

SUPPLEMENTARY MATERIAL

Anti-inflammatory isocoumarins from the bark of *Fraxinus chinensis* subsp. *rhynchophylla*

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ABSTRACT

A new isocoumarin (**1**) named fraxicoumarin was isolated from the bark of *Fraxinus chinensis* subsp. *rhynchophylla* along with three known compounds (**2–4**). The structure of the new compound was established by extensive spectroscopic studies and chemical evidence. The anti-inflammatory effects of the isolated compounds (**1–4**) on lipopolysaccharide (LPS)-induced RAW 264.7 macrophage cells were evaluated *in vitro*. Of the compounds tested, compounds **1** and **3** inhibited LPS-induced nitric oxide (NO) production in RAW 264.7 cells. Consistent with these findings, they also suppressed LPS-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) at the protein level in RAW 264.7 cells.

Keywords *Fraxinus chinensis* subsp. *rhynchophylla*; isocoumarins; anti-inflammation; nitric oxide; inducible nitric oxide synthase; cyclooxygenase-2

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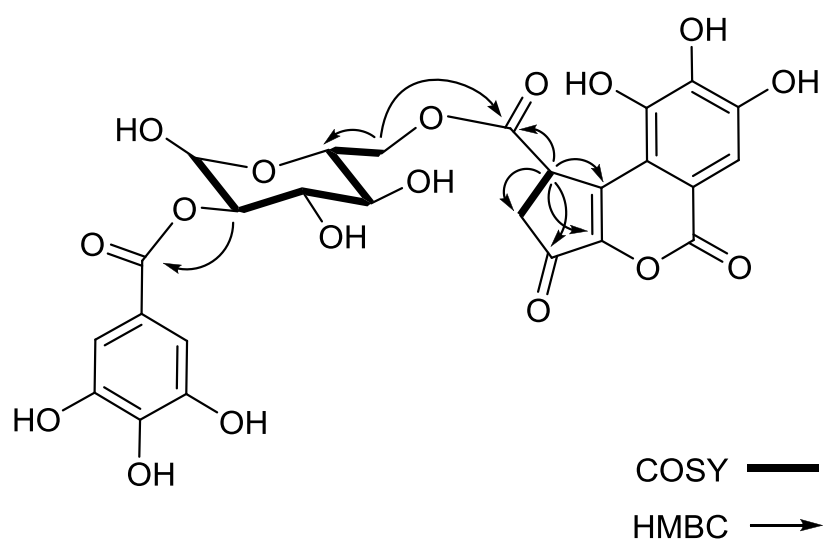


