

Supplementary Information

Flavin-Enabled Reductive and Oxidative Epoxide Ring Opening Reactions

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Supplementary Materials and Methods

1.1. General information

Commercially available compounds were purchased from Sigma-Aldrich, Aladdin, and Tokyo Chemical Industry Co., Ltd. (TCI) (Shanghai). Materials for column chromatography (CC) were silica gel (100–200 mesh; 300–400 mesh; Jiangyou Silica gel development, Inc.), Sephadex LH-20 (40–70 μm ; Amersham Pharmacia Biotech AB), and YMC*GEL ODS-A (12 nm S-50 μm ; YMC Company Ltd.). Thin-Layer-Chromatography (TLC, 0.1–0.2 mm or 0.3–0.4 mm) was conducted with pre-coated glass plates (silica gel GF254, 10–40 nm, Jiangyou Silica gel development, Inc.). Medium pressure liquid chromatography (MPLC) was performed on automatic flash chromatography (Cheetahtmmp 200, Bonna-Agela Technologies Co., Ltd.) with the wavelength at 304 nm. Semi-preparative HPLC was performed on a Hitachi HPLC station (Hitachi-L2130) with Diode Array Detector (Hitachi L-2455) using a Agilent Eclipse XDB-C18 (250 mm $\times$ 9.4 mm, 5 μm ; Agilent technology Ltd., USA) or Phenomenex ODS column (250 mm $\times$ 10.0 mm, 5 μm ; Phenomenex, USA), with a flow rate of 2.5 mL min⁻¹. HR-ESI-MS data were measured using a MaXis 4G UHR-TOFMS spectrometer (Bruker Daltonics Inc.). The optical rotations were recorded on a 341 Polarmeter (PerkinElme, Inc.). Deuterated NMR solvents were purchased from Cambridge Isotopes (Andover, MA). ¹H, ¹³C, and 2D NMR spectra were recorded on Bruker AV-500 MHz NMR spectrometer or Bruker AVANCE III HD 700 MHz NMR spectrometer (Bruker Biospin GmbH), with tetramethylsilane (TMS) as an internal standard.

PCR amplifications were performed on an Eppendorf Mastercycler nexus GX2 using Trans start Fast Pfu-DNA polymerase from TransGen biotech Co., LTD (Beijing, China). Restriction enzymes and DNA ligase were purchased from Thermo Fisher Scientific Inc (MA, USA). Unless otherwise stated, other biochemicals and chemicals were purchased from standard commercial sources. All DNA manipulations in *E. coli* and *Streptomyces* were performed according to standard procedures. DNA sequencing was performed in Guangzhou IGE Biotech Co., Ltd. All primers used in this work were synthesized by Tianyihuiyuan Biotech Co., Ltd (Guangzhou). Cofactors Flavin adenine dinucleotide (FAD), alloxazine, Riboflavin, Flavin mononucleotide (FMN), Nicotinamide adenine dinucleotide (NADH) are purchased from Sigma-Aldrich, USA.

1.2. Fermentation and isolation

The wild type strain *Micromonospora. rosaria* SCSIO N160 were used for the isolation of FST C (**1**), FST F (**15**), and FST D (**18**).^{1, 2} The strain *M. rosaria* SCSIO N160 were regularly propagated on Gauze's Medium No. 1 (soluble starch 2%, KNO₃ 0.1%, K₂HPO₄ 0.05%, MgSO₄·7H₂O 0.05%, FeSO₄·7H₂O 0.01%, pH 7.4) containing 3% sea salts. For preparing seed culture, a single colony of *M. rosaria* SCSIO N160 was inoculated into liquid production media consisting of starch 1%, glucose 2%, yeast extract 1%, corn powder 0.3%, beef extract 0.3%, MgSO₄·7H₂O 0.05%, K₂HPO₄ 0.05%, CaCO₃ 0.2%, and sea salt 3% (adjusted to pH 7.2 – 7.4 before sterilization) in a 250 mL Erlenmeyer flask containing 50 mL media in a rotary shaker (200 rpm) at 28 °C for 3 days. Freshly prepared seed cultures were used for inoculating a 24 liter-scale fermentation using the production media supplemented with polystyrene resin (Amberlite XAD-16; 5% vol, 200 mL) at 28 °C for 7 days.¹ The fermentation cultures were centrifuged at 3900 rpm. The mycelia and XAD-16 resins were filtered through a metal sieve (40 mesh) to be separated from the liquid portion. The products in the supernatants were absorbed by XAD-16 resins. The resins were washed twice with H₂O and transferred to a glass column to be extracted with 6 L acetone. The acetone fractions were concentrated by rotary vacuum evaporation to afford an aqueous residue, which was extracted four times with 2 L butanone. The mycelia cake was sonicated and extracted with 6 L butanone. After drying under vacuum, the crude extracts (20 g) were obtained. The crude extracts were dissolved in 20 mL of CH₃Cl/MeOH (8:2, v/v) and subjected to normal phase silica column (100-200 mesh) chromatography. The elution was performed by four gradient flow of CH₃Cl/MeOH (0:100; 95:5; 90:10 and 0:100) to yield four fractions (Fr.1-4). Fr. 1 was collected and further purified by semi-preparative C18 reverse phase Medium Pressure Liquid Chromatography (MPLC) (YMC*GEL ODS-A, 12 nm S-50 µm, 30 × 2.5 cm I.D.), eluting with a linear gradient (MeCN/H₂O, 20%-100%, 20 mL min⁻¹, 230 min). Fr.1-2 was further purified by semi-preparative HPLC using Phenomenex C18 column (5 µm, 250 mm × 10 mm, 2.5 mL min⁻¹). An isocratic elution gradient of 70% A (H₂O with 0.8% formic acid) /30% B (MeCN) with a flow rate of 2.5 mL min⁻¹ was applied to afford FST C (**1**) (250 mg). Fr. 2 was purified similar manner to afford FST F (**15**) (18 mg) and FST D (**18**) (3 mg). The identity and purity of isolated compounds were checked by comparing the HPLC retention time, UV spectrum, and LC-MS data with those of standard samples.

1.3. General analytical HPLC methods

HPLC analysis of non-enzyme reactions was carried out on the Agilent 1260 Infinity series instrument (Agilent Technologies Inc., USA) using a reversed phase C18 column (Kinetex® 5µm C18 100 Å, LC Column 150 × 4.6 mm, Phenomenex, USA) or a polar column (Comixsep®, P/N FMG-BPF5-EONU, Polar BiPFP 5u, 250 × 4.6 mm, China) with UV detection at 256 nm or 304 nm under the following program: solvent system (solvent A, 10% MeCN in water supplementing with 0.1% formic acid; solvent B, 90% MeCN in water); 5% B to 80% B (0 to 20 min), 80% B to 100% B (20 to 21 min), 100% B (21 to 24 min), 100% B to 5% B (24 to 25 min), 5% B (25 to 30 min); flow rate at 1 mL min⁻¹.

1.4. X-ray crystallographic data

Crystal data for FST B1 (**7**) mp 210 °C, C₁₈H_{15.54}O_{6.77} (M = 340.12 g/mol); orthorhombic, space group *P*2₁2₁2₁, *a* = 6.5747(2) Å, *b* = 12.7647(3), *c* = 17.4860(4) Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, *V* = 1467.50(7) Å³, *Z* = 4, *T* = 100.00(10) K, $\mu(\text{Cu K}\alpha) = 1.005 \text{ mm}^{-1}$, *D*_{calc} = 1.539 g/cm³, 6446 reflections measured ($8.576 \leq 2\theta \leq 148.056$), 2857 unique (*R*_{int} = 0.0199, *R*_{sigma} = 0.0176) which were used in all calculations. The final *R*₁ was 0.0404 (*I* > 2σ(*I*)) and *wR*₂ was 0.01105 (all data).

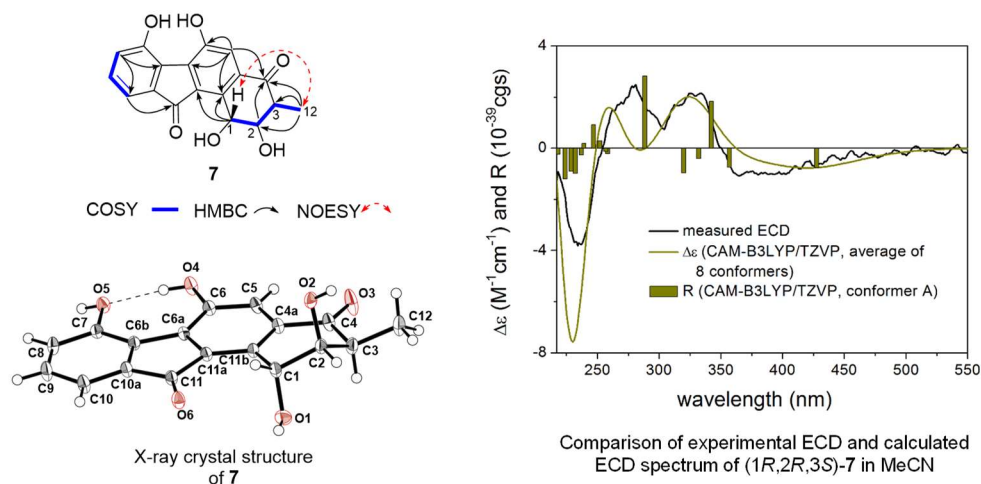
Crystal Data for FST C4 (**10**) mp 214 °C, C₁₈H₁₆O₉ (M = 376.31 g/mol): triclinic, space group *P*1 (no. 1), *a* = 8.0646(2) Å, *b* = 9.4581(4) Å, *c* = 12.3793(4) Å, $\alpha = 105.647(3)$, $\beta = 91.092(2)$, $\gamma = 110.786(3)$, *V* = 843.13(5) Å³, *Z* = 2, *T* = 100.00(10) K, $\mu(\text{Cu K}\alpha) = 1.035 \text{ mm}^{-1}$, *D*_{calc} = 1.482 g/cm³, 17982 reflections measured ($7.478 \leq 2\theta \leq 148.556$), 6389 unique (*R*_{int} = 0.0248, *R*_{sigma} = 0.0264) which were used in all calculations. The final *R*₁ was 0.0333 (*I* > 2σ(*I*)) and *wR*₂ was 0.0891 (all data).

Crystal Data for 1-*O*-methyl-FST B1 (**23**) mp 228 °C, C₂₀H₂₀O₇ (M = 372.36 g/mol): orthotriclinic, space group *P*2₁2₁2₁, *a* = 6.6367(4) Å, *b* = 14.0428(7) Å, *c* = 17.9812(12) Å, $\alpha = 90$, $\beta = 90$, $\gamma = 90$, *V* = 1675.81(17) Å³, *Z* = 4, *T* = 100.00(10) K, $\mu(\text{Cu K}\alpha) = 0.940 \text{ mm}^{-1}$, *D*_{calc} = 1.476 g/cm³, 9395 reflections measured ($7.988 \leq 2\theta \leq 148.046$), 3288 unique (*R*_{int} = 0.0623, *R*_{sigma} = 0.0592) which were used in all calculations. The final *R*₁ was 0.0663 (*I* > 2σ(*I*)) and *wR*₂ was 0.2017 (all data).

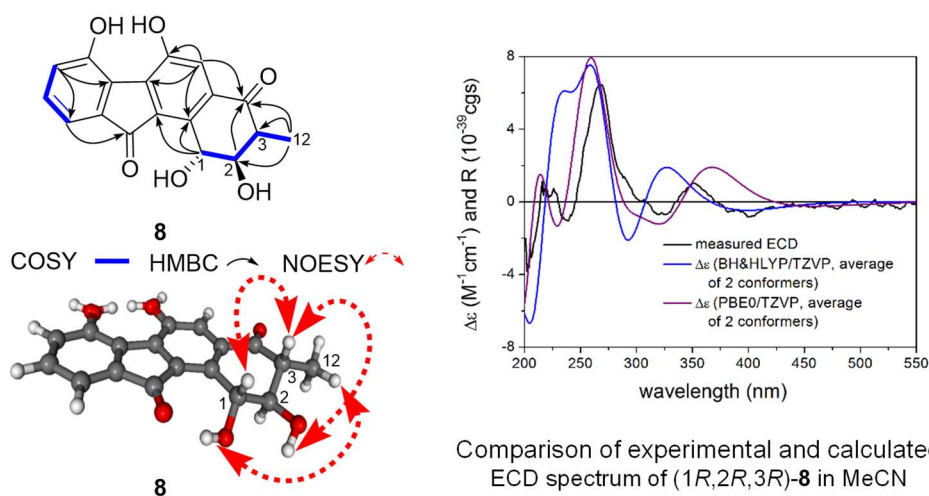
Crystal Data for Compound **40** mp 178 °C, C₁₁H₈O₃ (M = 188.17 g/mol): monoclinic, space group *P*2₁, *a* = 7.6523(5) Å, *b* = 4.7776(3) Å, *c* = 11.6829(9) Å, $\alpha =$

90° , $\beta = 90.544(8)^\circ$, $\gamma = 90^\circ$, $V = 427.10(5) \text{ \AA}^3$, $Z = 2$, $T = 100.01(12) \text{ K}$, $\mu(\text{Cu K}\alpha) = 427.10(5) \text{ mm}^{-1}$, $D_{\text{calc}} = 1.463 \text{ g/cm}^3$, 1758 reflections measured ($7.568^\circ \leq 2\Theta \leq 148.544^\circ$), 1084 unique ($R_{\text{int}} = 0.0445$, $R_{\text{sigma}} = 0.0497$) which were used in all calculations. The final R_1 was 0.0743 ($I > 2\sigma(I)$) and wR_2 was 0.1832 (all data).

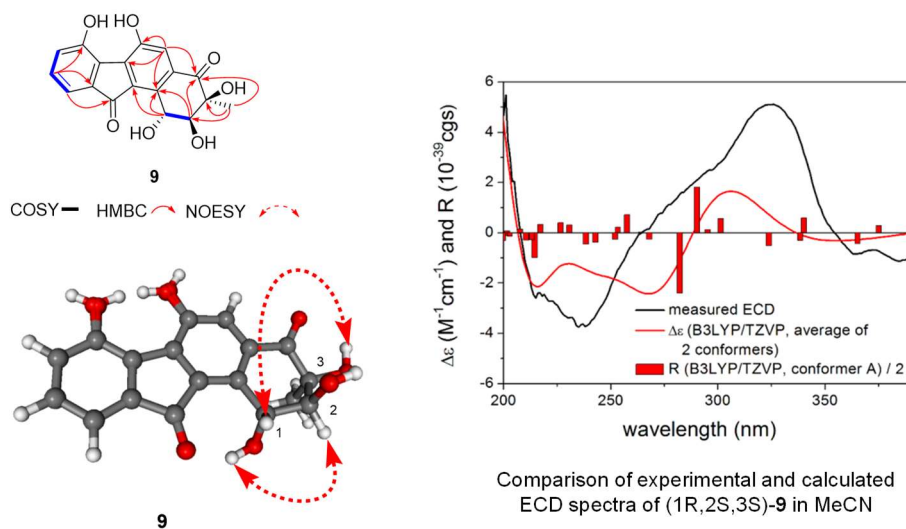
1.5. Structural elucidation of reaction products



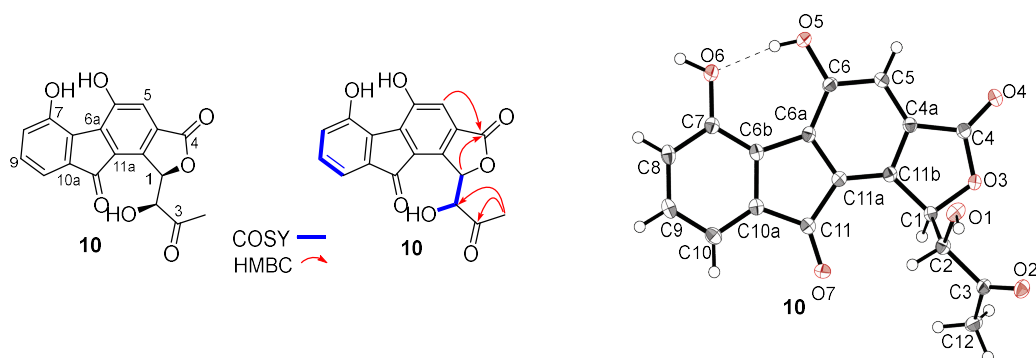
FST B1 (**7**) was isolated as yellow powder, $[\alpha]_D^{25} -9.4$ (c 0.05, MeOH). The molecular formula of **7** was established as $C_{18}H_{14}O_6$ according to HRESIMS m/z 325.0715 $[M - H]^-$ (calcd 325.0718, Supplementary Fig. 4). Extensive NMR analysis suggested that compound **7** had the same planar structure as FST B (Supplementary Table 2, Supplementary Fig. 4), however, the absolute configuration of FST B had not been previously established.³ NOESY correlations between H-1 & H₃-12 as well as H-1 & OH-2 (Supplementary Fig. 4) were consistent with the *cis*-configuration of H-2/H-3 in **7**. The electronic circular dichroism (ECD) spectrum of **7** was acquired and compared with that computed for **7** using the solution TDDFT-ECD methodology to support its assignment as the (1*R*,2*R*,3*S*) configuration (Supplementary Fig. 5). This assignment was confirmed by a single crystal X-ray analysis (CCDC 2036399) using Cu K α radiation with a Flack parameter value of 0.04(13) (Supplementary Table 3).



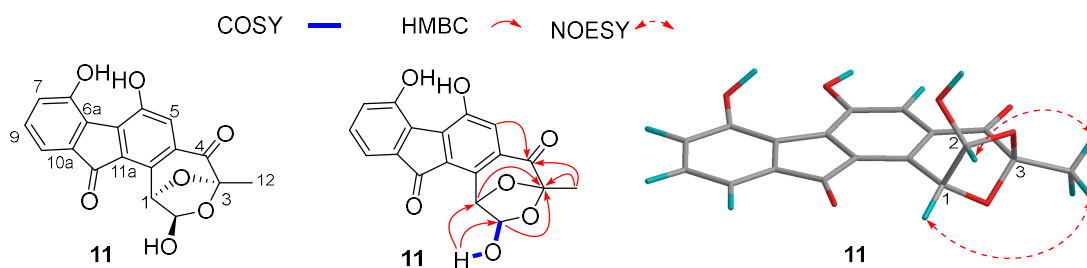
FST B2 (**8**) was isolated as yellow powder, $[\alpha]_D^{25}$ -8.8 (c 0.05, MeOH). The molecular formula was deduced to have $C_{18}H_{14}O_6$ based on HRESIMS m/z $[M - H]^-$ 325.0728 (calcd 325.0718) (Supplementary Fig. 6). The NMR spectra of **8** was essentially identical to that of **7** (Supplementary Table 2, Supplementary Fig. 6), the NOESY correlations of the 2-OH and H-3 indicated a *trans*-configuration of the H-2 and H-3 bond in **8**, which is different from that of **7** (Supplementary Fig. 4). The *trans* configuration of the 1-OH and 2-OH of **8** was further supported by NOESY correlations of H-1 and H-3 as well as 1-OH and 3-Me (Supplementary Fig. 6). Based on comparison of the experimental and calculated ECD spectra carried out on the (1*R*,2*R*,3*R*) stereoisomer of FST B1 (**7**), compound **8** was deduced to possess the (1*R*,2*R*,3*R*) configuration (Supplementary Fig. 7). In order to distinguish the two stereoisomers of FST B, **7** and **8** were named FST B1 and FST B2, respectively.



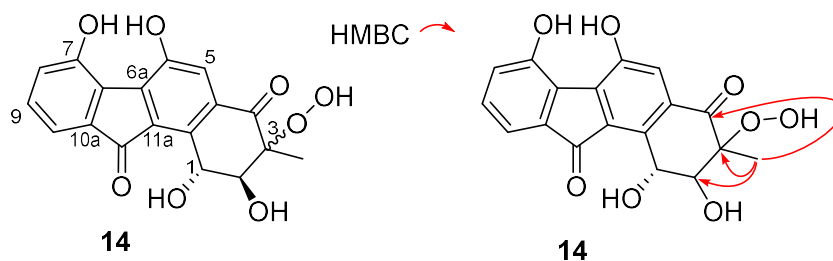
FST C3 (**9**) was obtained as yellow powder, $[\alpha]_D^{25}$ 7.4 (c 0.05, MeOH). The molecular formula of **9** was established as $C_{18}H_{14}O_7$ by HRESIMS m/z 341.0668 $[M - H]^-$ (calcd 341.0667), same as that of **3**. The 1H , ^{13}C , and 2D NMR data of **9** were highly similar to those of **3** (Supplementary Table 4, Supplementary Fig. 8). However, NOESY correlations of H-1 & 2-OH as well as 1-OH & 3-Me (Supplementary Fig. 8, Fig. 17) indicated a *cis* configuration of the 2-OH and 3-OH, differing from the *trans* configuration in **3**. Consequently, the absolute configuration of **9** was assigned as (1*R*,2*S*,3*S*) by means of the good agreement observed between the experimental ECD of **9** and the TDDFT-ECD spectrum of (1*R*,2*S*,3*S*)-**9** (Supplementary Fig. 9). Compound **9** was designated FST C3.



FST C4 (**10**) was obtained as yellow powder, $[\alpha]_D^{25} -12.6$ (c 0.05, MeOH). The molecular formula of **10** was established as $C_{18}H_{11}O_7$ by HRESIMS m/z 339.0519 $[M - H]^-$ (calcd 335.0510), requiring 13 degrees of unsaturation. Careful analysis of 1D and 2D NMR data of **10** (Supplementary Table 5, Supplementary Fig. 10) indicated that the rings B, C, and D of **10** were the same as those of **1**. These assignments were supported by the presence of characteristic aromatic ABC spin system (δ_H 6.59, 1H, dd, $J = 0.7, 8.2$ Hz; δ_H 6.93, 1H, dd, $J = 7.2, 8.2$ Hz; δ_H 6.67, 1H, dd, $J = 0.7, 8.1$ Hz) and HMBC correlations from H-5 to C-6/C-6a/C-11b, from H-8 to C-6b/C-10, from H-9 to C-7/C-10a, and from H-10 to C-8/C-6b. Besides the signals for these fragments, the remaining ^{13}C NMR data of **10** showed 4 carbons resonances for two sp^3 methine carbons (δ_C 81.2; δ_C 75.5), and two carbonyl carbons (δ_C 210.1; δ_C 170.4). Detailed analysis of 1D and 2D NMR data, these signals were assigned as a (1-hydroxy-2-oxopropyl)furan-2-one unit fused to the ring B at C-4a and C-11b, which were supported by the COSY correlations of H-1 and H-2, HMBC correlations from H₃-12 to C-2 (δ_C 210.1)/C-3, and from H-1/H-5 to C-4 (δ_C 170.4), as well as three less degrees of unsaturation. Finally, high-quality crystals of **10** were obtained to allow the successful performance of a single-crystal X-ray diffraction experiment using Cu K α radiation (Supplementary Table 6 CCDC 2129081), to confirm the planar structure of **10**, and to establish the absolute configuration of **10** as (1*R*,2*S*) with the Flack -0.01(5) (Supplementary Table 6).

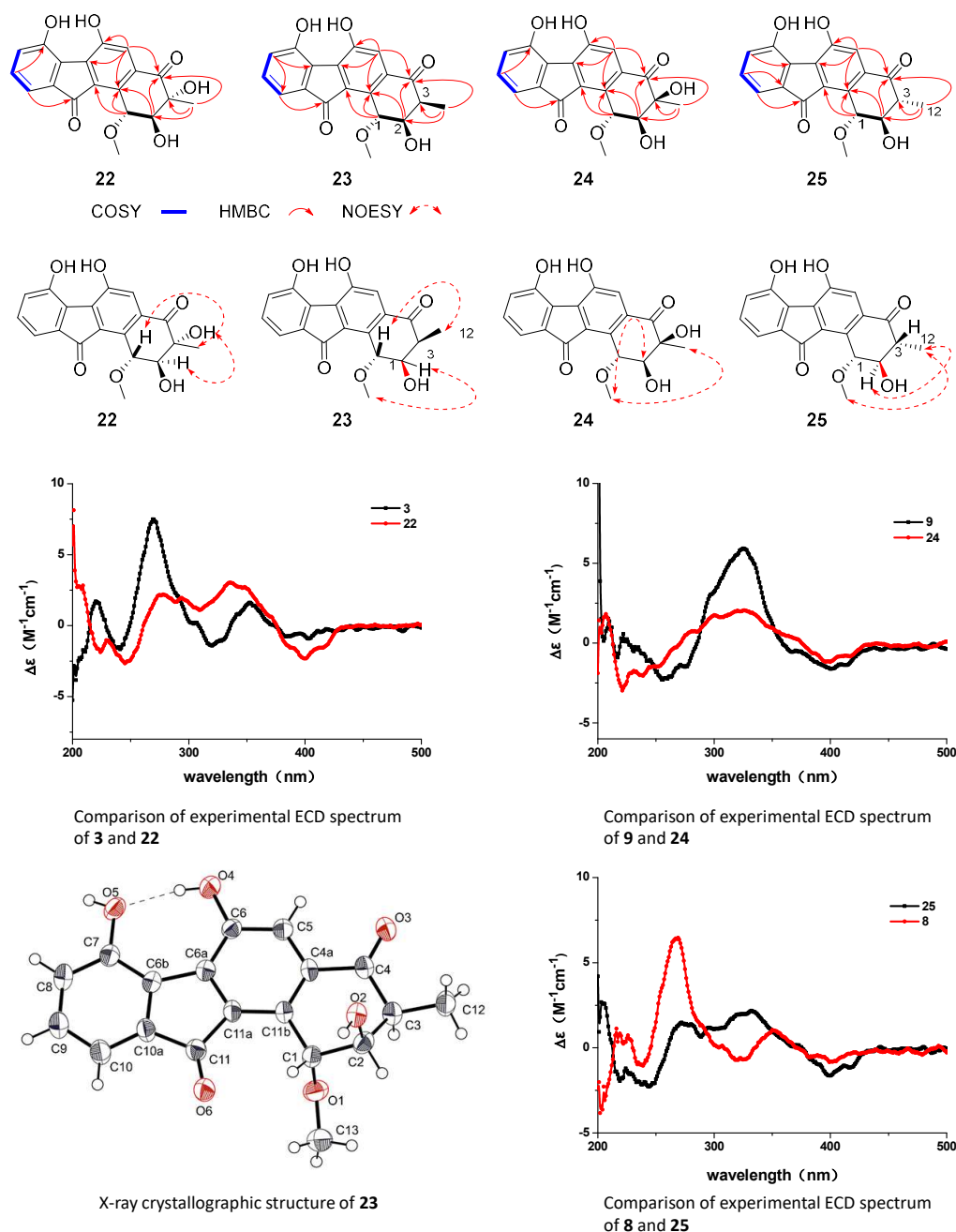


FST C5 (**11**) was obtained as yellow powder, $[\alpha]_D^{25} -3.2$ (c 0.05, MeOH). the molecular formula of **11** was established as $C_{18}H_{12}O_7$ by HRESIMS m/z 339.0512 $[M - H]^-$ (calcd 339.0510). Careful analysis of 1H NMR data of **11** (Supplementary Table 5, Supplementary Fig. 11) showed that the rings B, C, and D were the same as those of **1**, which was confirmed by detailed assignment of 2D NMR data of **11**. In additions to signals for fragments of rings B–D, the remaining ^{13}C NMR data of **11** showed 4 carbons resonances for two sp^3 methine carbons (δ_C 78.5; δ_C 98.0), one non-protonated sp^3 hybridized oxygenated carbon (δ_C 106.7), and one carbonyl carbons (δ_C 189.0), which were assigned as a 6,8-dioxabicyclo[3.2.1]oct-2-en-4-one unit fused to the ring B at C-4a and C-11b. This assignment was further supported by the COSY correlations of 2-OH and H-2, HMBC correlations from H₃-12 to C-3/C-4, from 2-OH to C-1/C-2, from H-1/H-2 to C-3, from H-5 to C-4, as well as three less degrees of unsaturation. Thus, the planar structure of **11** was assigned. The relative configuration of **11** was assigned by the NOESY correlations of H-1/H-2/H₃-12 (Supplementary Fig. 11), which indicated that H-1/H-2/H₃-12 located on the same side of the dioxolane ring. Given the configuration of C-1 remained unchanged, the absolute configuration of **11** was tentatively assigned as (1*R*,2*R*,3*R*).



Hydroperoxyfluostatin (**14**) was obtained as yellow powder and deduced the molecular formula $C_{18}H_{14}O_8$ by HRESIMS m/z 357.0613 $[M - H]^-$ (calcd 357.0616) (Supplementary Fig. 25), which is 34 mass units greater than that of **1**. Careful analysis of 1H NMR data (Supplementary Table 5, Supplementary Fig. 25) of **14** indicated the presence of characteristic aromatic ABC spin system (δ_H 6.56, 1H, dd, $J = 0.5, 8.0$ Hz; δ_H 6.91, 1H, dd, $J = 6.9, 8.0$ Hz; δ_H 6.68, 1H, d, $J = 0.5, 6.9$ Hz) and one singlet aromatic proton (δ_H 6.92, 1H, s), which suggested that the rings B, C, and D of **14** were the same as those of **1**. The deshielded C-2 (δ_C 72.0, Δ 8.0 ppm) and C-3 (δ_C 87.6, Δ 28.1 ppm) (assigned by HMBC correlations) in **14** suggested that the 2,3-epoxy ring in **1** is opened in **14**. The presence of a hydroperoxy group at C-3 in **14** was supported by the molecular formula of **14** and the chemical shift value of C-3 (δ_C 87.6). Thus, the planar structure of

14 was assigned. Given **14** was the ring opened product of **1** and the observed conversion of **14** to both **3** and **9**, compound **14** is likely a mixture of the (1*S*,2*S*,3*R*) and (1*S*,2*S*,3*S*) stereoisomers.



1-*O*-methyl-FST C2 (**22**) was obtained as yellow powder, $[\alpha]_D^{25}$ 6.4 (*c* 0.05, MeOH). The molecular formula of **22** was assigned as $C_{19}H_{16}O_7$ based on HRESIMS m/z 335.0830 $[M - H]^-$ (calcd 335.0823) 1H and ^{13}C NMR data (Supplementary Table 7, Supplementary Fig. 29) of **22** were highly similar to those of FST F (**15**). The deshielded C-2 (δ_C , 71.6, Δ 12.2 ppm) and C-3 (δ_C , 75.0, Δ 18.2 ppm) in **22** suggested the presence of

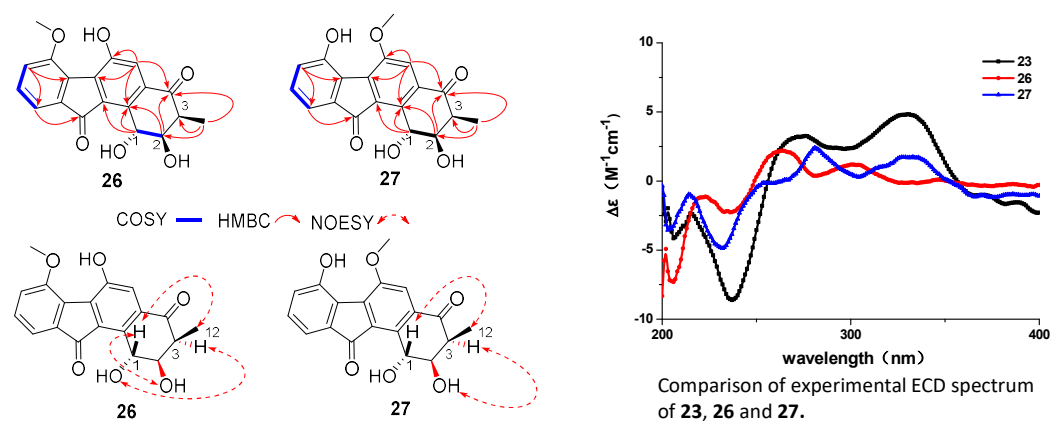
a vicinal diol subunit at C-2/C-3, instead of 2,3-epoxy ring in **15**. This assignment was supported by the HMBC correlations from H₃-12 to C-2/C-3/C-4. Further detailed analysis of 2D NMR data of **22** confirmed the its assigned planar structure. The relative configuration of **22** was deduced from the NOESY correlations of H-1 & H₃-12 as well as H-2 & OH-3 which suggested the *trans*-configuration of H-1/H-2, and the *cis*-configuration of H-2/OH-3 in **22** (Supplementary Fig. 29). Based on comparison of the experimental ECD spectra of **22** and **3**, the absolute configuration of **22** was assigned as (1*R*,2*S*,3*R*).

1-*O*-methyl-FST B1 (**23**) was obtained as yellow crystal, $[\alpha]_D^{25}$ -14.8 (*c* 0.05, MeOH). The molecular formula of **23** was assigned as C₁₉H₁₆O₆ based on HRESIMS *m/z* 339.0881 [M – H][–] (calcd 339.0874), corresponding to 12 degrees of unsaturation. ¹H and ¹³C NMR data of **23** (Supplementary Table 8, Supplementary Fig. 30) were similar to those of FST F (**15**).¹ The difference was that **23** had one more methine and one less non-protonated carbon than that of **15**, which suggested 2,3-epoxy ring in **15** has opened. These suggestions were supported the COSY correlations of H-3 and H₃-12, and HMBC correlations from H₃-12 to C-2/C-3/C-4. Further detailed assignment of 2D NMR data confirmed the structure of **23** and designated 1-*O*-methyl-FST B1 (**23**). The relative configuration of **23** was deduced from NOESY correlations of H-1 & H₃-12 as well as H₃-13 & H-2 (Supplementary Fig. 30), which suggested the *cis* configuration of H-1/H-2 in **23**. However, the relative configuration of H-2 and H-3 could not be assigned due to the lack of observed NOESY correlations. Finally, high-quality crystals of **23** were obtained to allow the successful performance of a single-crystal X-ray diffraction experiment using Cu K α radiation to establish the absolute configuration of **23** as (1*R*,2*R*,3*S*) based on the Flack parameter value of 0.0 (3) (Supplementary Table 3, CCDC 2036400).

1-*O*-methyl-FST C3 (**24**) was obtained as yellow-red powder, $[\alpha]_D^{25}$ 7.4 (*c* 0.05, MeOH). The molecular formula of **24** was assigned as C₁₉H₁₆O₇ based on HRESIMS *m/z* 355.0822 [M – H][–] (calcd 355.0823), the same as that of **22**. The high similarity of the 1D and 2D NMR spectra of compounds **24** (Supplementary Table 7, Supplementary Fig. 31) and **22** indicated that they shared the same planar structure. The NOESY correlations of H₃-13 & H-2 and H₃-13 & H₃-12 suggests the *trans*-configuration of H-1/H₃-12 (Supplementary Fig. 31), different from that of **22**. To determine the absolute

configurations of **24**, the ECD spectra was compared with that of **9**. The almost identical Cotton effects of **24** and **9** suggested that **24** should have the (1*R*,2*S*,3*S*) configuration.

1-*O*-methyl-FST B2 (**25**) was obtained as yellow powder, $[\alpha]_D^{25}$ 3.8 (*c* 0.05, MeOH). The molecular formula of **25** was assigned as C₁₉H₁₆O₆ based on HRESIMS *m/z* 339.0881 [M – H][–] (calcd 339.0874), the same as that of **23**. Extensive NMR analysis suggested that **25** (Supplementary Table 8, Supplementary Fig. 32) and **23** had the same planar structure. The NOESY correlations of the H₃-13 & H₃-12 indicated a *cis*-configuration of H-1 and H-3 in **25** (Supplementary Fig. 32), which is different from that in **23**. The *trans*-configuration of H-2 and H-3 in **25** was determined by the NOESY correlations of H-2 & H₃-12. Based on comparison of the experimental ECD spectra of **25** and **8**, the absolute configuration of **25** was assigned as (1*R*,2*R*,3*R*).

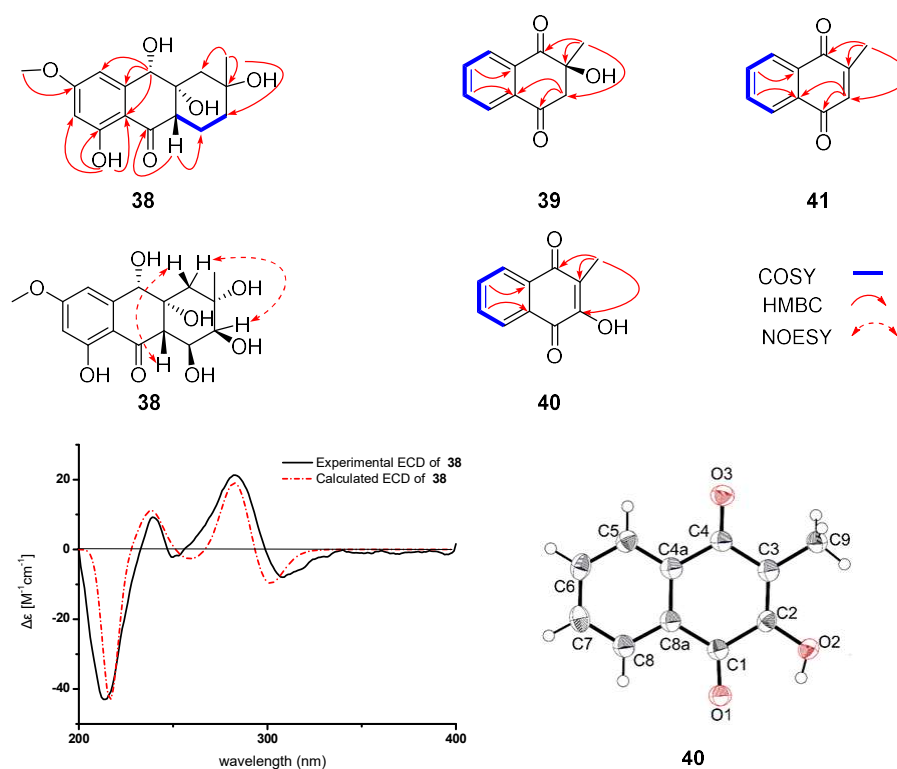


FST M (**26**) was obtained as yellow powder, $[\alpha]_D^{25}$ 4.4 (*c* 0.05, MeOH). The molecular formula of **26** was established as C₁₉H₁₆O₆ by HRESIMS *m/z* 339.0881 [M – H][–] (calcd 339.0874). The ¹H and ¹³C NMR data (Supplementary Table 9, Supplementary Fig. 38) of **26** were similar to that of **16**. The difference was that **26** has a doublet methyl signal, instead of the singlet methyl signal in **16**, suggesting that the 2,3-epoxy in **16** was opened and transformed to the secondary alcohol in **26**. Further detailed analysis of 2D NMR data assigned the structure of **26**. The *trans* configuration of H-1/H-2 and *cis* configuration of H-2/H-3 in **26** was deduced by ROESY correlations of H-1 & H₃-12, 2-OH & H₃-12, and H-1 & 2-OH (Supplementary Fig. 38).

6-*O*-methyl-FST B1 (**27**) was obtained as yellow powder, $[\alpha]_D^{25}$ 5.6 (*c* 0.05, MeOH). The molecular formula of **27** was established as C₁₉H₁₆O₆ by HRESIMS *m/z* 339.0885 [M – H][–] (calcd 339.0874). The ¹H, ¹³C NMR data (Supplementary Table 9,

Supplementary Fig. 39) of **27** were similar to that of **17**. The difference was that **27** has a doublet methyl signal, instead of the singlet methyl signal in **17**, which suggested that the 2,3-epoxy in **17** was opened and transformed to the secondary alcohol in **27**. Further detailed analysis of 2D NMR data assigned the structure of **27**. The relative configuration of **27** was deduced by NOESY correlations of H-1 & H₃-12 and H₃-12 & 2-OH, which supported the *trans*-configuration of H-1/H-2, and the *cis*-configuration of H-2/H-3 in **27** (Supplementary Fig. 39).

To determine the absolute configurations of **26** and **27**, their ECD spectra were compared with that of **23**. The almost identical cotton effects of **23**, **26** and **27** suggested that compounds **26** and **27** should have the (1*R*,2*R*,3*S*) configuration, the same as that of **23**.



