

Supplementary Material

**Structure elucidation of fucan sulfate from sea cucumber
Holothuria fuscopunctata through a bottom-up strategy and
the antioxidant activity analysis**

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Preparation of dFSI' by peroxide depolymerization

The depolymerized FSI (dFSI') were prepared using a modified protocol based on the previous report ([Shang et al., 2018](#)). In brief, 100 mg of FSI was dissolved in 4 mL of H₂O, 3.0 mg of copper acetate and 1 mL of 30% H₂O₂ solution was added and stirred at 35 °C for 3 h. Finally, the solution was precipitated with 2 mL of saturated sodium chloride solution and ethanol with a final concentration of 80% (v/v) and collected by centrifugation (4000 × g, 15 min). Then, the precipitate was dissolved, desalted by Sephadex G-25, concentrated and lyophilized to yield depolymerized product dFSI'. The average molecular weight of dFSI' was detected on a Shodex OHpak SB-804 HQ column and calculated as 4.6 kDa.

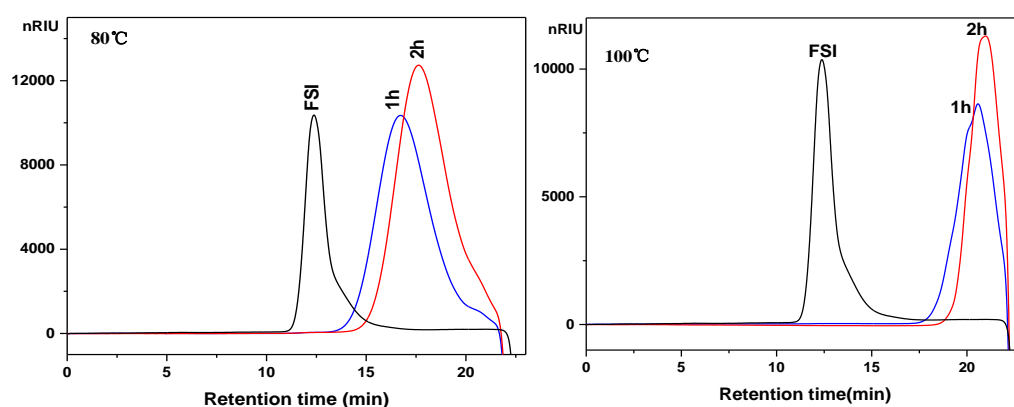


Figure S1. HPGPC profiles of acid hydrolysis hydrolysates of FSI at 80°C and 100°C, respectively.