Figure S1. Key ^1H - ^1H COSY and HMBC correlations of compound **1**

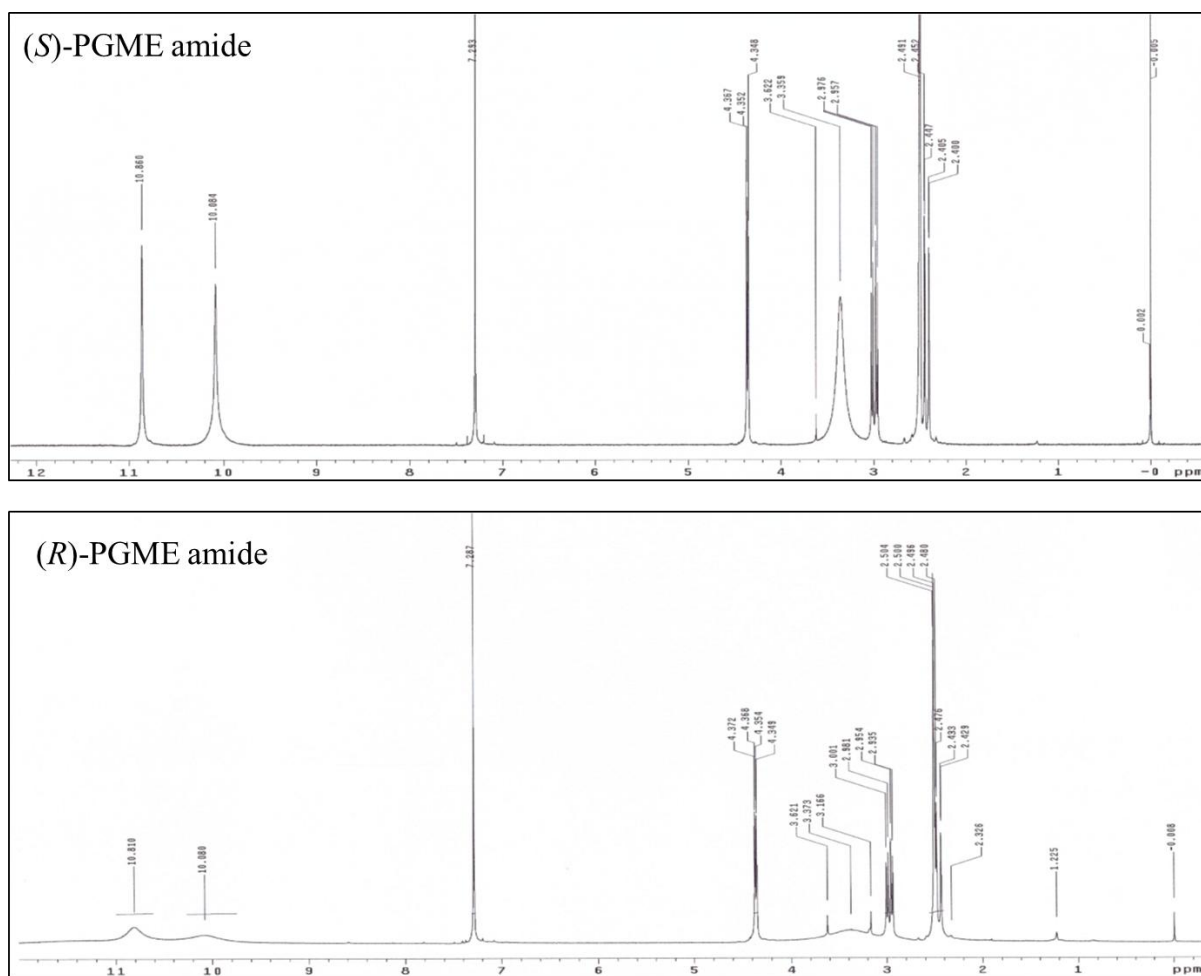
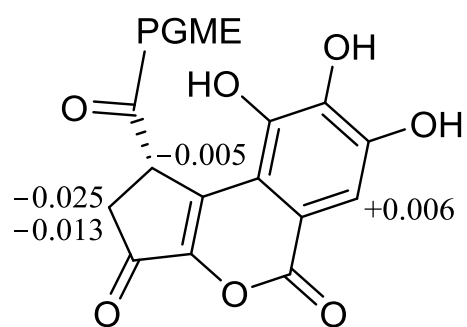


Figure S2. ^1H -NMR spectra of (*S*)- and (*R*)-PGME amides **1c** and **1d** (400 MHz, CD_3OD)



1c : R = (*S*)-PGME amide

1d : R = (*R*)-PGME amide

Figure S3. ^1H -NMR chemical-shift differences ($\Delta\delta$ ppm) between (*S*)- and (*R*)-PGME amides