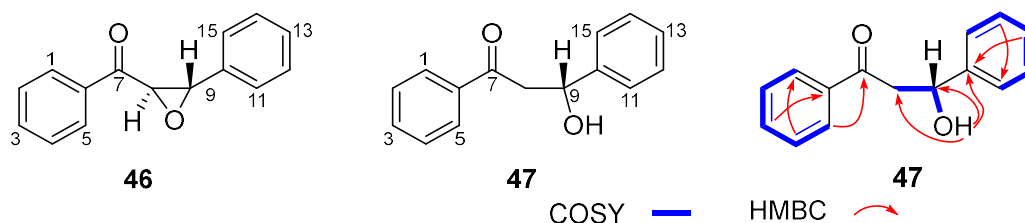
Compound **38** was obtained as pale-white powder, $[\alpha]_{\text{D}}^{25}$ 2.8 (*c* 0.05, MeOH). The molecular formula of **38** was assigned as C₁₆H₂₀O₈ based on HRESIMS *m/z* 339.1089 [M – H][–] (calcd 339.1085), indicating 7 degrees of unsaturation. The ¹H and ¹³C NMR data of **38** (Supplementary Table 10, Supplementary Fig. 48) displayed high similarity to those of **36**. The difference was that **38** had one more methine, instead of a non-protonated carbon in **36**, which suggested that the ring of 4a,9a-epoxy in **36** was opened in **38** and transformed to the secondary alcohol. The careful analysis 2D NMR data assigned the structure of **38**. According to the NOESY correlations of H-4a/H-1a, and H-1b/H-3 was

deduced to locate on the same side as 3-OH (Supplementary Fig. 48). Consequently, the absolute configurations of H-4a and H-9a were assigning to (4a*S*,9a*R*), which were deduced by comparison of the experimental and calculated ECD spectra of (4a*S*,9a*R*)-**38**.

3-Hydroxy-3-methyl-2,3-dihydronaphthalene-1,4-dione (**39**) was obtained as white powder, $[\alpha]_{\text{D}}^{25}$ 4.6 (*c* 0.05, MeOH). The molecular formula of **39** was assigned as C₁₁H₁₀O₃ based on HRESIMS *m/z* 189.0550 [M – H][–] (calcd 189.0557), indicating 7 degrees of unsaturation. The ¹H, ¹³C, and HSQC spectra of **39** (Supplementary Table 11, Supplementary Fig. 51) indicated the presence of methylene group, which suggested the absence of the 2,3-epoxy ring of **37** in **39**. This suggestion was supported by HMBC correlations from H₃-11 to C-2/C-3/C-4 and from H-2 to C-2/C-4/C-8a. Detailed 2D NMR analysis assigned the planar structure of **39**. Given that **39** was epoxide ring-opening product of **37**, the (3*S*) absolute configuration of C-3 was tentatively assigned.

2-Hydroxy-3-methyl-1,4-naphthoquinone (phthiocol, **40**) was obtained as white powder, $[\alpha]_{\text{D}}^{25}$ 5.2 (*c* 0.05, MeOH). The molecular formula of **40** was assigned as C₁₁H₉O₃ based on HRESIMS *m/z* 189.0550 [M – H][–] (calcd 189.0546), indicating 8 degrees of unsaturation. A comparison of ¹H and ¹³C NMR spectral data of **40** (Supplementary Table 11, Supplementary Fig. 52) and **39** revealed their high structural similarity. Compound **40** differed from **39** in the presence of resonances at δ_{C} 158.9 and δ_{C} 119.1, which were assigned to the $\Delta^{2,3}$ double bond in **40**. Detailed 2D NMR analysis confirmed the structure of **40**. The structure of **40** was further confirmed by a single-crystal X-ray structure analysis (Supplementary Table 12, CCDC 2036401) as 2-hydroxy-3-methyl-1,4-naphthoquinone.

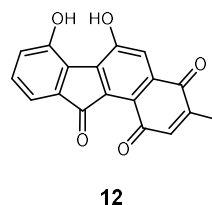
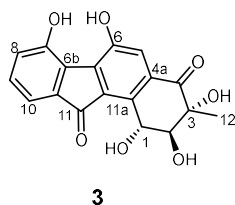
Compound **41** was obtained as white powder. The molecular formula of **41** was assigned as C₁₁H₈O₂ based on HRESIMS *m/z* 173.0601 [M – H][–] (calcd 173.0597), indicating 8 degrees of unsaturation. A comparison of ¹H and ¹³C NMR spectral data of **41** (Supplementary Table 11, Supplementary Fig. 53) and **40** revealed their high structural similarity. The difference was that the hydroxyl group at C-2 in **40** was replaced by a hydrogen in **41**, which was supported by 2D NMR data. Thus, the structure of **41** was assigned to be 3-methylnaphthalene-1,4-dione.



Compound **47** was obtained as yellow powder, $[\alpha]_D^{25}$ -8.6 (*c* 0.05, MeOH). The molecular formula of **47** was established as $C_{15}H_{14}O_2$ by HRESIMS m/z 249.0894 $[M + Na]^+$ (calcd 249.0886). The 1H , ^{13}C and HSQC spectra of **47** (Supplementary Table 13, Supplementary Fig. 57) indicated the presence of one methine (δ_H 5.14, m, H-9; δ_C 69.8 C-9) and one methylene proton signals (δ_H 3.19, dd, $J = 4.4, 15.8$, H-8a, δ_H 3.43, dd, $J = 8.5, 15.8$, H-8b; δ_C 69.8, C-8), which suggested that 8,9-epoxy ring in **46** was opened in **47**. This suggestion was supported by COSY correlations of H₂-8 & H-9 and HMBC correlations from 9-OH to C-8/C-9/C-10. Further detailed analysis of 2D NMR data assigned the structure of **47**. Given that **47** was epoxide ring-opening product of **46**, the absolute configuration of C-9 was tentatively assigned as (9*R*).

Supplementary Tables

Supplementary Table 1. ^1H and ^{13}C NMR spectroscopic data of **3** and **12** in $\text{DMSO-}d_6$.

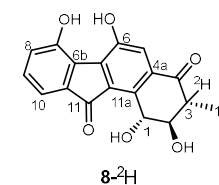
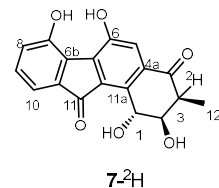
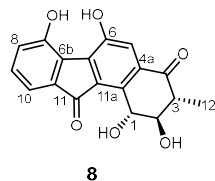
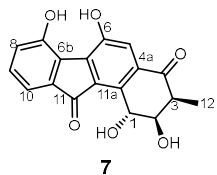


Position	3^a		12^b	
	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)
1	67.3, CH	5.12, dd (4.4, 4.4)	181.8, C	
2	75.8, CH	3.74, dd (4.5, 4.5)	137.8, CH	6.66, s
3	75.2, C		144.4, C	
4	197.6, C		186.3, C	
4a	131.0, C		133.3, C	
5	122.1, CH	6.93, s	122.0, CH	7.01, overlapping
6	159.5, C		167.3, C	
6a	140.6, C		140.5, C	
6b	129.7, C		128.2, C	
7	160.1, C		156.2, C	
8	126.2, CH	6.55, d (8.3)	127.7, CH	6.69, d (7.9)
9	130.5, CH	6.90, dd (8.3, 7.1)	130.4, CH	7.01, overlapping
10	111.4, CH	6.65, d (7.1)	113.3, CH	6.80, d (7.0)
10a	134.9, C		135.7, C	
11	196.6, C		192.3, C	
11a	131.0, C		134.6, C	
11b	129.2, C		118.1, C	
12	20.8, CH ₃	1.19, s	15.8, CH ₃	1.99, s
1-OH		5.76, d (4.4)		
2-OH		5.38, d (4.6)		
3-OH		5.68, s		

^aData were recorded on a Bruker Avance 700 MHz NMR spectrometer in $\text{DMSO-}d_6$ with TMS as an internal standard.

^bData were recorded on a Bruker Avance 600 MHz NMR spectrometer in $\text{DMSO-}d_6$ with TMS as an internal standard.

Supplementary Table 2. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of 7, 8, 7- ^2H and 8- ^2H in $\text{DMSO-}d_6$.



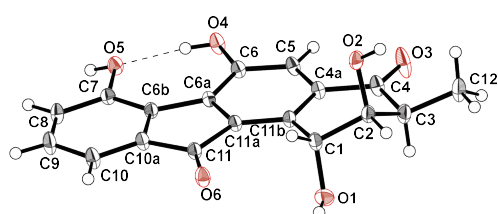
Position	7		8		7-^2H		8-^2H	
	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)
1	63.6, CH	5.45, d (3.4)	69.2, CH	4.94, dd (6.1, 3.0)	64.1, CH	5.44, d (3.5)	68.3, CH	5.20, d (4.9)
2	74.8, CH	4.01, dd (3.4, 2.4)	74.2, CH	3.60, m	75.3, CH	4.00, d (3.5)	74.3, CH	3.80, d (4.9)
3	41.6, CH	3.17, overlapped	47.2, CH	2.52, overlapped	41.7, CH	7.44, s	47.4, CH	7.29, s
4	197.7, C		198.4, C		198.3, C		199.1, C	
4a	132.4, C		131.5, C		132.8, C		132.4, C	
5	119.6, CH	7.48, s	121.4, CH	6.92, s	120.1, CH	7.05, d (8.1)	121.5, CH	6.90, d (7.3)
6	149.2, C	7.31, dd (8.1, 7.0)	158.3, C	6.89, dd (8.1, 7.0)	150.4, C		155.5, C	
6a	133.7, C		140.2, C		134.4, C		138.5, C	
6b	125.5, C		129.2, C		120.3, C		128.3, C	
7	150.7, C		159.7, C		152.0, C	7.29, dd (8.1, 7.1)	156.8, C	7.17, dd (7.3, 6.8)
8	124.0, CH		126.2, CH		124.4, CH		125.9, CH	
9	131.7, CH		130.1, CH		132.0, CH		131.3, CH	

10	116.3, CH	7.21, d (7.0)	111.2, CH	6.66, d (7.0)	116.2, CH	7.16, d (7.1)	113.7, CH	7.01, d (6.8)
10a	135.4, C		134.2, C		135.8, C		135.1, C	
11	192.4, C		197.1, C		193.1, C		198.5, C	
11a	134.1, C		130.4, C		134.5, C		131.3, C	
11b	134.3, C		131.0, C		134.6, C		131.7, C	
12	11.4, CH ₃	1.16, d (6.8)	13.1, CH ₃	1.18, d (7.1)	11.8, CH ₃	1.15, s	14.3, CH ₃	1.22, s
1-OH				5.58, d (3.0)				5.46, s
2-OH				5.24, d (3.1)				5.28, s

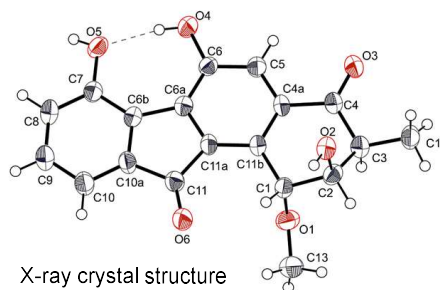
Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

Supplementary Table 3. Crystal data and structure refinement for 7 and 23

Identification code	7	23
Empirical formula	C ₁₈ H _{15.54} O _{6.77}	C ₂₀ H ₂₀ O ₇
Formula weight	340.12	372.36
Temperature/K	99.9(7)	99.8(9)
Crystal system	orthorhombic	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
a/Å	6.5747(2)	6.6367(4)
b/Å	12.7647(3)	14.0428(7)
c/Å	17.4860(4)	17.9812(12)
α /°	90	90
β /°	90	90
γ /°	90	90
Volume/Å ³	1467.50(7)	1675.81(17)
Z	4	4
$\rho_{\text{calc}}/\text{cm}^3$	1.539	1.476
μ/mm^{-1}	1.005	0.940
F(000)	711.0	784.0
Crystal size/mm ³	0.5 × 0.5 × 0.5	0.2 × 0.05 × 0.05
Radiation	CuK α (λ = 1.54184)	CuK α (λ = 1.54184)
2 θ range for data collection/°	8.576 to 148.056	7.988 to 148.046
Index ranges	-7 ≤ h ≤ 7, -15 ≤ k ≤ 15, -19 ≤ l ≤ 21	-8 ≤ h ≤ 5, -17 ≤ k ≤ 17, -21 ≤ l ≤ 22
Reflections collected	6446	9395
Independent reflections	2857 [R _{int} = 0.0199, R _{sigma} = 0.0176]	3288 [R _{int} = 0.0623, R _{sigma} = 0.0592]
Data/restraints/parameters	2857/0/235	3288/0/251
Goodness-of-fit on F ²	1.044	1.092
Final R indexes [I ≥ 2 σ (I)]	R ₁ = 0.0404, wR ₂ = 0.1102	R ₁ = 0.0663, wR ₂ = 0.1863
Final R indexes [all data]	R ₁ = 0.0407, wR ₂ = 0.1105	R ₁ = 0.0815, wR ₂ = 0.2017
Largest diff. peak/hole / e Å ⁻³	0.26/-0.22	0.30/-0.41
Flack parameter	0.04(13)	0.0(3)



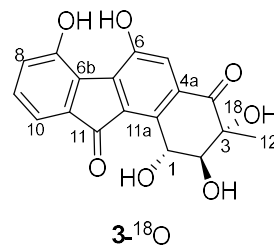
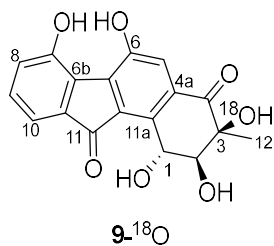
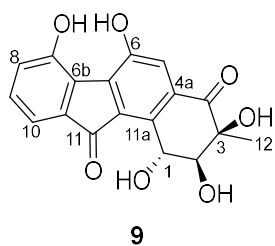
X-ray crystal structure
of **7**



X-ray crystal structure
of **23**

The ellipsoids of non-hydrogen atoms are shown at 50% probability levels for **7** and **23**.

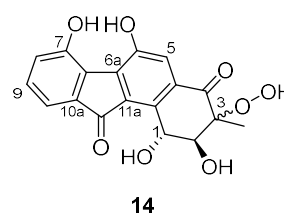
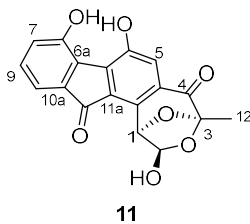
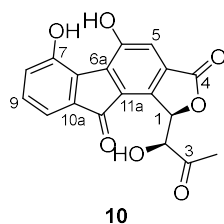
Supplementary Table 4. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of **9, **9- ^{18}O** and **3- ^{18}O** .**



Position	9^a		9-^{18}O^b		3-^{18}O^c	
	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)
1	66.3, CH	5.37, d (4.0)	67.1, CH	5.14, d (4.0, 4.0)	67.3, CH	5.11, dd (4.4, 4.4)
2	77.9, CH	3.84, d (4.0)	78.0, CH	3.82, dd (4.0, 4.0)	75.8, CH	3.74, dd (4.5, 4.5)
3	76.7, C		76.4, C		75.2, C	
4	200.2, C		200.3, C		197.6, C	
4a	133.2, C		131.3, C		131.0, C	
5	120.6, CH	7.48, s	121.6, CH	6.87, s	122.1, CH	6.92, s
6	149.9, C		159.3, C		159.5, C	
6a	135.1, C		140.3, C		140.6, C	
6b	125.9, C		129.8, C		129.7, C	
7	151.3, C		160.2, C		160.1, C	
8	124.6, CH	7.11, dd (8.2, 0.9)	126.1, CH	6.53, dd (8.1, 1.1)	126.2, CH	6.54, d (8.3)
9	132.4, CH	7.32, dd (8.2, 7.2)	130.5, CH	6.88, dd (8.1, 7.2)	130.5, CH	6.89, dd (8.3, 7.1)
10	116.9, CH	7.22, dd (7.2, 0.9)	111.3, CH	6.63, dd (7.2, 1.1)	111.4, CH	6.64, dd (7.1)
10a	135.7, C		134.8, C		134.9, C	
11	192.8, C		196.5, C		196.6, C	
11a	133.1, C		131.1, C		131.0, C	
11b	134.4, C		129.1, C		129.2, C	
12	24.0, CH ₃	1.33, s	23.8, CH ₃	1.29, s	20.8, CH ₃	1.20, s
1-OH				5.06, d (4.0)		5.74, d (4.4)
2-OH				5.03, d (4.0)		5.41, d (4.6)
3-OH				4.90, s		5.67, s

^aMeasured in DMSO-*d*₆-trifluoroacetic acid (TFA)-*d*₁ (100:1), ^bIn DMSO-*d*₆. Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

Supplementary Table 5. ¹H (700 MHz) and ¹³C (175 MHz) NMR spectroscopic data of 10, 11 and 14 in DMSO-*d*₆.

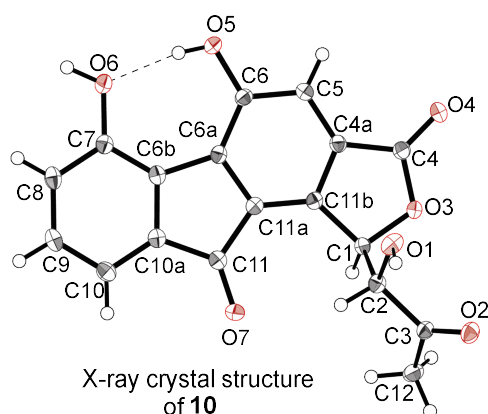


Position	10^a		11^a		14^b	
	δ_C , type	δ_H , mult. (<i>J</i> in Hz)	δ_C , type	δ_H , mult. (<i>J</i> in Hz)	δ_C , type	δ_H , mult. (<i>J</i> in Hz)
1	81.2, CH	5.87, d (2.0)	78.5, CH	5.79, br s	N ^c	5.72, dd (4.4, 4.4)
2	75.5, CH	4.78, dd (6.7, 2.0)	98.0, CH	5.10, d (5.6)	72.0, CH	4.83, dd (7.1, 4.4)
3	210.1, C		106.7, C		87.6, C	
4	170.4, C		189.0, C		195.0, C	
4a	127.9, C		128.6, C		131.0, C	
5	118.6, CH	6.66, s	122.1, CH	6.89, s	N ^c	6.92, s
6	162.0, C		160.4, C		159.3, C	
6a	140.6, C		138.5, C		140.8, C	
6b	130.4, C		130.2, C		129.4, C	
7	159.7, C		159.7, C		160.6, C	
8	126.2, CH	6.59, dd (8.7, 0.7)	125.9, CH	6.58, dd (8.1, 0.5)	126.9, CH	6.56, dd (8.3, 0.7)
9	130.6, CH	6.93, dd (8.2, 7.2)	130.6, CH	6.93, dd (7.9, 7.0)	N ^c	6.91, dd (8.0, 6.9)
10	112.0, CH	6.67, dd (6.9, 0.7)	111.7, CH	6.68, dd (7.2, 0.7)	117.1, CH	6.68, dd (7.2, 0.7)
10a	134.3, C		134.7, C		134.9, C	
11	195.0, C		195.8, C		N ^c	
11a	N ^c		127.5, C		N ^c	
11b	130.6, C		126.3, C		129.4, C	
12	27.1, CH ₃	2.24, s	18.2, CH ₃	1.57, s	N ^c	1.12, s
2-OH		5.59, d (6.7)		6.97, d (5.4)		

^a Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments. ^b Assignments were based on ¹H and HMBC experiments. ^cN, no signal was observed.

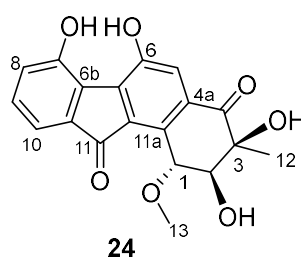
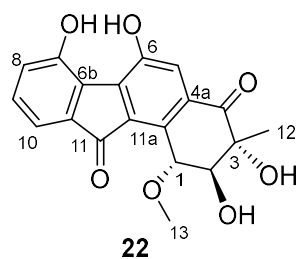
Supplementary Table 6. Crystal data and structure refinement for 10.

Identification code	10
Empirical formula	C ₁₈ H ₁₆ O ₉
Formula weight	376.31
Temperature/K	100.00(10)
Crystal system	triclinic
Space group	P1
a/Å	8.0646(2)
b/Å	9.4581(4)
c/Å	12.3793(4)
α /°	105.647(3)
β /°	91.092(2)
γ /°	110.786(3)
Volume/Å ³	843.13(5)
Z	2
$\rho_{\text{calc}}/\text{cm}^3$	1.482
μ/mm^{-1}	1.035
F(000)	392.0
Crystal size/mm ³	0.02 × 0.02 × 0.01
Radiation	Cu K α (λ = 1.54184)
2 θ range for data collection/°	7.478 to 148.556
Index ranges	-10 ≤ h ≤ 10, -11 ≤ k ≤ 11, -15 ≤ l ≤ 15
Reflections collected	17982
Independent reflections	6389 [R _{int} = 0.0248, R _{sigma} = 0.0264]
Data/restraints/parameters	6389/3/516
Goodness-of-fit on F ²	1.050
Final R indexes [I ≥ 2 σ (I)]	R ₁ = 0.0333, wR ₂ = 0.0882
Final R indexes [all data]	R ₁ = 0.0343, wR ₂ = 0.0891
Largest diff. peak/hole / e Å ⁻³	0.40/-0.34
Flack parameter	-0.01(5)



The ellipsoids of non-hydrogen atoms are shown at 50% probability levels for **10**.

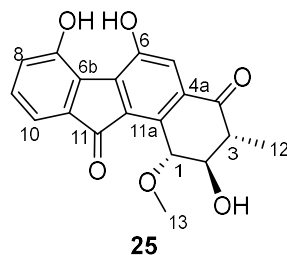
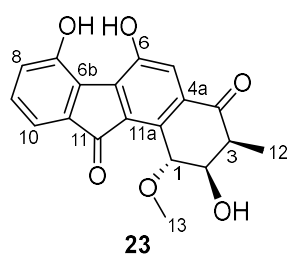
Supplementary Table 7. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of **22 and **24** in $\text{DMSO}-d_6$.**



Position	22		24	
	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)
1	75.5, CH	4.99, d (3.5)	75.8, CH	4.84, d (2.5)
2	71.6, CH	4.00, dd (3.5, 3.4)	72.8, CH	4.02, m
3	75.0, C		76.3, CH	
4	196.7, C		200.8, C	
4a	131.6, C		134.9, C	
5	121.8, CH	6.91, s	121.1, CH	6.85, s
6	160.3, C		159.7, C	
6a	140.1, C		140.9, C	
6b	129.6, C		129.6, C	
7	159.7, C		158.7, C	
8	125.8, C	6.54, d (8.2)	125.7, CH	6.53, d (8.1)
9	130.4, CH	6.89, dd (8.2, 7.0)	130.4, CH	6.89, dd (8.1, 6.7)
10	111.3, CH	6.65, d (7.0)	111.1, CH	6.63, d (6.7)
10a	135.0, C		135.2, C	
11	195.7, C		195.5, C	
11a	131.4, C		131.6, C	
11b	124.8, C		125.3, C	
12	21.3, CH_3	1.23, s	24.4, CH_3	1.26, s
13	58.3, CH_3	3.56, s	57.8, CH_3	3.54, s
2-OH		5.49, br d (4.4)		5.12, br s
3-OH		5.04, s		4.90, s

Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

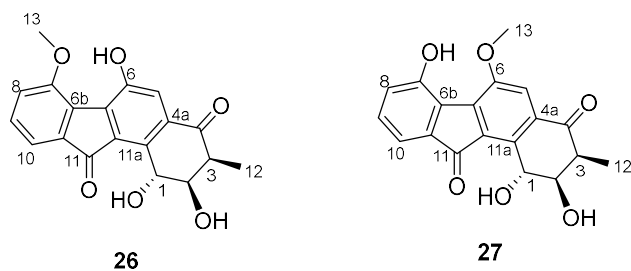
Supplementary Table 8. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of **23** and **25** in $\text{DMSO}-d_6$.



Position	23		25	
	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)
1	72.3, CH	5.08, d (3.4)	74.2, CH	4.98, br d (3.1)
2	71.2, CH	4.22, br s	69.9, CH	4.15, dd (3.1, 2.6)
3	42.4, CH	3.00, m	48.2, CH	2.50, overlapping
4	198.2, C		199.8, C	
4a	133.9, C		131.6, C	
5	120.8, CH	7.19, s	121.3, CH	6.88, s
6	155.1, C		159.6, C	
6a	135.4, C		139.8, C	
6b	128.0, C		129.7, C	
7	155.9, C		160.1, C	
8	125.3, CH	6.81, d (8.1)	125.8, CH	6.52, dd (6.5, 0.6)
9	131.2, CH	7.09, dd (8.1, 6.9)	130.3, CH	6.88, dd (7.0, 6.5)
10	113.8, CH	6.90, d (6.9)	110.9, CH	6.62, dd (7.0, 0.6)
10a	136.8, C		135.1, C	
11	194.8, C		195.9, C	
11a	132.5, C		131.8, C	
11b	128.7, C		126.4, C	
12	12.0, CH_3	1.16, d (6.9)	16.5, CH_3	1.21, d (7.9)
13	57.9, CH_3	3.48, s	57.4, CH_3	3.42, s

Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

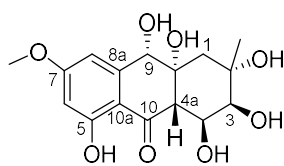
Supplementary Table 9. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of 26 and 27 in $\text{DMSO-}d_6$.



Position	26		27	
	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult. (<i>J</i> in Hz)
1	64.1, CH	5.47, d (3.3)	64.1, CH	5.50, d (2.8)
2	75.3, CH	4.01, br s	75.2, CH	4.04, dd (2.8, 2.3)
3	42.1, CH	3.19, m	42.1, CH	3.20, dq (6.7, 1.9)
4	198.2, C		198.0, C	
4a	134.5, C		134.5, C	
5	121.0, CH	7.40, s	115.8, CH	7.69, s
6	150.9, C		151.3, C	
6a	132.9, C		136.3, C	
6b	128.4, C		124.5, C	
7	152.3, C		152.1, C	
8	120.3, CH	7.46, overlapping	125.3, CH	7.09, d (8.1)
9	132.3, CH	7.48, overlapping	132.8, CH	7.35, dd (8.1, 6.8)
10	118.2, CH	7.36, d (6.3)	116.8, CH	7.21, d (6.8)
10a	135.8, C		135.9, C	
11	192.6, C		192.5, C	
11a	132.1, C		132.9, C	
11b	135.2, C		136.2, C	
12	11.8, CH_3	1.17, d (7.1)	11.9, CH_3	1.18, d (7.3)
6-OMe			58.0, CH_3	4.14, s
7-OMe	58.0, CH_3	4.12, s		
1-OH		5.42, s		
2-OH		5.35, s		

Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

Supplementary Table 10. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of **38 in methanol- d_4 .**

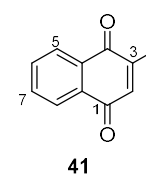
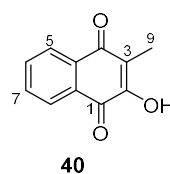
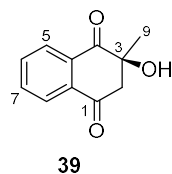


38

Position	38	
	δ_{C} , type	δ_{H} , mult. (J in Hz)
1	38.1, CH_2	2.02, d (14.5) 1.92, d (14.5)
2	72.8, C	
3	75.2, CH	3.65, br s
4	66.6, CH	4.58, d (3.0)
4a	52.1, CH	2.93, d (10.0)
5	164.4, C	
6	99.0, CH	6.34, d (2.7)
7	166.9, C	
8	105.3, CH	6.79, m
8a	147.4, C	
9	73.7, CH	
9a	77.7, C	
10	204.9, C	
10a	110.1, C	
11	54.7, CH_3	3.85, s
12	26.0, CH_3	1.31, s

Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

Supplementary Table 11. ¹H (700 MHz) and ¹³C (175 MHz) NMR spectroscopic data for 39, 40 and 41 in DMSO-*d*₆.

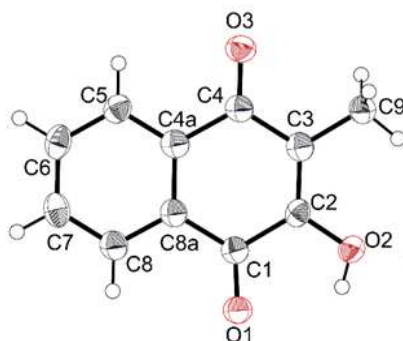


Position	39		40		41	
	δ_C , type	δ_H , mult. (<i>J</i> in Hz)	δ_C , type	δ_H , mult. (<i>J</i> in Hz)	δ_C , type	δ_H , mult. (<i>J</i> in Hz)
1	195.5, C		184.1, C		183.0, C	
2	52.3, CH ₂	3.05, d (16.5) 3.26, d (16.5)	158.9, C		133.6, C	6.98, m
3	74.8, C		119.1, C		146.4, C	
4	197.4, C		182.3, C		183.4, C	
4a	134.1, C		130.7, C		130.2, C	
5	127.5, CH	7.99, dd (7.1, 1.6)	132.9, CH	7.95, dd (7.7, 1.1)	124.4, C	8.02, m
6	134.5, CH	7.87, ddd (7.6, 7.1, 1.6)	125.9, CH	7.73, dd (7.7, 7.3)	132.3, C	7.86, m, overlapping
7	134.9, CH	7.85, ddd (7.6, 7.1, 1.6)	125.9, CH	7.79, ddd (7.3, 7.2, 1.1)	132.4, C	7.86, m, overlapping
8	126.1, CH	7.90, dd (7.1, 1.6)	134.6, CH	7.94, d (7.2)	123.9, C	7.97, m
8a	135.3, C		133.3, C		130.1, C	
9	24.5, CH ₃	1.38, s	9.2, CH ₃	1.91, s	14.3, CH ₃	2.12, s

Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

Supplementary Table 12. Crystal data and structure refinement for 40.

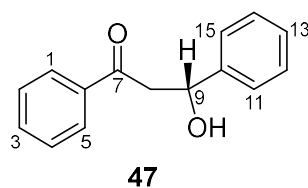
Identification code	40
Empirical formula	C ₁₁ H ₈ O ₃
Formula weight	188.17
Temperature/K	100.01(12)
Crystal system	monoclinic
Space group	P2 ₁
a/Å	7.6523(5)
b/Å	4.7776(3)
c/Å	11.6829(9)
α/°	90
β/°	90.544(8)
γ/°	90
Volume/Å ³	427.10(5)
Z	2
ρ _{calc} /cm ³	1.463
μ/mm ⁻¹	0.893
F(000)	196.0
Crystal size/mm ³	0.05 × 0.01 × 0.01
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	7.568 to 148.544
Index ranges	-9 ≤ h ≤ 9, -3 ≤ k ≤ 5, -14 ≤ l ≤ 8
Reflections collected	1758
Independent reflections	1084 [R _{int} = 0.0445, R _{sigma} = 0.0497]
Data/restraints/parameters	1084/1/129
Goodness-of-fit on F ²	1.025
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0743, wR ₂ = 0.1797
Final R indexes [all data]	R ₁ = 0.0779, wR ₂ = 0.1832
Largest diff. peak/hole / e Å ⁻³	0.56/-0.34
Flack parameter	0.6(5)



X-ray crystal structure of **40**

The ellipsoids of non-hydrogen atoms are shown at 50% probability levels for **40**.

Supplementary Table 13. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of **47 in $\text{DMSO-}d_6$.**



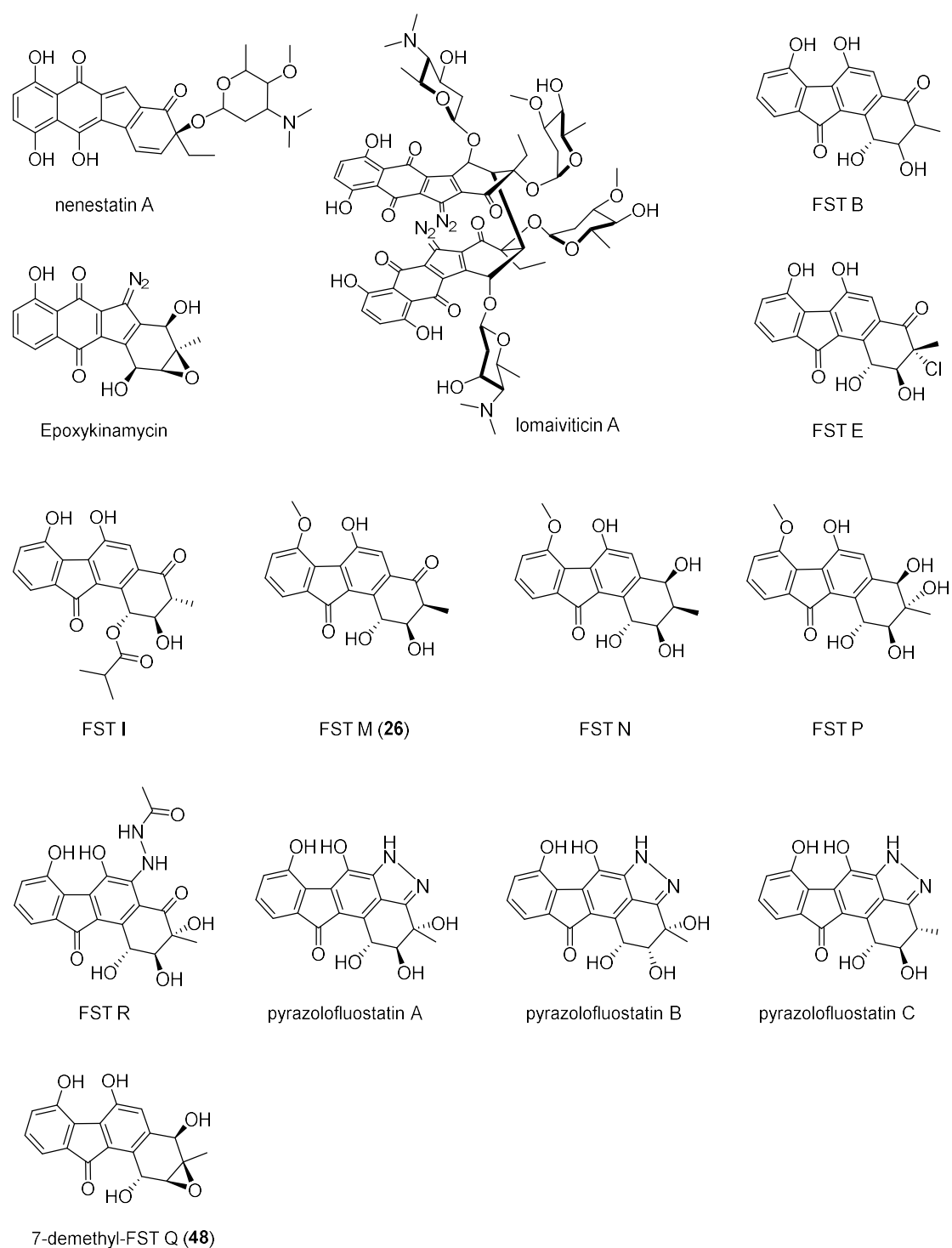
47		
Position		
	δ_{C} , type	δ_{H} , mult.(J in Hz)
1/5	128.6, CH	7.98, dd (7.3, 1.3)
2/4	133.5, C	7.64, tt (7.3, 1.3)
3	129.1, C	7.53, t (7.3)
6	137.6, C	
7	198.8, CH	
8	48.4, CH_2	3.19, dd (15.8, 4.4) 3.43, dd (15.8, 8.5)
9	69.8, CH	5.14, m
10	145.9, C	
11/15	126.4, C	7.42, d (6.8)
12/14	128.5, C	7.34, t (6.8)
13	127.4, C	7.25, tt (6.8, 1.2)
9-OH		5.38, br d (4.3)

Assignments were based on DEPT, HSQC, COSY, HMBC, and NOESY experiments.

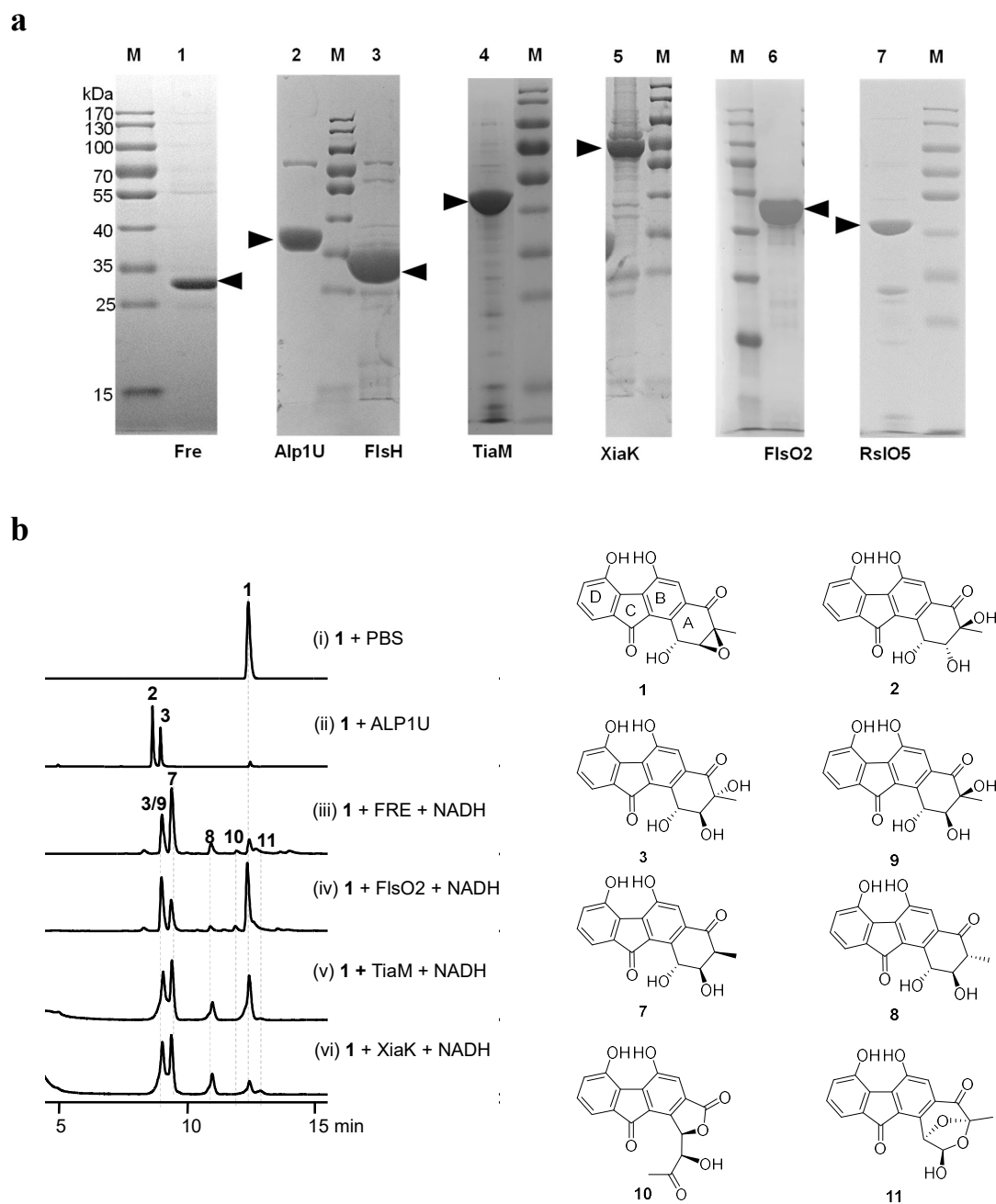
Supplementary Table 14. Strains and plasmids used in this study.