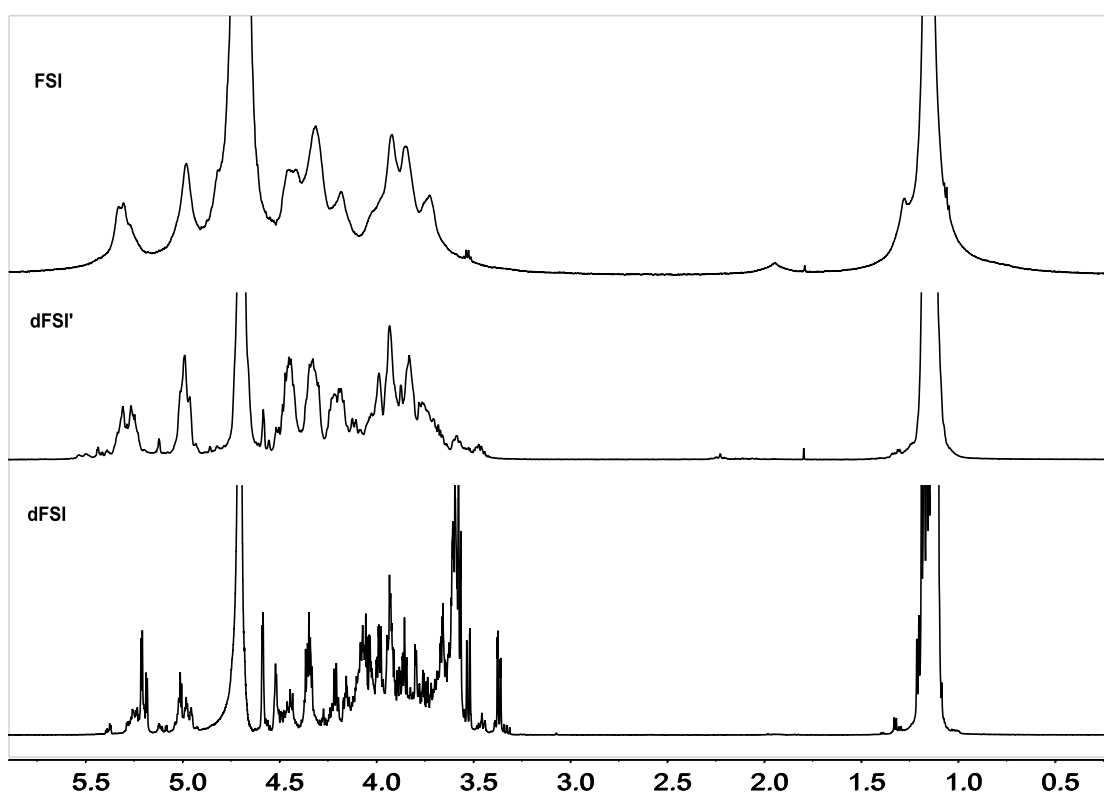
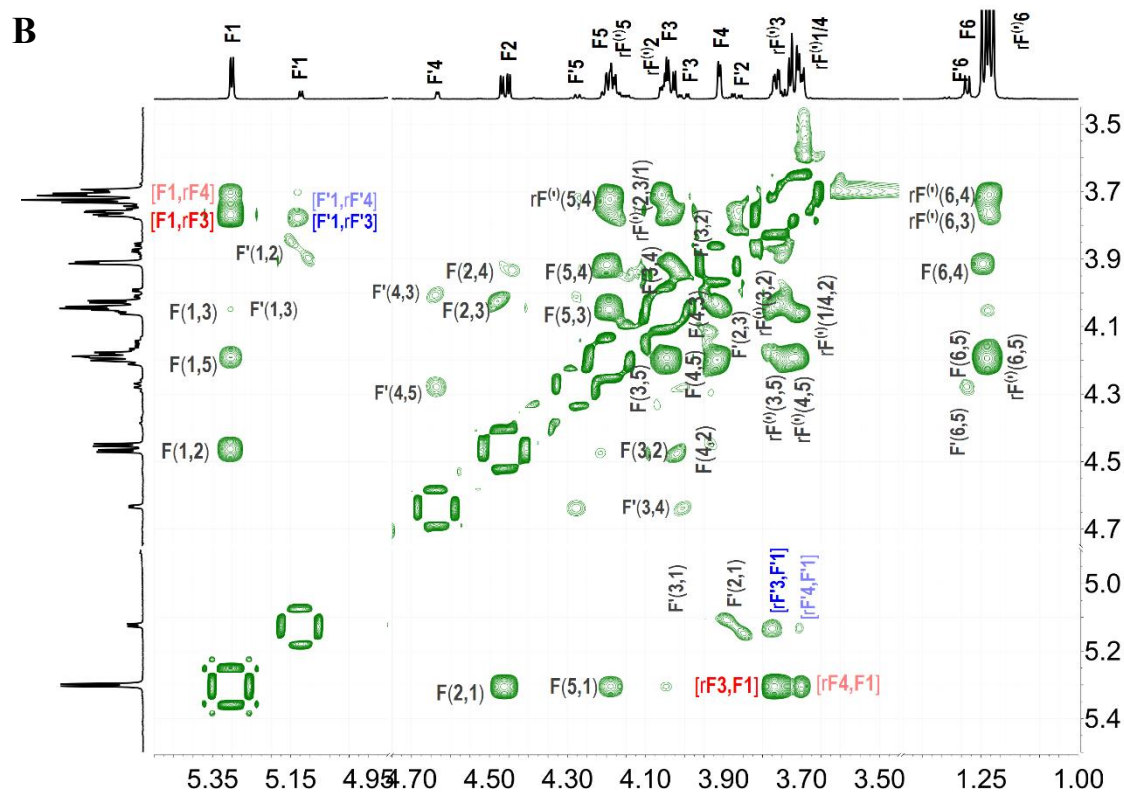
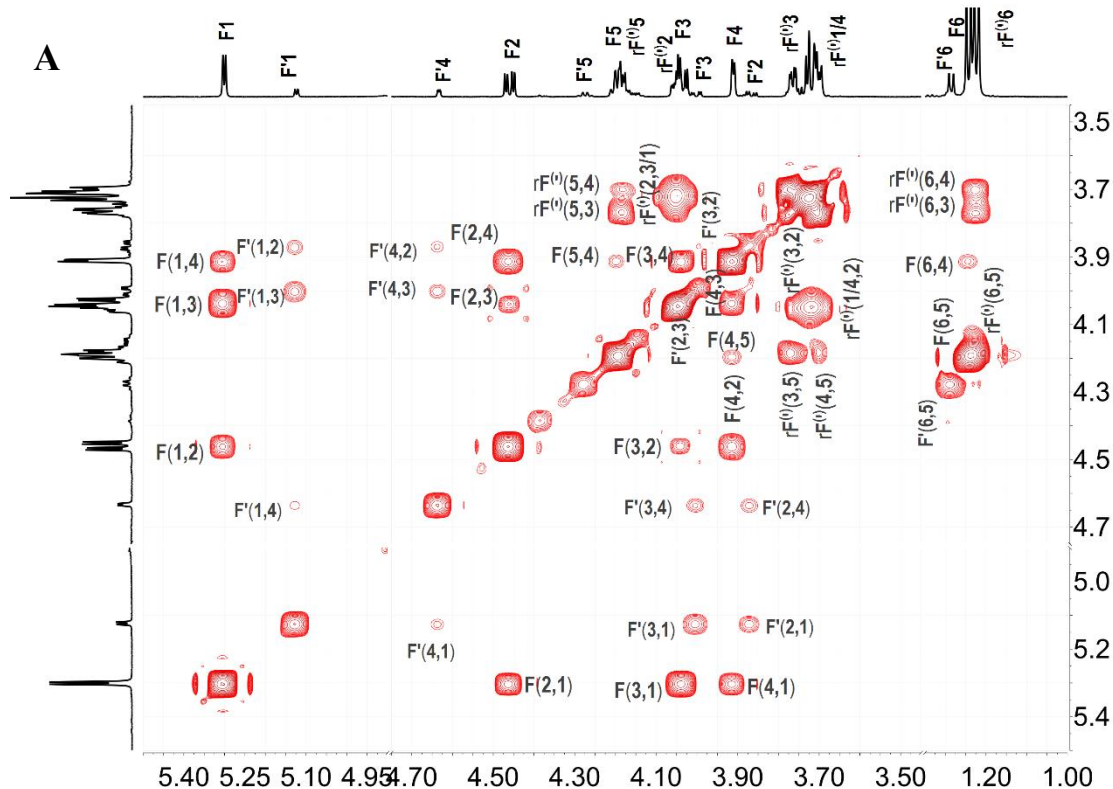


Figure S2. ^1H NMR spectra of native FSI from *H. fuscopunctata*, dFSI' obtained by peroxide depolymerization and dFSI obtained by mild acid hydrolysis.