1c and **1d**

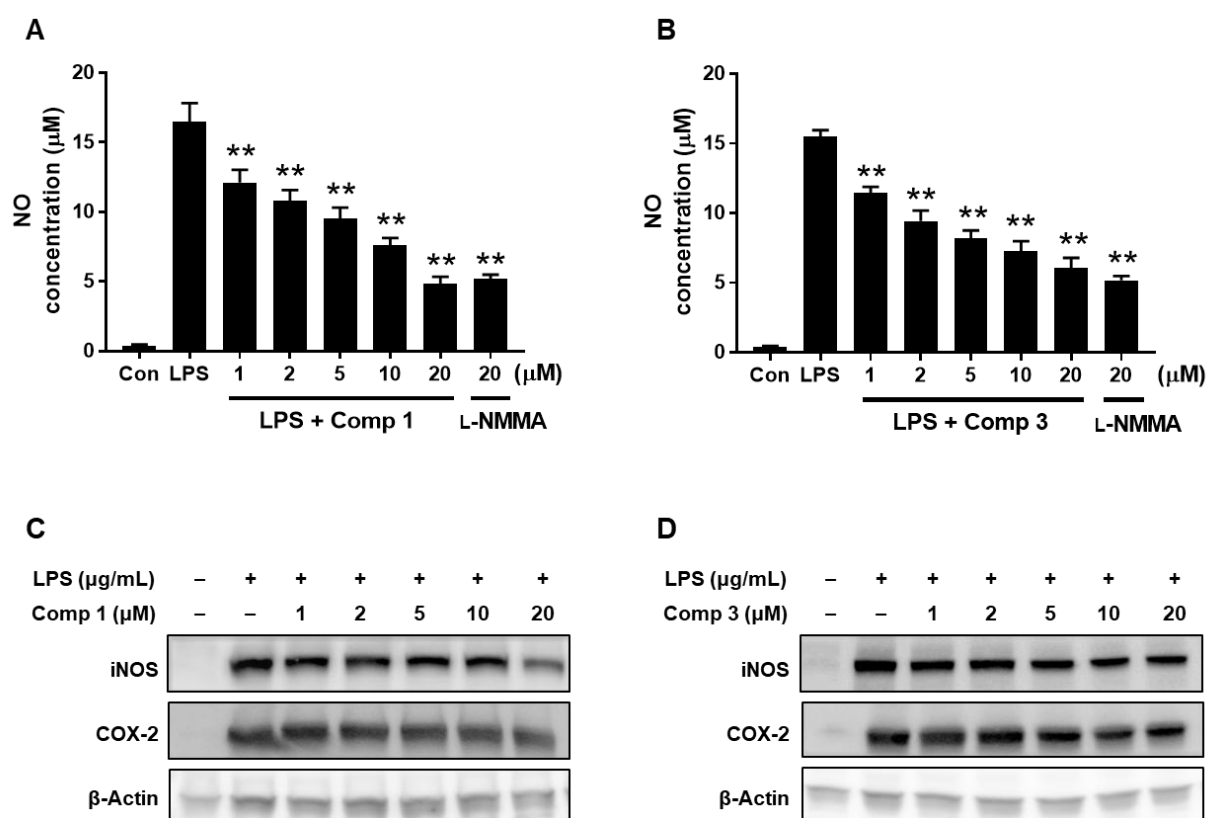


Figure S4. Anti-inflammatory activities of compounds **1** and **3**. Effects of compounds **1** and **3** on LPS-induced NO production (A and B) and LPS-induced iNOS and COX-2 protein expression (C and D) in RAW 264.7 cells. The values are expressed as means \pm SD from three independent experiments. * $P < 0.05$ vs. LPS, ** $P < 0.01$ vs. LPS

