Iduronic acid (IdoA) and N-acetyl glucosamine (GlcNAc) units, respectively.

Sugar residues	Chemical Shifts / ppm							
	1	2	3	4	5	6	7	8
I; $\rightarrow 4)-\alpha-IdoA\rightarrow 1$	99.233	73.281	63.721	69.743	66.397	174.801	–	–
G; $\rightarrow 4)-\alpha-GlcNAc\rightarrow 1$	93.927	53.680	68.573	77.250	71.220	59.679	174.801	22.217

Table S2. Determination of anti-SARS-ACE2 binding for the unfractionated polysaccharide inhibitors.

Inhibitors	IC_{50}	IC_{50} 95% CI	Ref.
AS-GAGs[a]	28.4 μg mL^{-1}	4.33 – 5.29 μg mL^{-1}	
Enoxaparin[a]	91.5 μg mL^{-1}	5.20 – 5.65 μg mL^{-1}	
Pentosan polysulfate[a]	57.1 μg mL^{-1}	4.31 – 4.84 μg mL^{-1}	
Heparan sulfate[a]	132.4 μg mL^{-1}	2.11 – 2.14 μg mL^{-1}	
Heparin	0.12 μg mL^{-1}	0.09 – 0.15 μg mL^{-1}	[21]
Heparan sulfate	62 μg mL^{-1}	>15 μg mL^{-1}	[21]
LMWH	5 – 12 μg mL^{-1}	–	[22]
UFH	1 – 4 μg mL^{-1}	–	[22]
	5.99 μg L^{-1}	2.90 – 11.96 μg L^{-1}	[25]
Enoxaparin	1.08 mg L^{-1}	247 – 4164 μg L^{-1}	[25]

[a] Values from this work.

Figure S1.

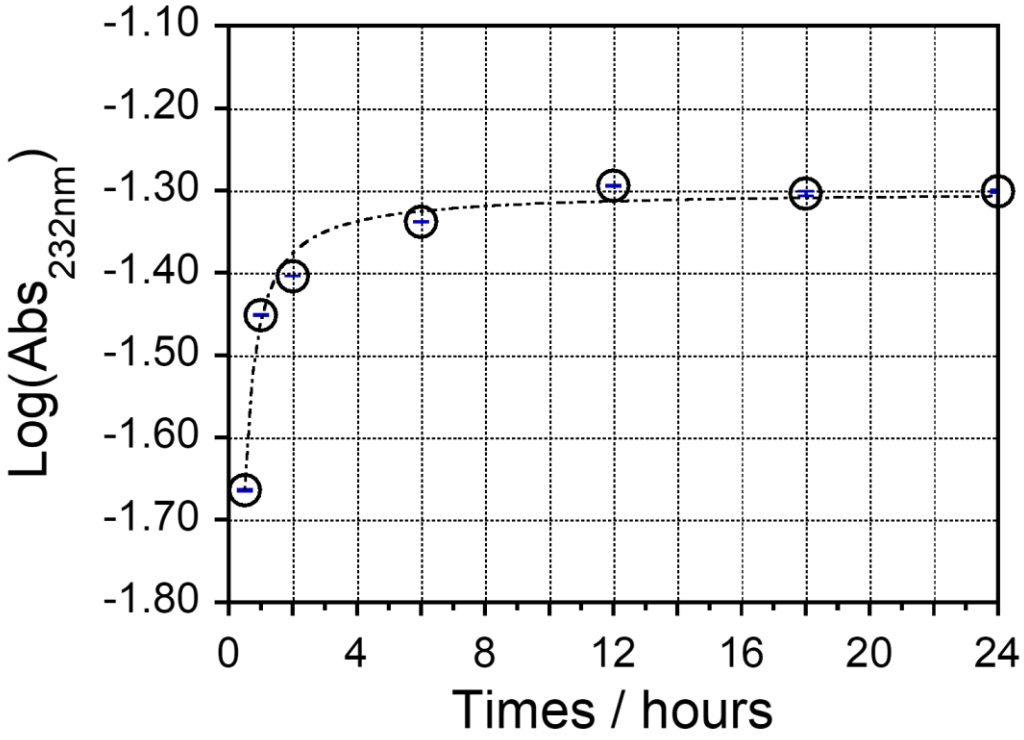


Fig. S1. Effect of hydrolysis time on the relative change (Log(Abs_{232nm})) of the unsaturated disaccharide content of mGAG-F3 enzymatic hydrolysis by treatment with a combination of heparin lyase I (EC 4.2.2.7), II (Heparitinase II), and III (EC 4.2.2.8). The digested mixture was in 0.3 mL of 50 mM Tris-HCl buffer (pH 7.2) and 10 mM CaCl2 at 37°C for 24 hrs, after which the reaction samples were heated in a boiling water for 3 minutes. Results are reported as mean ± SD. Error bar denotes standard deviation.

