

Supplementary data

Fucose-rich sulfated polysaccharides from two Vietnamese sea cucumbers *Bohadschia argus* and *Holothuria (Theelothuria) spinifera*: structures and anticoagulant activity

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A



B

Figure S1. Sea cucumbers (A) *Bohadschia argus* (Jaeger, 1833) and (B) *Holothuria (Theelothuria) spinifera* (Theel, 1886)

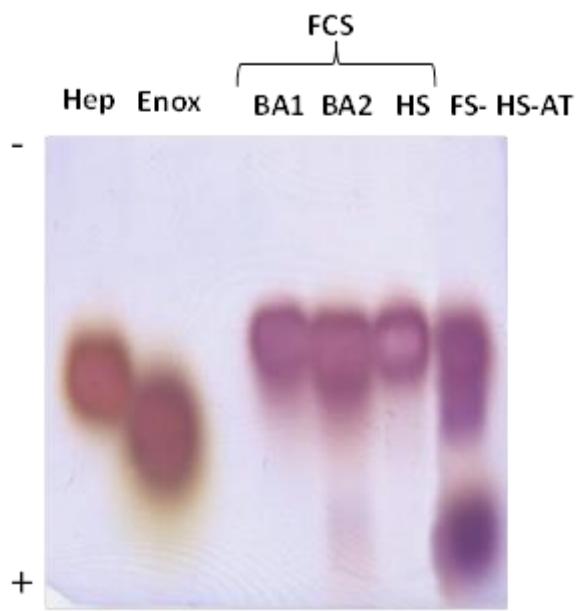


Figure S2. Agarose gel electrophoresis of polysaccharides. Abbreviations: **Hep** – unfractionated heparin from Sigma; **Enox** – enoxaparin (Clexane®, Sanofi); **BA1, BA2** – *Bohadschia argus* FCS fractions eluted from DEAE-Sephacel with 0.75 M and 1.0 M NaCl, respectively; **HS** – the corresponding *Holothuria spinifera* FCS fraction eluted from DEAE-Sephacel with 1 M NaCl; **FS-HS-AT** – fraction of **FS** obtained from crude polysaccharide of *Holothuria spinifera* after mild acid treatment and ion-exchange chromatography.