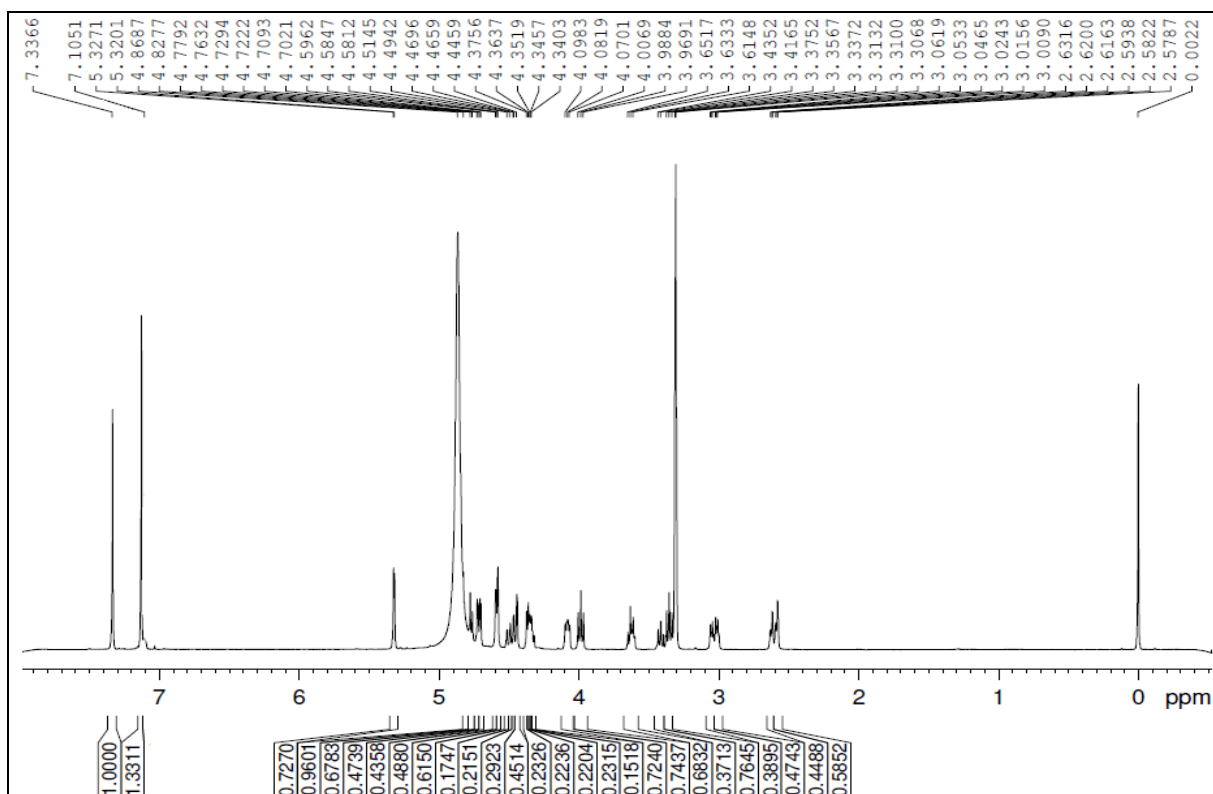


Figure S5. ¹H NMR spectrum of **1** (400 MHz, CD₃OD)