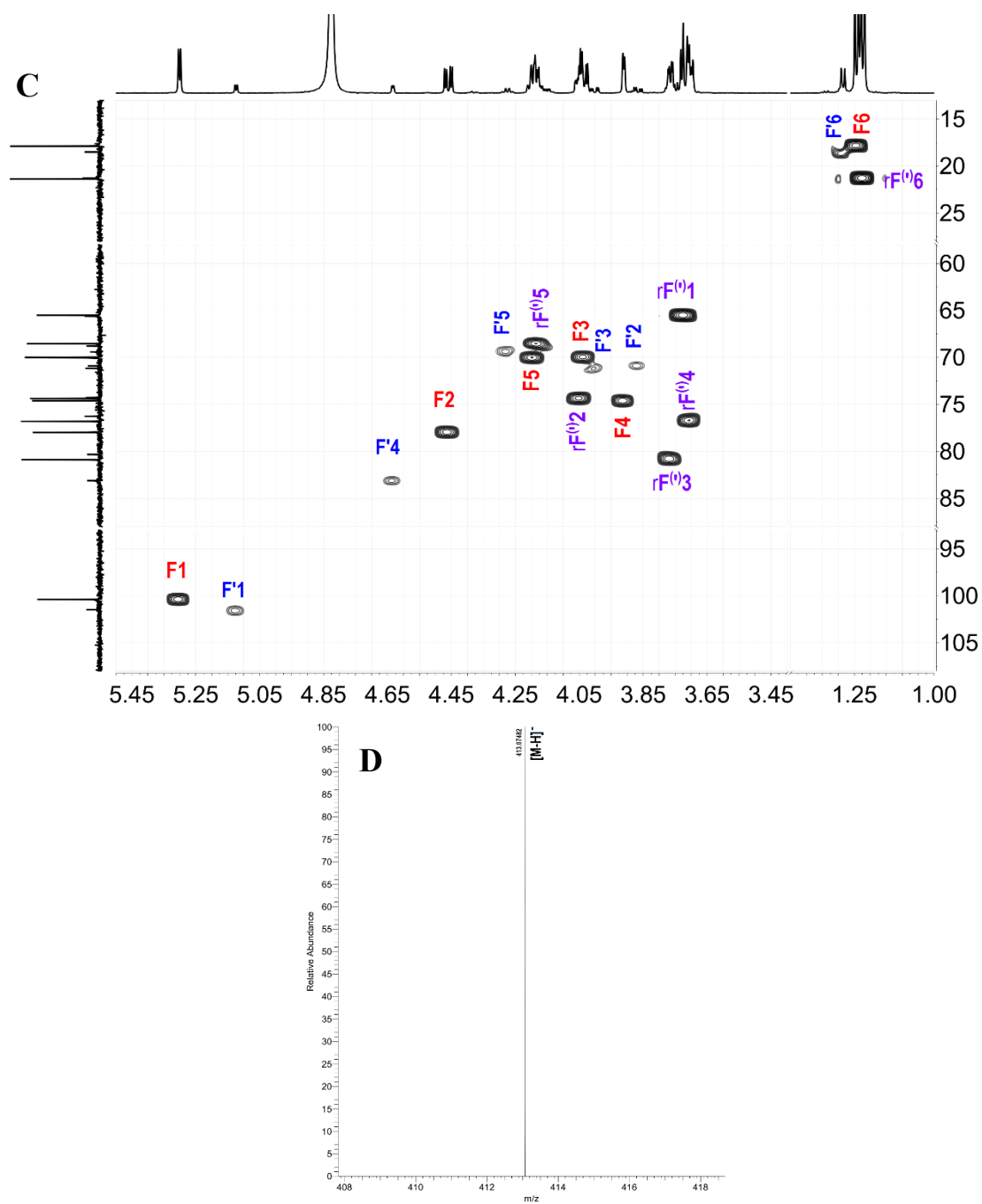


Figure S3. ^1H - ^1H TOCSY (A), ^1H - ^1H ROESY (B), ^{13}C - ^1H HSQC (C) spectra of **FI** and its MS spectrum (D).