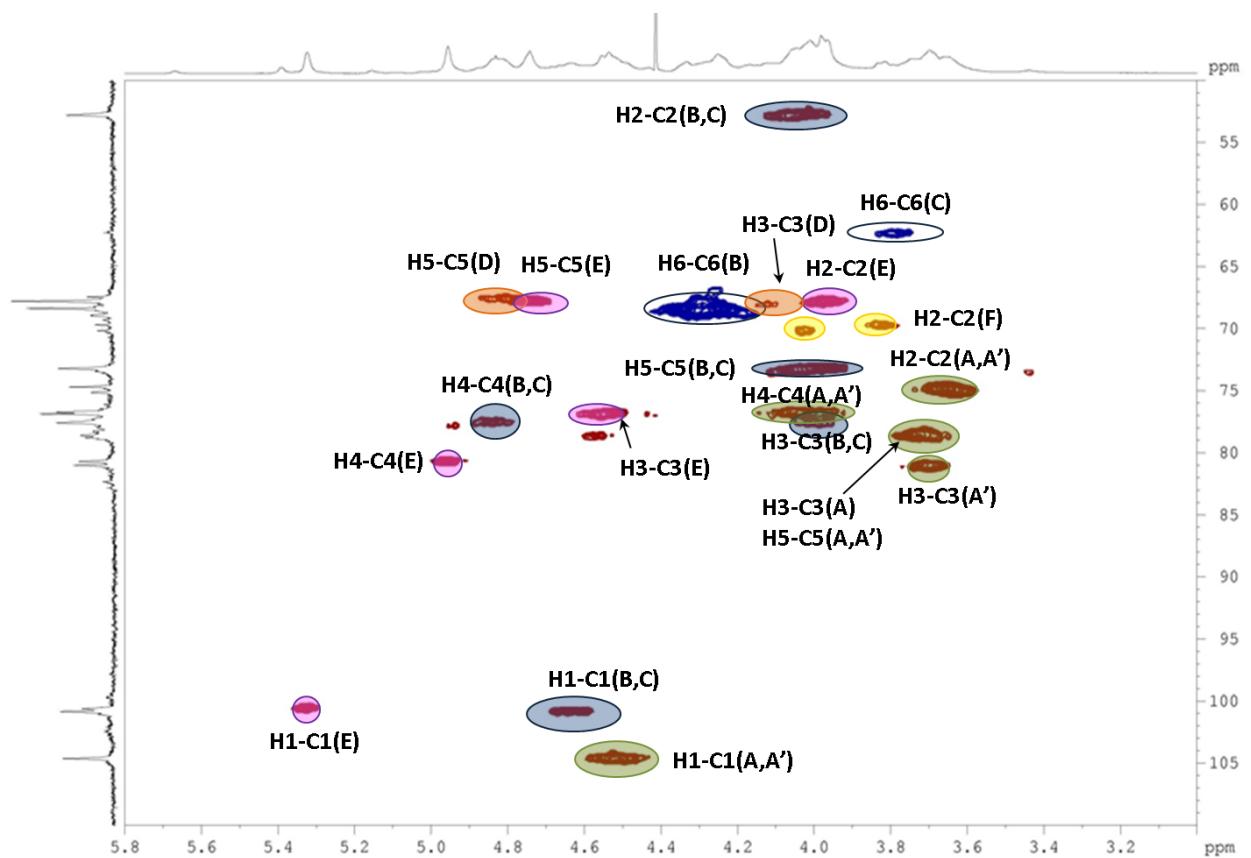


Figure S3. The HSQC NMR spectrum of FCS-BA

Table S1. The data of the ^1H and ^{13}C NMR spectra (chemical shifts, ppm) of fucosylated chondroitin sulfates

Residue	H-1 (C-1)	H-2 (C-2)	H-3 (C-3)	H-4 (C-4)	H-5 (C-5)	H-6 (C-6)
A →4)- β -D-Glc p A-(1→	4.48 (105.0)	3.64 (75.0)	3.71 (78.2)	3.96 (76.6)	3.71 (78.2)	- (176.0)
A' →4)- β -D-Glc p A-(1→	4.48 (105.0)	3.60 (75.0)	3.68 (80.7)	4.00 (76.6)	3.71 (78.2)	- (176.0)
B →3)- β -D-Gal p NAc4S6S-(1→	4.58 (100.9)	4.07 (52.7)	3.95 (77.9)	4.81 (77.2)	4.00 (73.2)	4.33, 4.20 (68.5)
C →3)- β -D-Gal p NAc4S-(1→	4.58 (100.9)	4.07 (52.7)	3.95 (77.9)	4.81 (77.2)	4.02 (76.2)	3.81 (62.3)
D α -L-Fuc p 2S4S-(1→	5.69 (97.7)	4.48 (76.6)	4.17 (67.8)	4.86 (82.5)	4.90 (67.5)	1.37 (16.9)
E α -L-Fuc p 3S4S-(1→	5.34 (100.5)	3.95 (67.6)	4.53 (76.6)	5.01 (80.6)	4.85 (67.6)	1.37 (17.2)
F α -L-Fuc p 4S-(1→	5.41 (99.6)	3.82 (69.7)	4.04 (70.0)	4.77 (82.4)	4.85 (67.6)	1.37 (17.2)