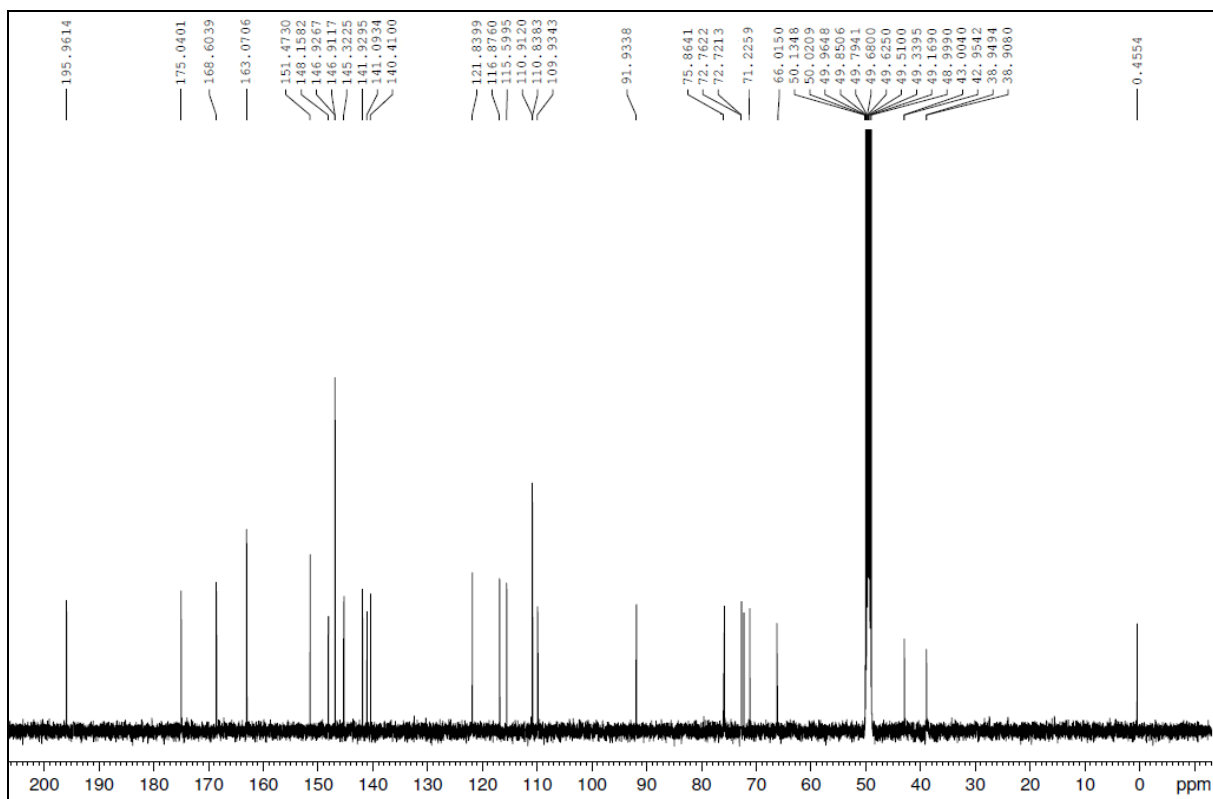


Figure S6. ¹³C NMR spectrum of **1** (100 MHz, CD₃OD)