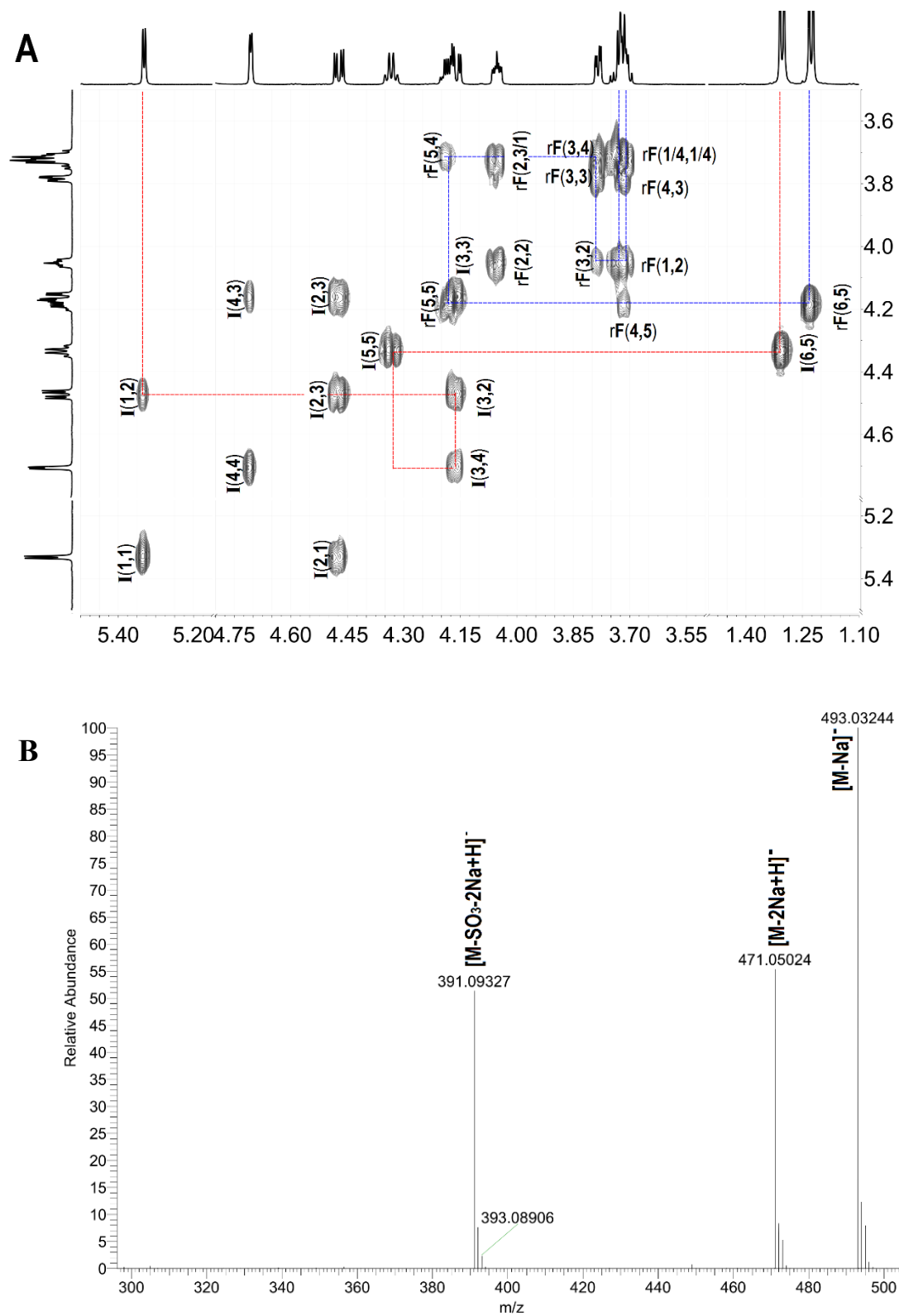
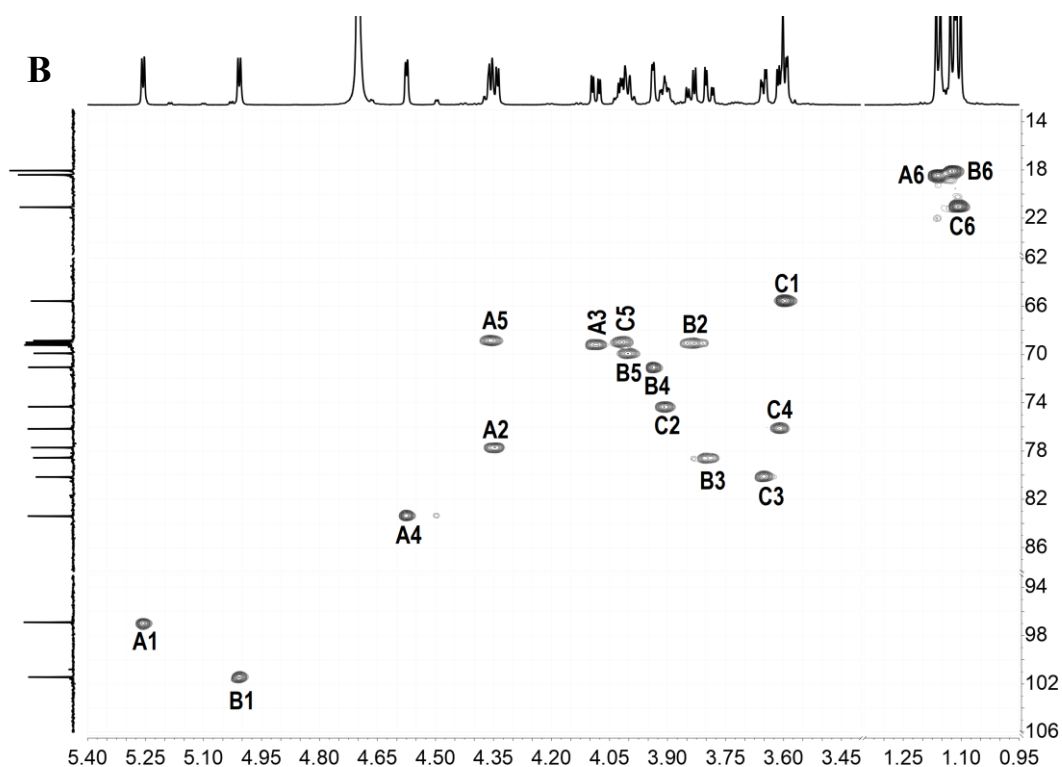