Figure S2.

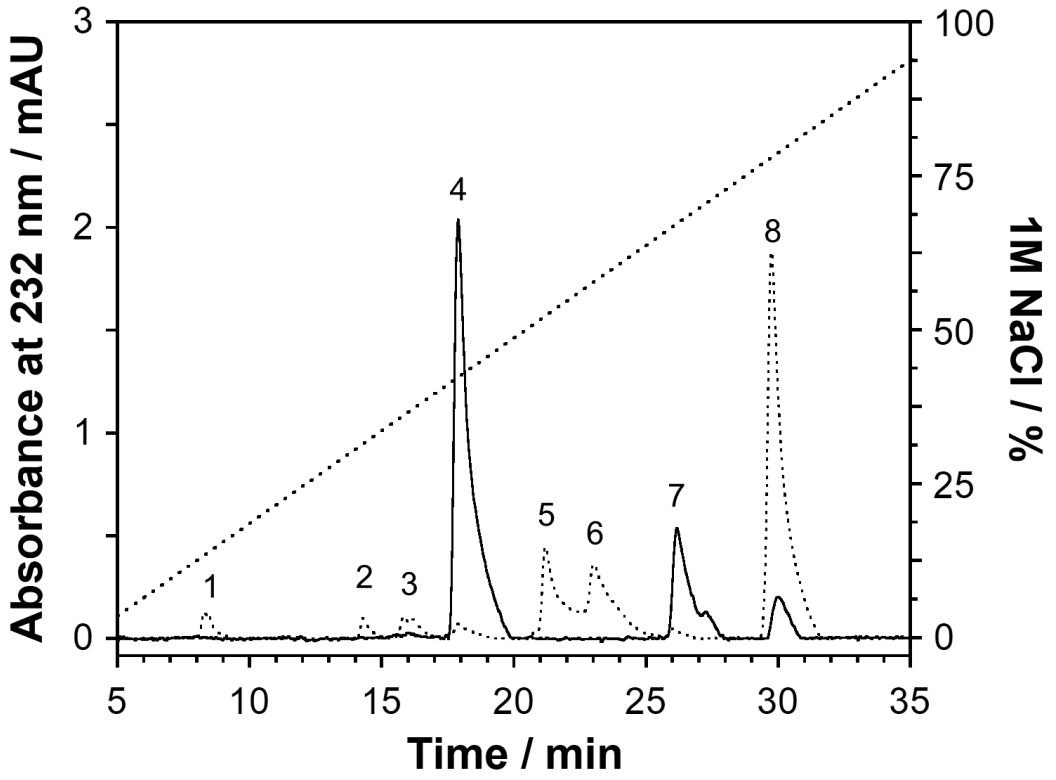
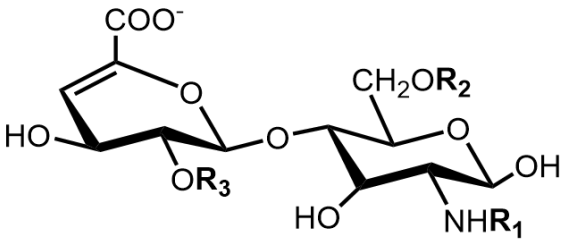


Fig. S2. SAX-HPLC chromatogram of mGAG-F3 (solid line) and heparin (dashed line) disaccharides by treatment with a combination of heparin lyase I (EC 4.2.2.7), II (Heparitinase II), and III (EC 4.2.2.8), eluting with a linear gradient of 0.1 – 1.0 M NaCl (dotted line) at a flow rate of 1.0 mL/min. The elutions were monitored by absorbance at 232 nm for detecting the unsaturated non-reducing ends of the disaccharides. The standard disaccharide positions are indicated by number labels; 1: ΔUA-GlcNAc, 2: ΔUA-GlcNSO$_3$, 3: ΔUA-GlcNAc(6S), 4: ΔUA(*2S*)-GlcNAc, 5: ΔUA-GlcNSO$_3$(*6S*), 6: ΔUA(*2S*)-GlcNSO$_3$, 7: ΔUA(*2S*)-GlcNAc(*6S*), 8: ΔUA(*2S*)-GlcNSO$_3$(*6S*).

Figure S3.