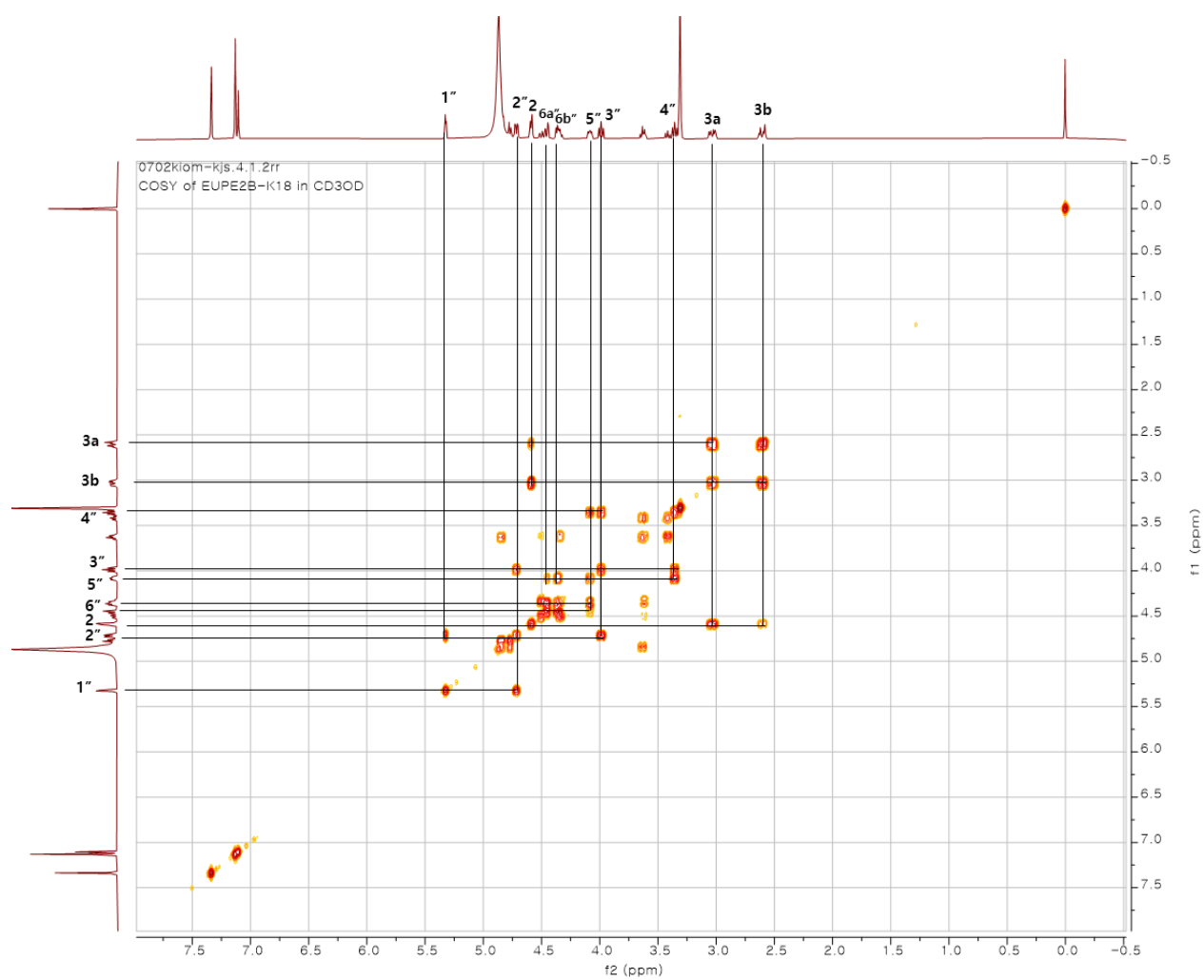


Figure S7. ^1H - ^1H COSY spectrum of **1** (500 MHz, CD_3OD)