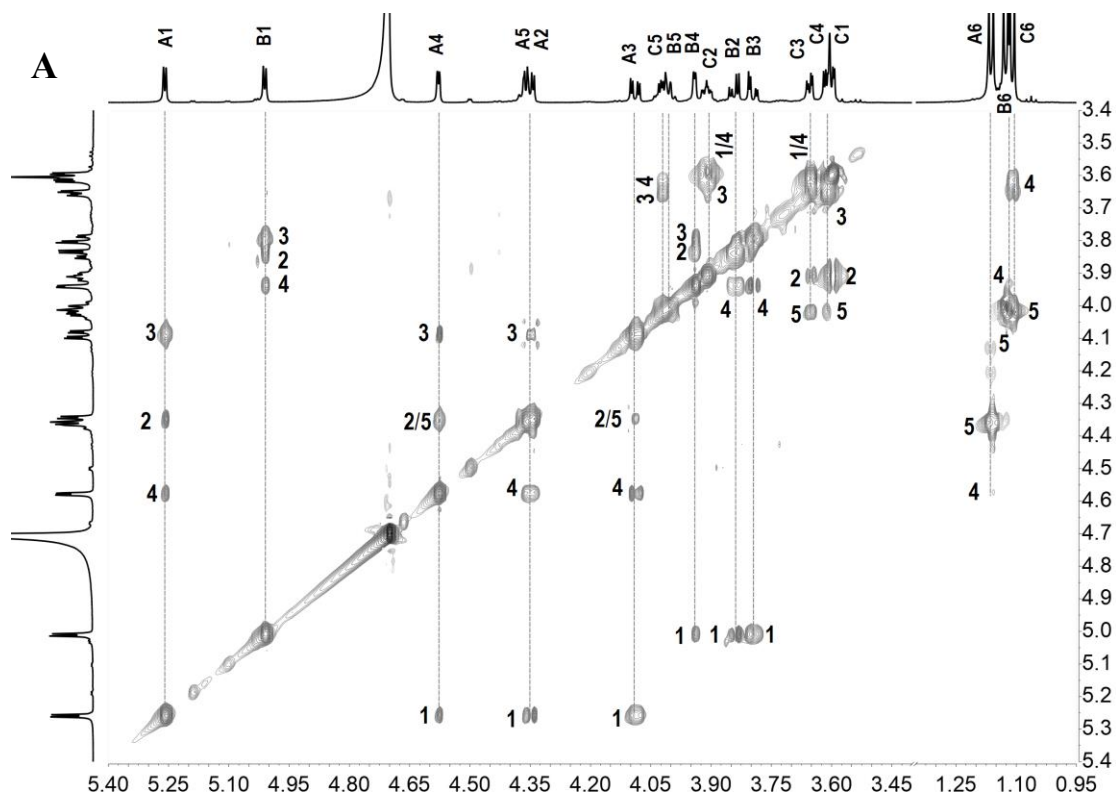