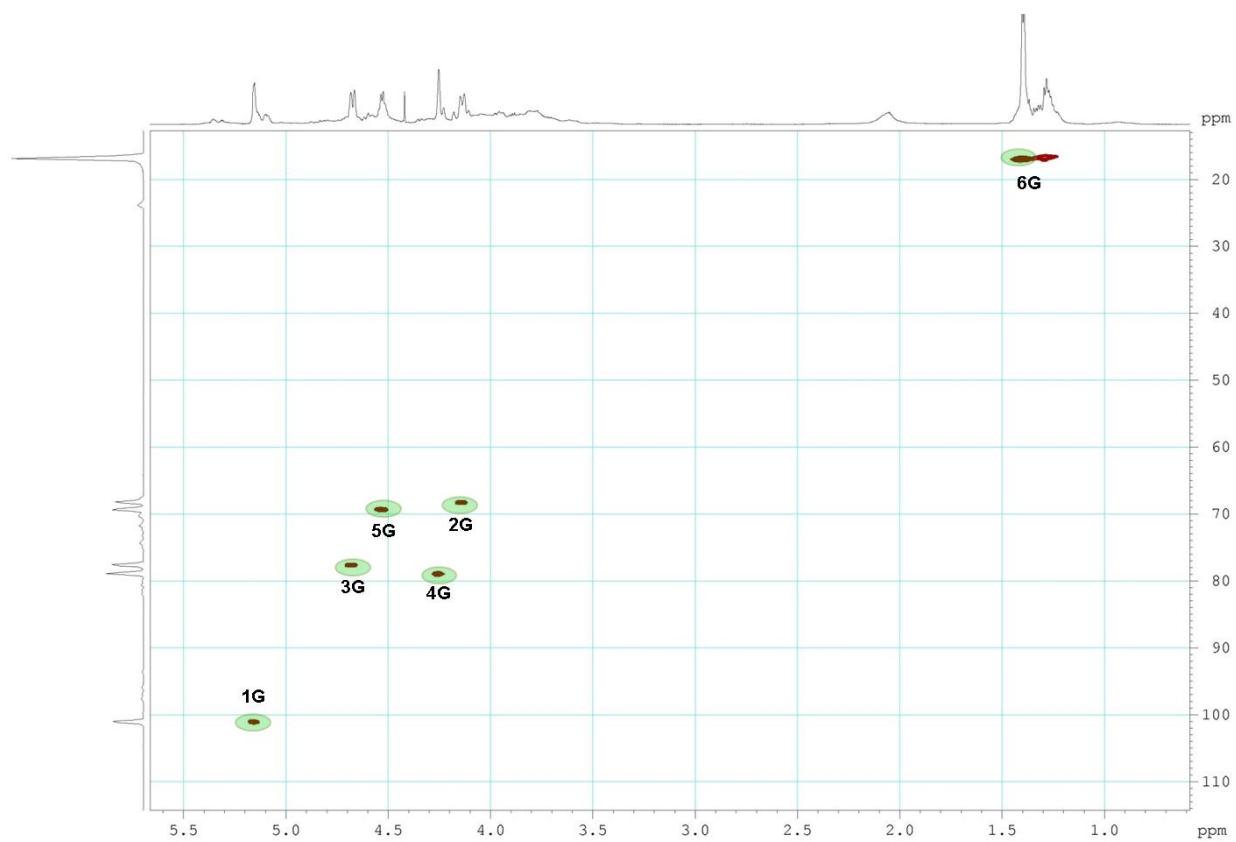


Figure S4. The HSQC NMR spectrum of **FS-BA-AT**

Table S2. The data of ^1H and ^{13}C NMR spectra (chemical shifts, ppm) of sulfated fucan **FS-BA-AT** and its desulfated derivative **FS-BA-AT-DS**

Species, sample	Structural unit	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
<i>Holothuria fuscopunctata</i> [22]	-4)- α -L-Fucp3S-(1-	5.22 103.00	4.20 69.88	4.73 79.28	4.21 80.71	4.62 71.17	1.47 18.68
FS-BA-AT	G -4)- α -L-Fucp3S-(1-	5.15 101.00	4.14 68.3	4.67 77.6	4.26 78.9	4.53 69.3	1.40 16.9
	α -L-Fucp3S-(1-	5.10 101.1	3.97 67.9	4.61 79.0	4.18 71.7	4.57 68.0	1.28 16.5
FS-BA-AT-DS	-4)- α -L-Fucp-(1-	5.11 96.83	4.03 69.71	3.96 67.58	4.00 76.22	4.31 67.79	1.22 16.29