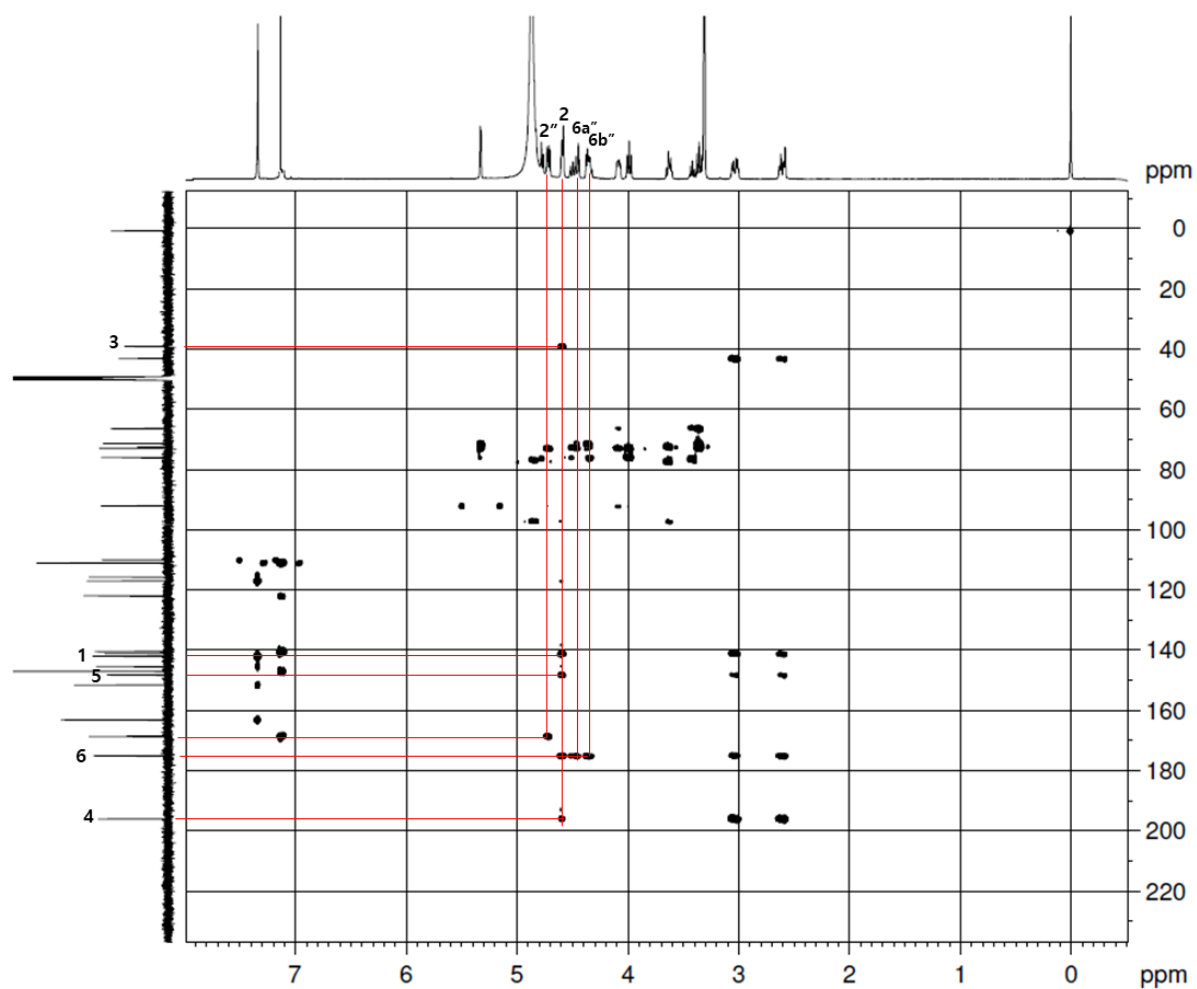


Figure S8. HMBC spectrum of **1** (500 MHz, CD₃OD)