Strains/plasmids	Description	Reference/sources
<i>E. coli</i>		
DH5α	Host strain for cloning	Invitrogen
BL21 (DE3)	Host strain for protein expression	Novagen
Rosetta (DE3)	Host strain for protein expression	Novagen
Actinomycete		
<i>Micromonospora rosaria</i> SCSIO N160	Native producer of FSTs	1
Plasmids		
pET28a	Kanamycin (Kmr), expression vector	Novagen
pCSG118	0.7 kb <i>fre</i> <i>NdeI/BamHI</i> PCR fragment from genomic DNA of <i>E. coli</i> BL21(DE3) into pET28a	4
pCSG5102	1467 bp <i>flsO2</i> <i>NdeI/BamHI</i> PCR fragment from genomic DNA of <i>M. rosaria</i> SCSIO N160 into pET28a	5
pCSG5213	A 780 bp <i>NdeI/BamHI</i> fragment of <i>flsH</i> by PCR from genomic DNA of <i>M. rosaria</i> SCSIO N160 inserted into pET28a	5
pCSG5225	A 957 bp <i>alp1U</i> fragment was synthesized and digested with <i>NdeI/EcoRI</i> and then was inserted into pET28a	5
pCSG2607	1.8 kb <i>xiaK</i> PCR fragment (<i>NdeI/BglII</i>) from SCSIO 02999 inserted into pET28a (<i>NdeI/BamHI</i>)	6
pCSG99	1.56 kb <i>NdeI/EcoRI</i> <i>tiaM</i> PCR fragment from genomic DNA of <i>Dactylosporangium aurantiacum</i> subsp. <i>hamdenensis</i> NRRL 18085 into pET28a	7
pCSG8112	A 1083 bp <i>rslO5</i> fragment was synthesized and digested with <i>NdeI/BamHI</i> and then was inserted into pET28a	This study
pRSF-BmGDH	Expression vector for D-glucose dehydrogenase from <i>Bacillus megaterium</i> (BmGDH)	8
pCSG3622	1.06 kb <i>NdeI/BamHI</i> PCR fragment encoding TaGDH from genomic DNA of <i>Thermoplasma acidophilum</i> in pET28a	8

Supplementary Figures

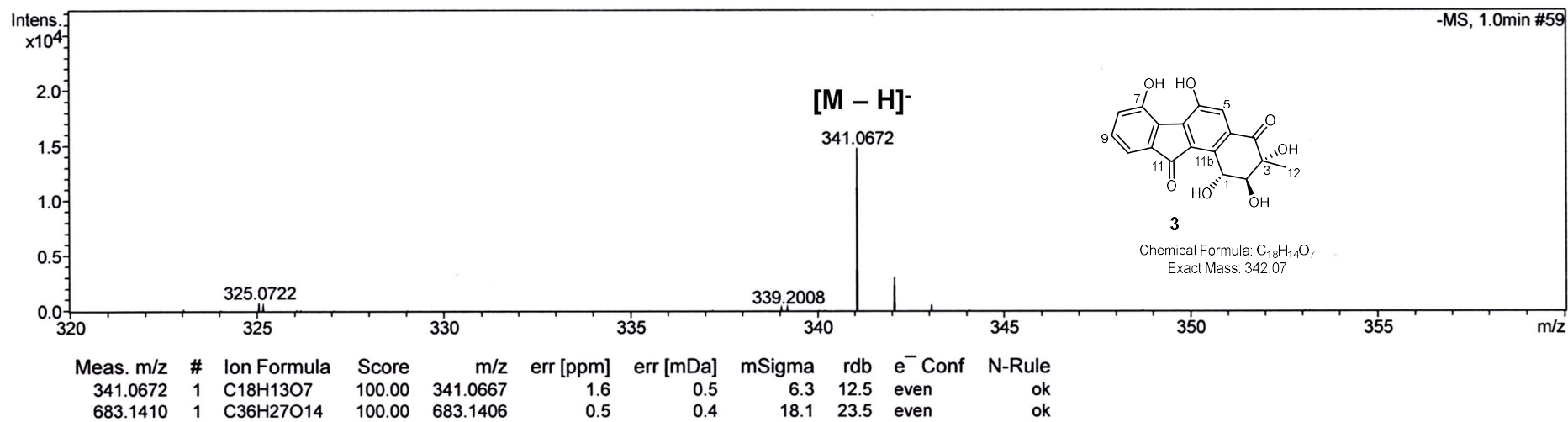


Supplementary Fig. 1. Naturally-occurring atypical angucyclines and FSTs proposed to be derived from epoxide precursors.

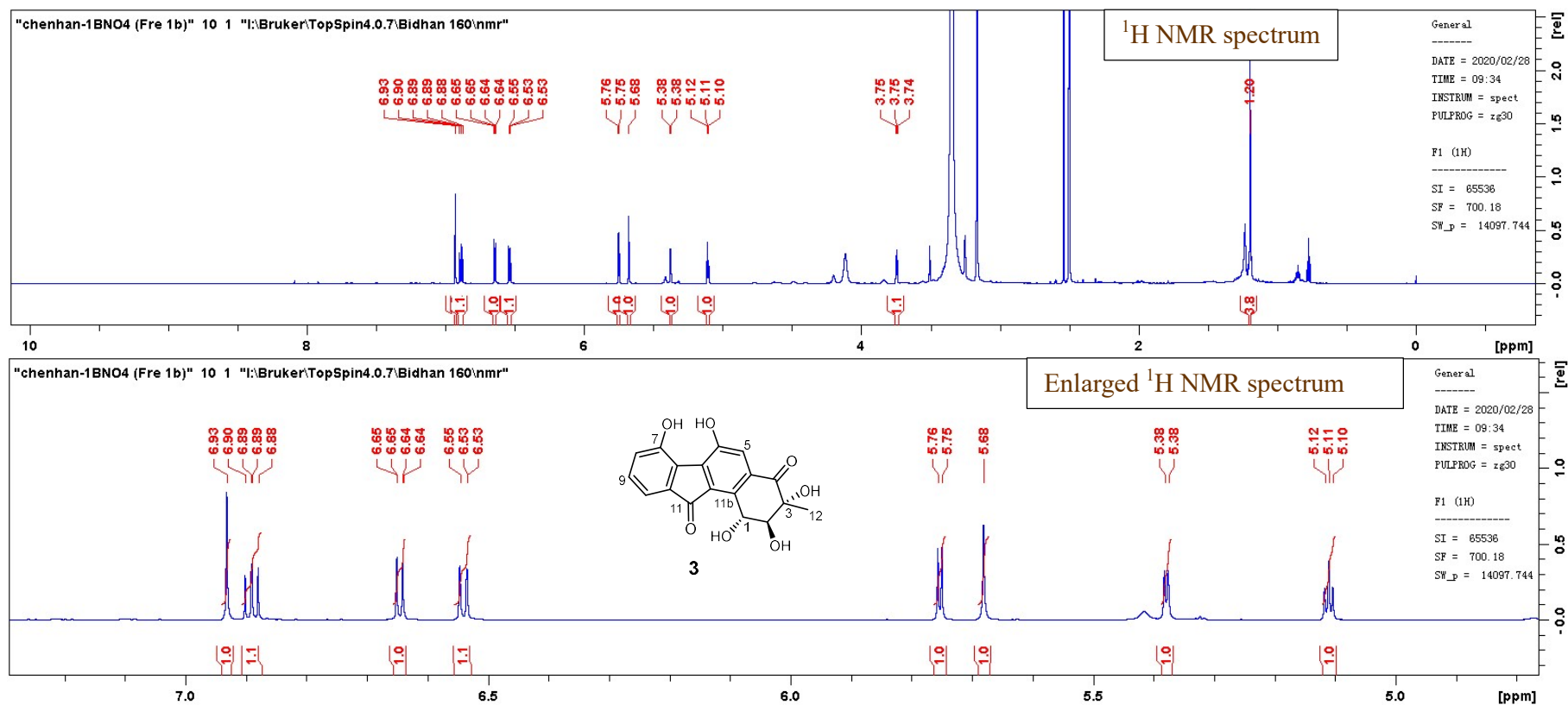


Supplementary Fig. 2. SDS-PAGE analysis of recombinant enzymes and HPLC analysis of their reactions with FST C (1).

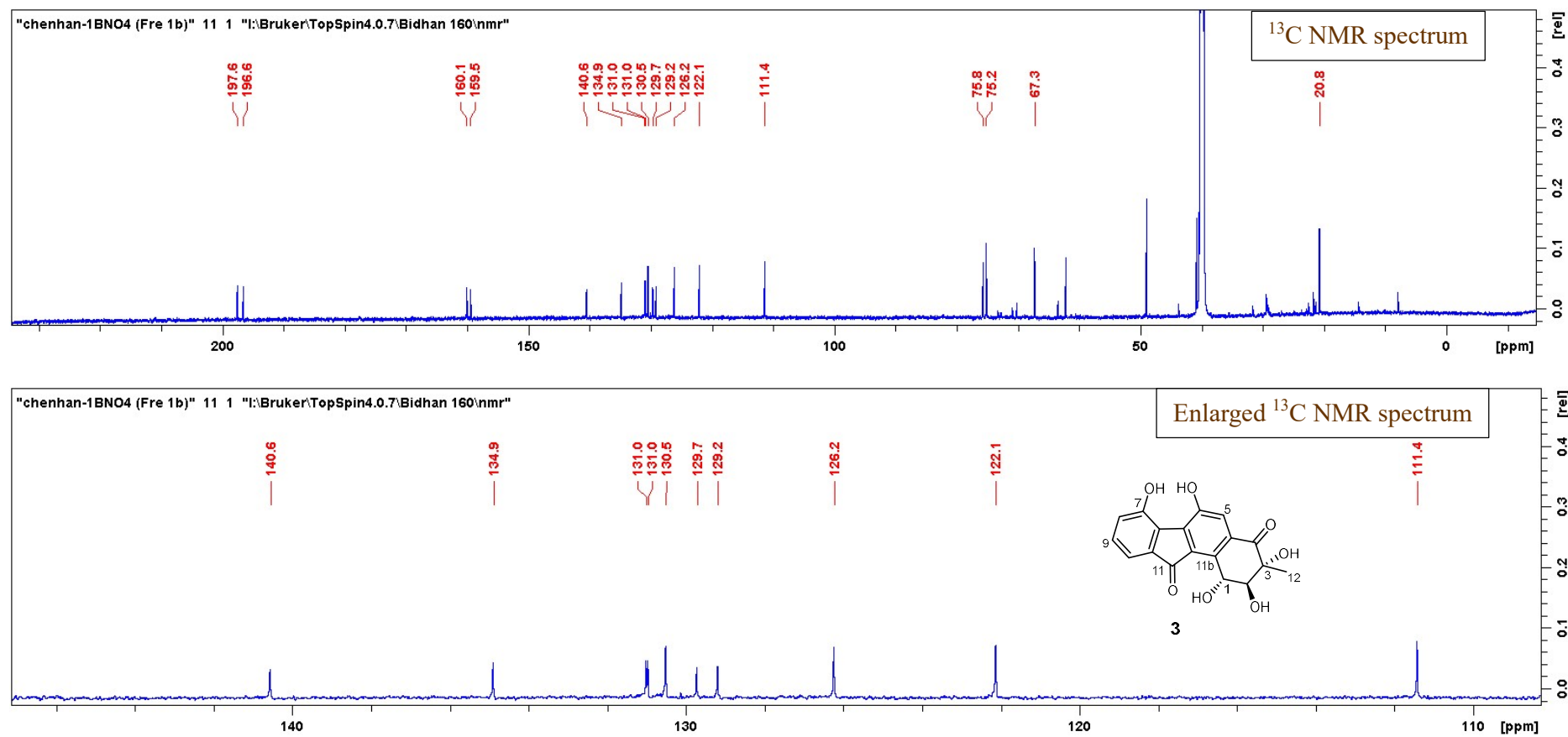
(a) SDS-PAGE analysis of purified recombinant enzymes. The expected sizes of the recombinant proteins are 30 kDa for Fre (lane 1), 36.3 kDa for Alp1U (lane 2), 30 kDa for FlsH (lane 3), 55.6 kDa for TiaM (lane 4), 65.4 kDa for XiaK (lane 5), 51 kDa for FlsO2 (lane 6), and 38.4 kDa for RslO5 (lane 7); protein molecular weight standard (lane M). (b) HPLC analysis of reactions of 10 μ M Alp1U (or Fre, FlsO2, TiaM, XiaK), 100 μ M **1**, and 10 mM NADH in 50 mM PBS buffer (pH 7) for 2 h at 30 °C.



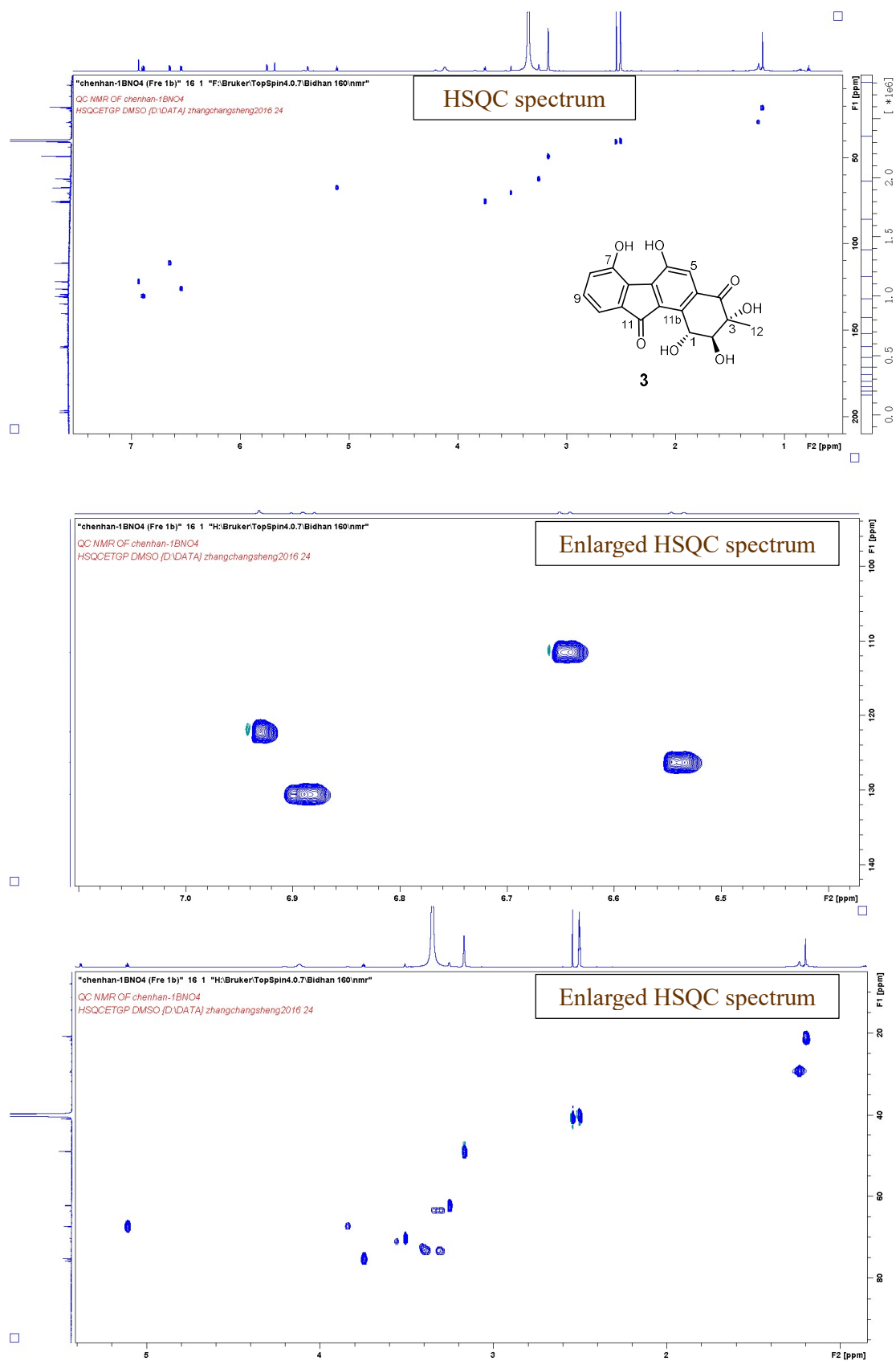
Supplementary Fig. 3. Spectroscopic data for FST C2 (3). (a) HRESIMS spectrum.



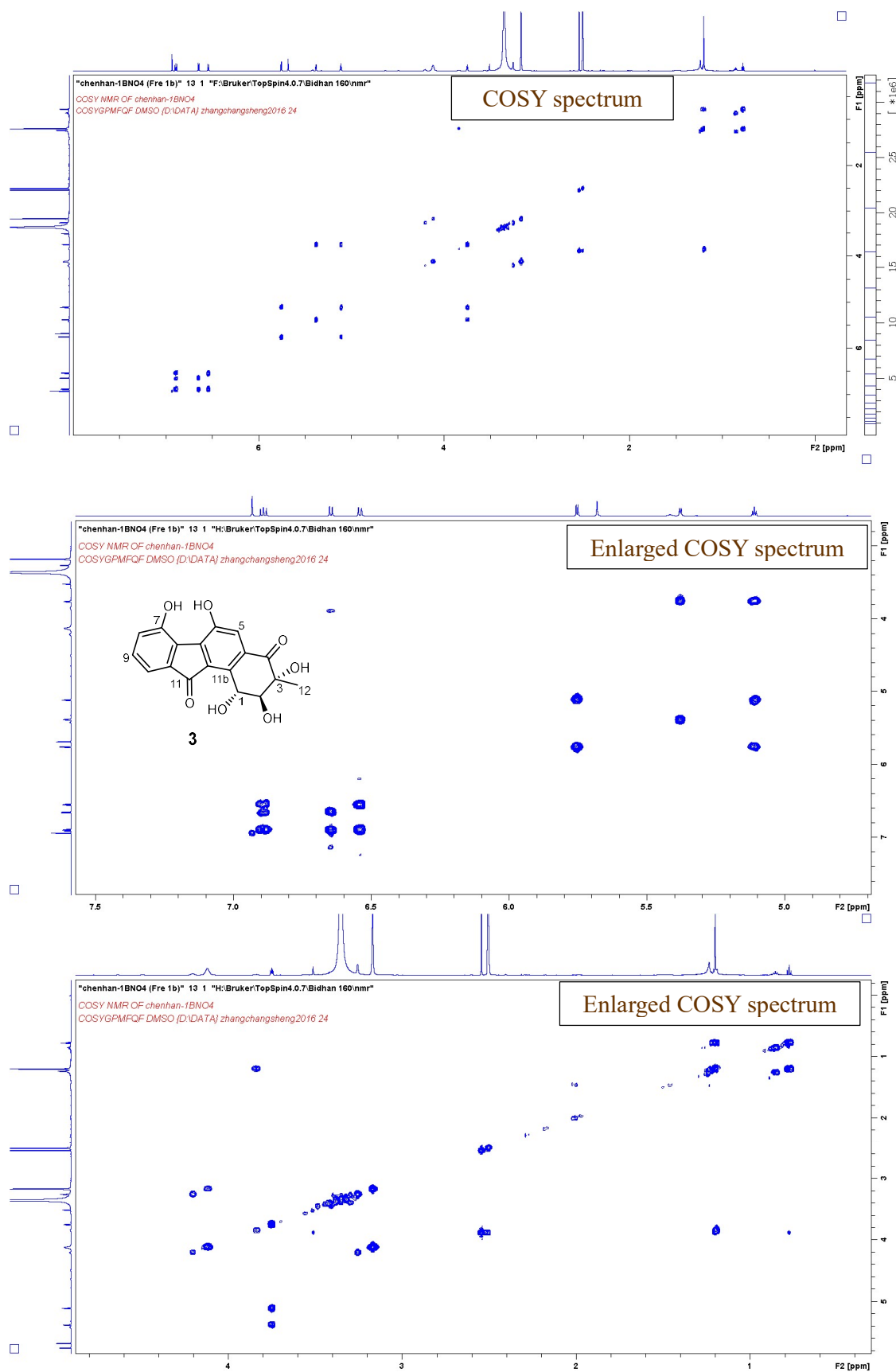
Supplementary Fig. 3. Spectroscopic data for FST C2 (**3**). (b) The ¹H and enlarged ¹H NMR spectrum of **3** in DMSO-*d*₆.



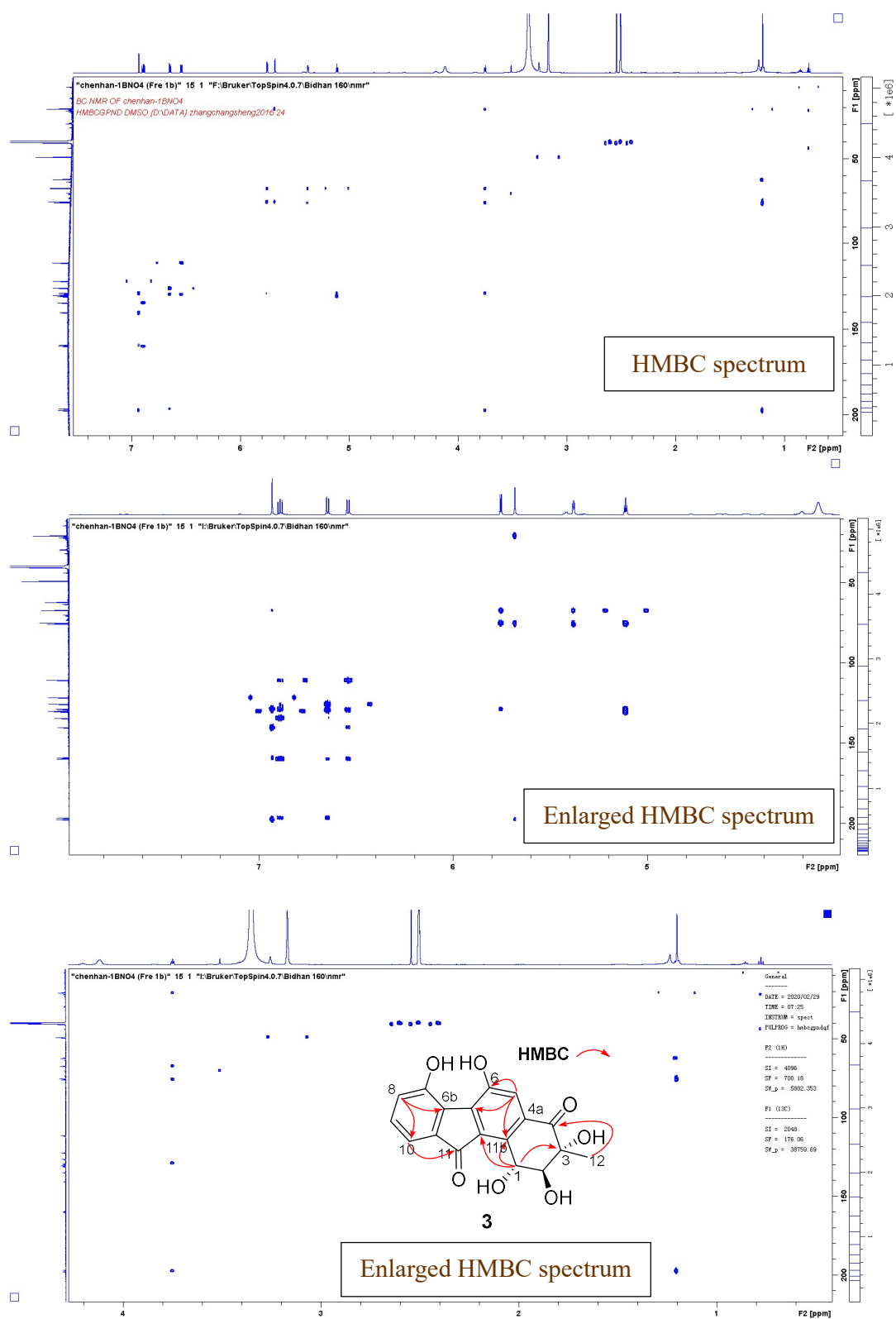
Supplementary Fig. 3. Spectroscopic data for FST C2 (3). (c) The ¹³C and enlarged ¹³C NMR spectrum of 3 in DMSO-*d*₆.



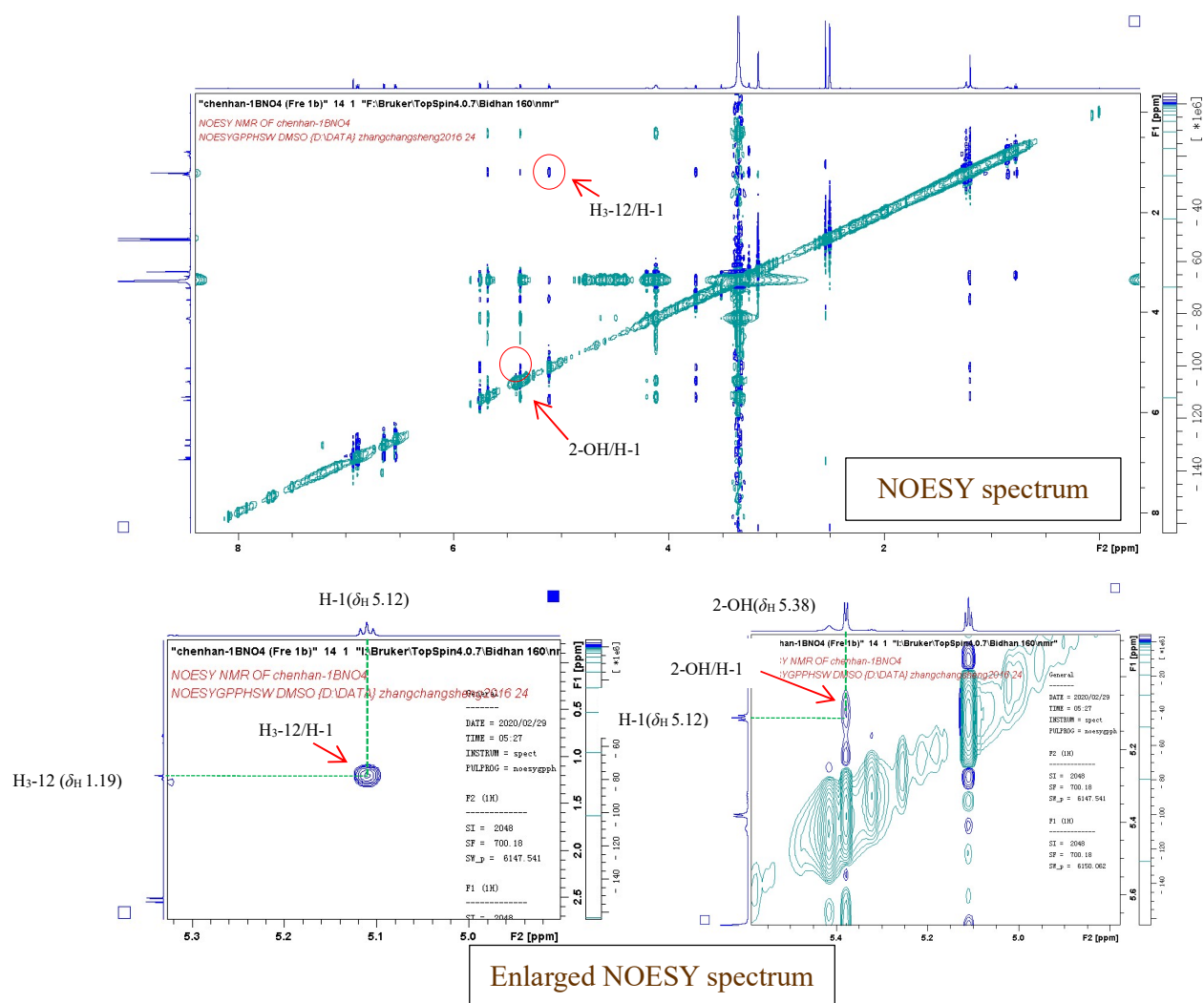
Supplementary Fig. 3. Spectroscopic data for FST C2 (3). (d) The HSQC and enlarged HSQC spectrum of 3 in DMSO- d_6 .



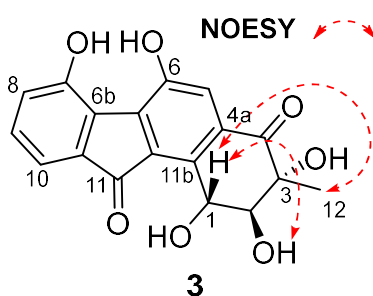
Supplementary Fig. 3. Spectroscopic data for FST C2 (3). (e) The COSY and enlarged COSY spectrum of 3 in DMSO- d_6 .

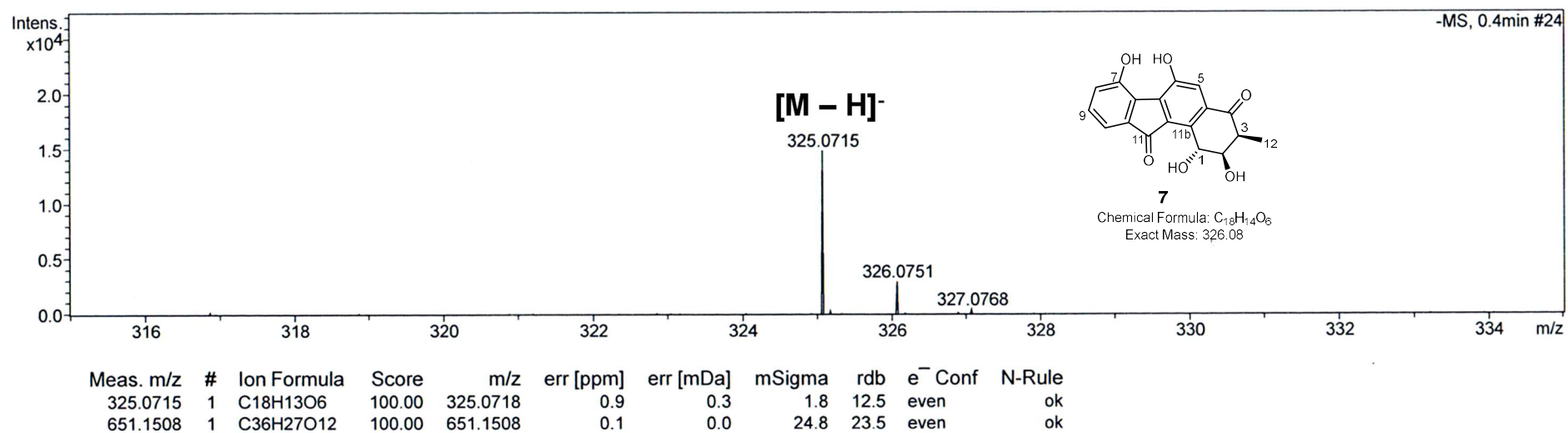


Supplementary Fig. 3. Spectroscopic data for FST C2 (3). (f) The HMBC and enlarged HMBC spectrum of 3 in DMSO- d_6 .

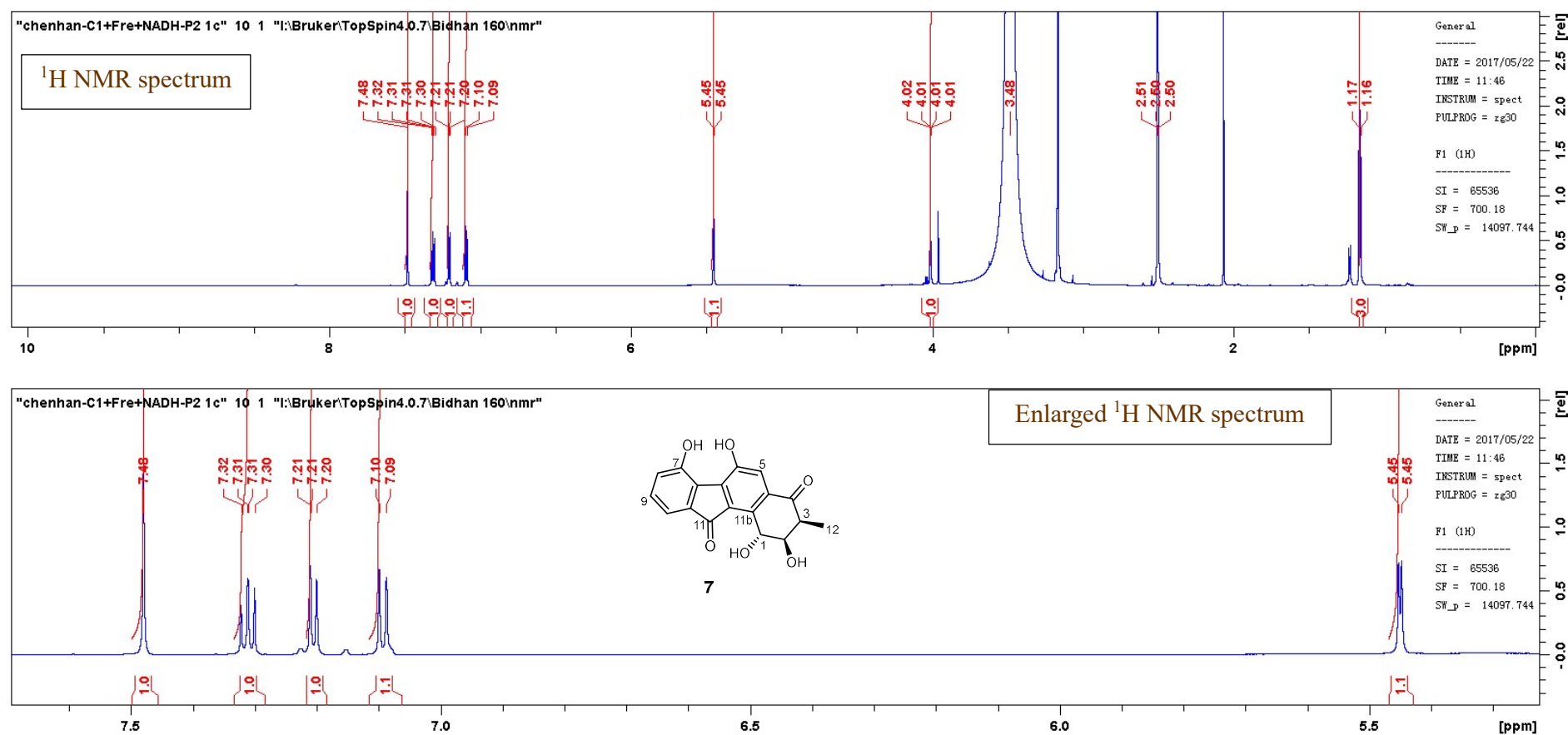


Supplementary Fig. 3. Spectroscopic data for FST C2 (3). (g) The NOESY and enlarged NOESY spectrum of **3 in DMSO-*d*₆.**

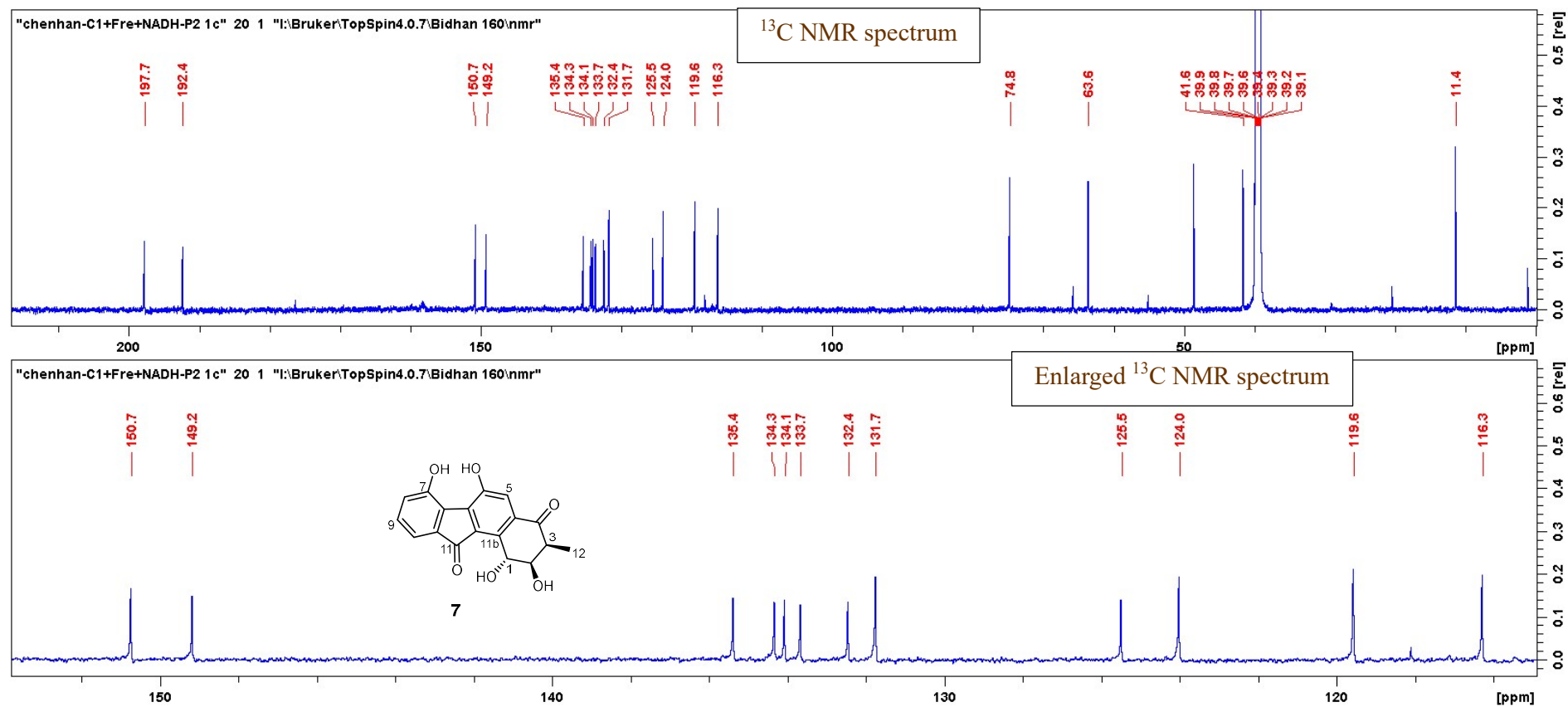




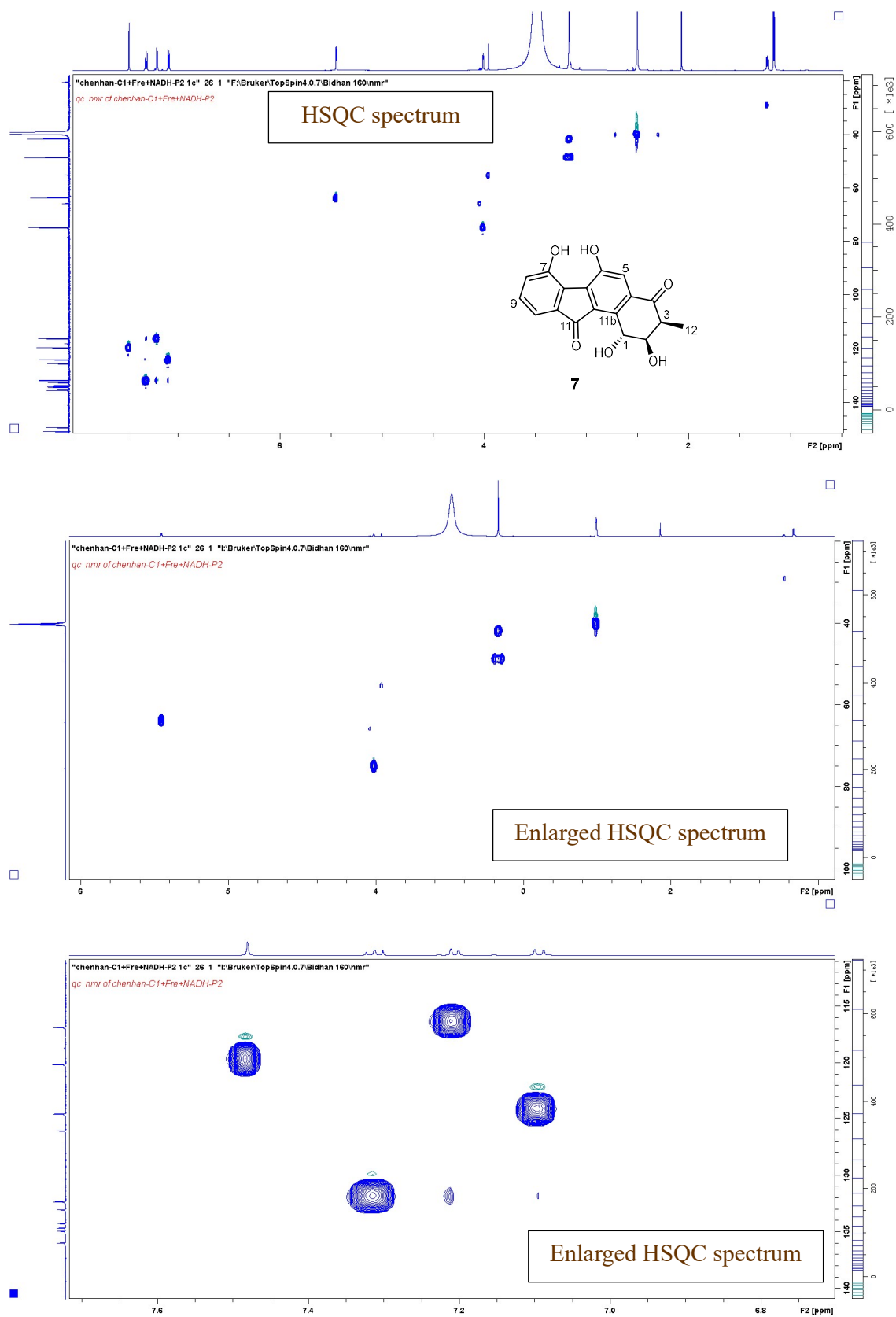
Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (a) HRESIMS spectrum.