Figure S4. ^1H - ^1H COSY (A) spectrum of **FII** and its MS spectrum (B).



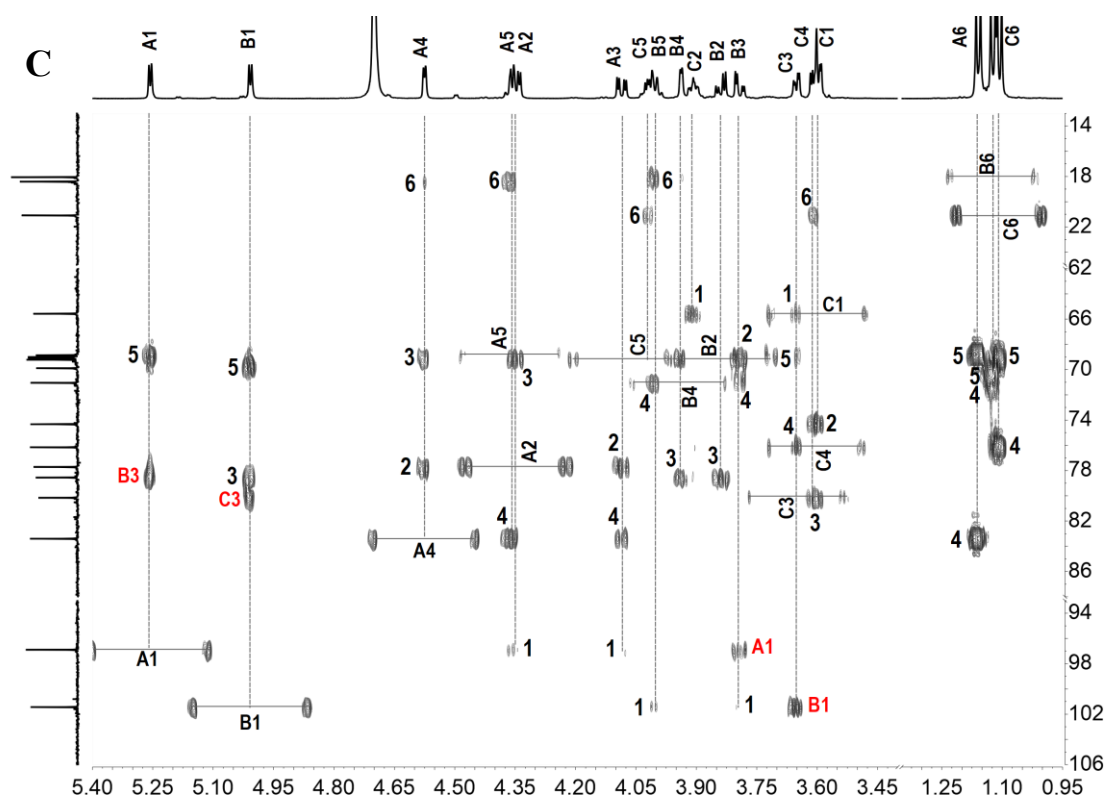
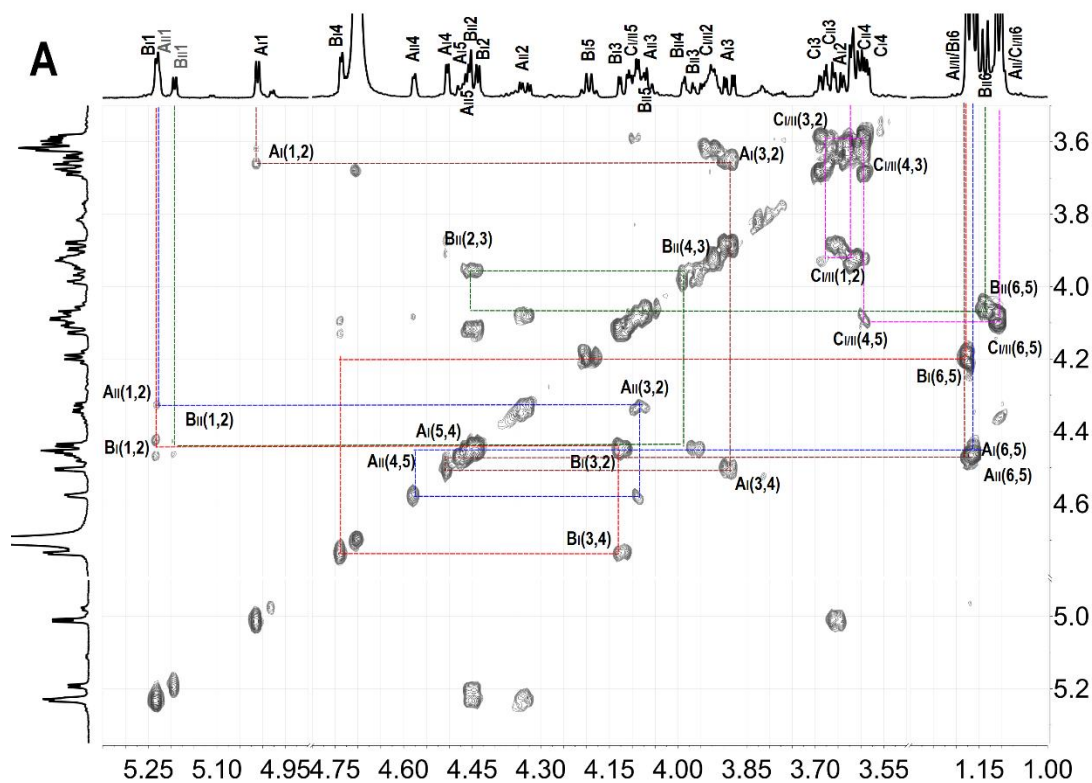


Figure S5. ^1H - ^1H TOCSY (A), ^1H - ^{13}C HSQC (B) and ^1H - ^{13}C HMBC (C) spectra of **III**.