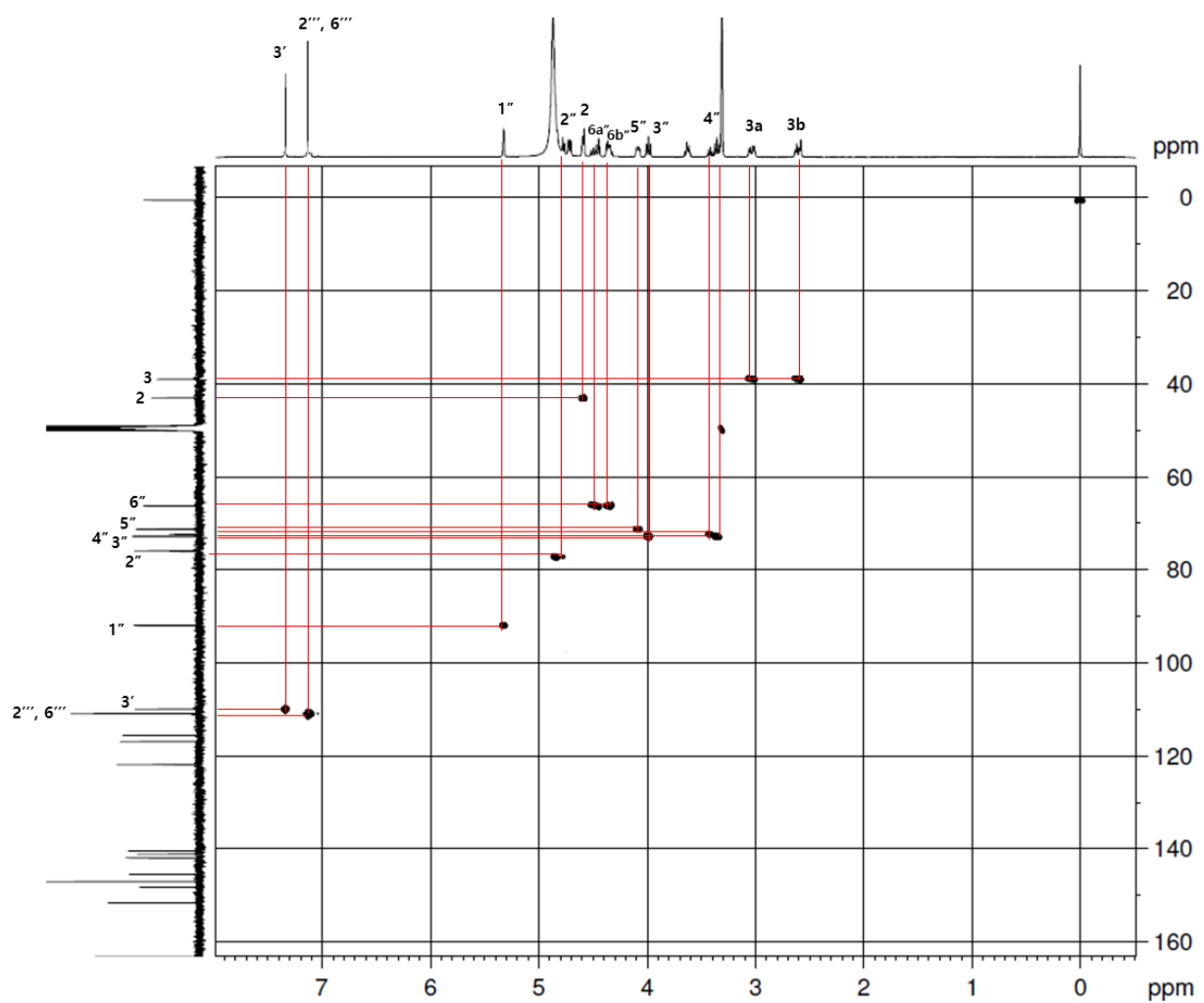


Figure S9. HMQC spectrum of **1** (500 MHz, CD₃OD)

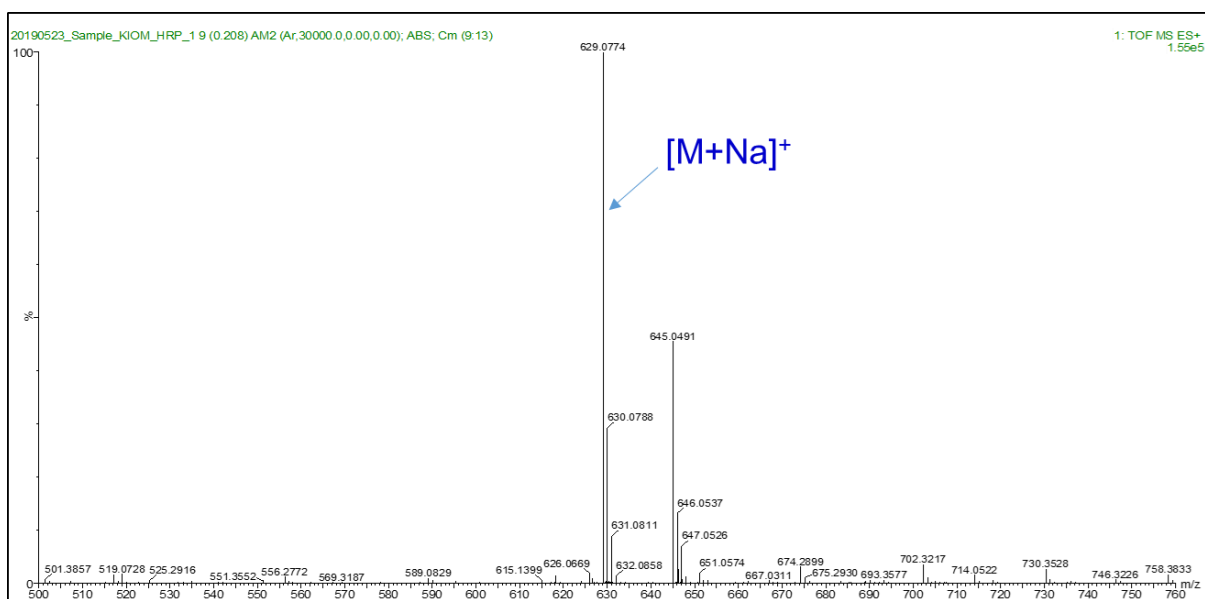


Figure S10. HRESIMS spectrum of **1**