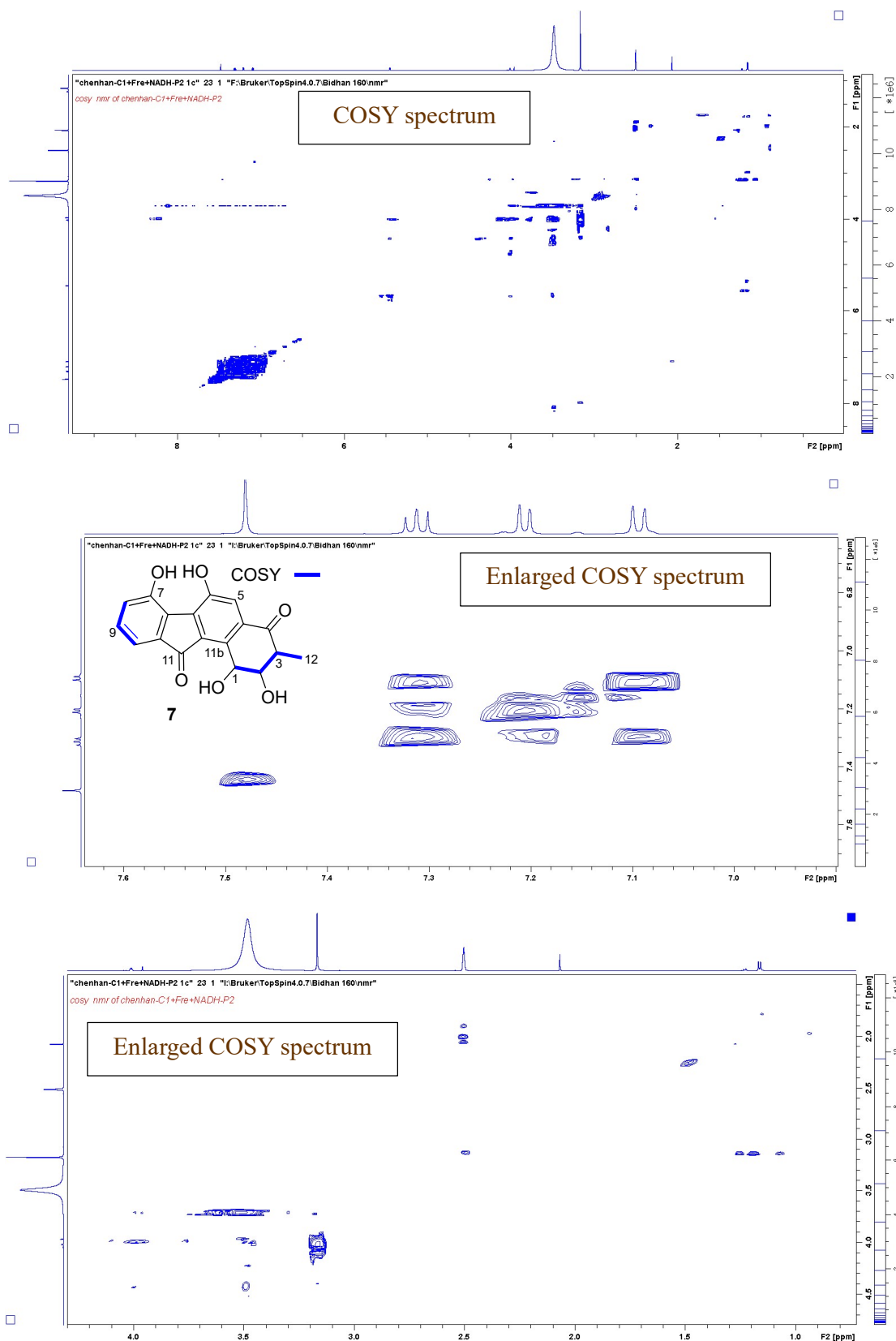
Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (b) The ¹H and enlarged ¹H NMR spectrum of 7 in DMSO-*d*₆.



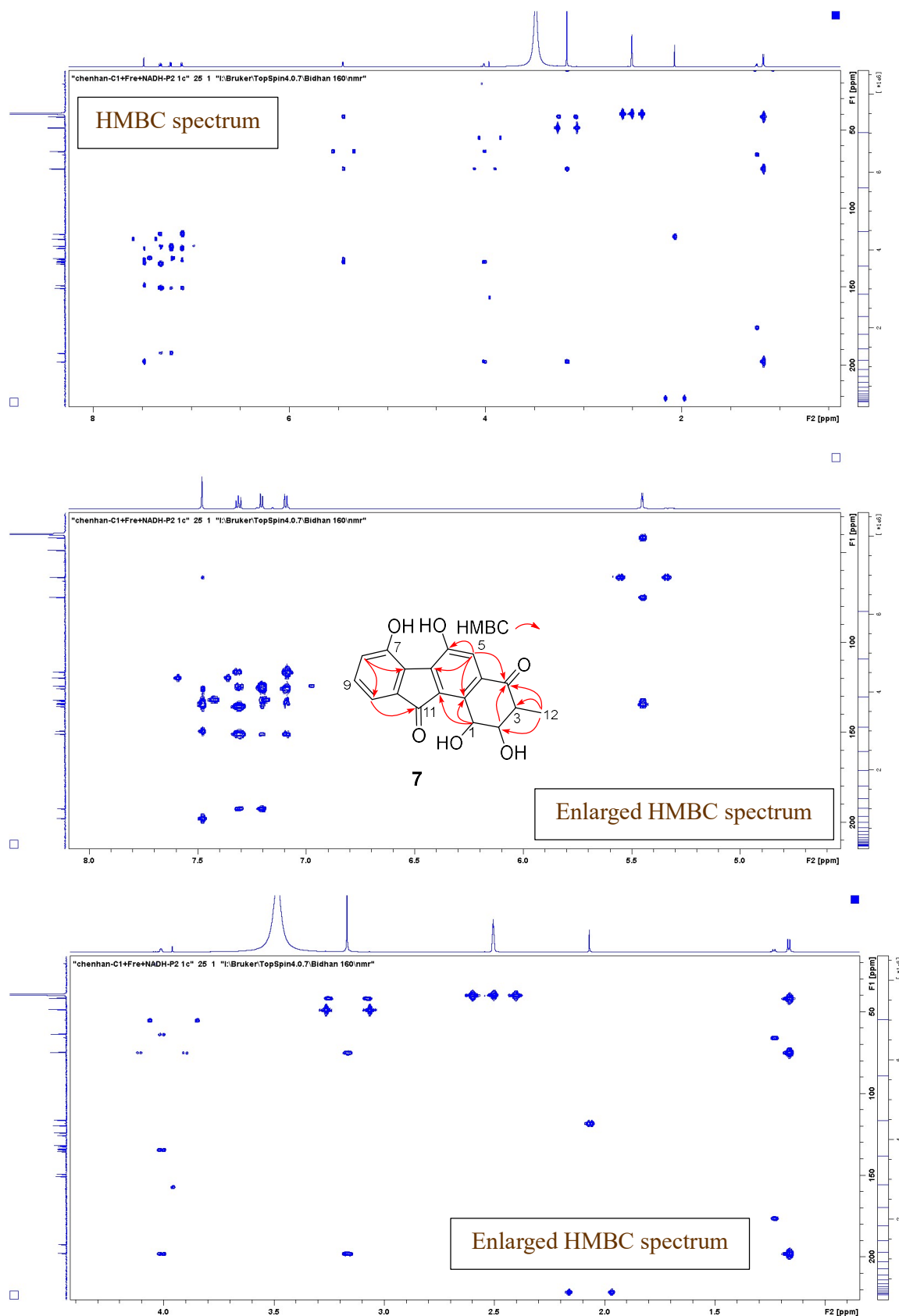
Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (c) The ¹³C and enlarged ¹³C NMR spectrum of 7 in DMSO-*d*₆.



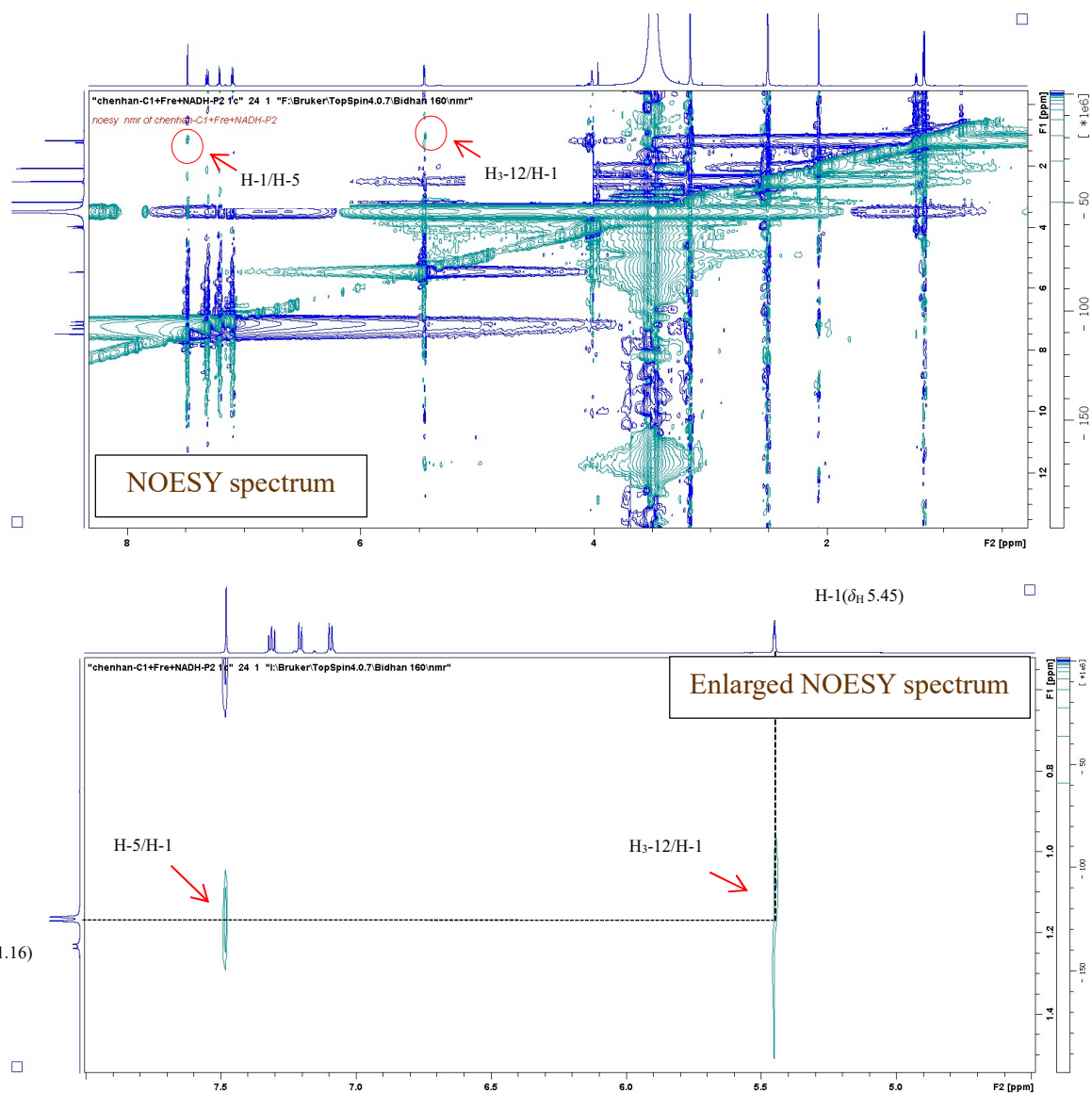
Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (d) The HSQC and enlarged HSQC spectrum of 7 in DMSO-*d*₆.



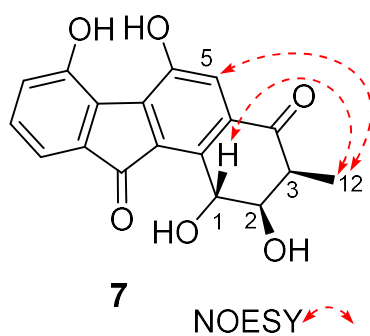
Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (e) The COSY and enlarged COSY spectrum of **7** in DMSO- d_6

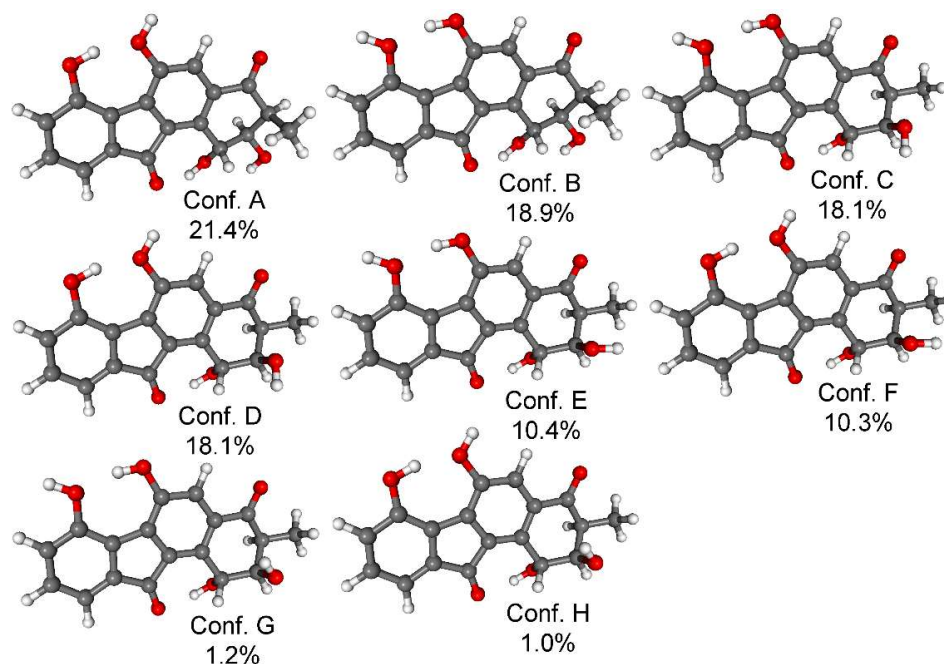
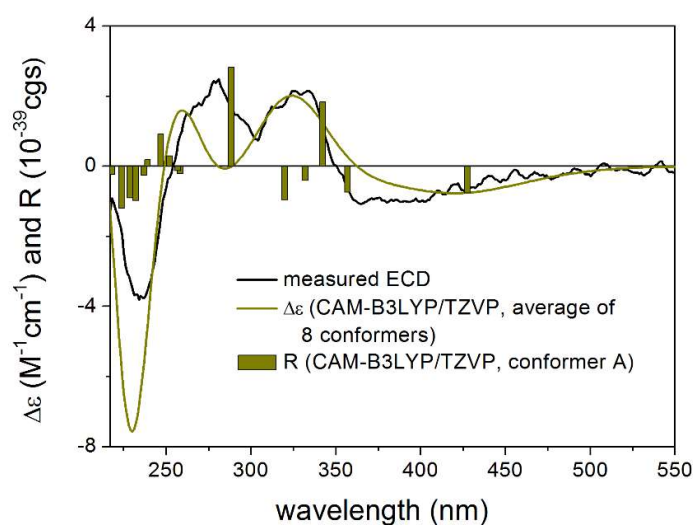


Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (f) The HMBC and enlarged spectrum of 7 in DMSO-*d*₆.

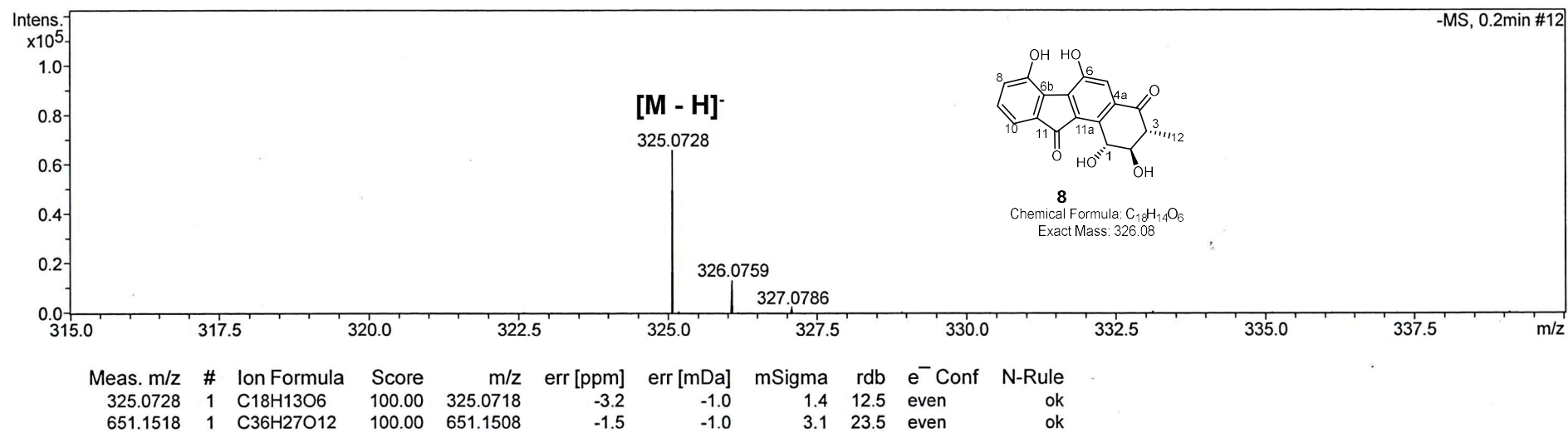


Supplementary Fig. 4. Spectroscopic data for FST B1 (7). (g) The NOESY and enlarged NOESY spectrum of **7** in DMSO- d_6 .

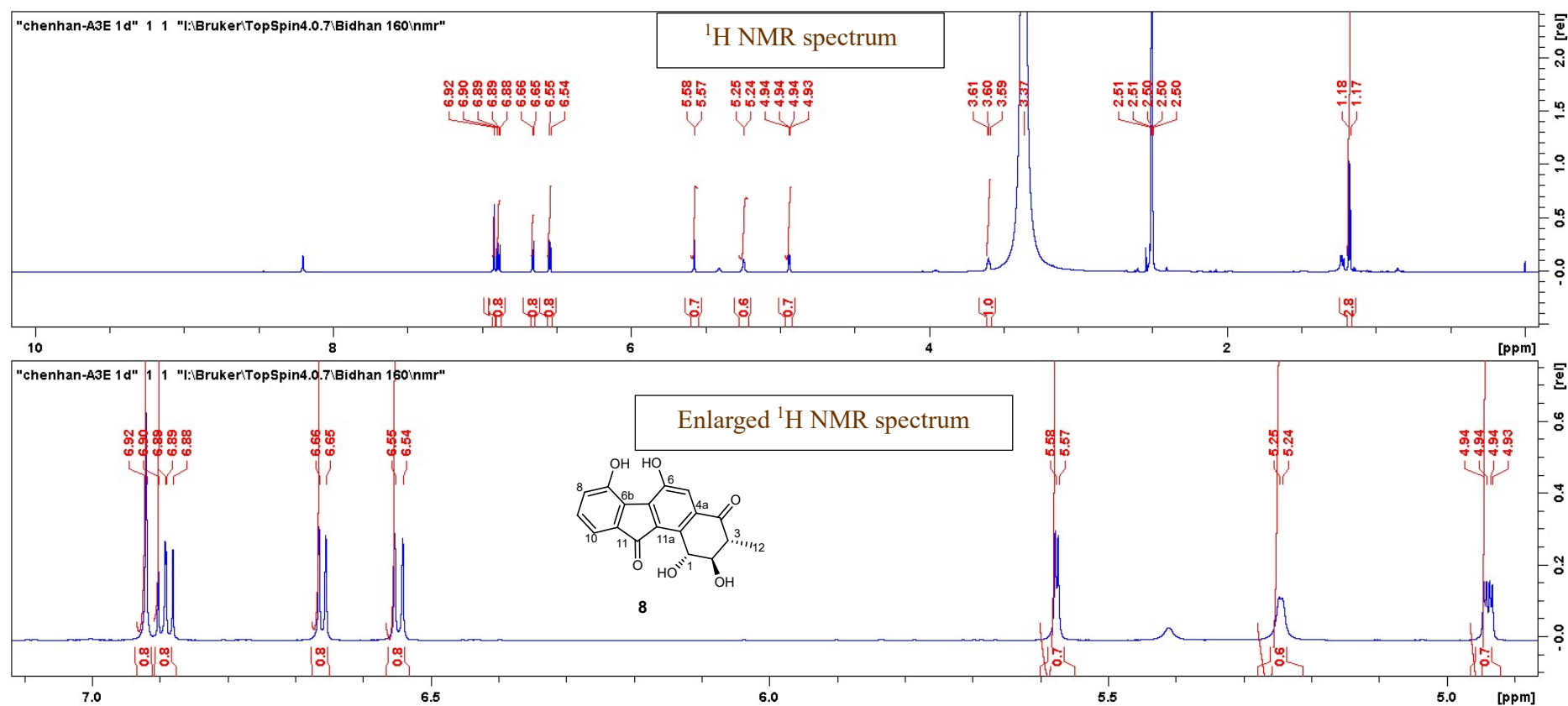


a**b**

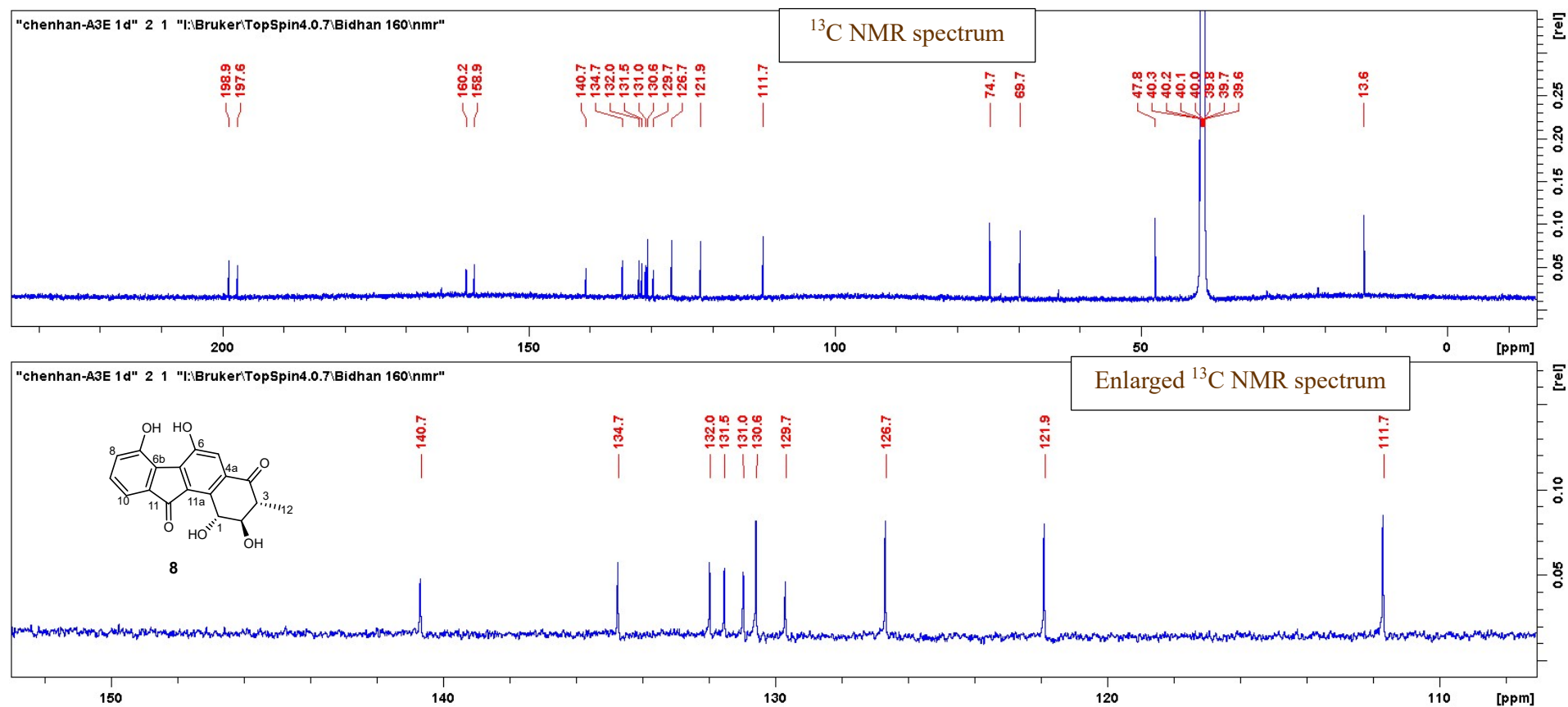
Supplementary Fig. 5. Comparison of experimental ECD and calculated ECD spectra of (1*R*,2*R*,3*S*)-7 in MeCN. (a) Structure and population of the low-energy ω B97X/TZVP PCM/MeCN conformers ($> 1\%$) of (1*R*,2*R*,3*S*)-7. (b) Experimental ECD spectrum of 7 in MeCN compared with the Boltzmann-weighted CAM-B3LYP/TZVP PCM/MeCN ECD spectrum of (1*R*,2*R*,3*S*)-7 computed for the ω B97X/TZVP PCM/MeCN conformers. Bars represent the rotational strength of the lowest-energy conformer.



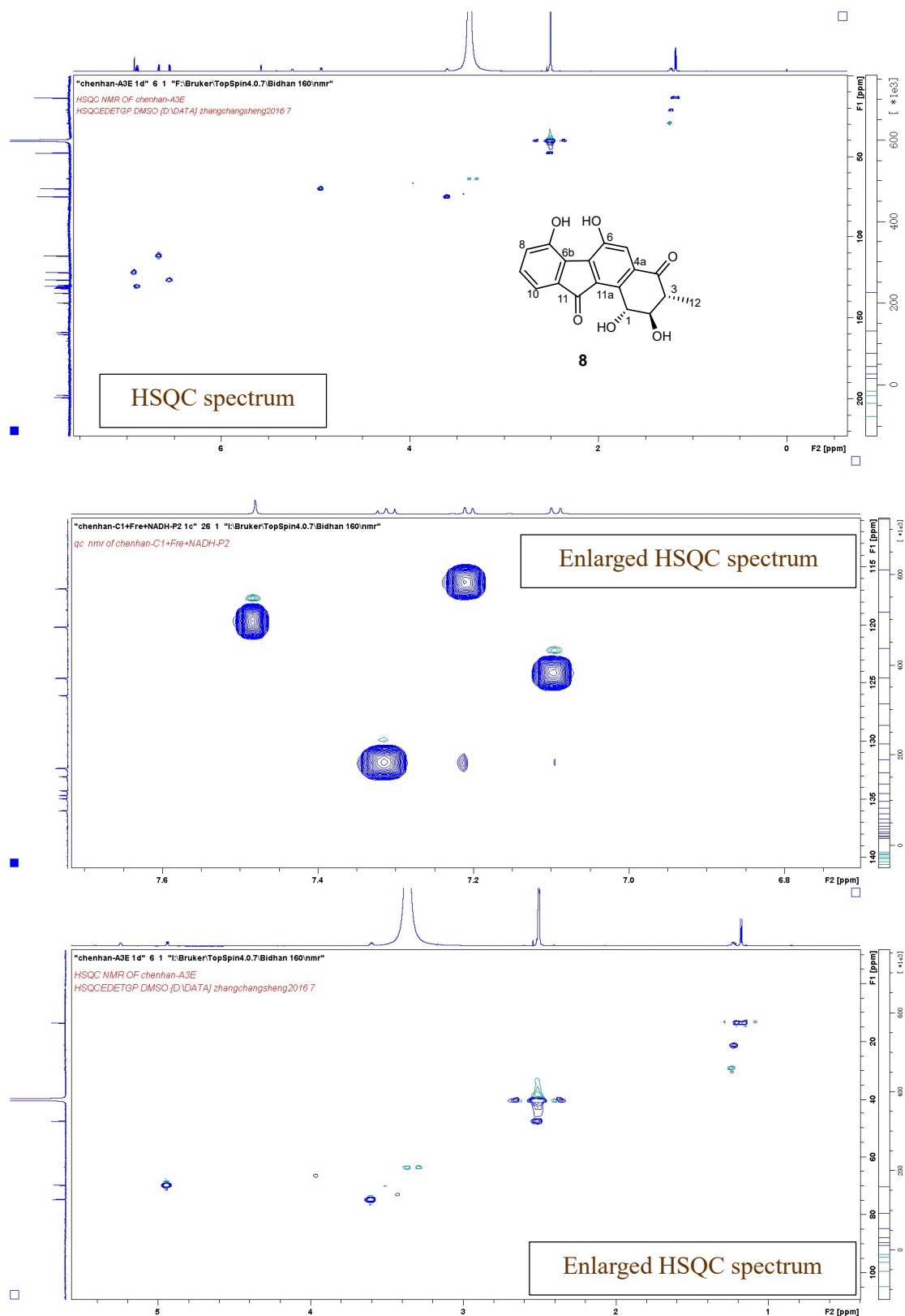
Supplementary Fig. 6 Spectroscopic data for FST B2 (8). (a) HRESIMS spectrum.



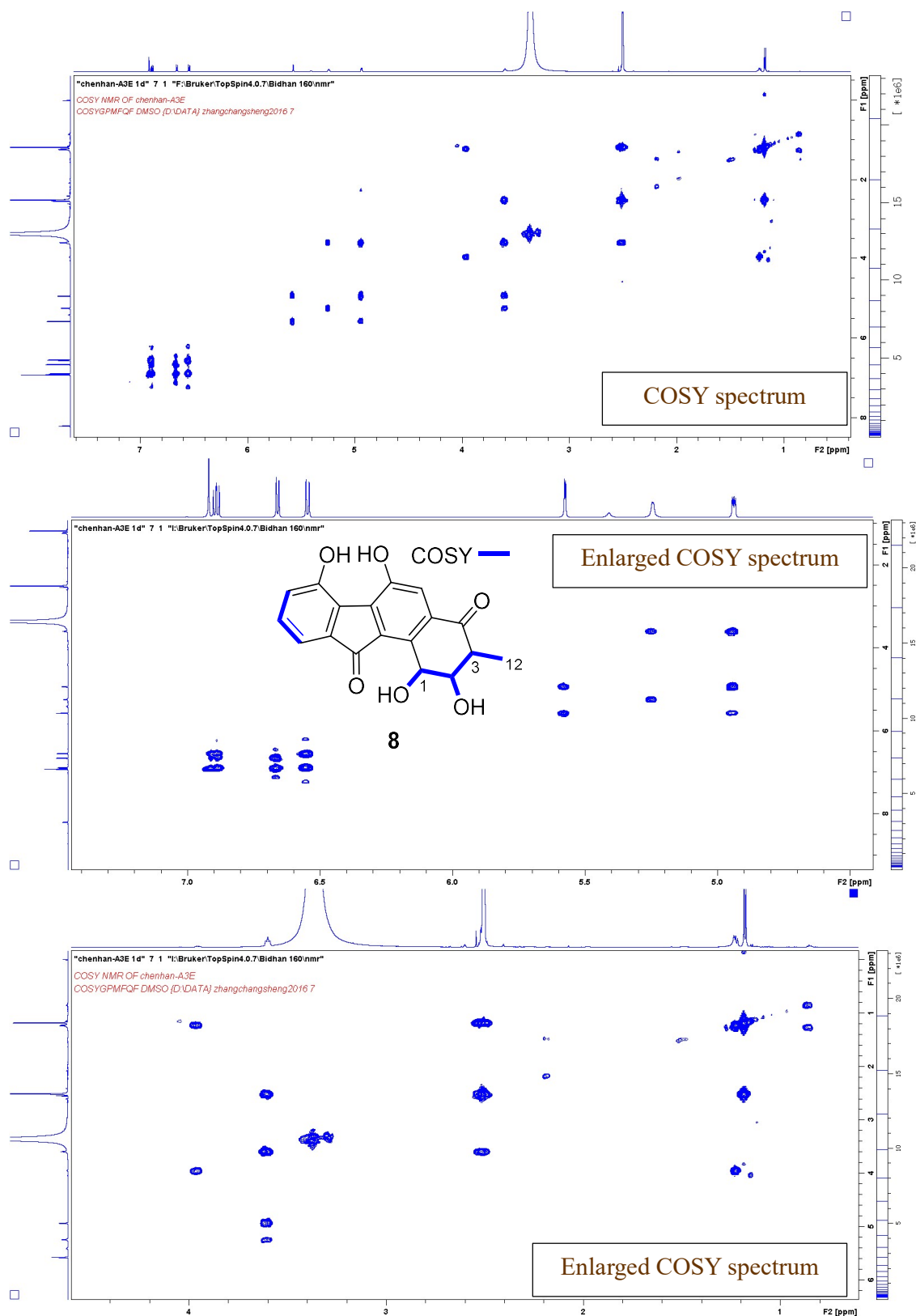
Supplementary Fig. 6 Spectroscopic data for FST B2 (**8**). (b) The ¹H and enlarged ¹H NMR spectrum of **8** in DMSO-*d*₆.

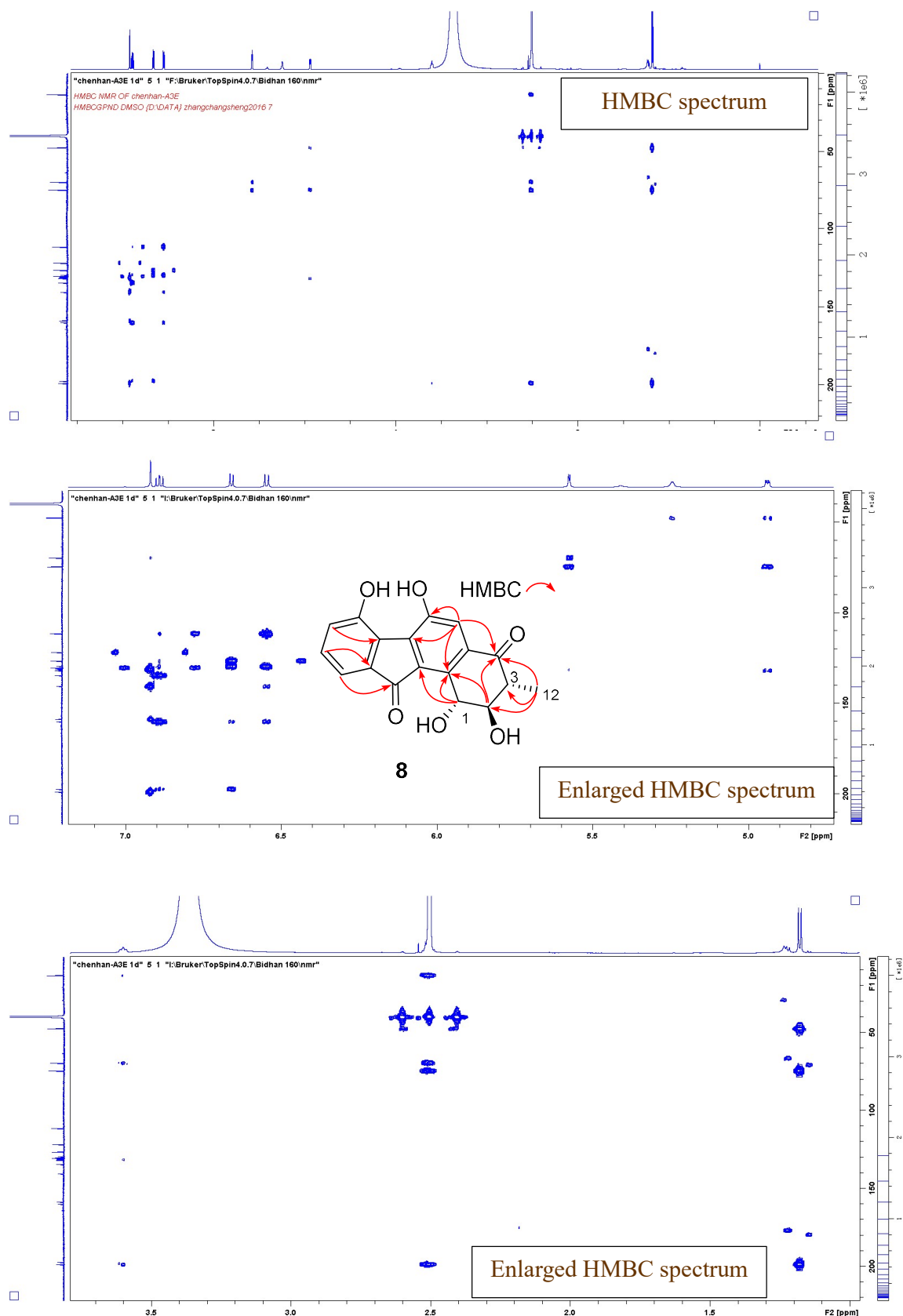


Supplementary Fig. 6 Spectroscopic data for FST B2 (**8**). (c) The ¹³C and enlarged ¹³C NMR spectrum of **8** in DMSO-*d*₆.

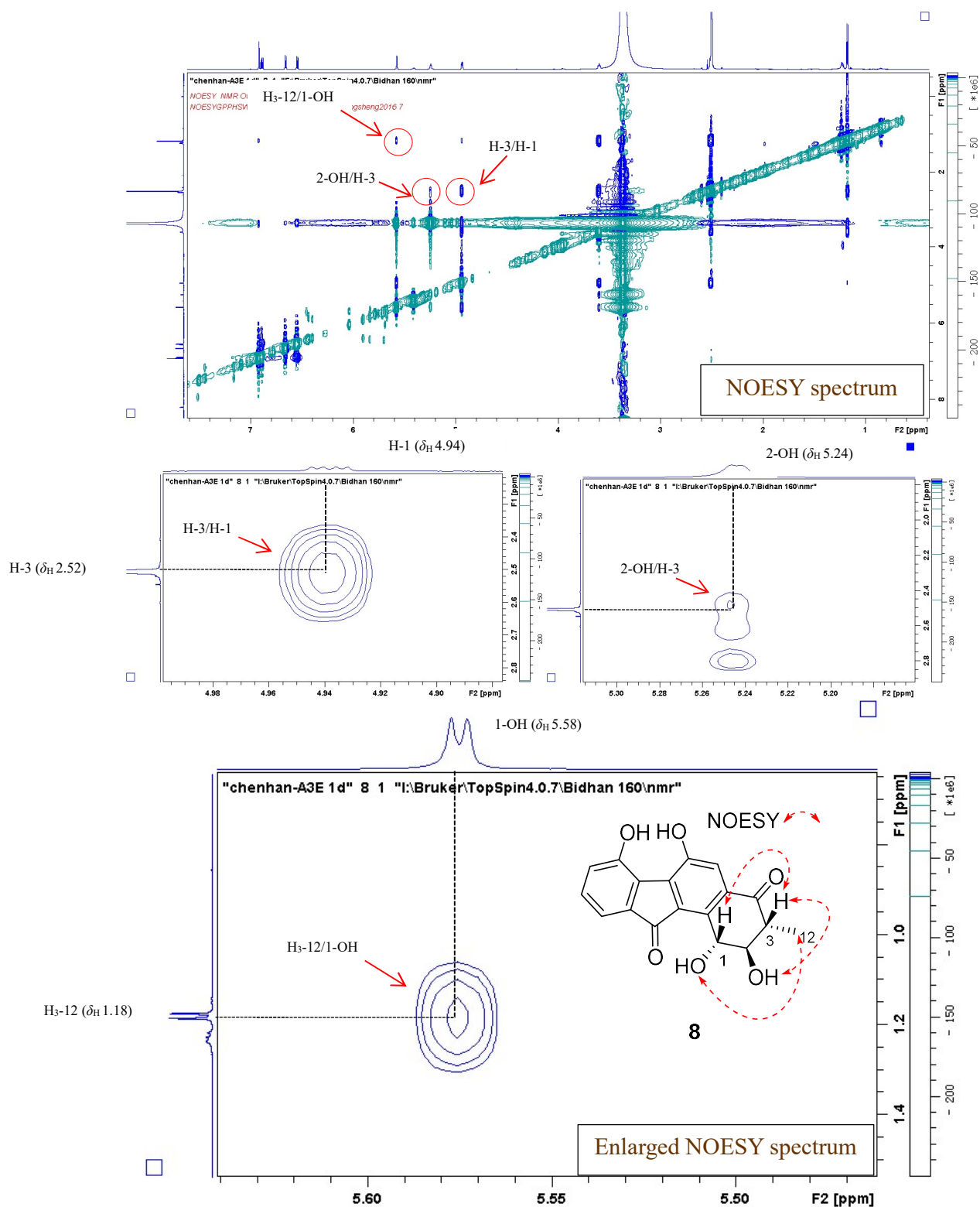


Supplementary Fig. 6 Spectroscopic data for FST B2 (8). (d) The HSQC and enlarged HSQC spectrum of 8 in DMSO-*d*₆.