No.	Disaccharide	R1	R2	R3
1	ΔUA-GlcNAc	Ac	H	H
2	ΔUA-GlcNSO$_3$	SO$_3$	H	H
3	ΔUA-GlcNAc(6S)	Ac	SO$_3$	H
4	ΔUA(2S)-GlcNAc	Ac	H	SO$_3$
5	ΔUA-GlcNSO$_3$(6S)	SO$_3$	SO$_3$	H
6	ΔUA(2S)-GlcNSO$_3$	SO$_3$	H	SO$_3$
7	ΔUA(2S)-GlcNAc(6S)	Ac	SO$_3$	SO$_3$
8	ΔUA(2S)-GlcNSO$_3$(6S)	SO$_3$	SO$_3$	SO$_3$

Fig. S3. 2D Structure of the commonly occurring unsaturated disaccharides derived from digestion with heparinase enzymes. Abbreviations: Ac, acetyl; ΔUA, Δ(4,5) unsaturated hexuronic acid; GlcNAc, *N*-acetylated glucosamine; GlcNSO$_3$, *N*-sulfated glucosamine; H, hydrogen; 2S and 6S, 2-O-sulfate and 6-O-sulfate, respectively.

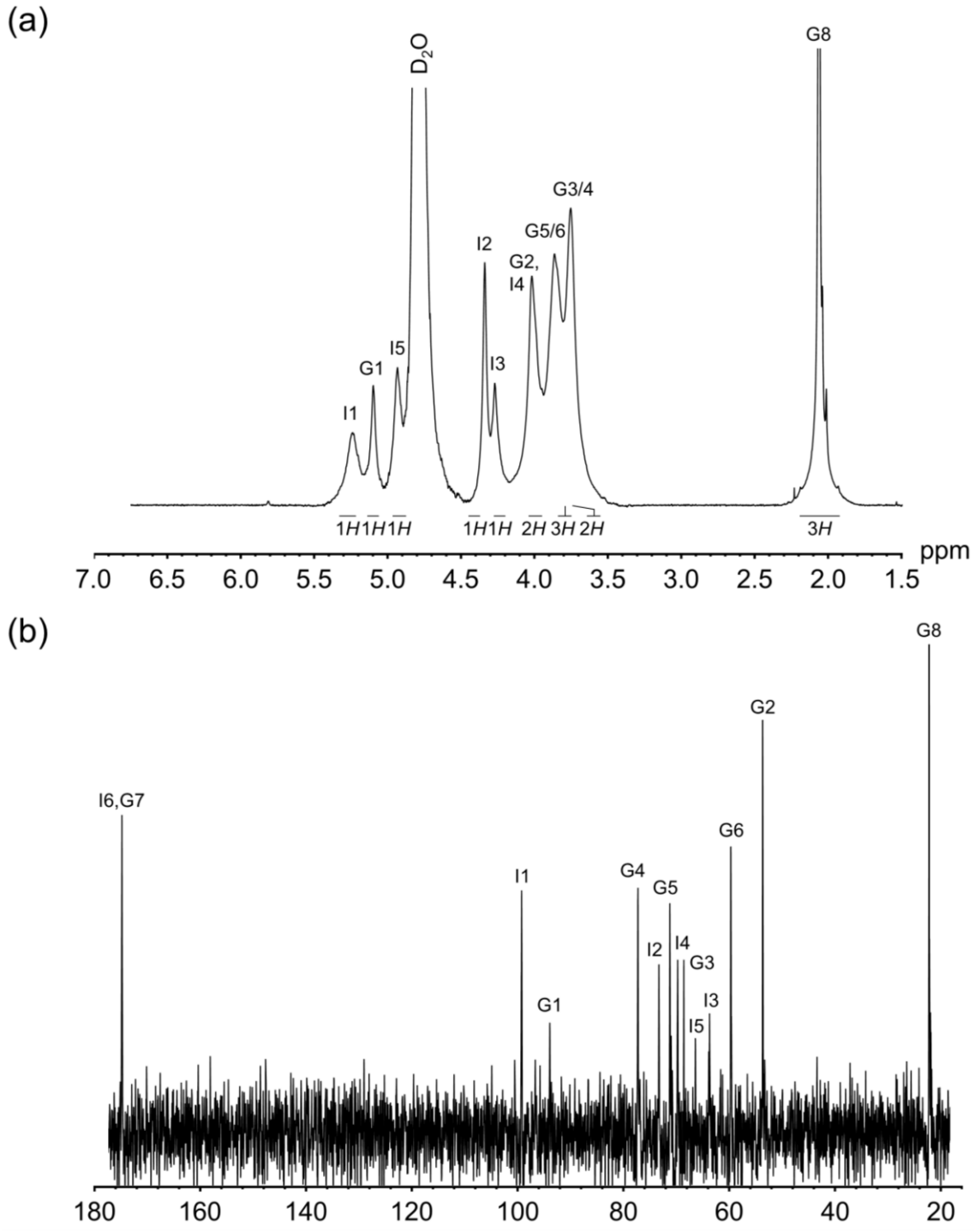


Fig. S4. (a) ^{1}H– and (b) ^{13}C–NMR spectra of the AS-GAG from the mucus of *A. fulica*. The assignment was based on comparison with the previously described acharan sulfate. The peaks are labeled as I or G for Iduronic acid (IdoA) and *N*-acetyl glucosamine (GlcNAc) units, respectively. The number after these letters indicate the positions of ^{1}H and ^{13}C.