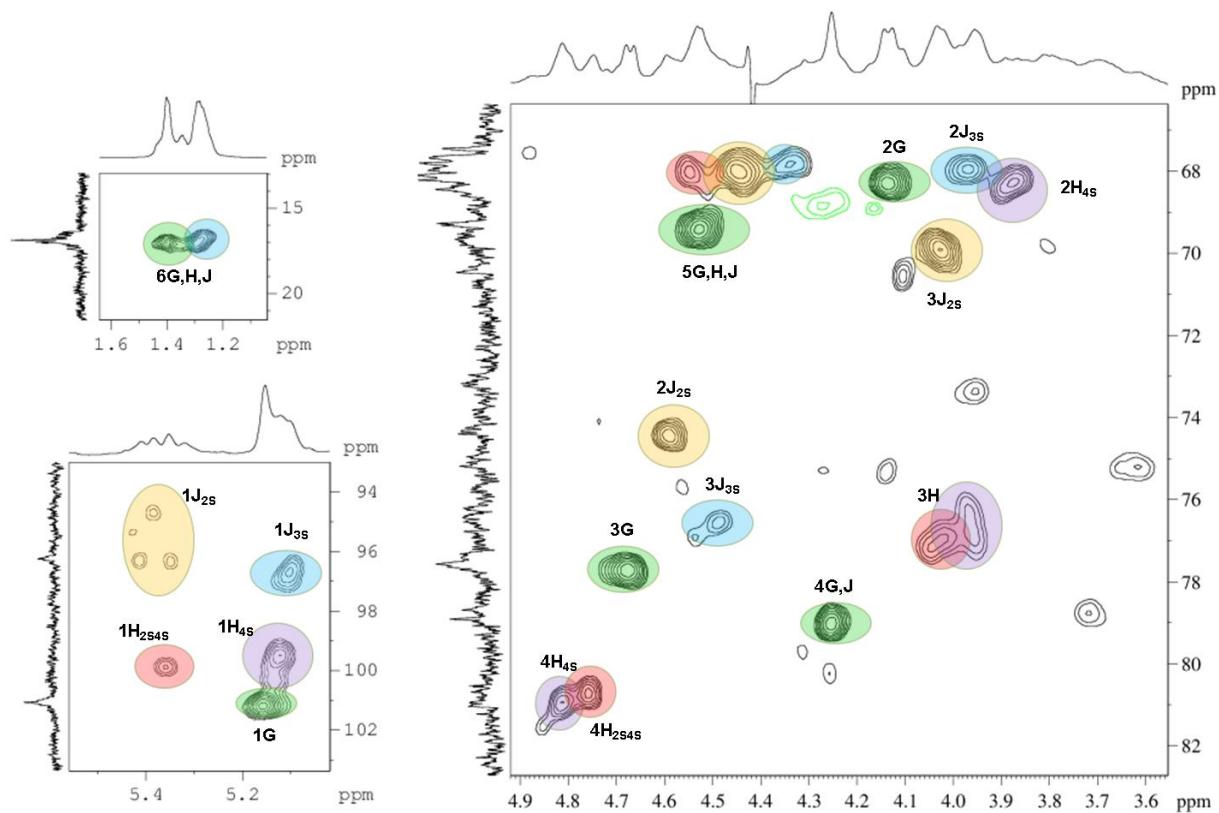


Figure S5. The HSQC NMR spectrum of **FS-HS-AT**

Table S3. ^{13}C and ^1H chemical shifts δ , ppm of anomeric atoms in the NMR spectra of fucan sulfate **FS-HS-AT** (see Figure 6)

Residue	$\mathbf{J}_{2S} \rightarrow \mathbf{H}_{2,4S}$	$\mathbf{H}_{2,4S} \rightarrow \mathbf{J}_{2S} \rightarrow \mathbf{H}_{4S}$	$\mathbf{H}_{4S} \rightarrow \mathbf{J}_{2S} \rightarrow \mathbf{H}_{4S}$	$\mathbf{J}_{3S} \rightarrow \mathbf{H}$	$\mathbf{H}_{2,4S} \rightarrow \mathbf{J}$	$\mathbf{H}_{4S} \rightarrow \mathbf{J}_{3S}$	$\mathbf{H}_{4S} \rightarrow \mathbf{J}_{2S}$
C-1	94.7	96.3	96.3	96.7	99.9	99.5	100.4
H-1	5.39	5.41	5.32	5.10	5.36	5.12	5.14