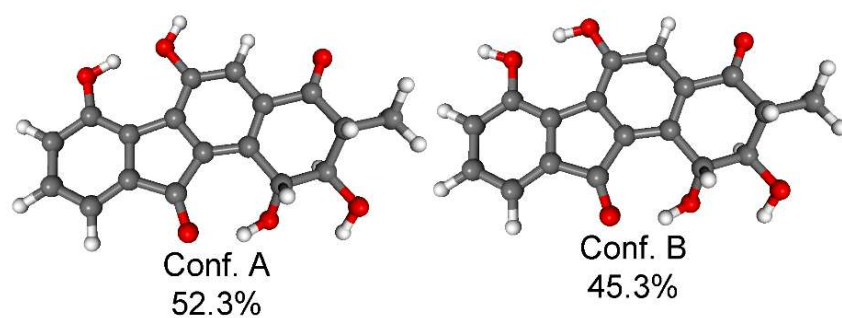


Supplementary Fig. 6 Spectroscopic data for FST B2 (8). (f) The HMBC and enlarged HMBC spectrum of 8 in DMSO-*d*₆.

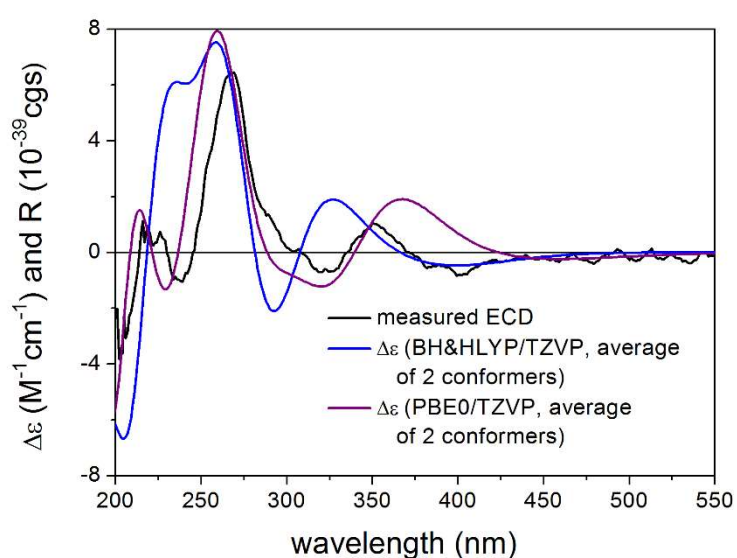


Supplementary Fig. 6 Spectroscopic data for FST B2 (**8**). (g) The NOESY and enlarged NOESY spectrum of **8** in DMSO- d_6 .

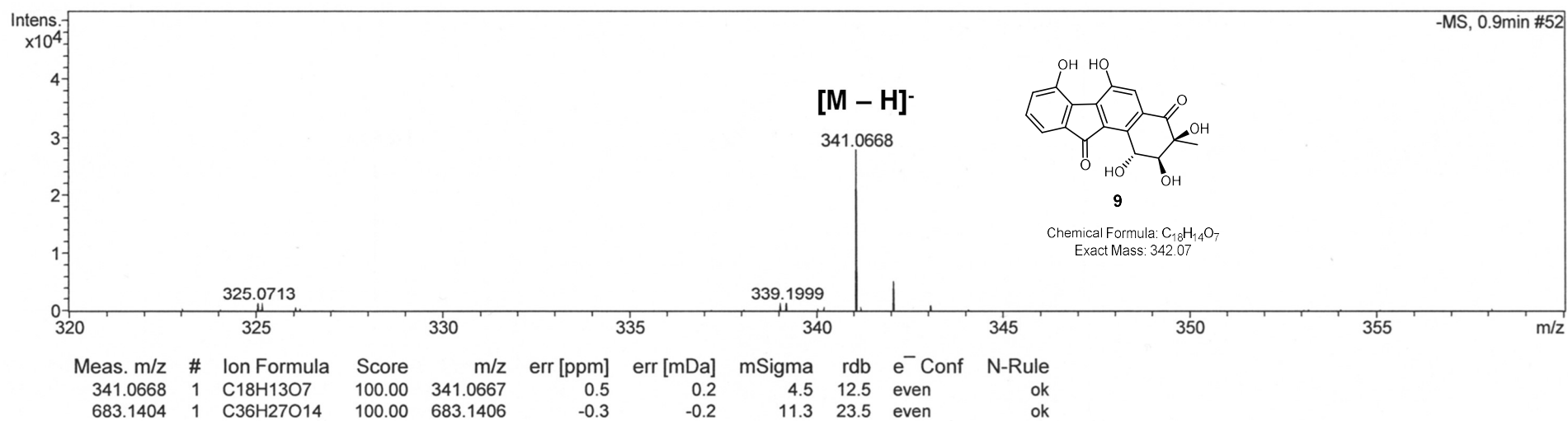
a



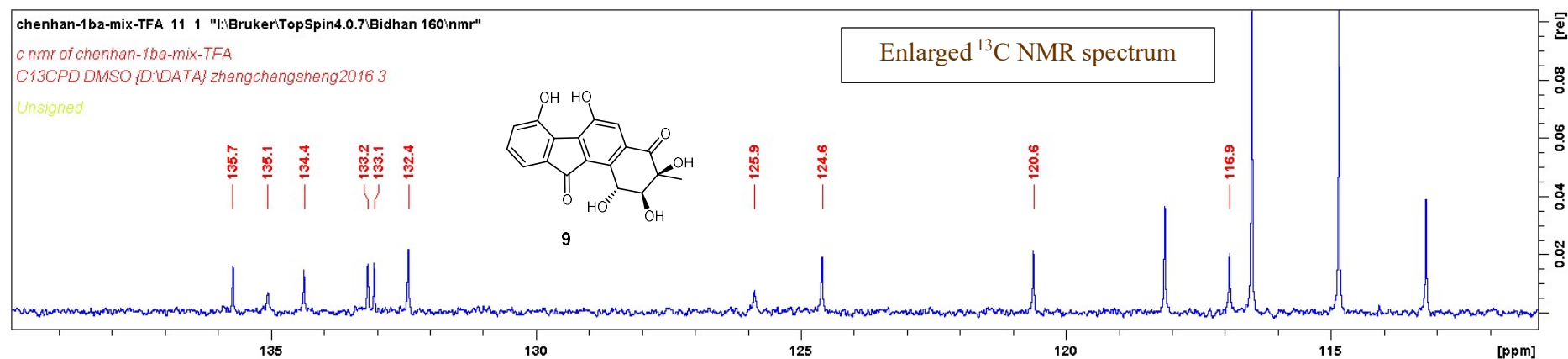
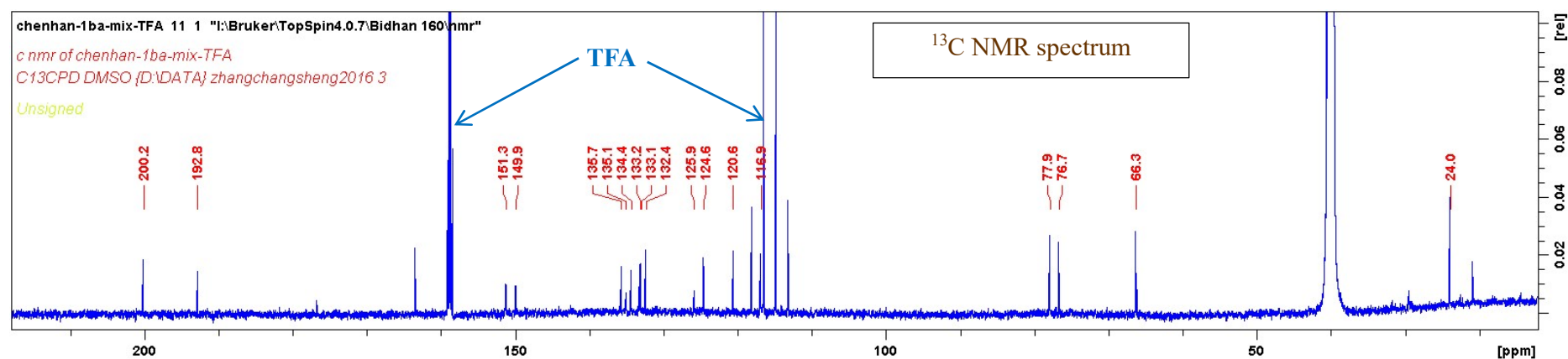
b



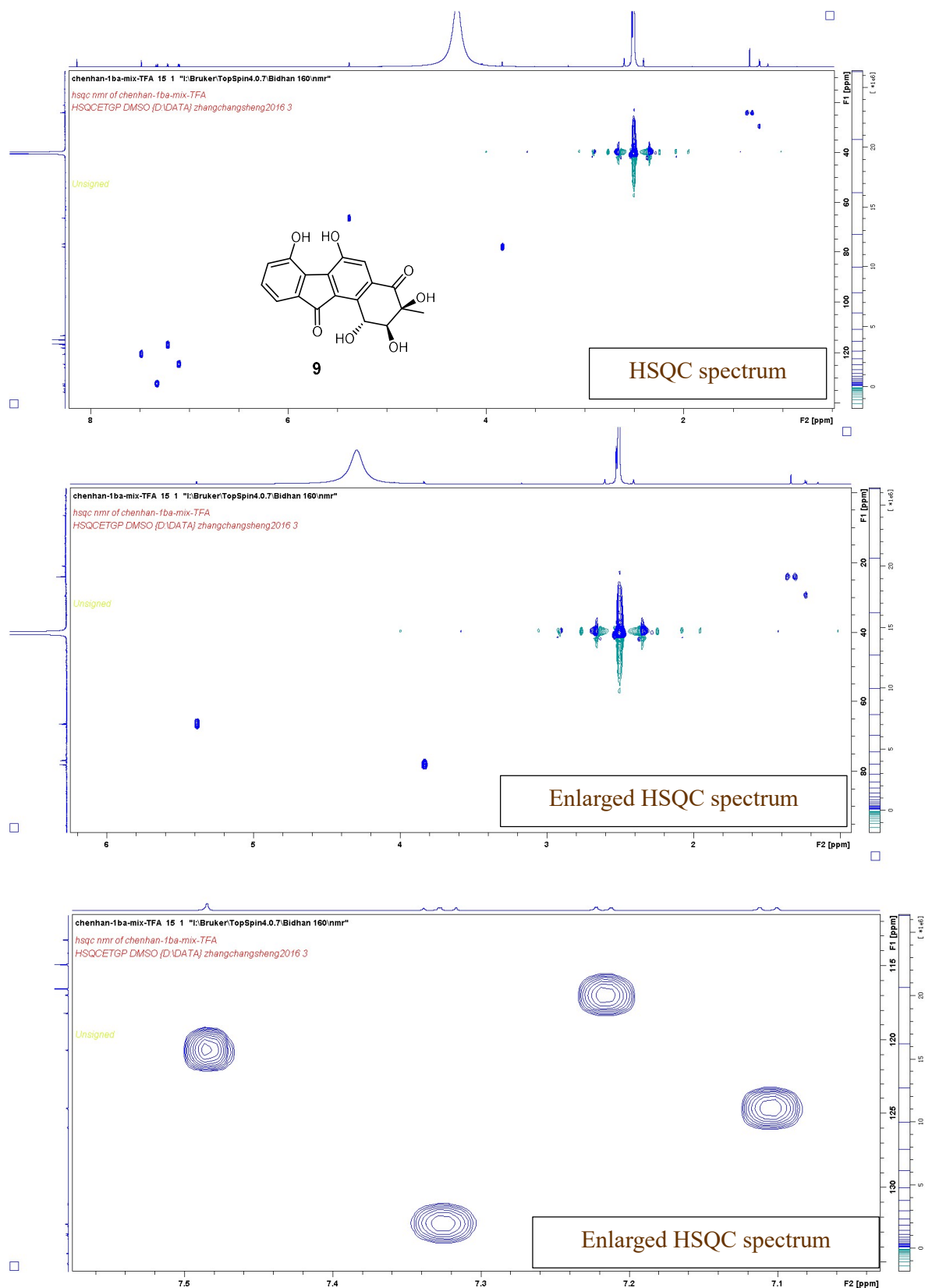
Supplementary Fig. 7. Comparison of experimental and calculated ECD spectra of **8 in MeCN. (a)** Structure and population of the low-energy ω B97X/TZVP PCM/MeCN conformers ($> 1\%$) of (1*R*,2*R*,3*R*)-**8**. **(b)** Experimental ECD spectrum of **8** in MeCN compared with the Boltzmann-weighted BH&HLYP/TZVP PCM/MeCN and PBE0/TZVP PCM/MeCN ECD spectra of (1*R*,2*R*,3*R*)-**8** computed for the ω B97X/TZVP PCM/MeCN conformers.



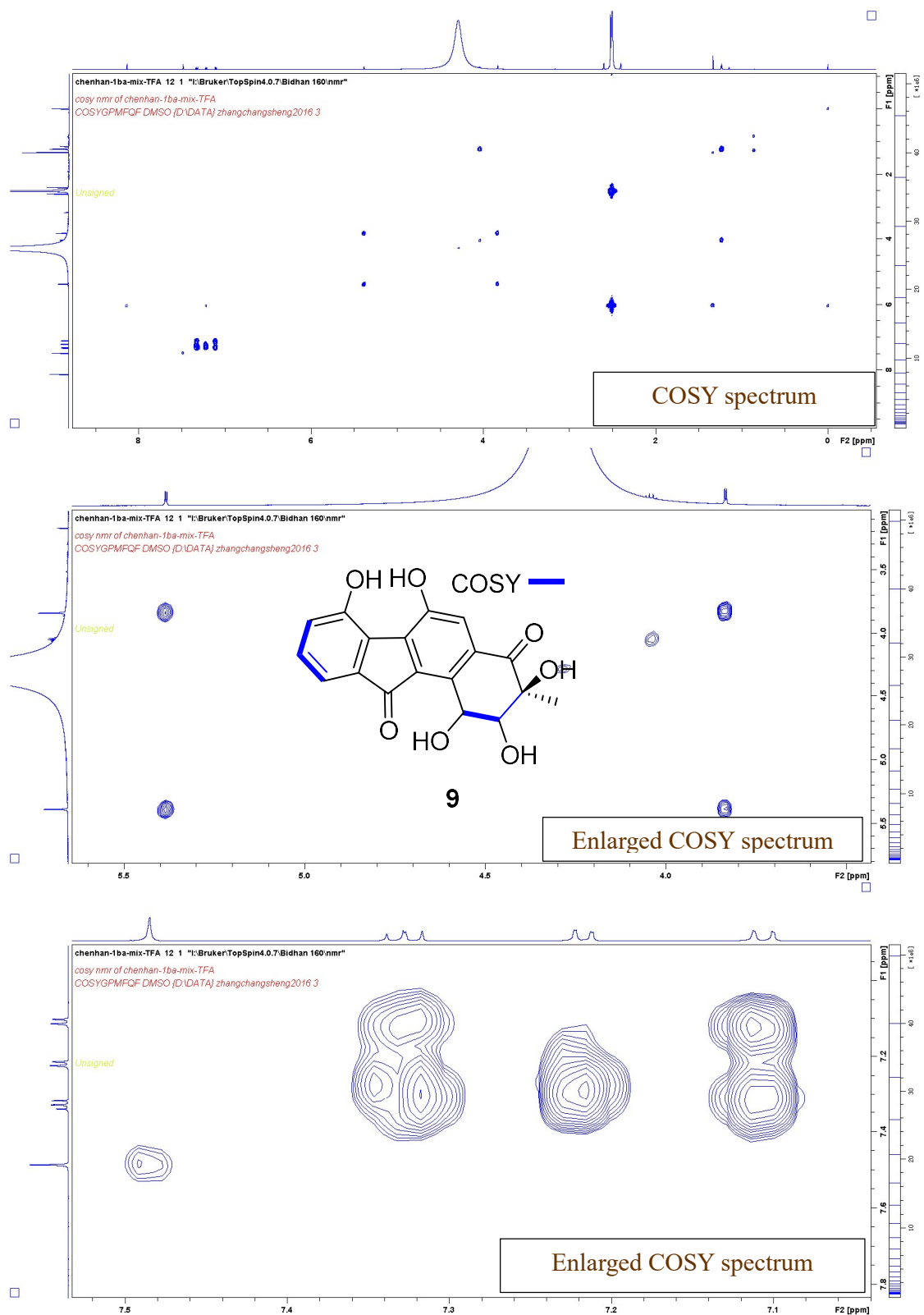
Supplementary Fig. 8. Spectroscopic data for 9. (a) HRESIMS spectrum.



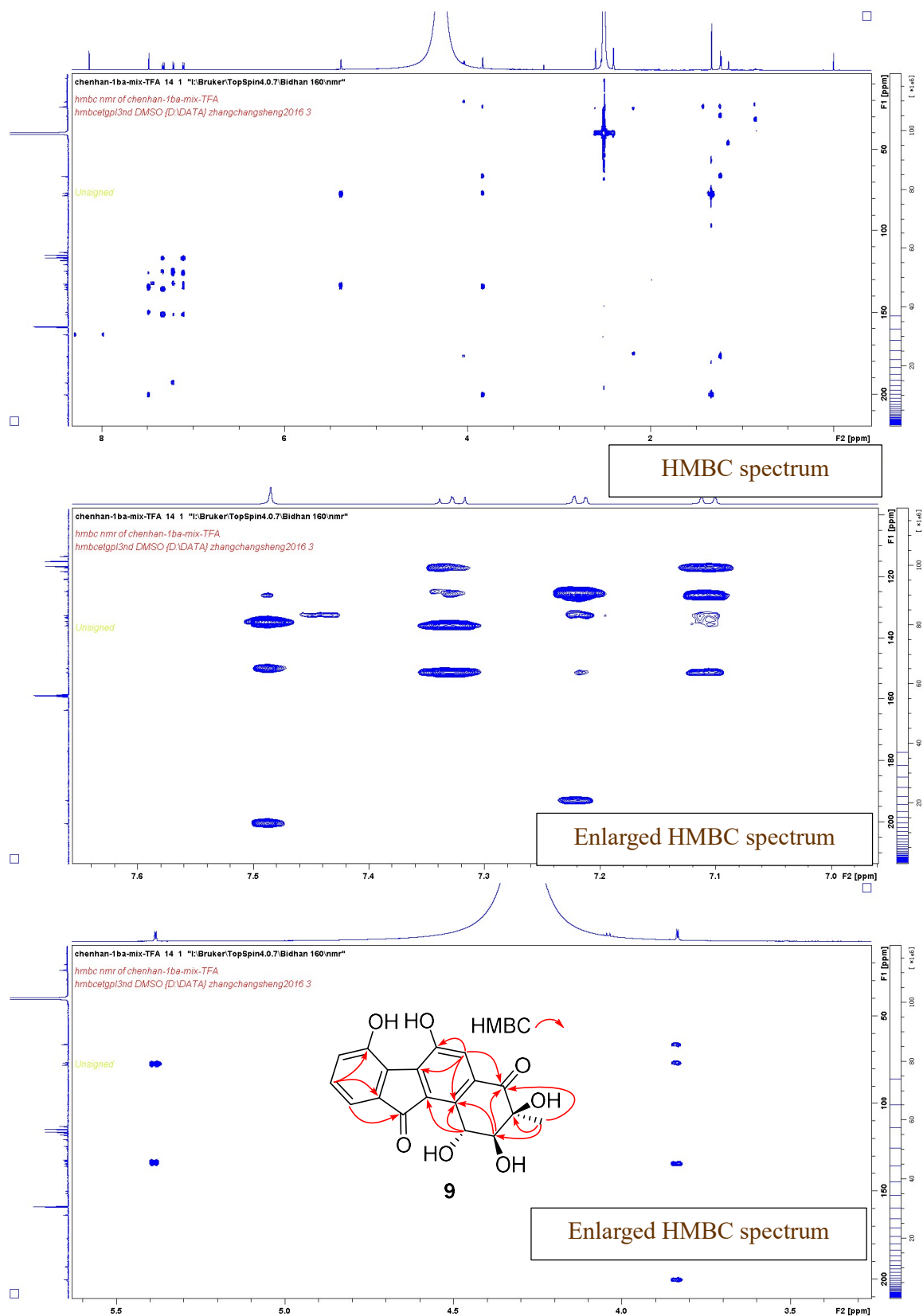
Supplementary Fig. 8. Spectroscopic data for 9. (c) The ¹³C and enlarged ¹³C NMR spectrum of **9** in DMSO-*d*₆:trifluoroacetic acid (TFA)-*d*₁ (100:1).



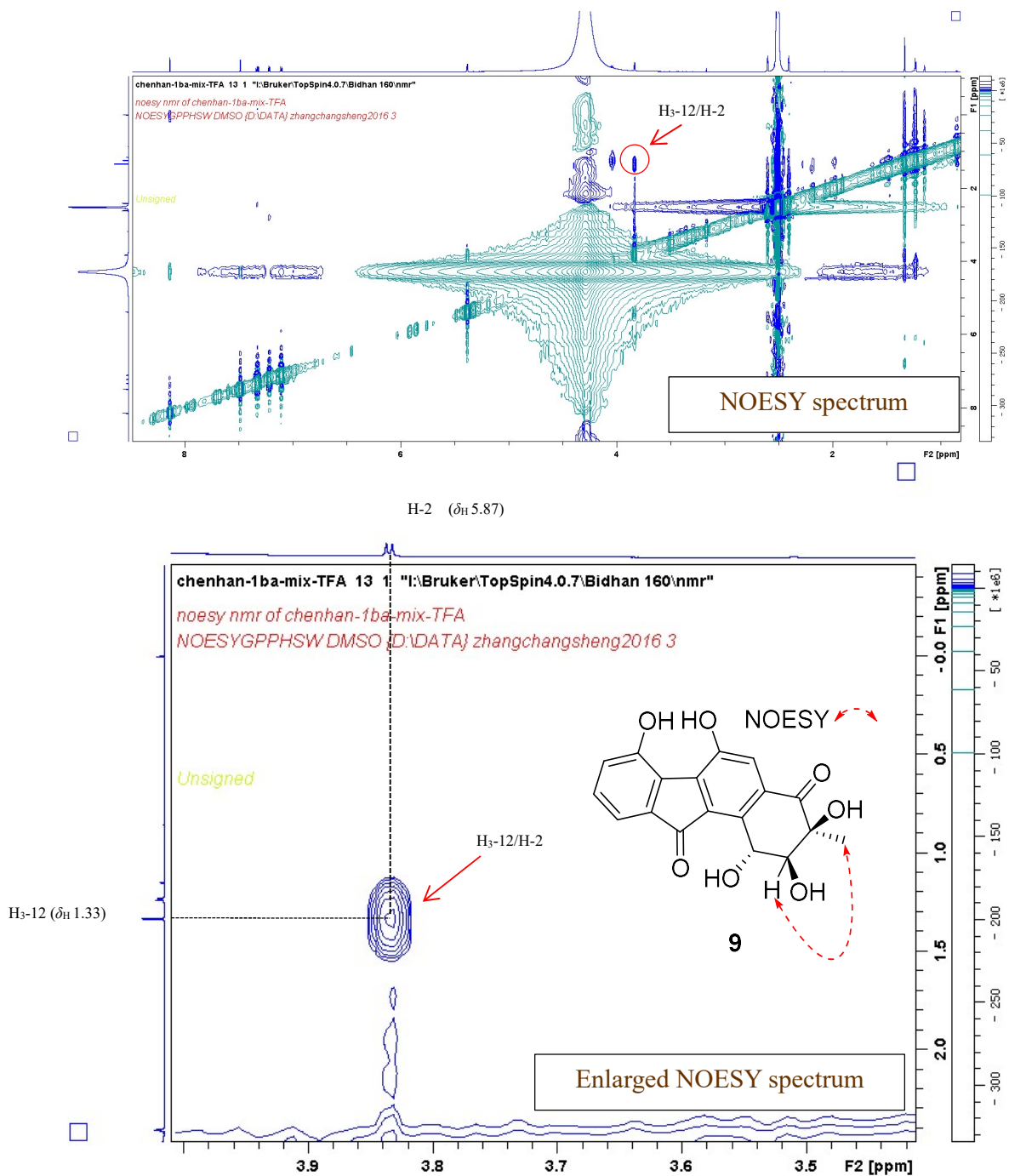
Supplementary Fig. 8. Spectroscopic data for 9. (d) The HSQC and enlarged HSQC spectrum of **9** in DMSO-*d*₆.



Supplementary Fig. 8. Spectroscopic data for 9. (e) The COSY and enlarged COSY spectrum of **9** in DMSO- d_6 .

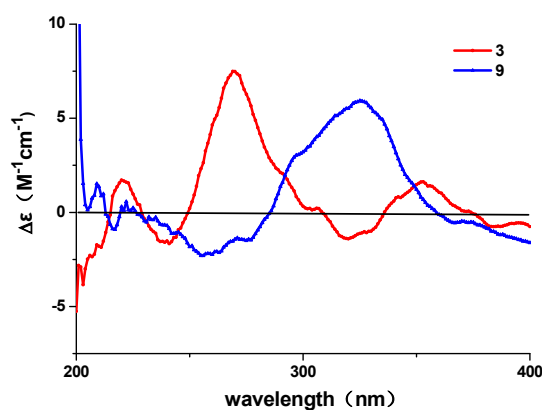


Supplementary Fig. 8. Spectroscopic data for 9. (f) The HMBC and enlarged HMBC spectrum of 9 in DMSO- d_6 .

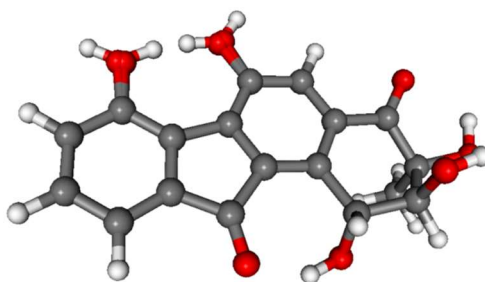


Supplementary Fig. 8. Spectroscopic data for 9. (g) The NOESY and enlarged NOESY spectrum of **9** in DMSO-*d*₆.

a

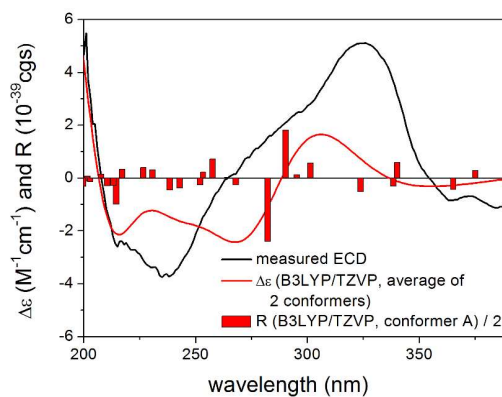


b

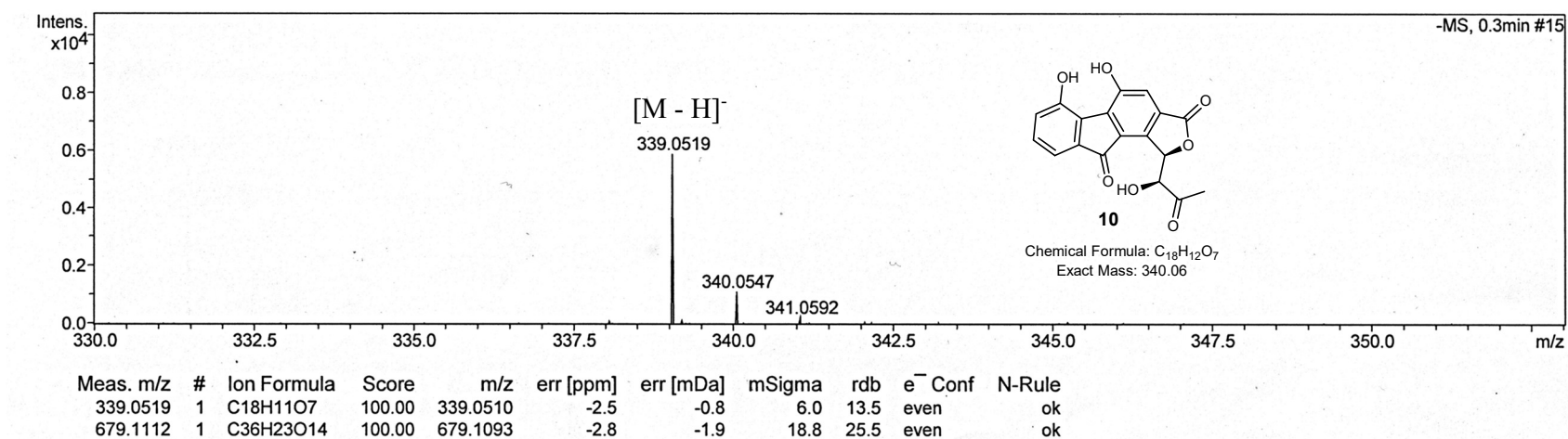


Overlapped conformers A and B (99%)

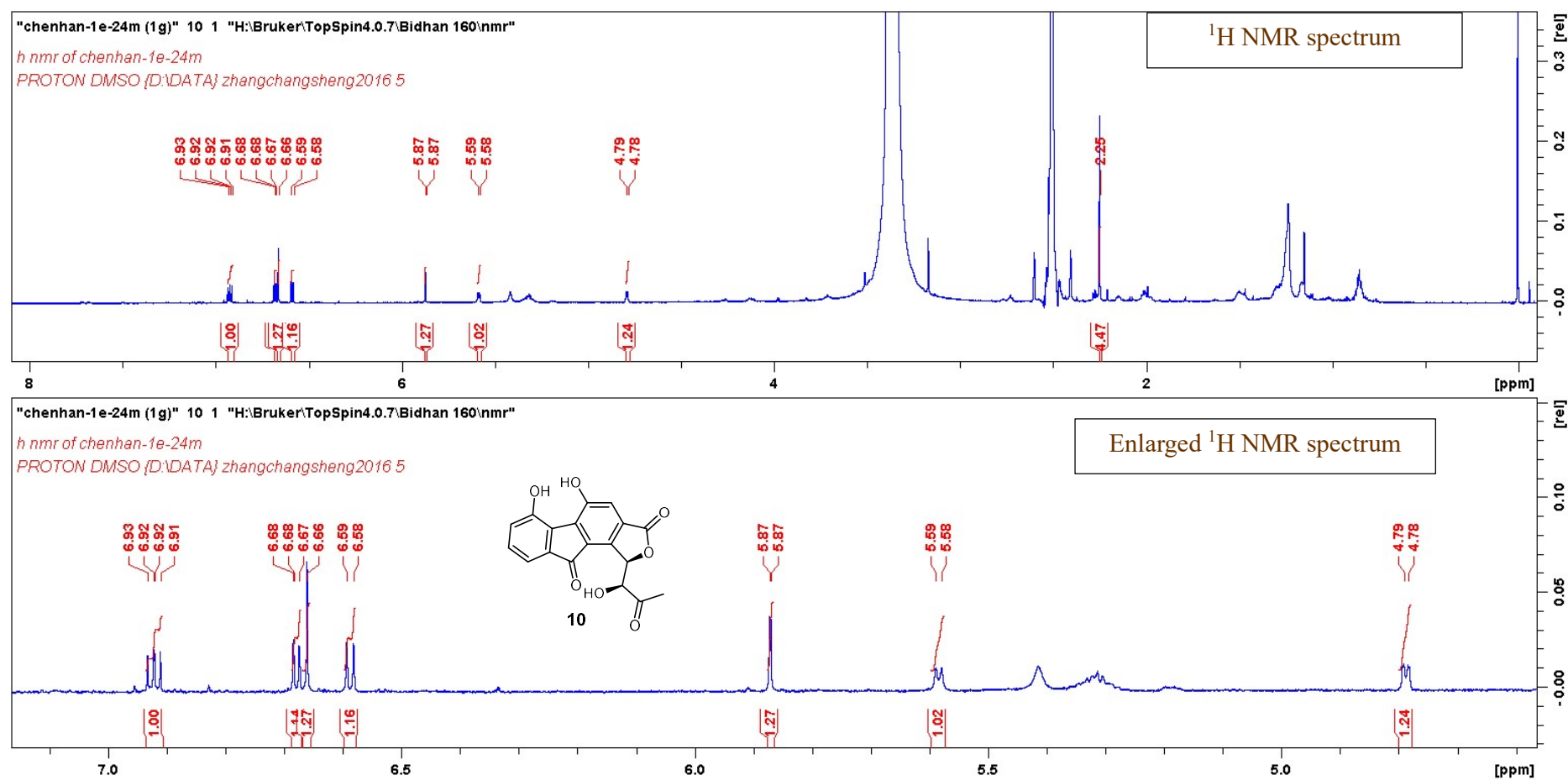
c



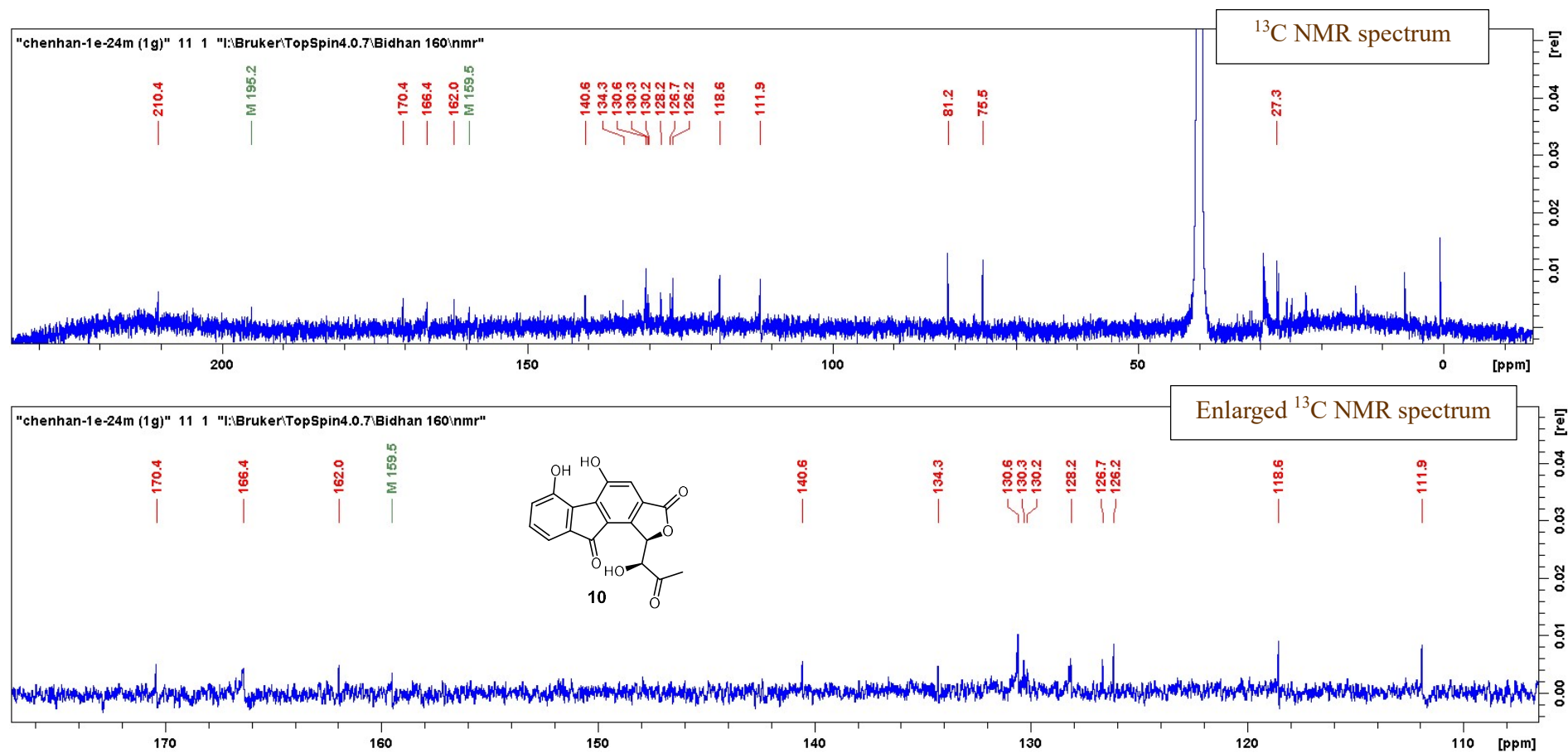
Supplementary Fig. 9. Comparison of the experimental ECD spectra of 3 with 9 and comparison of experimental and calculated ECD spectra of 9 in MeCN. (a) Comparison of the experimental ECD spectrum of 9 with 3. **(b)** Structure and population of the low-energy ω B97X/TZVP PCM/MeCN conformers ($> 1\%$) of (1*R*,2*S*,3*S*)-9. **(c)** Experimental ECD spectrum of 9 in MeCN compared with the Boltzmann-weighted B3LYP/TZVP PCM/MeCN ECD spectrum of (1*R*,2*S*,3*S*)-9 computed for the ω B97X/TZVP PCM/MeCN conformers.



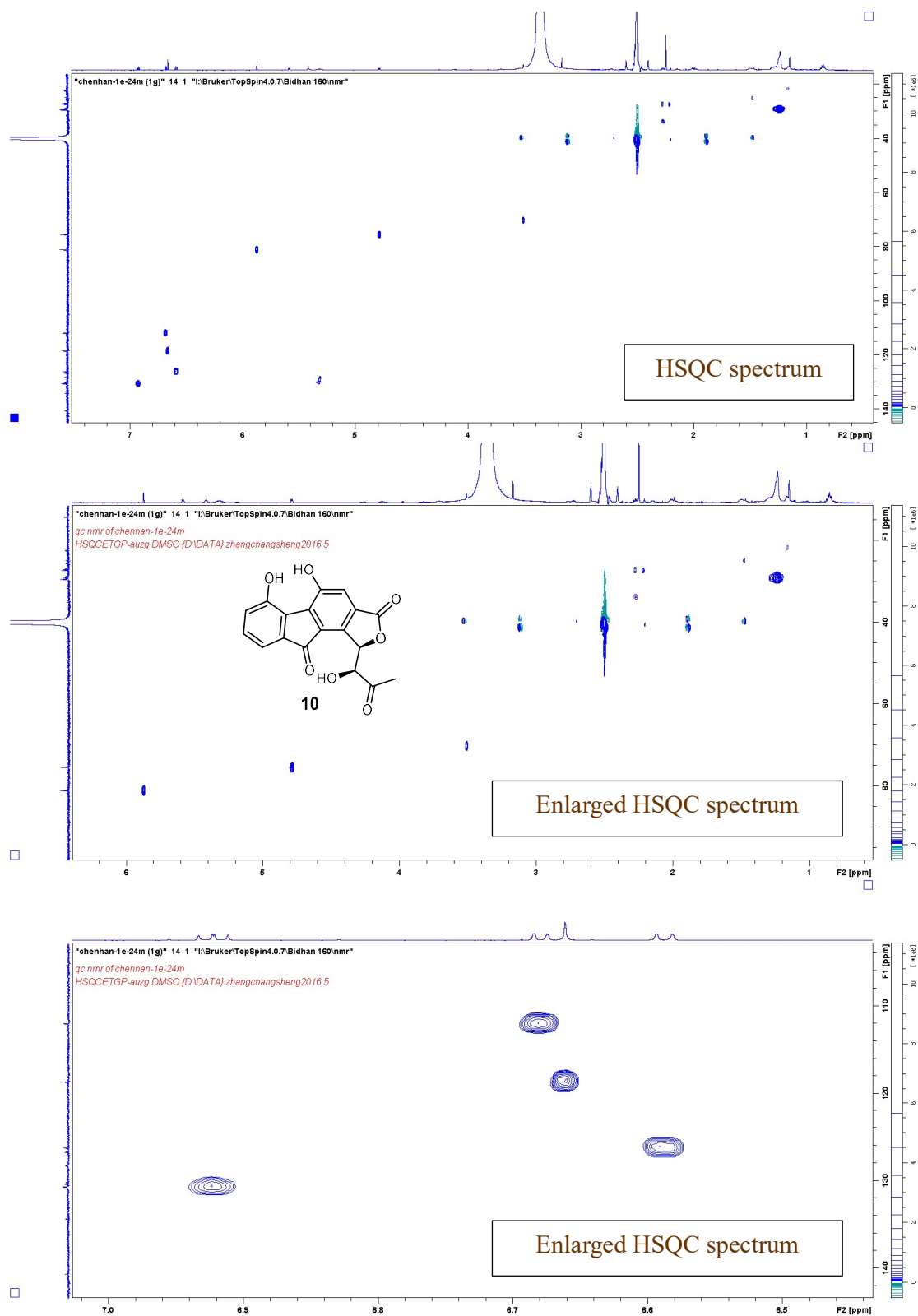
Supplementary Fig. 10. Spectroscopic data for 10. (a) HRESIMS spectrum.