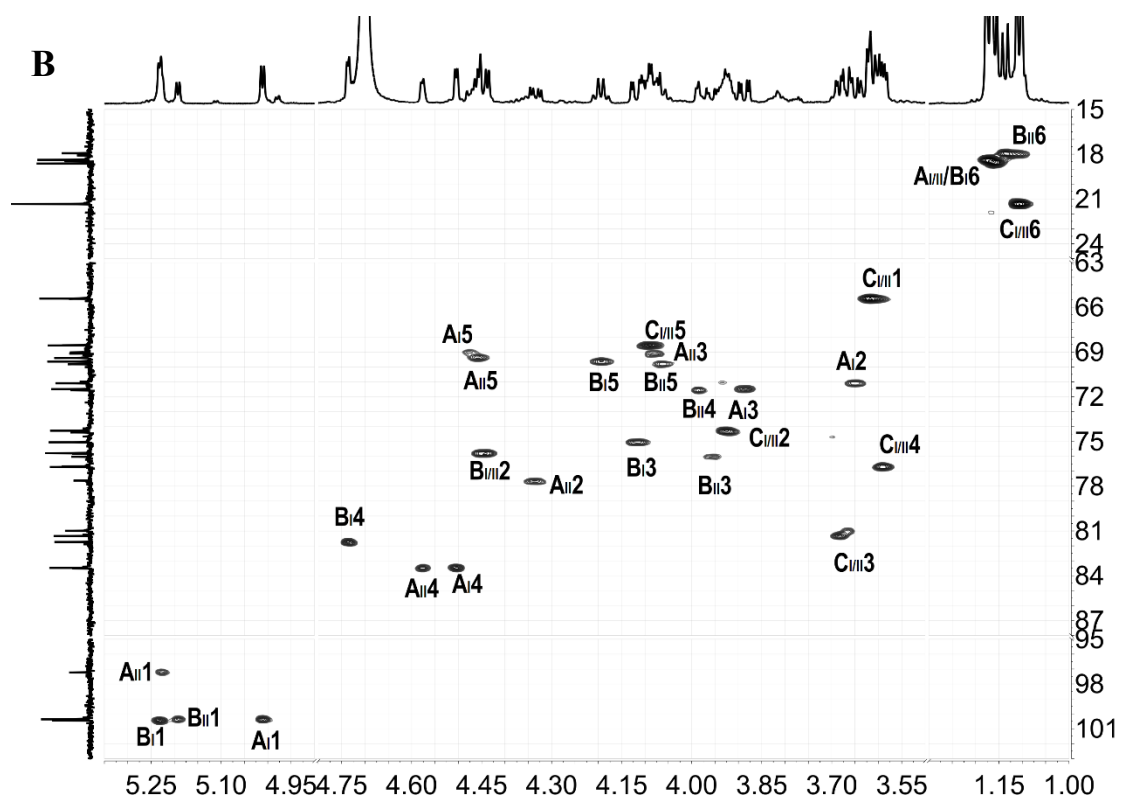


Figure S6. The ^1H - ^1H COSY (A) and ^1H - ^{13}C HSQC (B) spectra of oligosaccharides **FIV**.

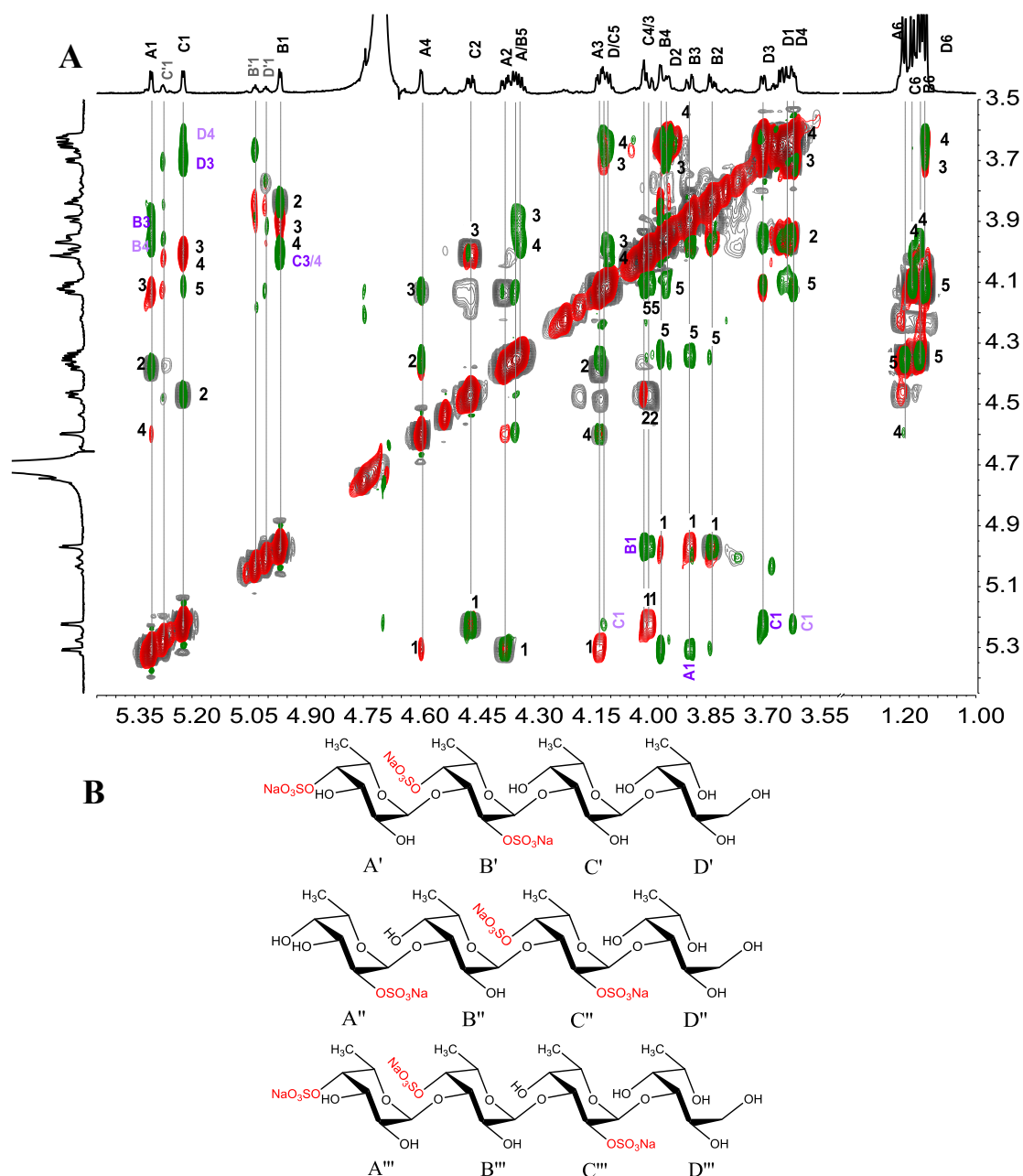


Figure S7. The overlapped spectra (A) of ^1H - ^1H COSY (gray), TOCSY (red) and ROESY (green) of oligosaccharides **FV** and the structure of the minor components in **FV** (B).