Figure S4.

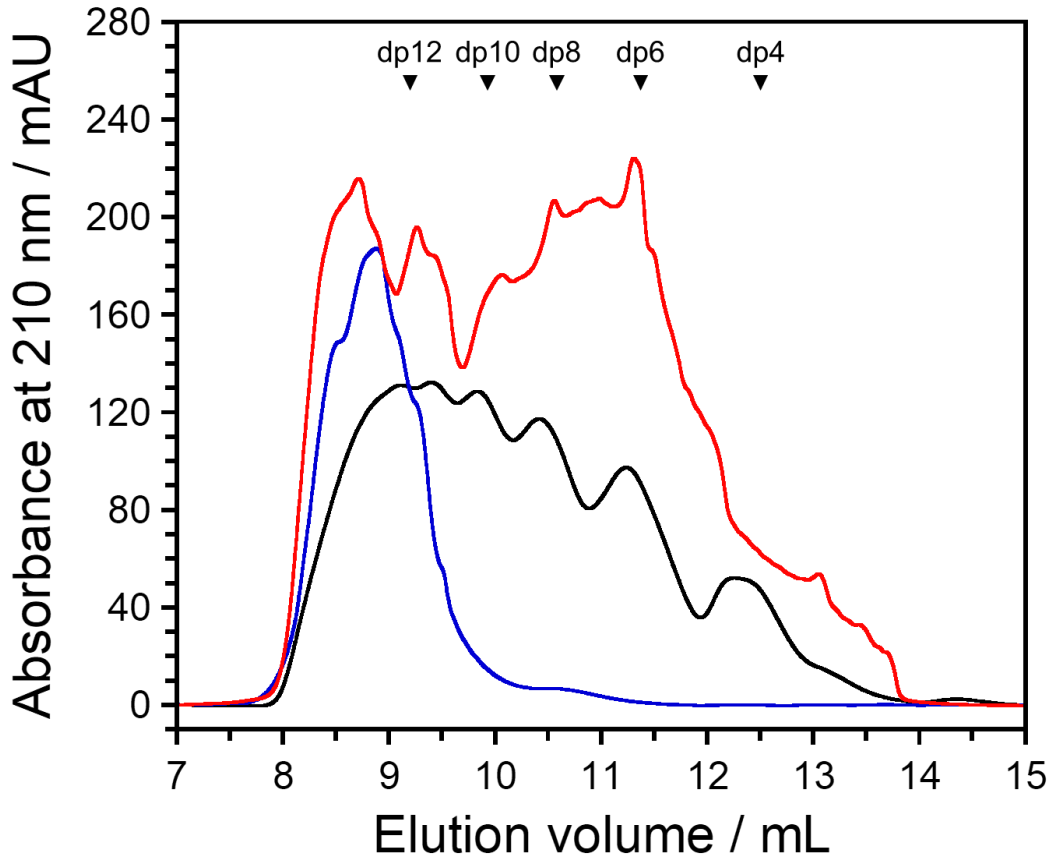


Fig. S5. Size exclusion chromatography analysis of the partial digest product of sulfated polysaccharide; mGAG-F3 between pre- (blue) and post- (red) depolymerization, in comparison to LMWH (black). The mGAG-F3 was depolymerized by partial digestion with a combination of heparin lyase I (EC 4.2.2.7), II (Heparitinase II), and III (EC 4.2.2.8). The digests were chromatographed on a Superdex 30 Increase 30/100 column using 0.1 M ammonium acetate (pH 7.0) as the eluent at 1.5 CV at flow rate 0.5 ml/min. The centers of tetrasaccharide, hexasaccharide, octasaccharide, decasaccharide, and decasaccharide peaks are marked with arrows. A calibration curve was plotted by the logarithm of molecular weights versus retention time in each standard position. The molecular weight of mGAG-F3 fraction was estimated by compared with the calibration curve.