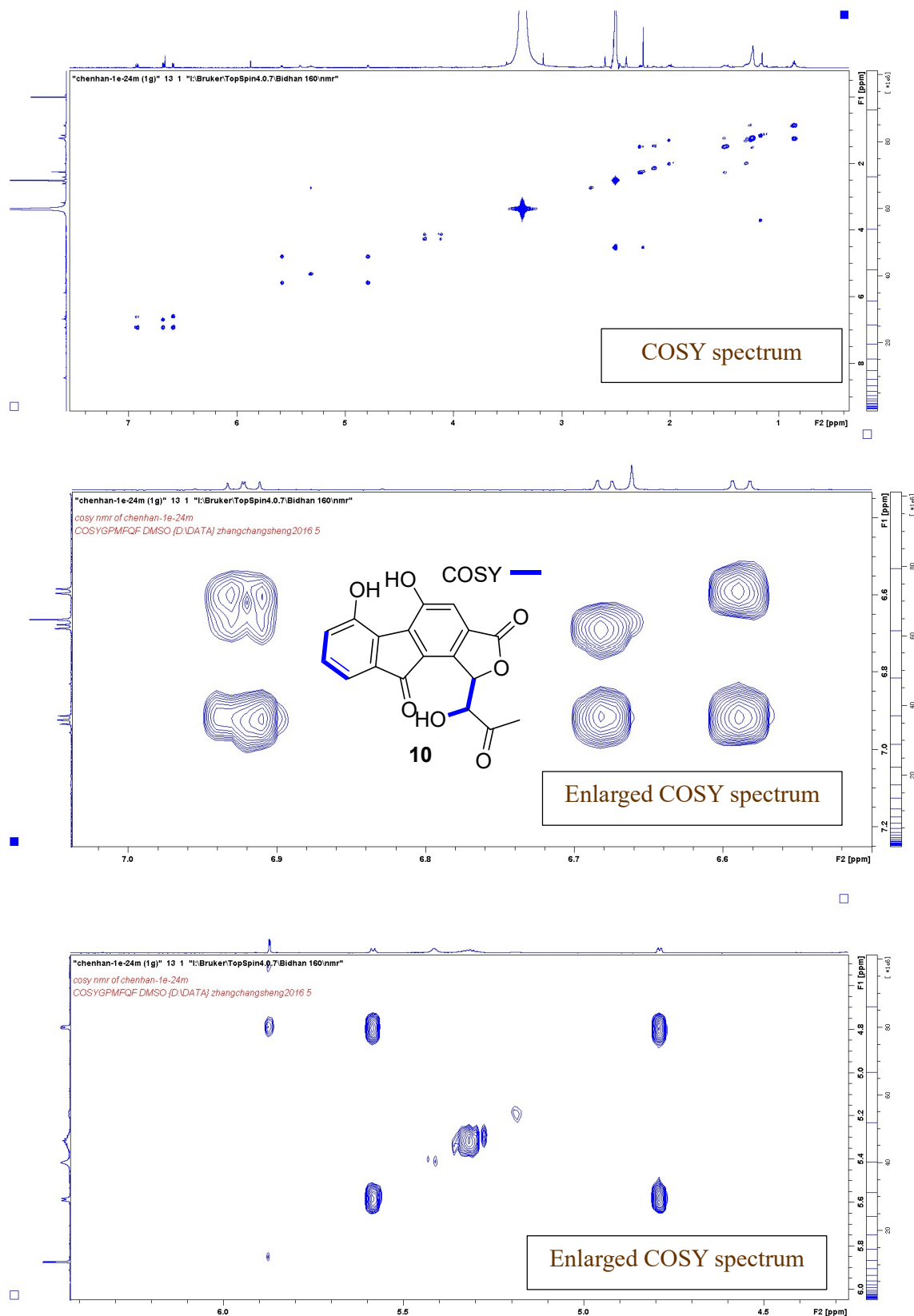
Supplementary Fig. 10. Spectroscopic data for 10. (b) The ¹H and enlarged ¹H NMR spectrum of 10 in DMSO-*d*₆.



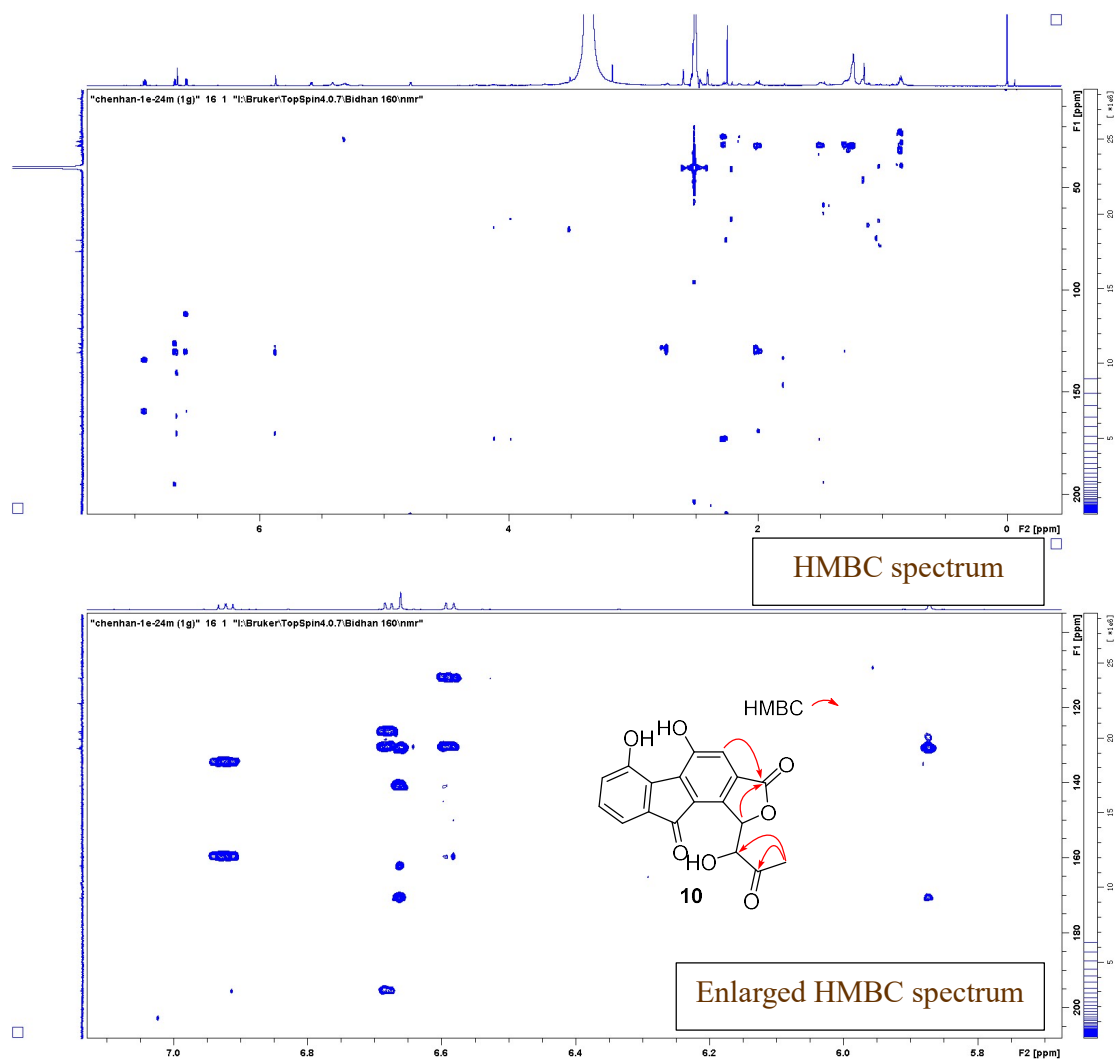
Supplementary Fig. 10. Spectroscopic data for 10. (B) (c) The ¹³C and enlarged ¹³C NMR spectrum of 10 in DMSO-*d*₆.



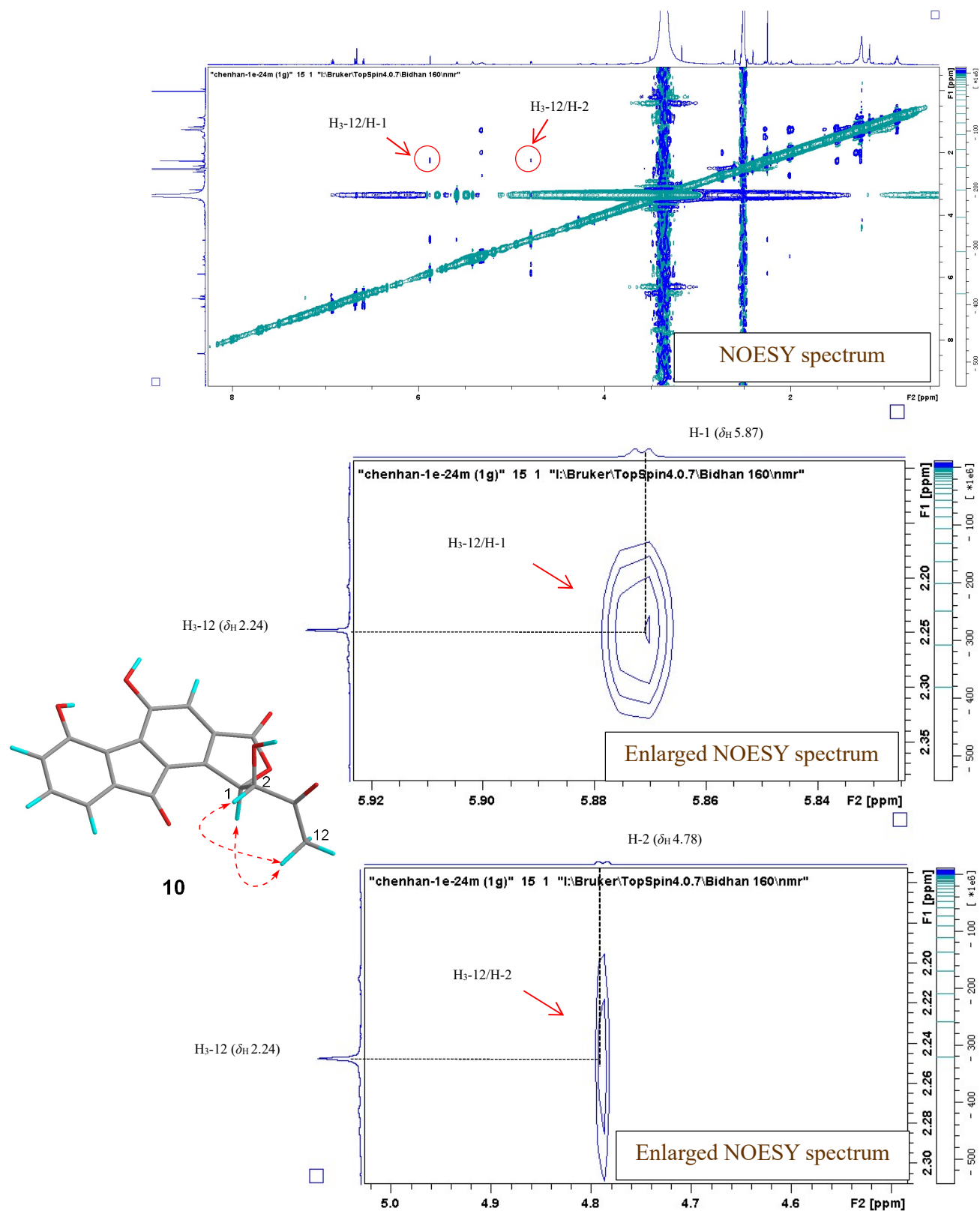
Supplementary Fig. 10. Spectroscopic data for 10. (d) The HSQC and enlarged HSQC spectrum of 10 in DMSO-*d*₆.



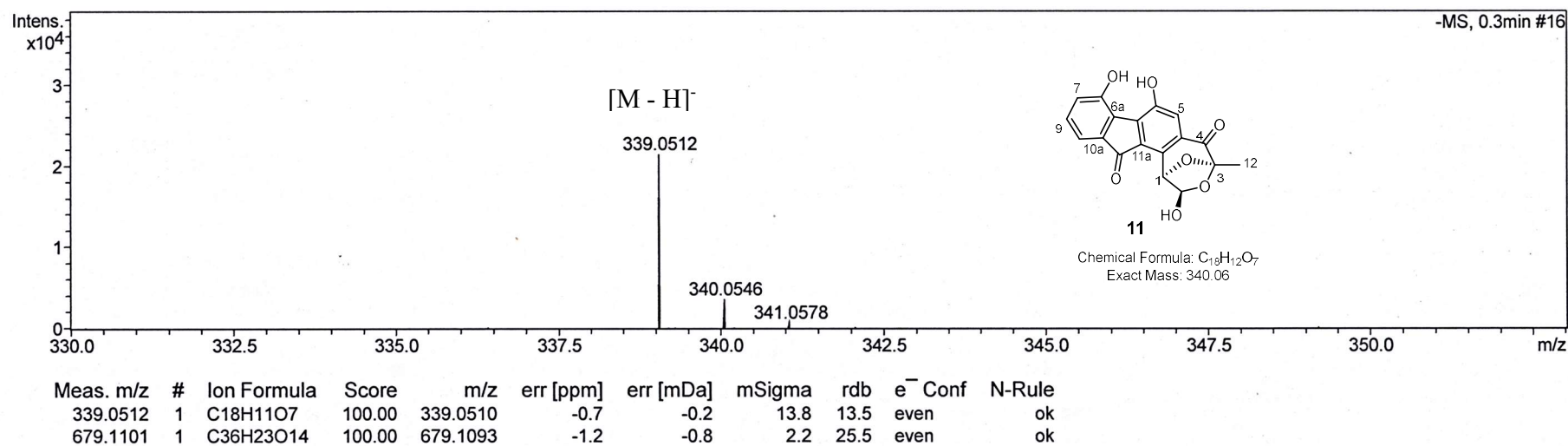
Supplementary Fig. 10. Spectroscopic data for 10. (e) The COSY and enlarged COSY spectrum of 10 in DMSO-*d*₆.



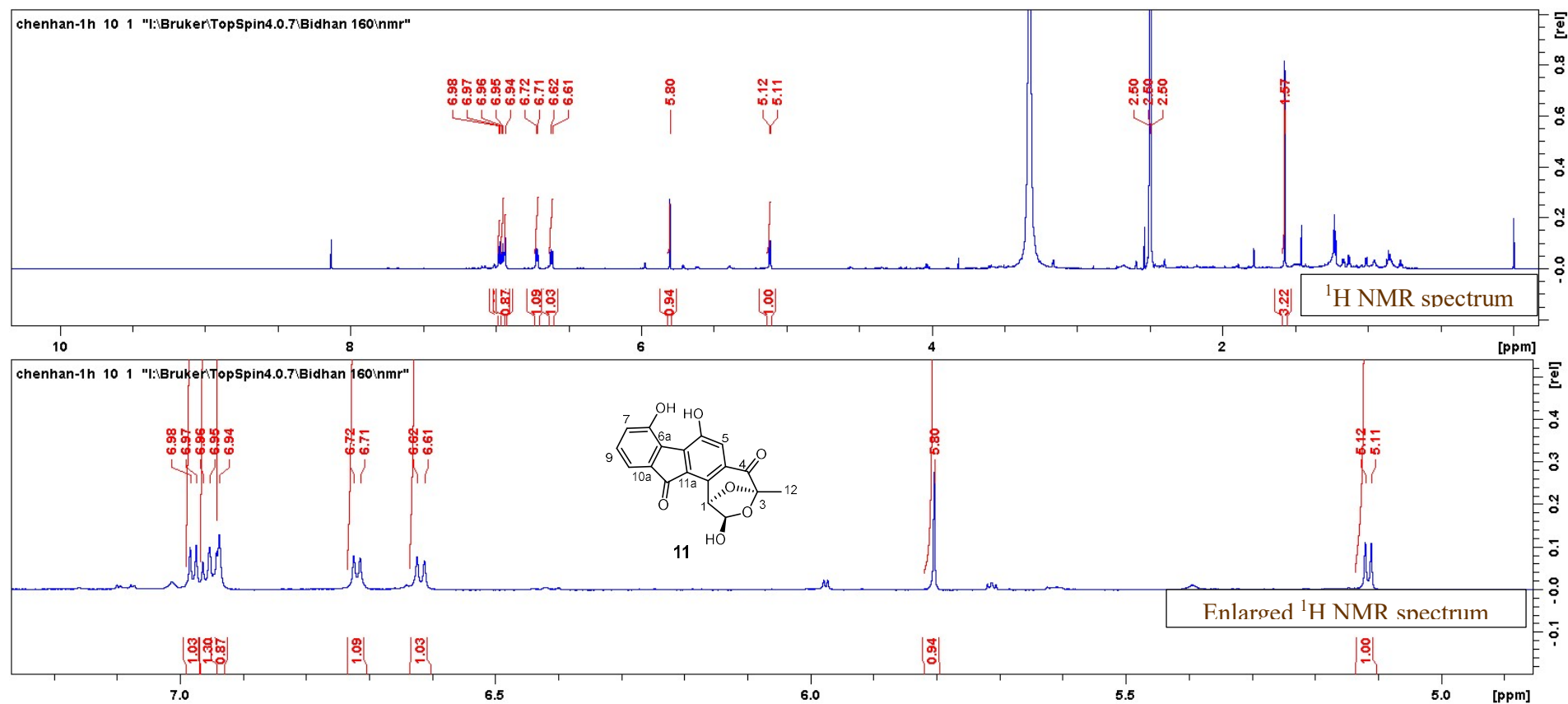
Supplementary Fig. 10. Spectroscopic data for 10. (f) The HMBC and enlarged HMBC spectrum of **10** in DMSO-*d*₆.



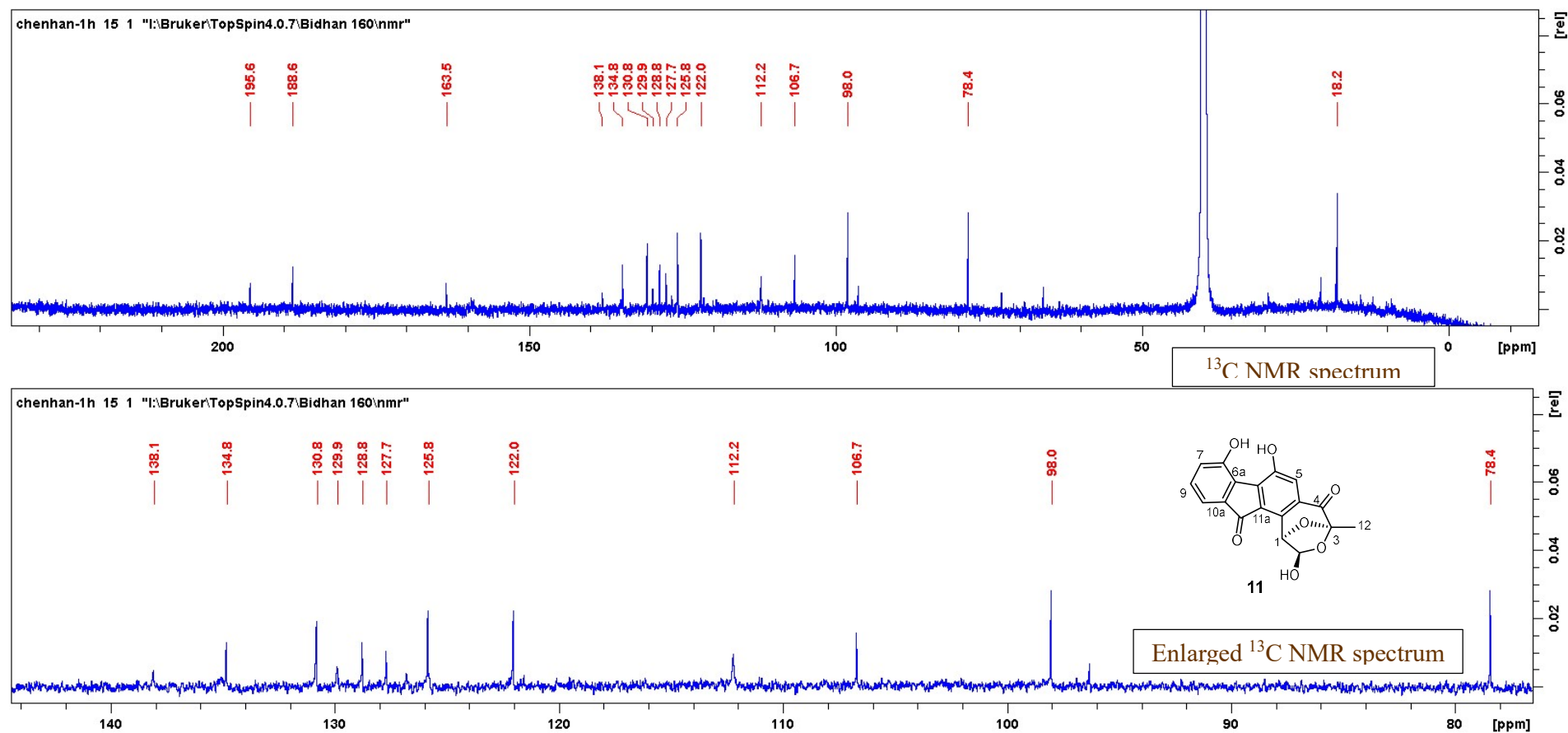
Supplementary Fig. 10. Spectroscopic data for 10. (g) The NOESY and enlarged NOESY spectrum of **10** in DMSO-*d*₆.



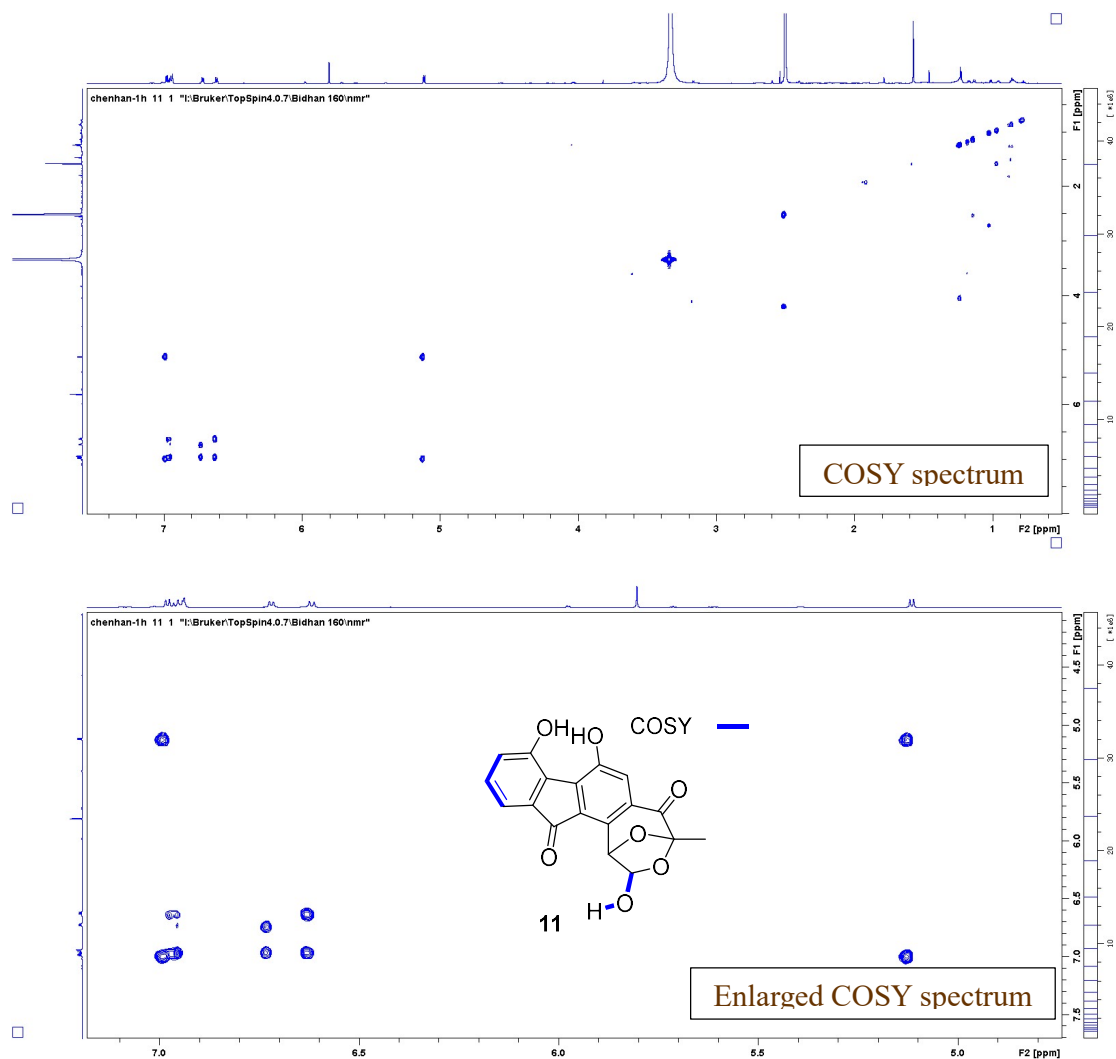
Supplementary Fig. 11 Spectroscopic data for 11. (a) HRESIMS spectrum.



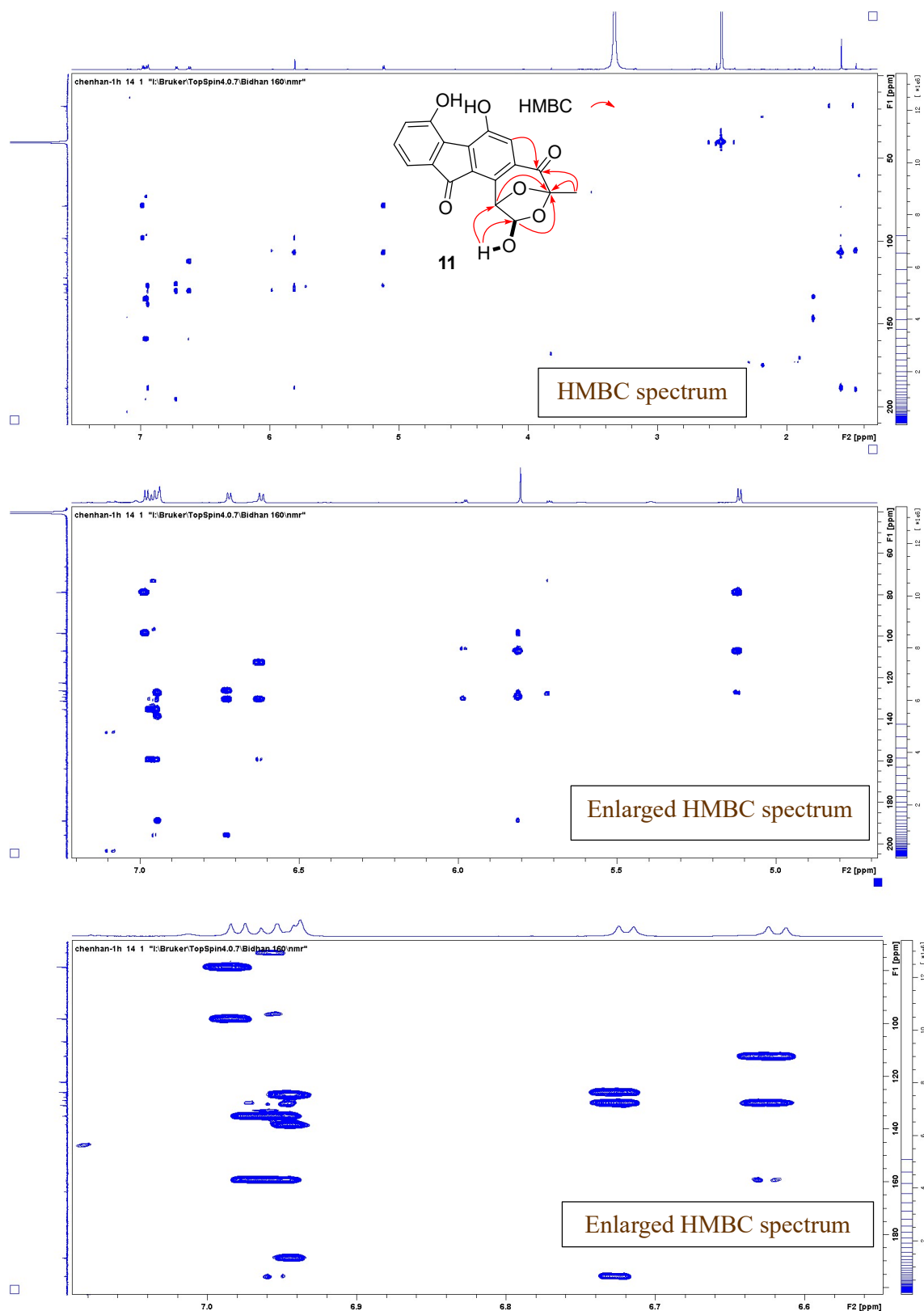
Supplementary Fig. 11 Spectroscopic data for **11**. (b) The ¹H and enlarged ¹H NMR spectrum of **11** in DMSO-*d*₆.



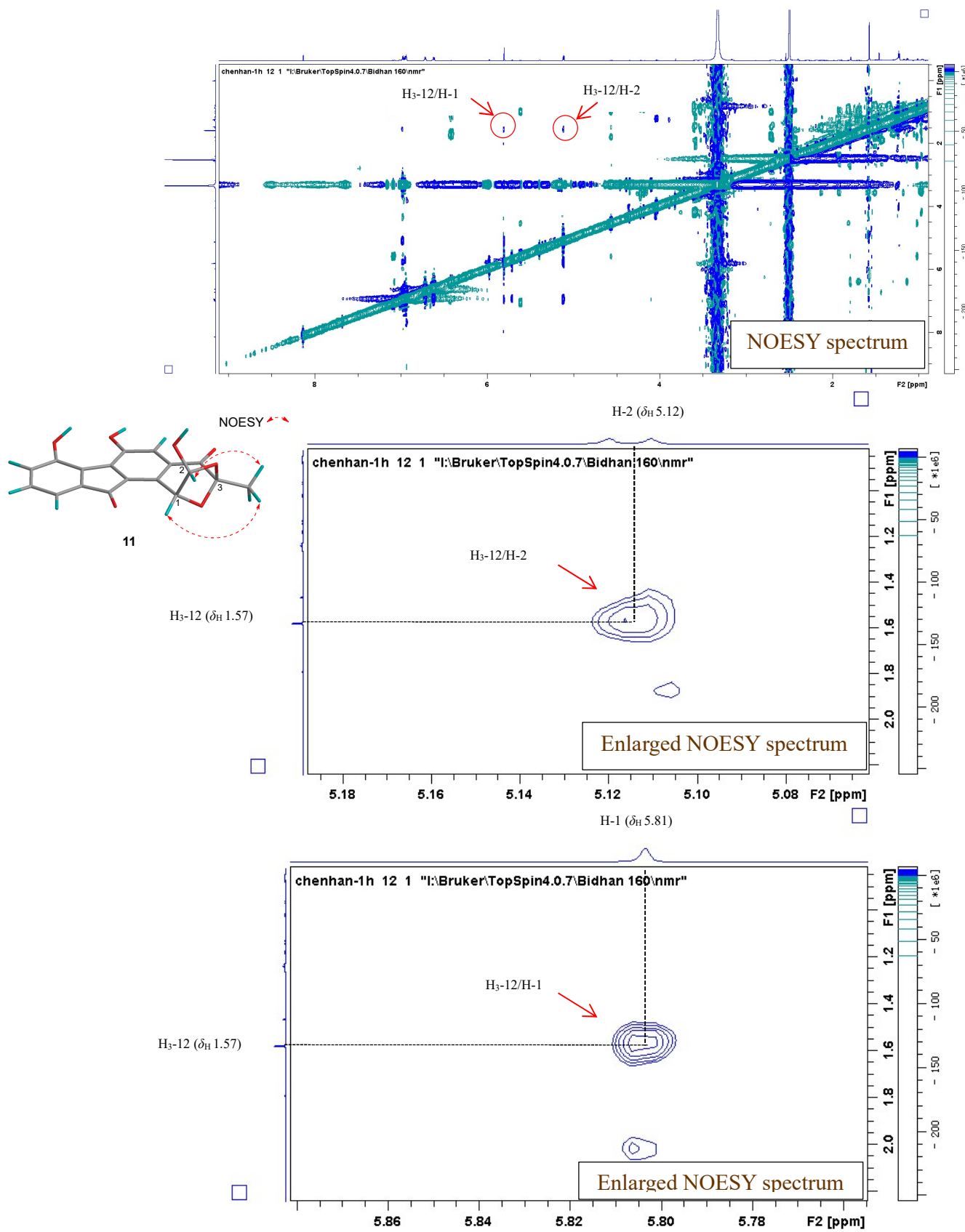
Supplementary Fig. 11 Spectroscopic data for 11. (c) The ¹³C and enlarged ¹³C NMR spectrum of 11 in DMSO-*d*₆.



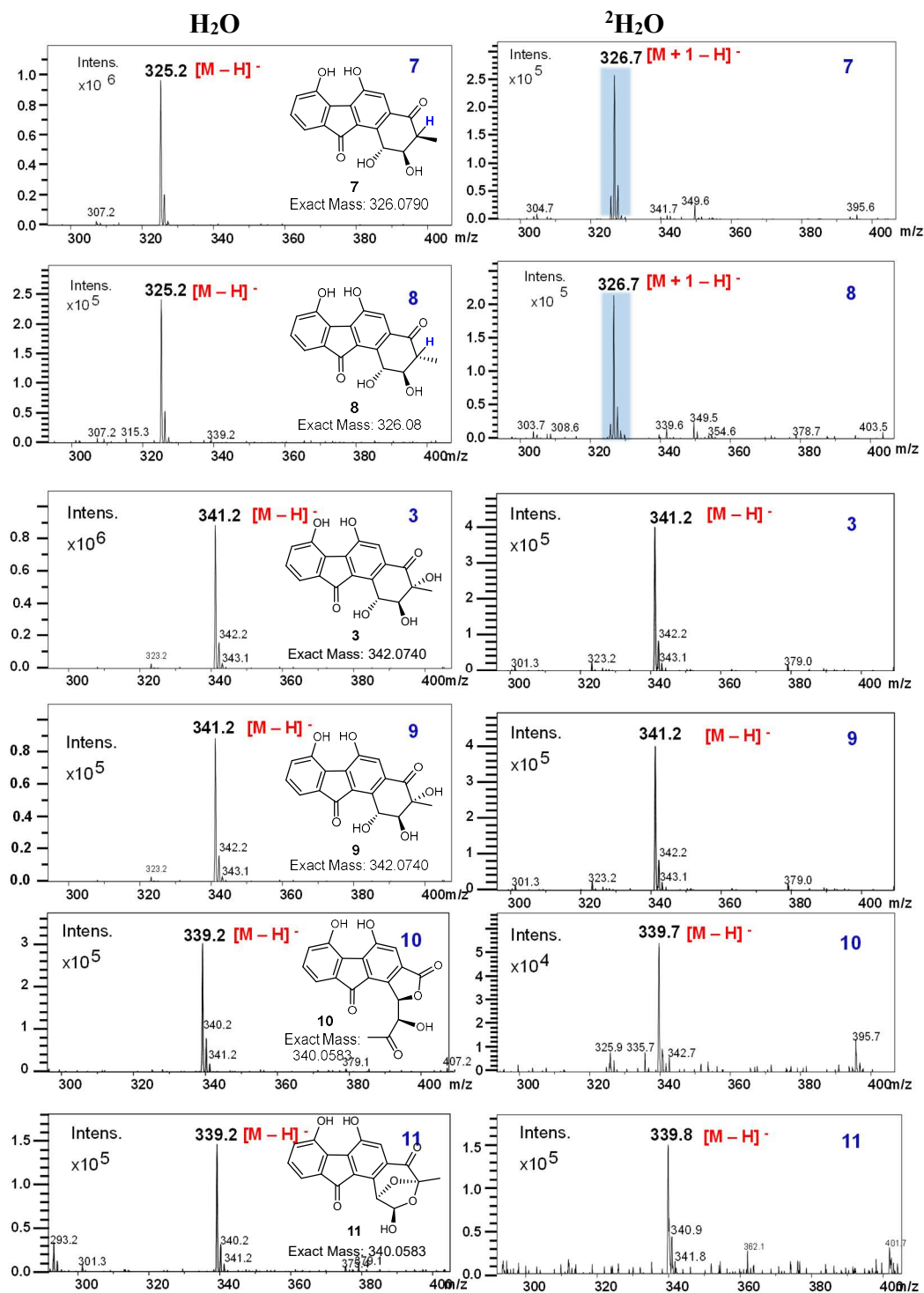
Supplementary Fig. 11 Spectroscopic data for 11. (e) The COSY and enlarged COSY spectrum of 11 in DMSO- d_6 .



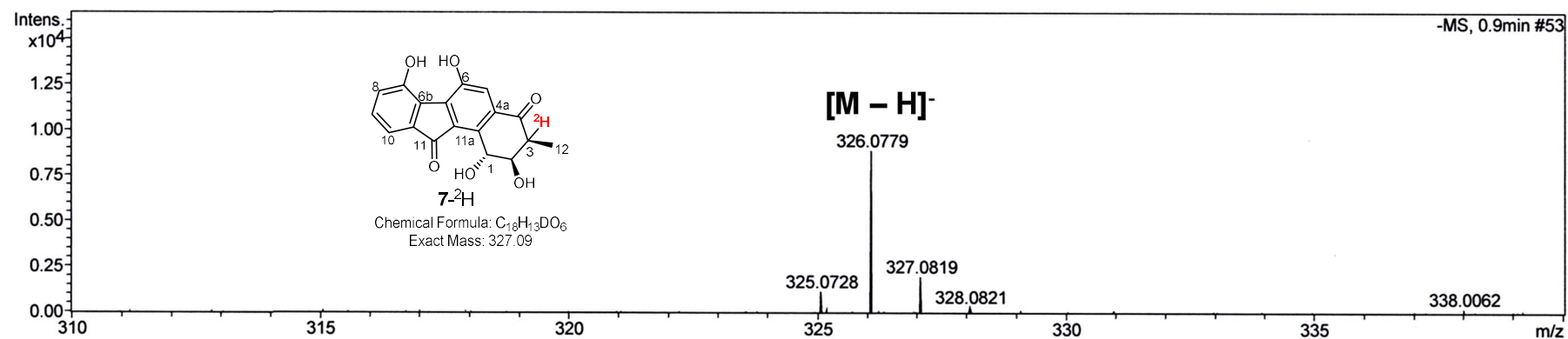
Supplementary Fig. 11 Spectroscopic data for 11. (f) The HMBC and enlarged HMBC spectrum of 11 in DMSO-*d*₆.



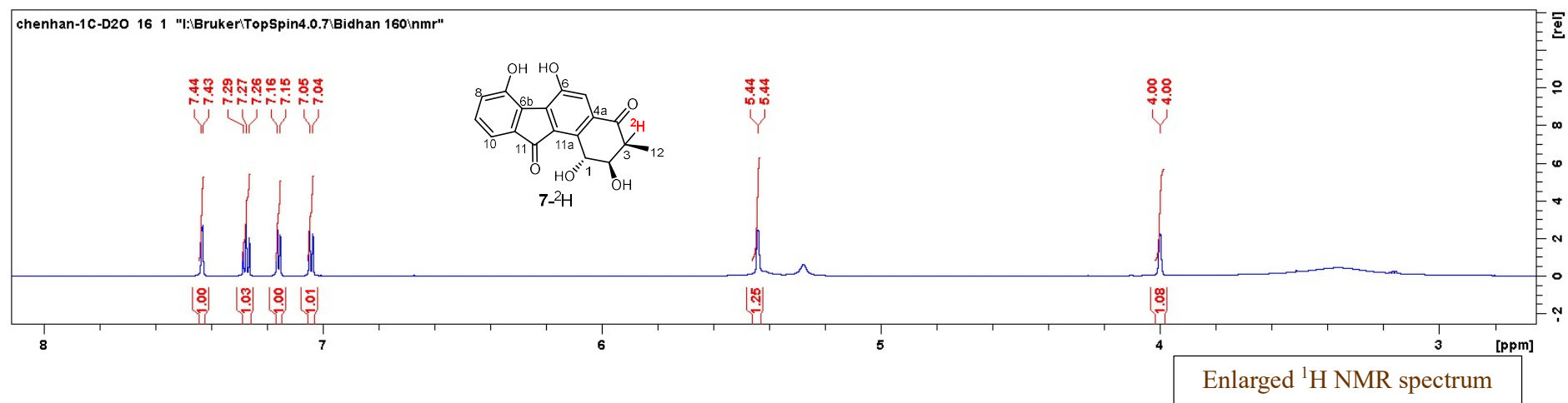
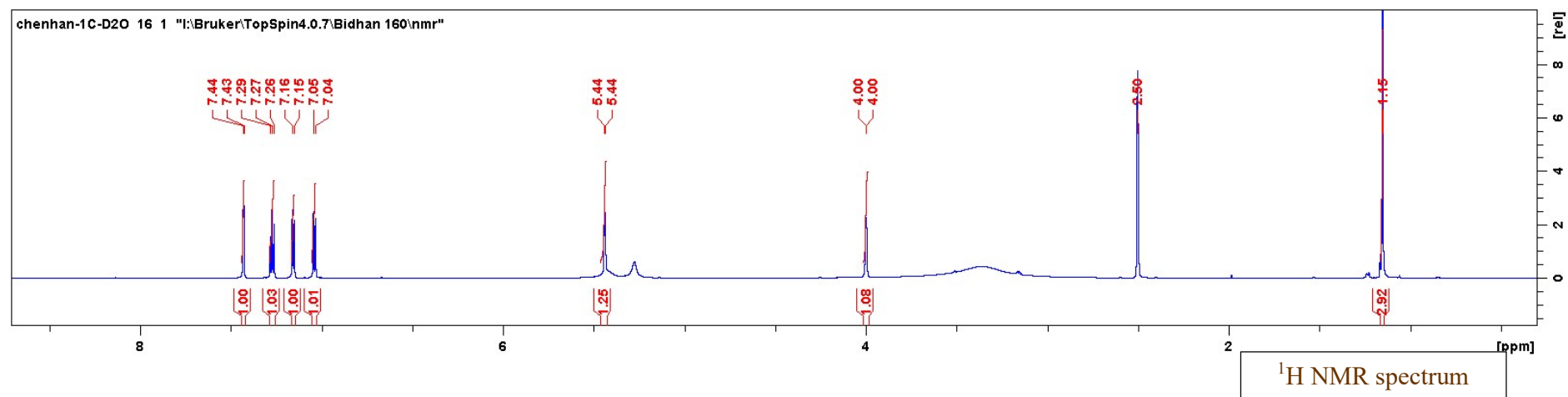
Supplementary Fig. 11 Spectroscopic data for **11**. (g) The NOESY and enlarged NOESY spectrum of **11** in DMSO- d_6 .