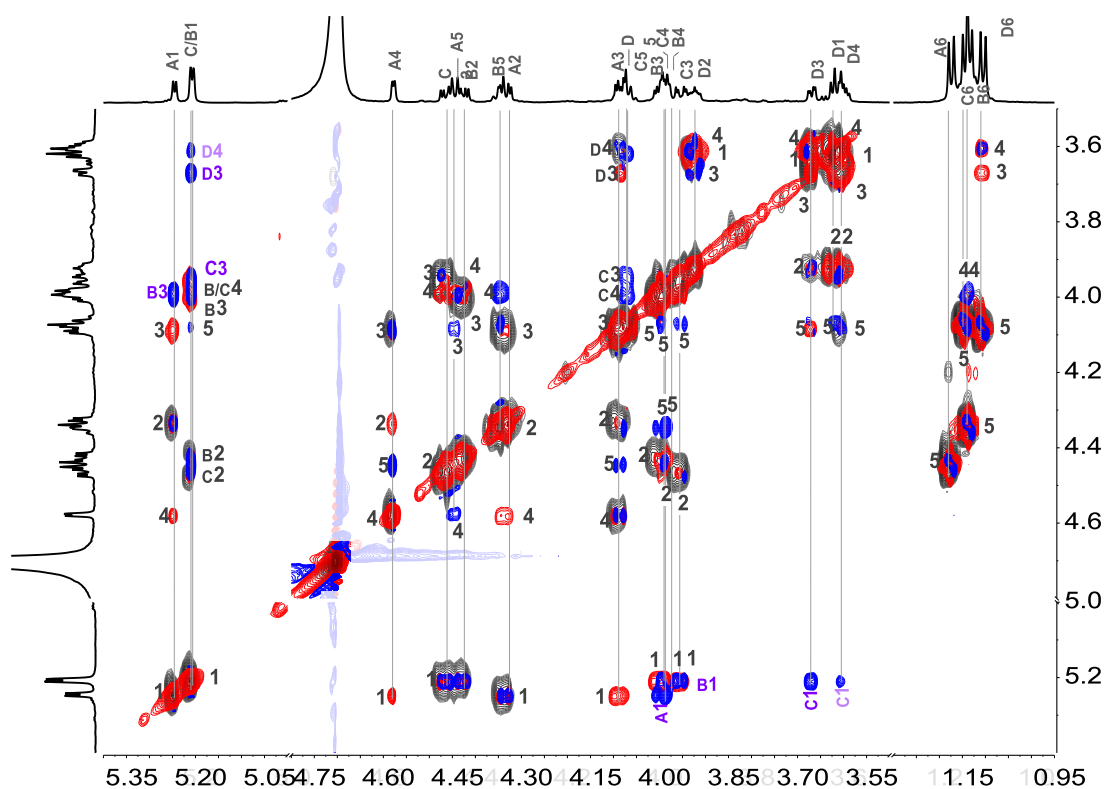


Figure S8. The overlapped spectra of ^1H - ^1H COSY (gray), TOCSY (red) and ROESY (blue) of oligosaccharides **FVI**.

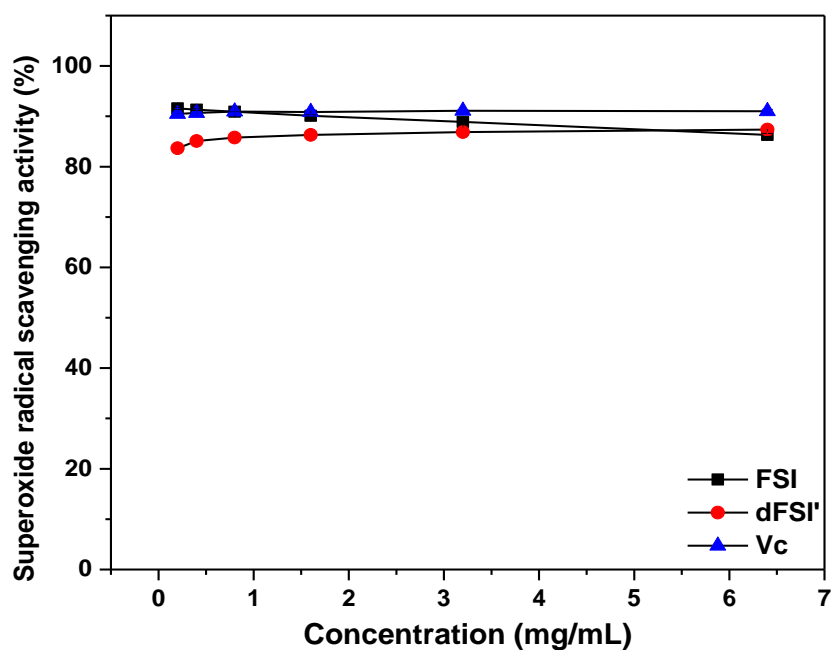


Figure S9. Scavenging effect of FSI, dFSI' and Vc on superoxide radicals at the concentration range of 0.2 - 6.4 mg/mL.

Shang, F., Mou, R., Zhang, Z., Gao, N., Lin, L., Li, Z., et al. (2018). Structural analysis and anticoagulant activities of three highly regular fucan sulfates as novel intrinsic factor Xase inhibitors. *Carbohydrate Polymers*, 195, 257-266.