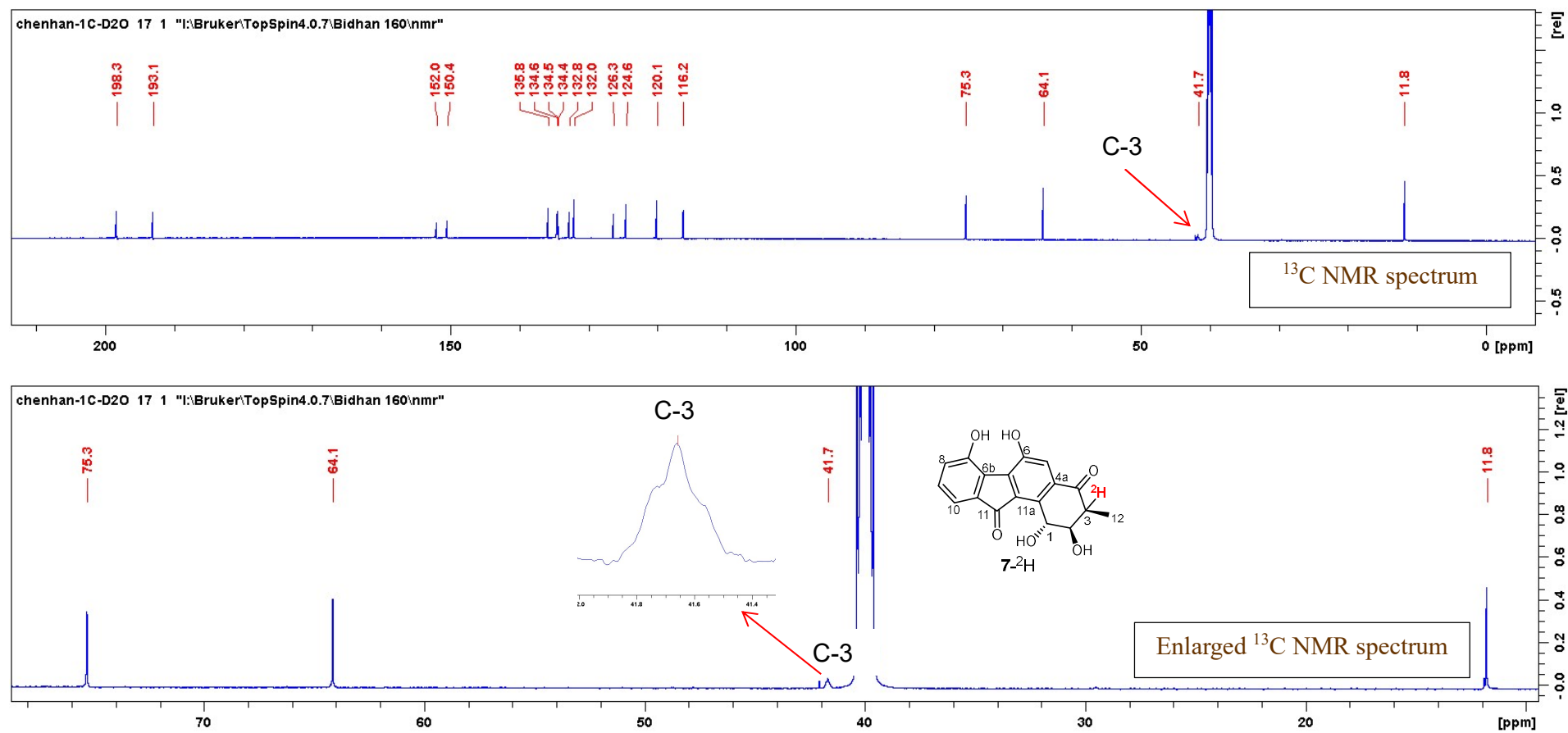
Supplementary Fig. 12. LC-MS analysis of reactions of 1 with FAD/NADH using PBS buffers prepared with H₂O (control) or ²H₂O.



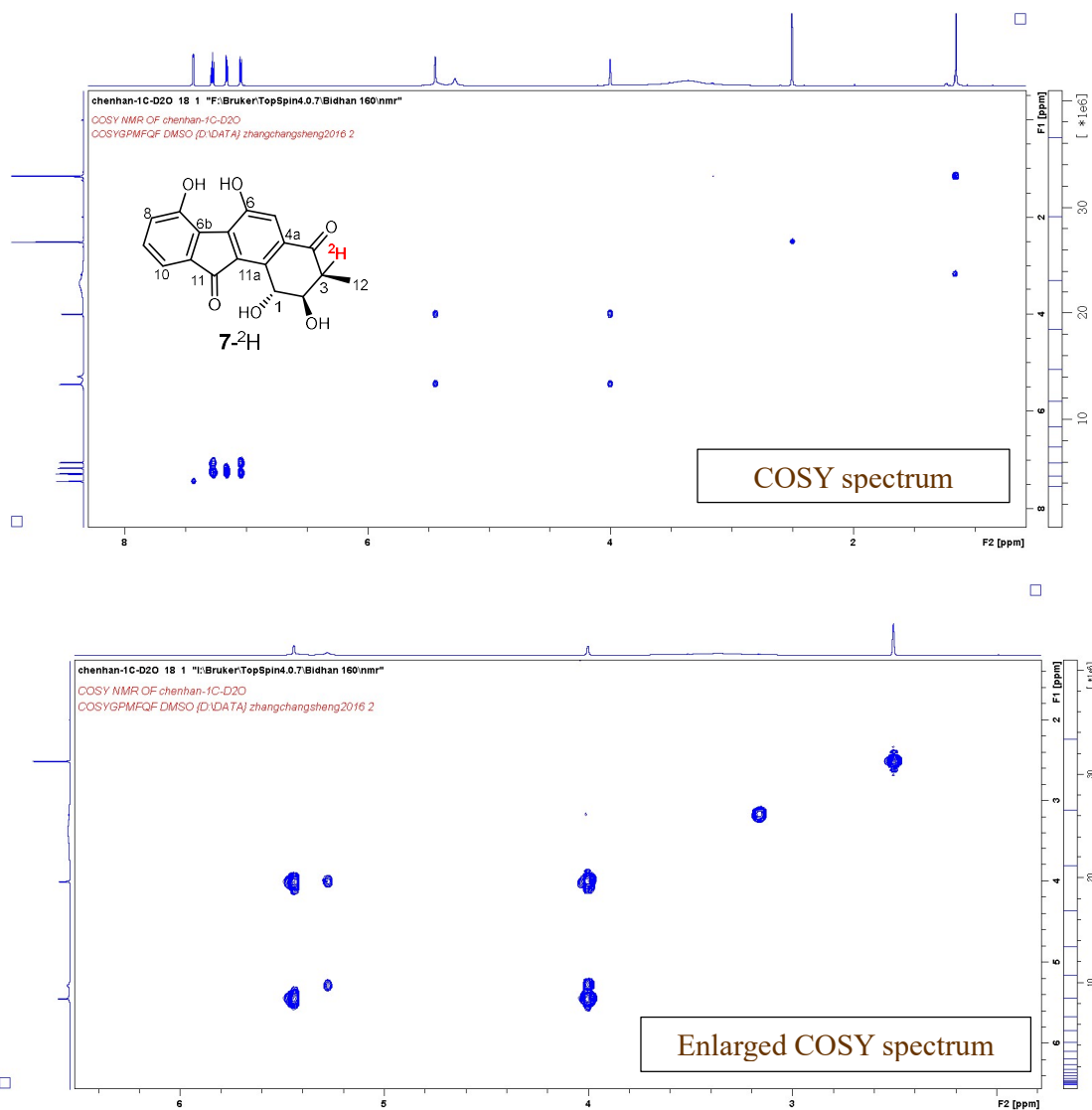
Supplementary Fig. 13. Spectroscopic data for 7-²H. (a) HRESIMS spectrum.



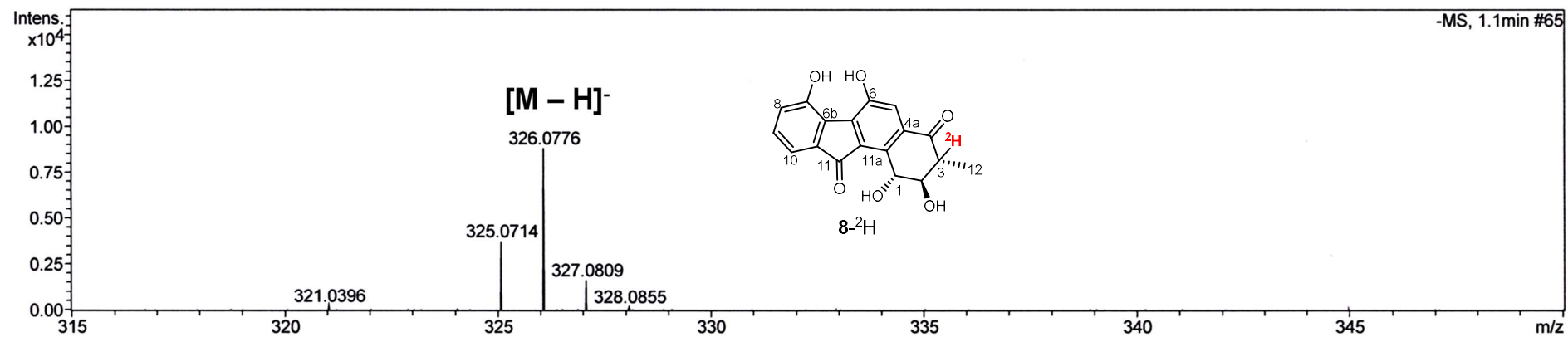
Supplementary Fig. 13. Spectroscopic data for 7-²H. (b) The ¹H and enlarged ¹H NMR spectrum of 7-²H in DMSO-*d*₆.



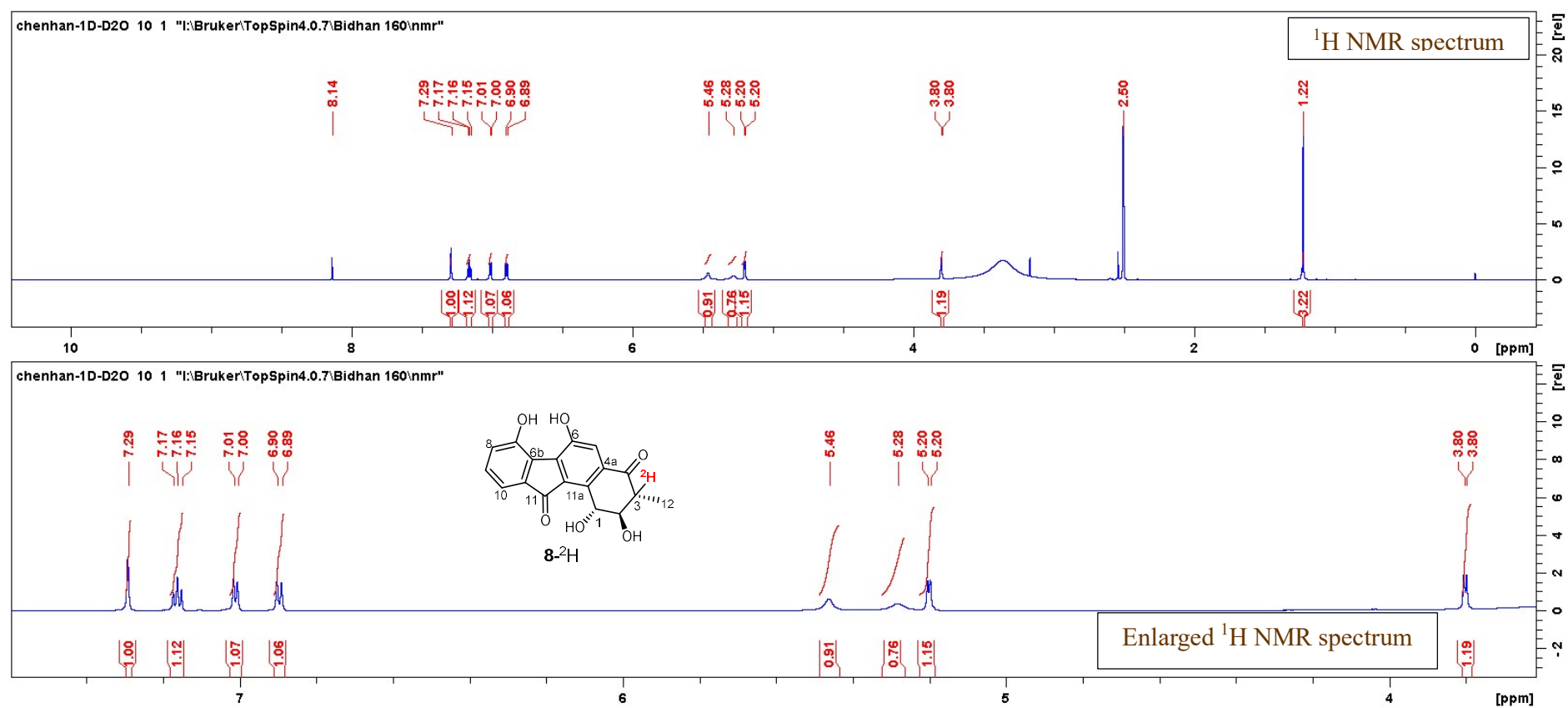
Supplementary Fig. 13. Spectroscopic data for 7- ^2H . (c) The ^{13}C NMR and enlarged ^{13}C NMR spectrum of 7- ^2H in $\text{DMSO}-d_6$.



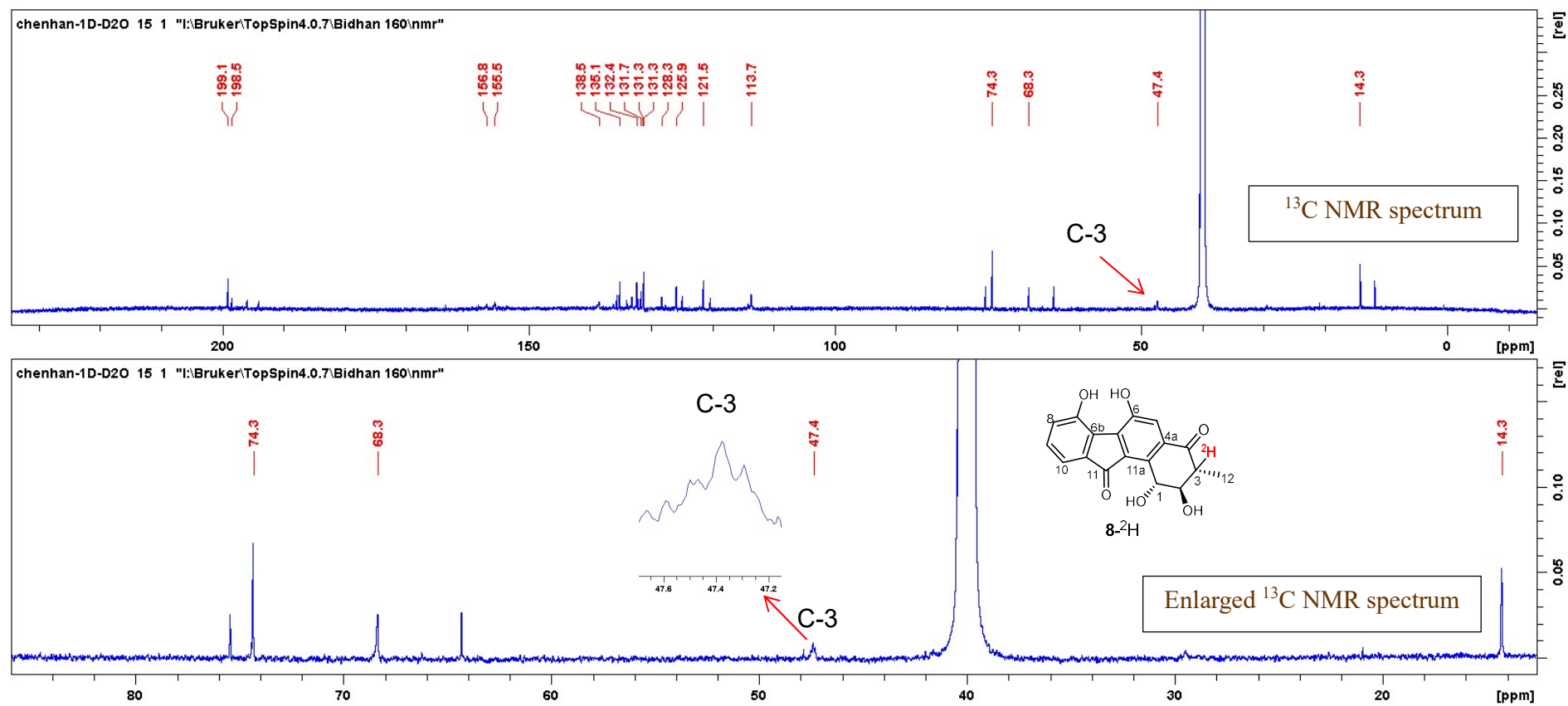
Supplementary Fig. 13. Spectroscopic data for 7-²H. (d) The COSY and enlarged COSY spectrum of 7-²H in DMSO-*d*₆.



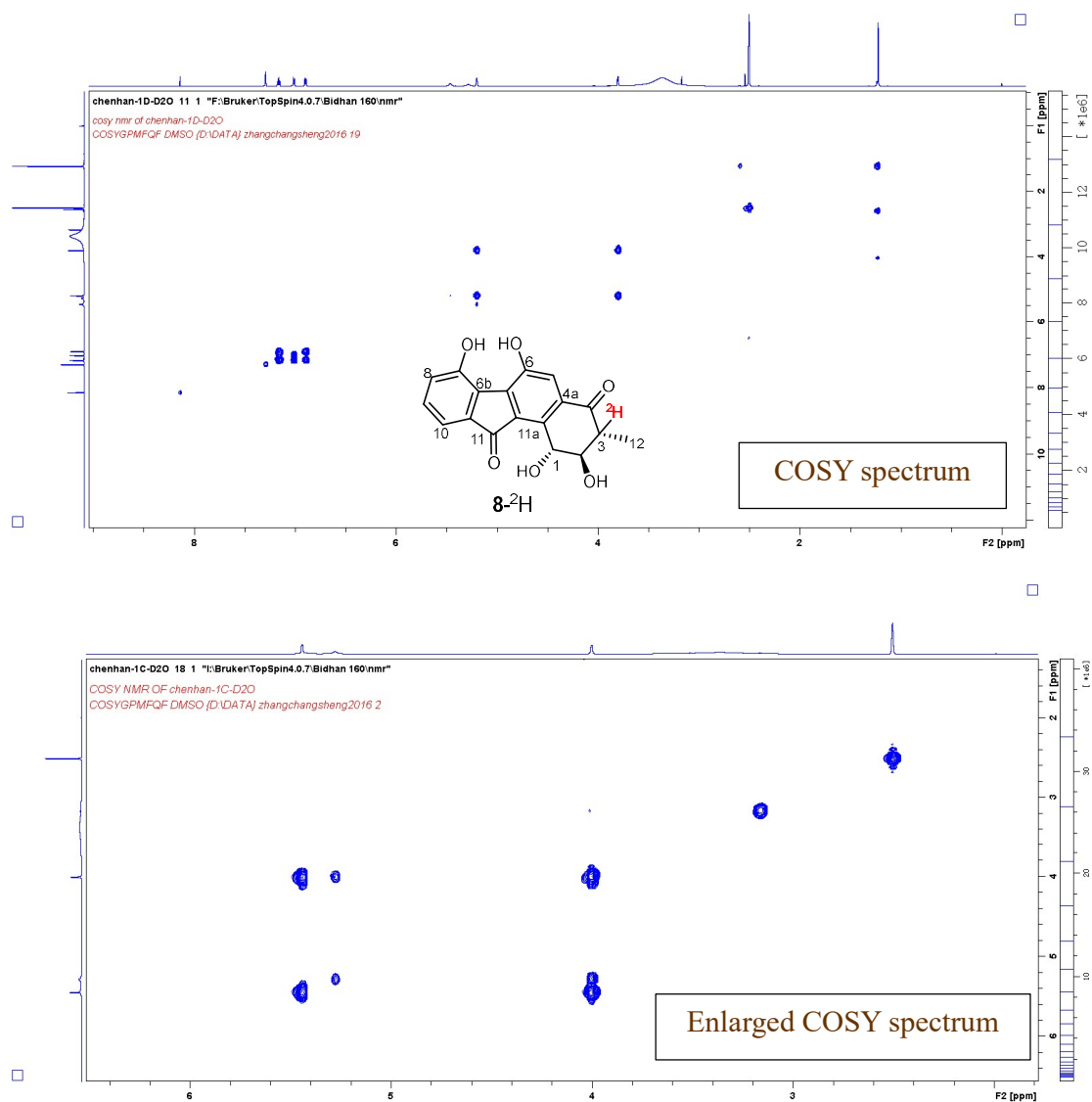
Supplementary Fig. 14. Spectroscopic data for 8-²H. (a) HRESIMS spectrum.



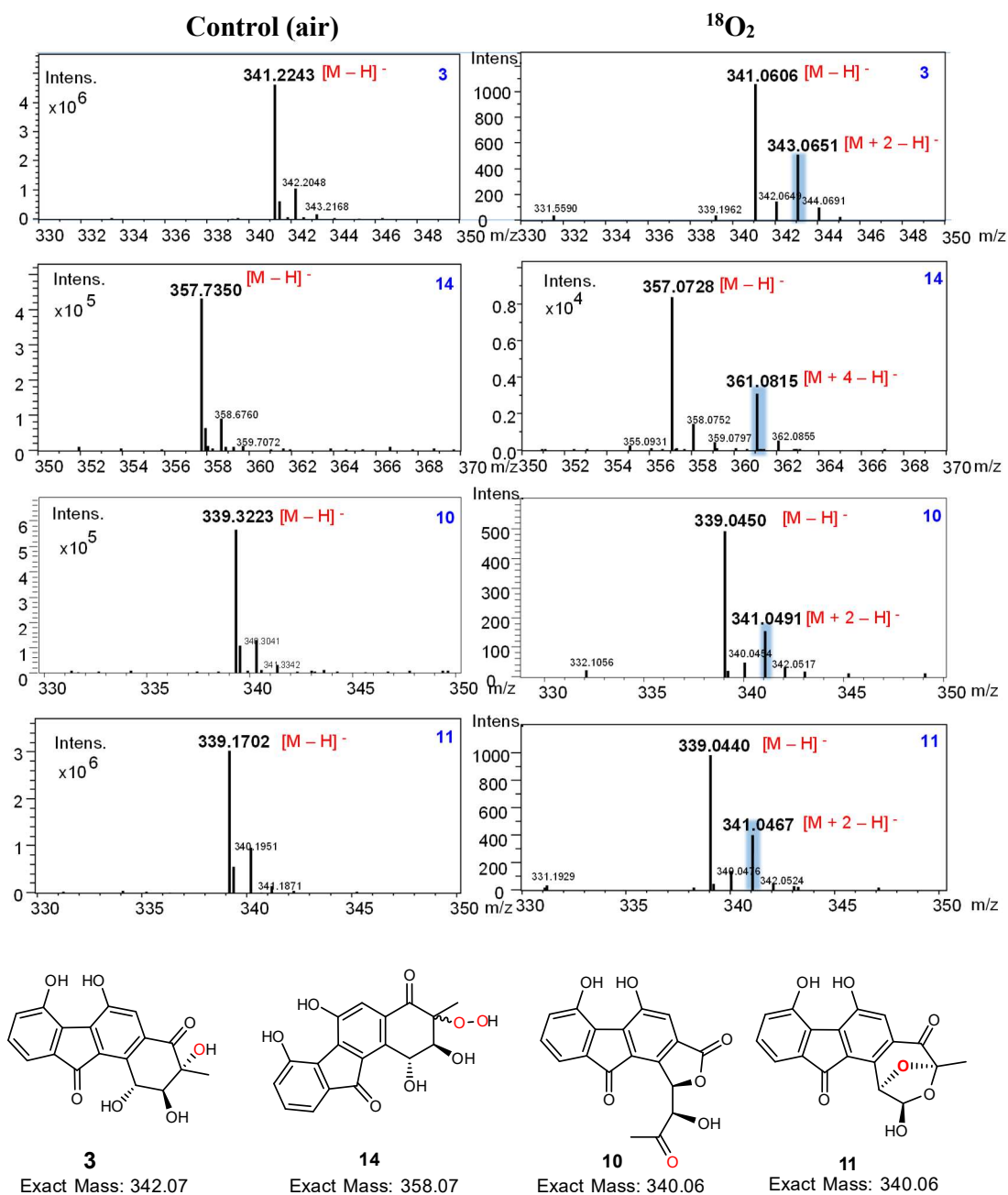
Supplementary Fig. 14. Spectroscopic data for **8-²H**. (b) The ¹H and enlarged ¹H NMR spectrum of **8-²H** in DMSO-*d*₆.



Supplementary Fig. 14. Spectroscopic data for 8-²H. (c) The ^{13}C and enlarged ^{13}C NMR spectrum of 8-²H in DMSO- d_6 .

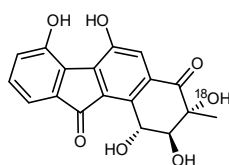
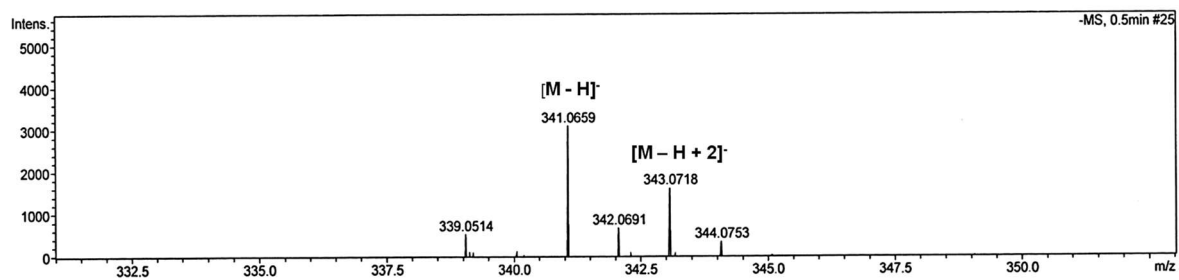


Supplementary Fig. 14. Spectroscopic data for 8-²H. (d) The COSY and enlarged COSY spectrum of 8-²H in DMSO-*d*₆.



Supplementary Fig. 15. LC-HRMS analysis of reactions of FST C (1) with FAD/NADH under air (control) or under $^{18}\text{O}_2$.

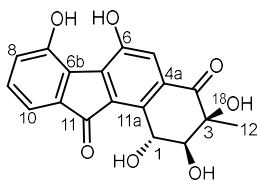
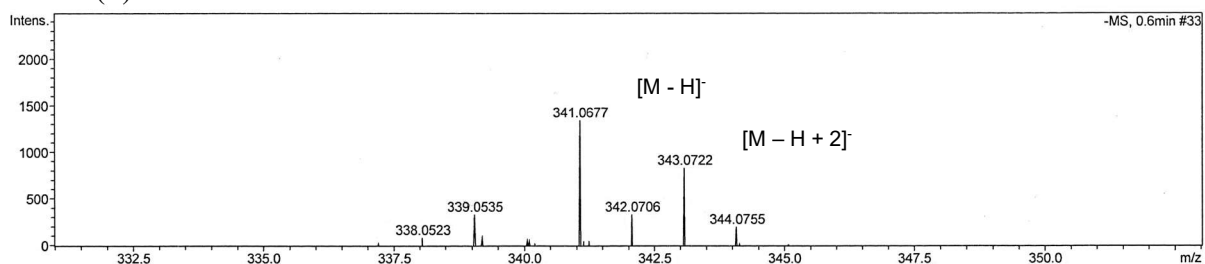
(a) HRESIMS for **3**-¹⁸O.



3-¹⁸O

Chemical Formula: C₁₈H₁₄O₆¹⁸O
Exact Mass: 344.08

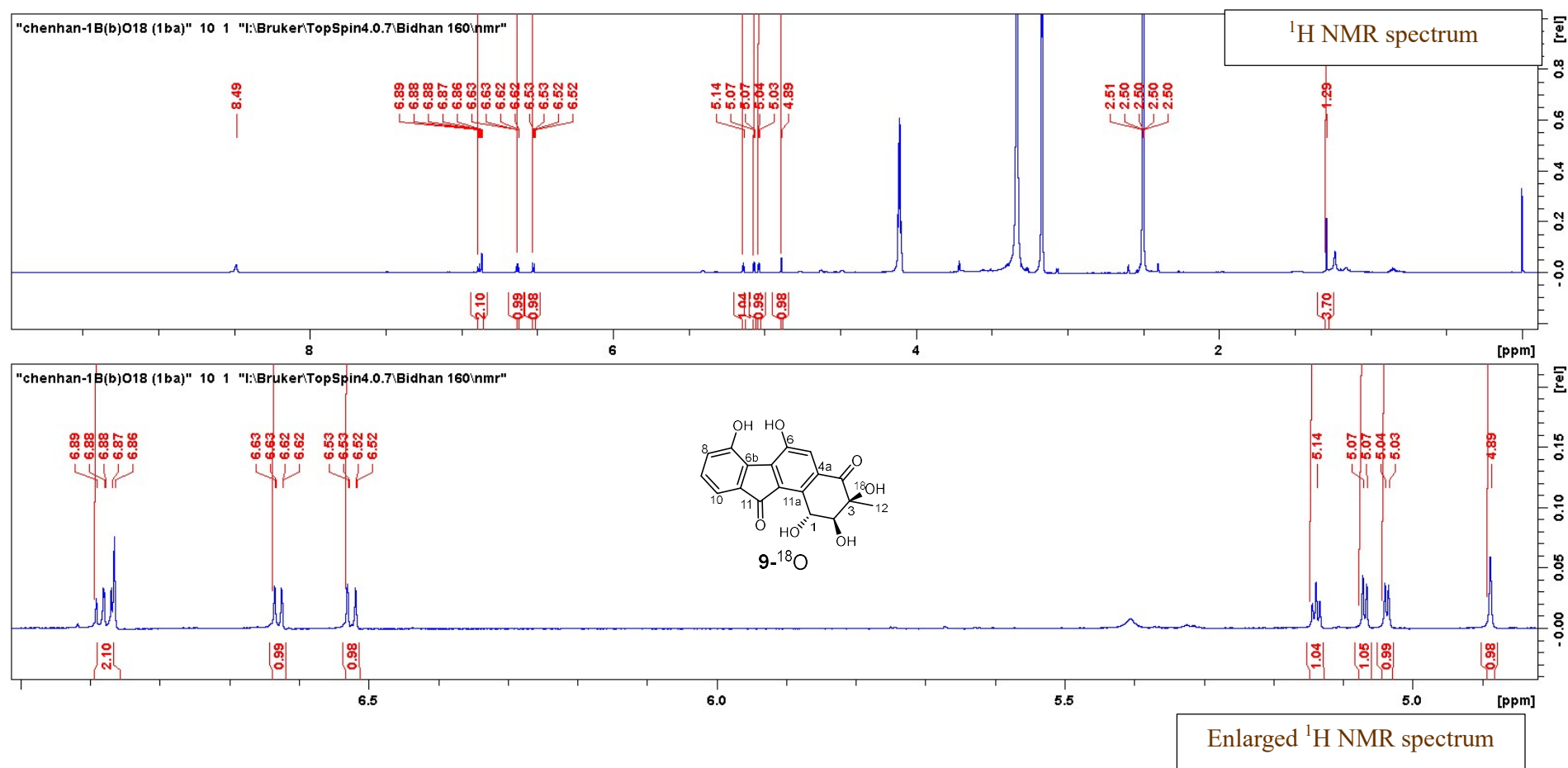
(b) HRESIMS for **9**-¹⁸O.



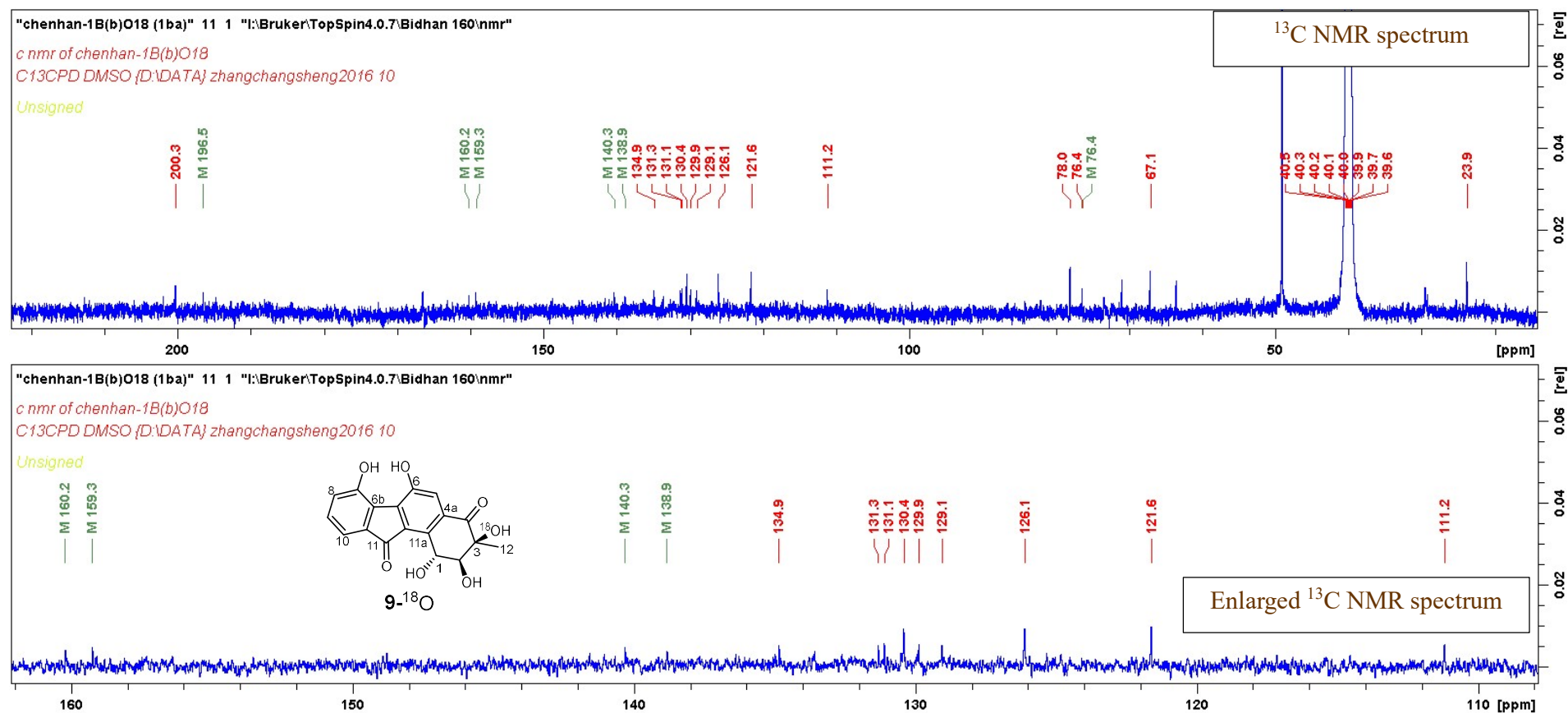
9-¹⁸O

Chemical Formula: C₁₈H₁₄O₆¹⁸O
Exact Mass: 344.08

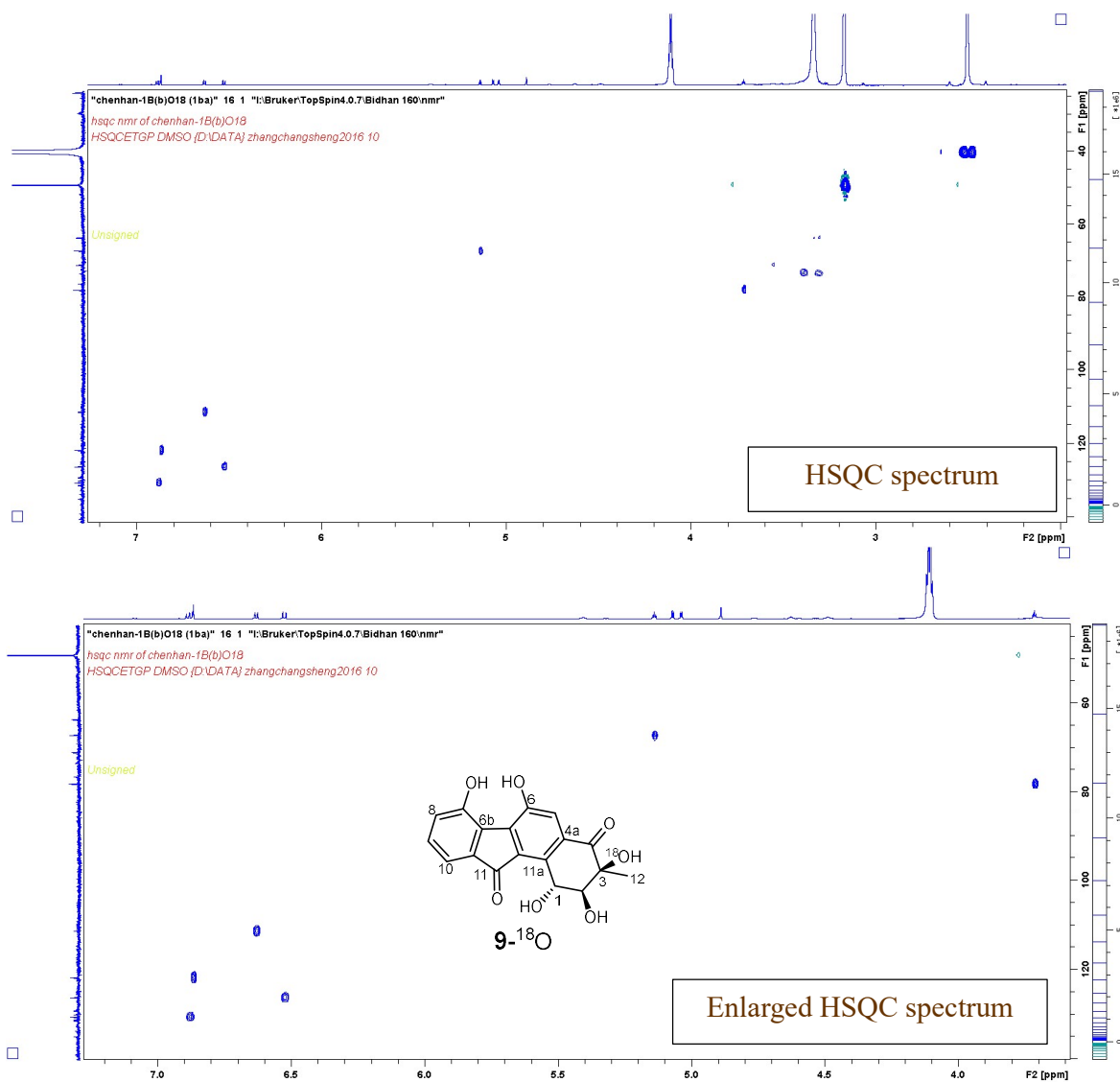
Supplementary Fig. 16. HRESIMS spectroscopic data for 3-¹⁸O and 9-¹⁸O.



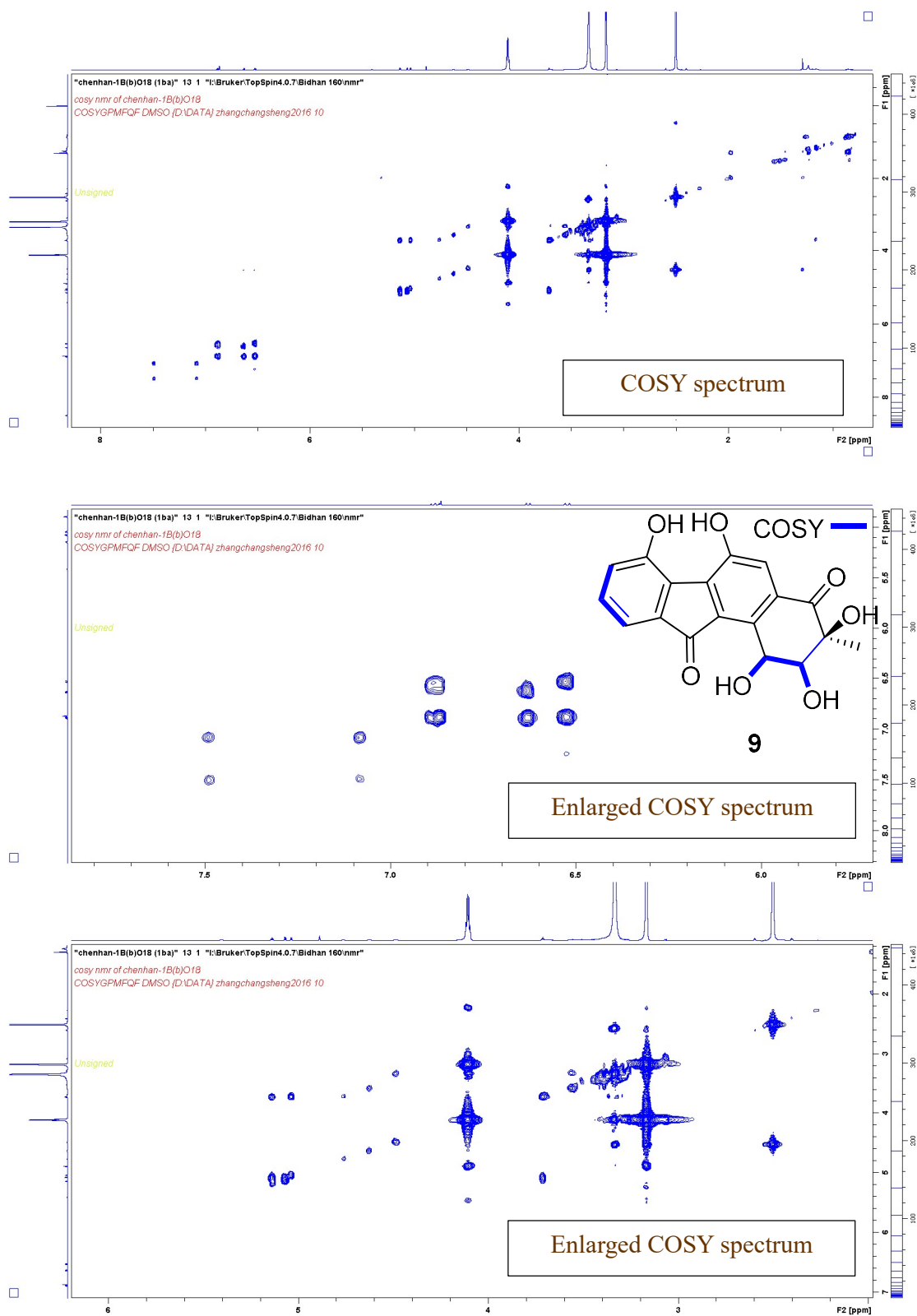
Supplementary Fig. 17. Spectroscopic data of **9-¹⁸O. (a) The ¹H and enlarged ¹H NMR spectrum of **9-¹⁸O** in DMSO-*d*₆.**



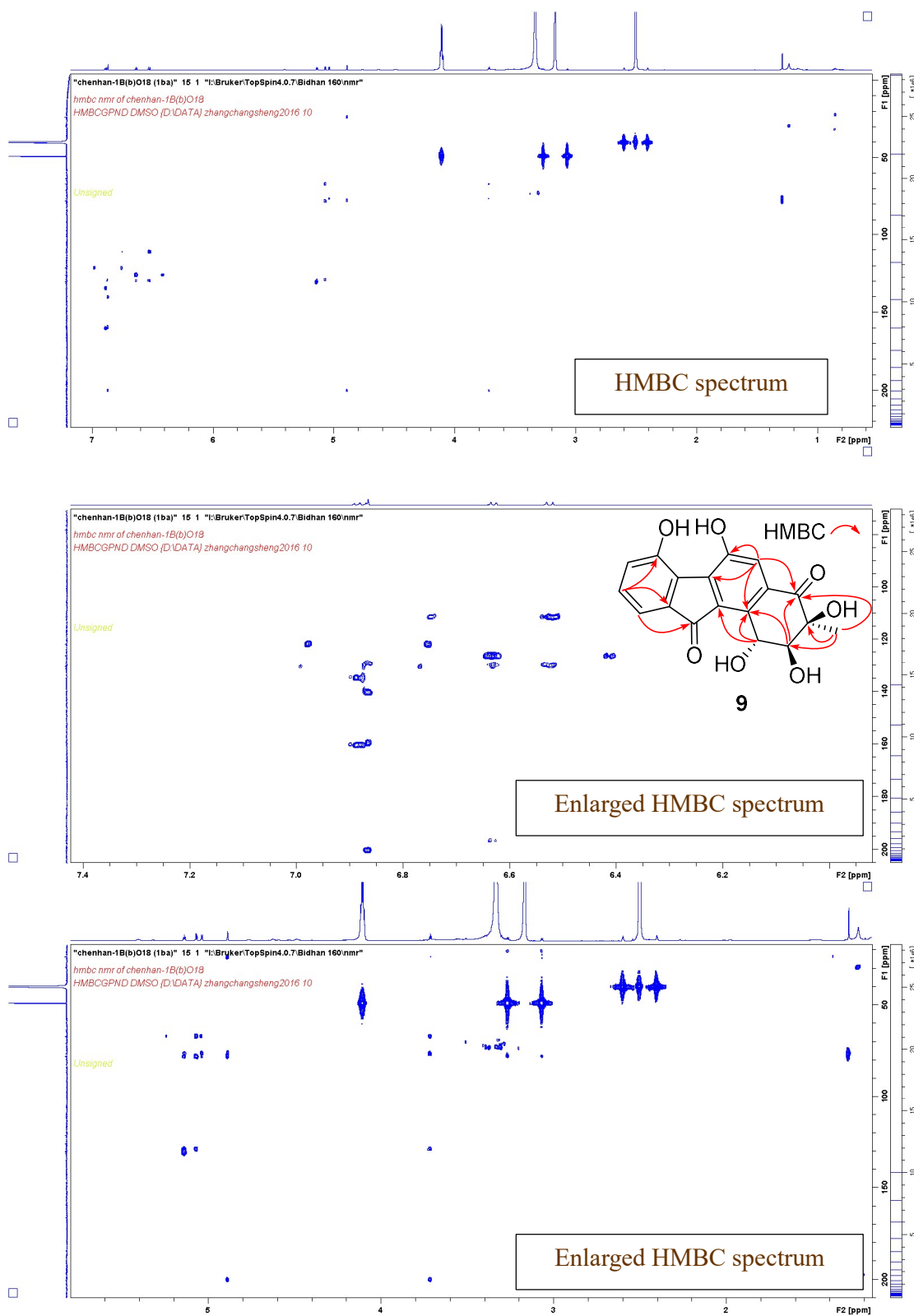
Supplementary Fig. 17. Spectroscopic data of **9- ^{18}O** . (b) The ^{13}C and enlarged ^{13}C NMR spectrum of **9- ^{18}O** in $\text{DMSO-}d_6$.



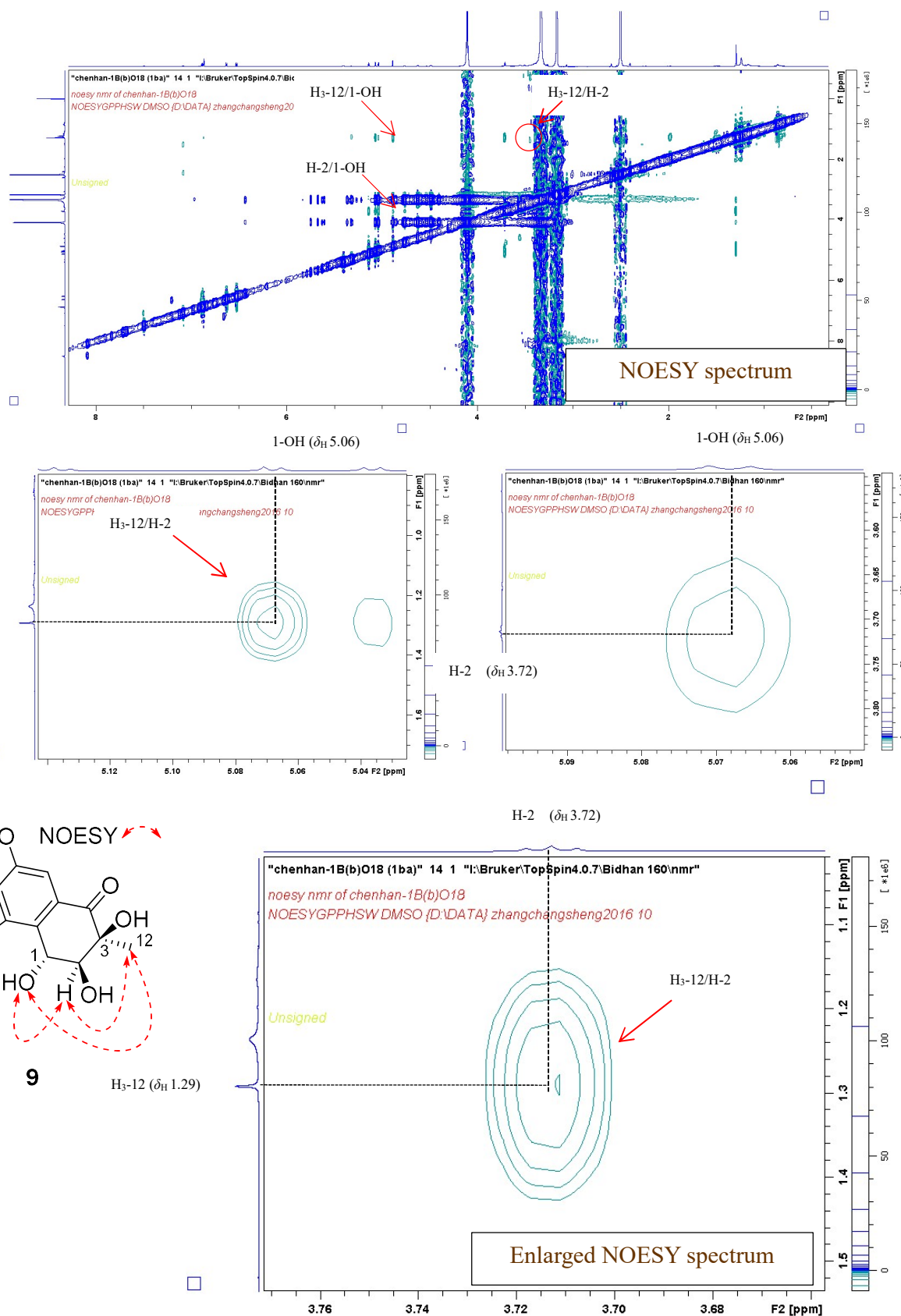
Supplementary Fig. 17. Spectroscopic data of 9-¹⁸O. (c) The HSQC and enlarged HSQC spectrum of 9-¹⁸O in DMSO-*d*₆.



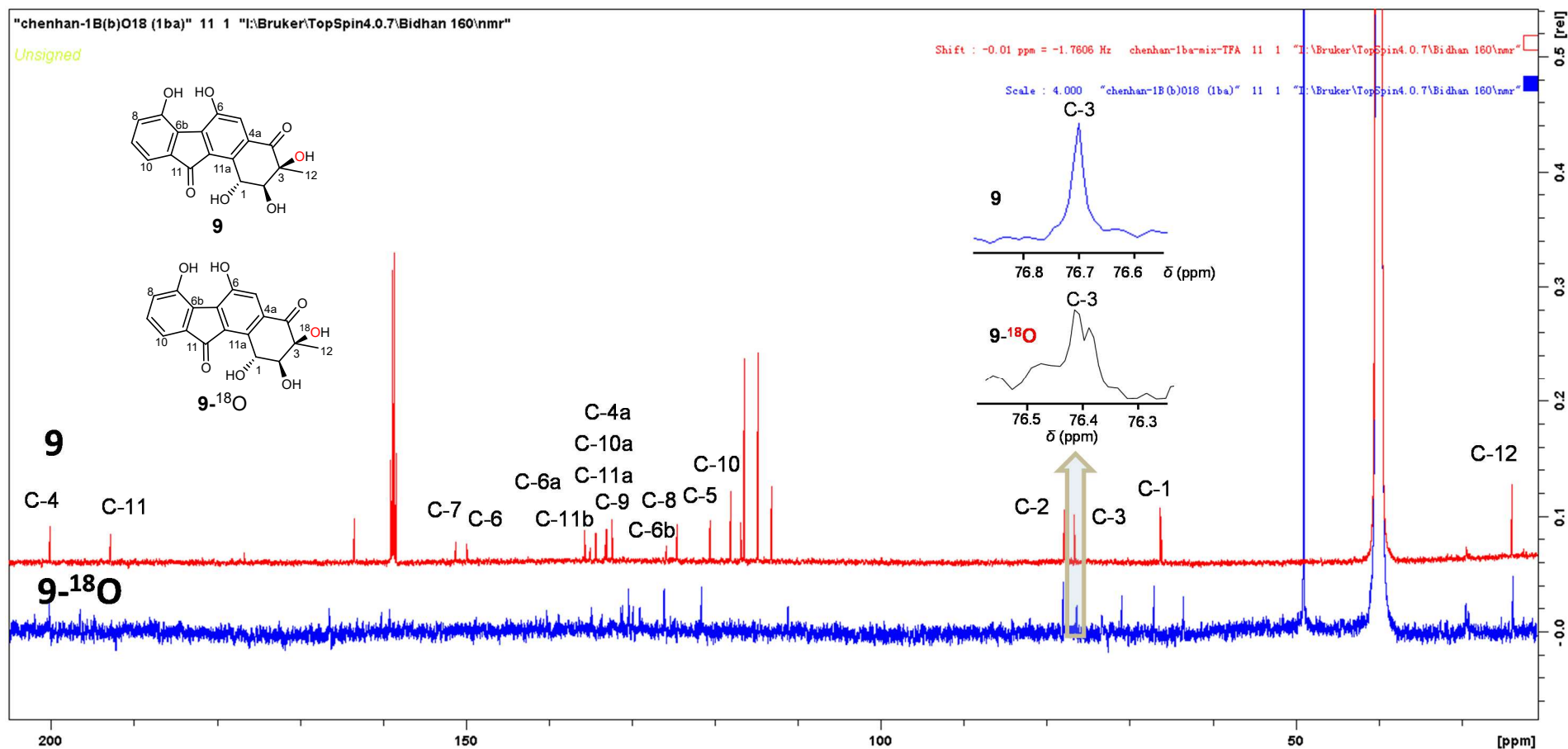
Supplementary Fig. 17. Spectroscopic data of 9-¹⁸O. (d) The COSY and enlarged COSY spectrum of 9-¹⁸O in DMSO-*d*₆.



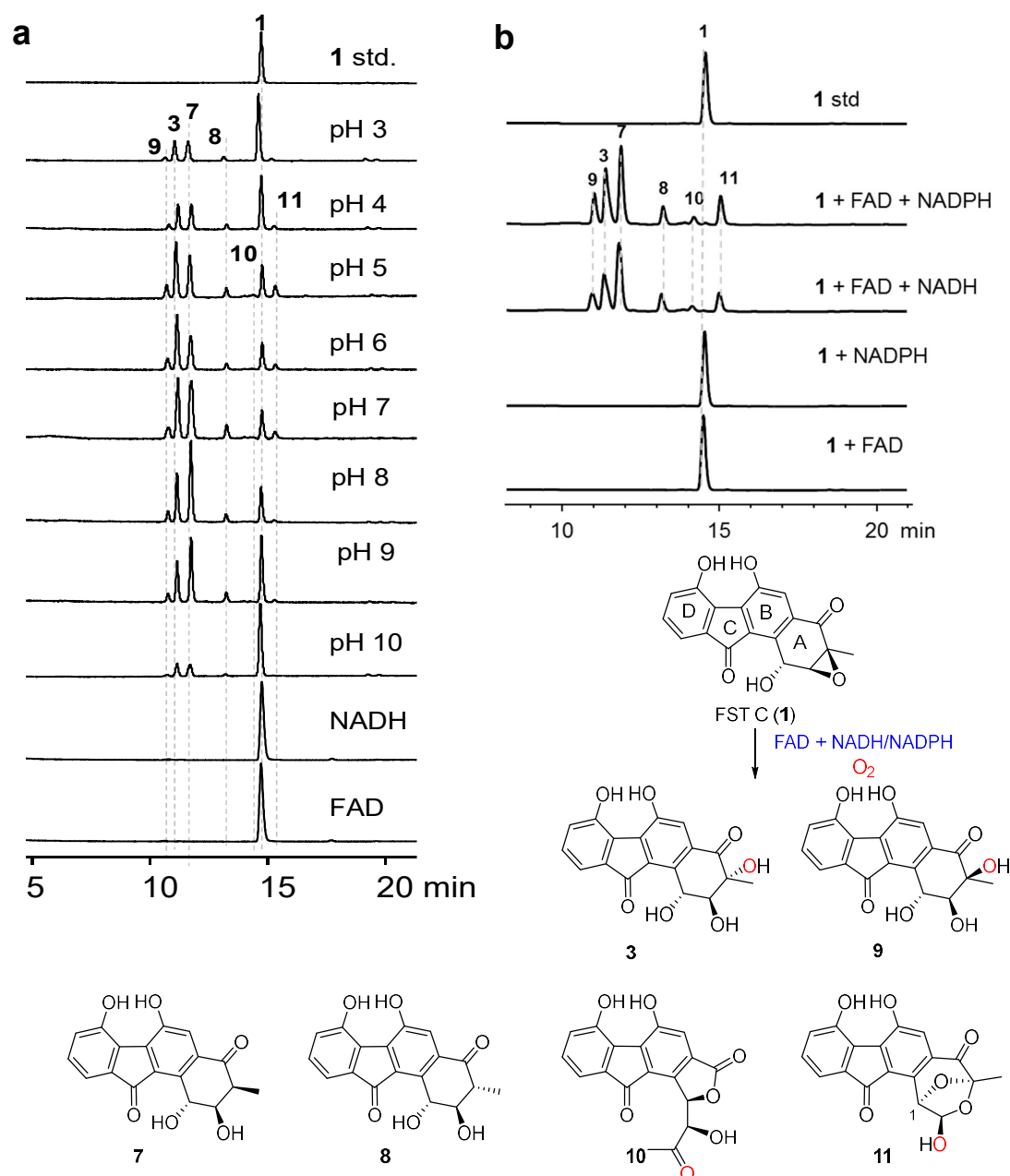
Supplementary Fig. 17. Spectroscopic data of **9-¹⁸O.** (e) The HMBC and enlarged HMBC spectrum of **9**-¹⁸O in DMSO-*d*₆.



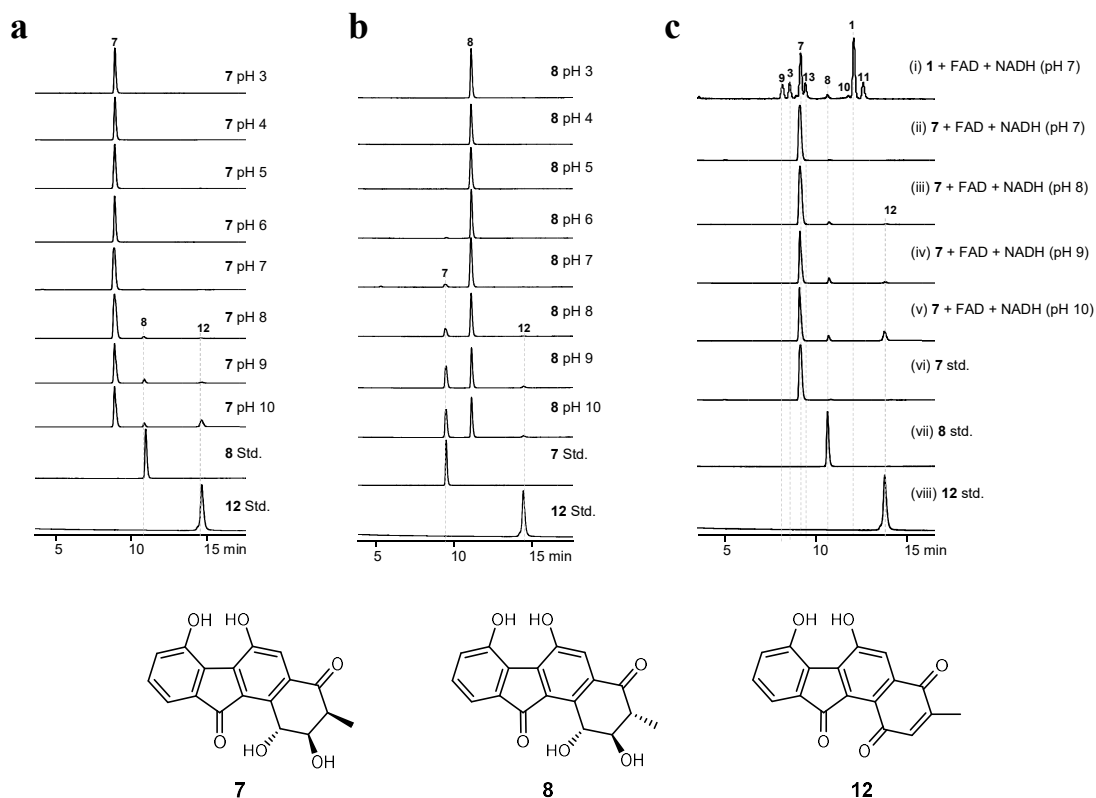
Supplementary Fig. 17. Spectroscopic data of $9\text{-}^{18}\text{O}$. (f) The NOESY and enlarged NOESY spectrum of $9\text{-}^{18}\text{O}$ in $\text{DMSO-}d_6$.



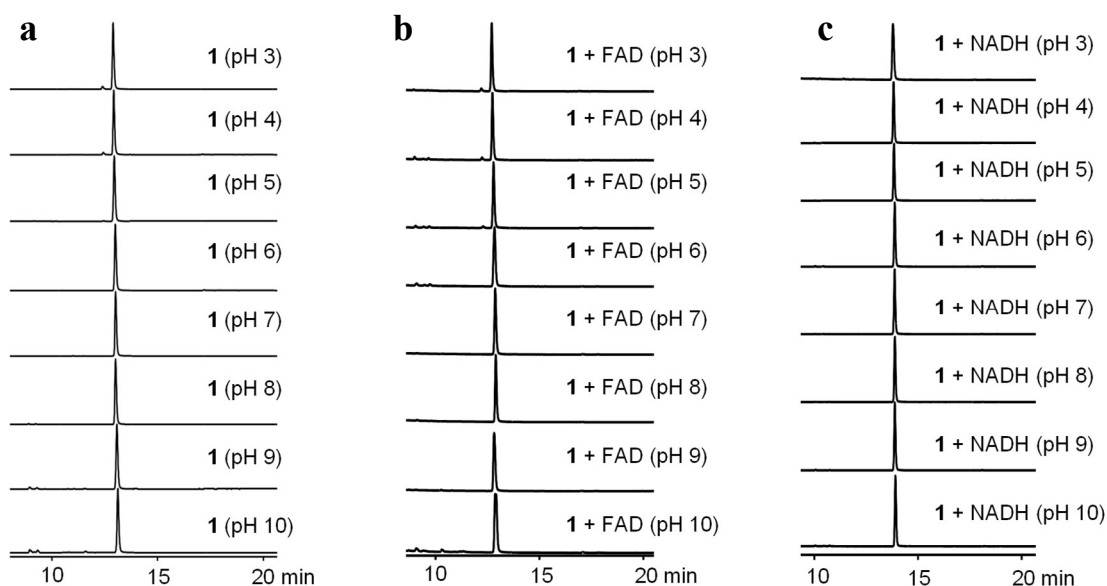
Supplementary Fig. 18. Comparison the ^{13}C NMR spectroscopic data of 9- ^{18}O in $\text{DMSO-}d_6$ and 9 in $\text{DMSO-}d_6$:trifluoroacetic acid (TFA)- d_1 (100:1).



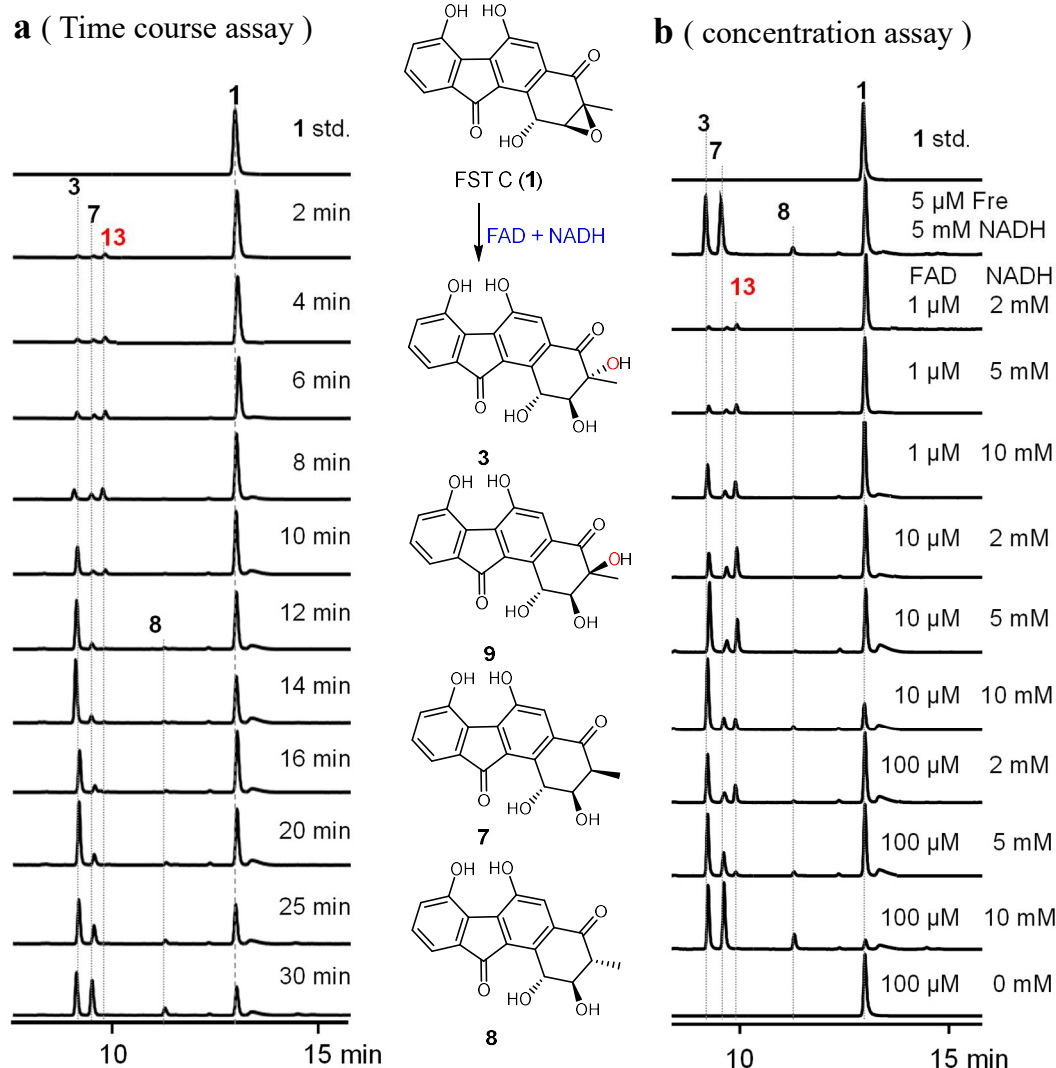
Supplementary Fig. 19. HPLC analysis of reactions of 1 and FAD/NAD(P)H in buffers of varying pH values. (a) A standard assay contains 100 μ M 1, 100 μ M FAD and 10 mM NADH in buffers from pH 3 to pH 10. The buffers include citric acid/ Na_2HPO_4 buffer (50 mM, pH 3 to 6); PBS buffer (50 mM, pH 7); boric acid/borax buffer (50 mM, pH 8 and 9); borax/ NaOH buffer (50 mM, pH 10). The reaction mixtures were incubated at 30 $^\circ\text{C}$ for 2 h. (b) the assay contains 100 μ M 1, 100 μ M FAD and 10 mM NADH or NADPH in PBS buffer (50 mM, pH 7) with incubation at 30 $^\circ\text{C}$ for 2hrs. HPLC analysis was performed on the Agilent 1260 Infinity series instrument (Agilent Technologies Inc., USA) using a polar column (Comixsep®, P/N FMG-BPF5-EONU, Polar BiPPF 5u, 250 $\times$ 4.6 mm, China) with UV detection at 304 nm.



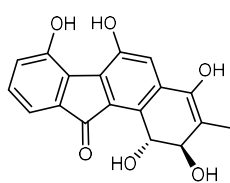
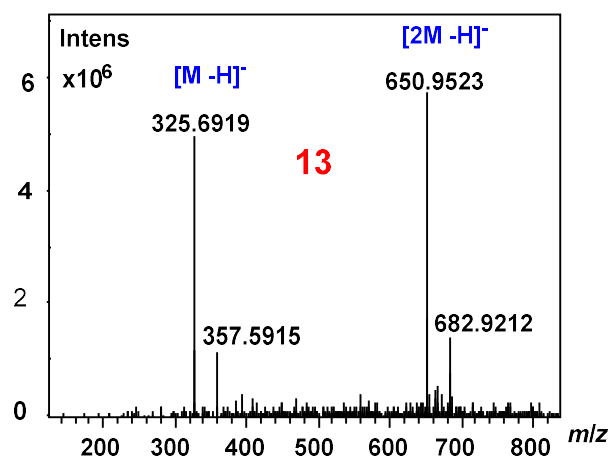
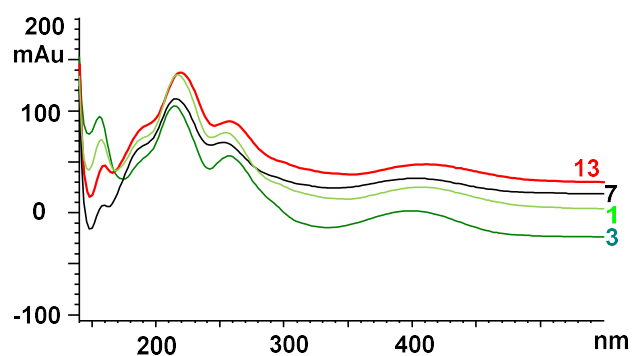
Supplementary Fig. 20. The spontaneous conversion of 7 to 8 (or 8 to 7) in buffers with varying pH values. (a) HPLC analysis of spontaneous conversion of 7 to 8 and 12. Compound 7 was incubated in 50 mM buffers with varying pHs from 3 to 10 for 30 min at 30 °C. (b) HPLC analysis of spontaneous conversion of 8 to 7 and 12. Compound 8 was incubated in 50 mM buffers with varying pHs from 3 to 10 for 30 min at 30 °C. (c) HPLC analysis of 7 with FAD/NADH. (i) The assay contains 100 μ M 1, 10 μ M FAD and 2 mM NADH at 30 °C for 20 min (pH 7); (ii) 7 + 10 μ M FAD and 2 mM NADH (pH 7); (iii) 7 + FAD and NADH (pH 8); (iv) 7 + FAD and NADH (pH 9); (v) 7 + FAD and NADH (pH 10); (vi) 7 std.; (vii) 8 std.; (viii) 12 std. The reaction mixtures of (i–v) were incubated at 30 °C for 30 min and the HPLC analysis were performed using polar column.



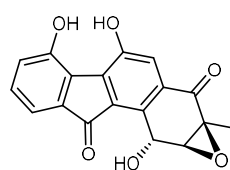
Supplementary Fig. 21. The stability of FST C (1) under varying conditions. (a) HPLC analysis of 100 μ M FST C (1) in 50 mM buffers with varying pHs from 3 to 10. (b) HPLC analysis of 100 μ M 1 + 100 μ M FAD in 50 mM buffers with varying pH from 3 to 10. (c) HPLC analysis of 100 μ M 1 + 10 mM NADH in 50 mM buffers with varying pH from 3 to 10. The buffers include citric acid/ Na_2HPO_4 buffer (50 mM, pH 3 to 6); PBS buffer (50 mM, pH 7); boric acid/borax buffer (50 mM, pH 8 and 9); borax/ NaOH buffer (50 mM, pH 10). The mixtures were incubated at 30 $^\circ\text{C}$ for 2 h.



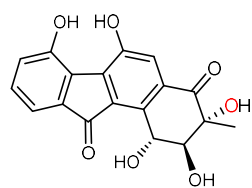
Supplementary Fig. 22. HPLC analysis for a time course assay of **1 and FAD/NADH and reactions of **1** with varying concentrations of FAD and NADH. (a)** HPLC analysis of a time course assay of 100 μ M **1** in 50 mM PBS buffer (pH 7) with 100 μ M FAD and 10 mM NADH. The reactions at 30 $^{\circ}$ C were sampled at 2, 4, 6, 8, 10, 12, 14, 16, 20, 25, and 30 min. The instable intermediate **13** was observed between 2 to 10 min. **(b)** HPLC analysis of the reactions of 100 μ M **1** in 50 mM PBS buffer (pH 7) with varying concentrations of FAD (1/ 10 / 100 μ M) and NADH (2 / 5 / 10 mM) at 30 $^{\circ}$ C for 30 min. The best production of **13** was observed with 10 μ M FAD and 2 mM NADH. HPLC analysis was performed on the Agilent 1260 Infinity series instrument (Agilent Technologies Inc., USA) using a reversed phase C18 column (Kinetex[®] 5 μ m C18 100 $\text{\AA}$, LC Column 150 $\times$ 4.6 mm, Phenomenex, USA) with UV detection at 304 nm.



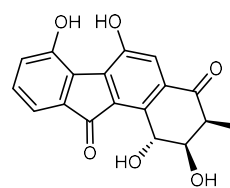
13



1

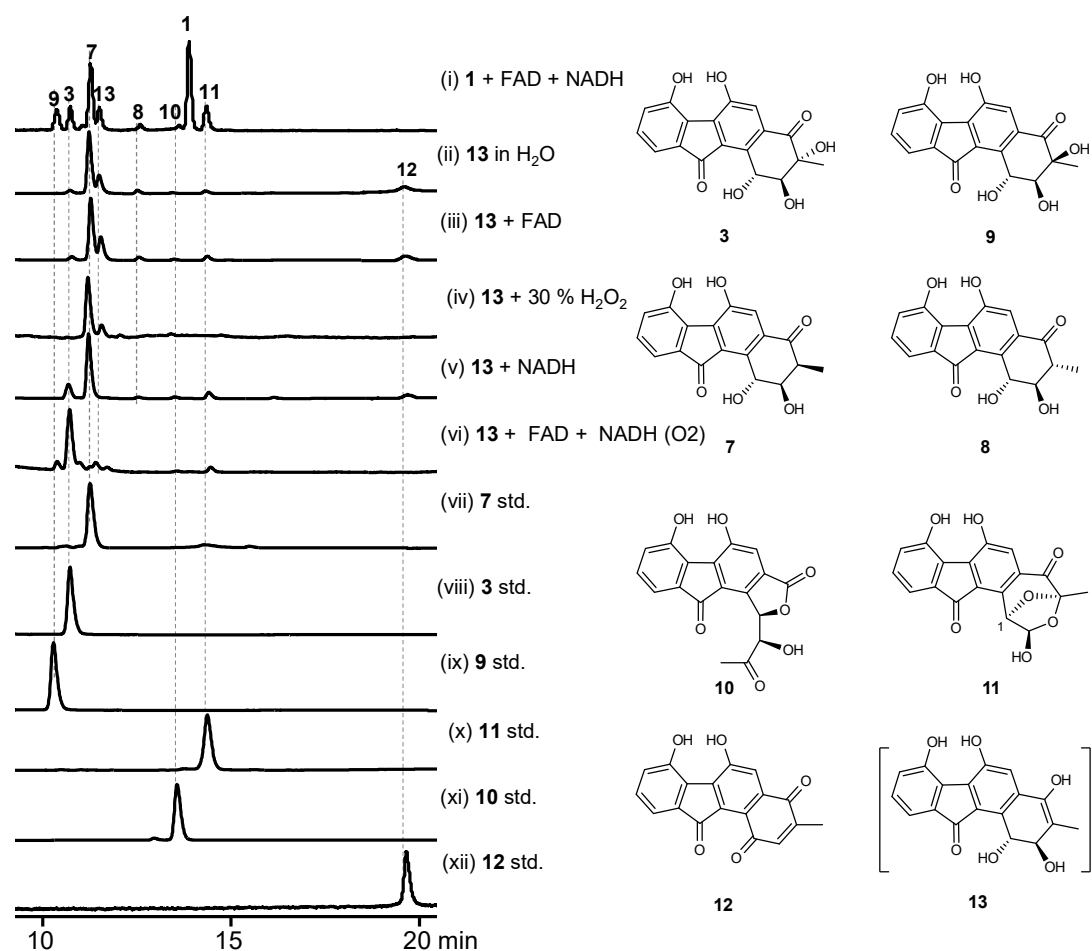


3

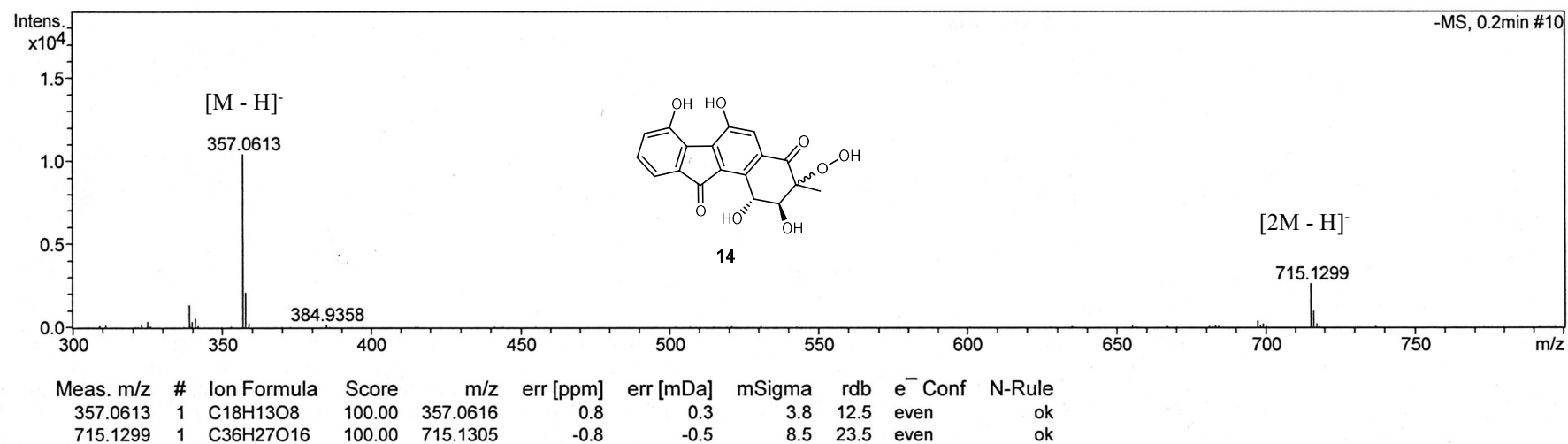


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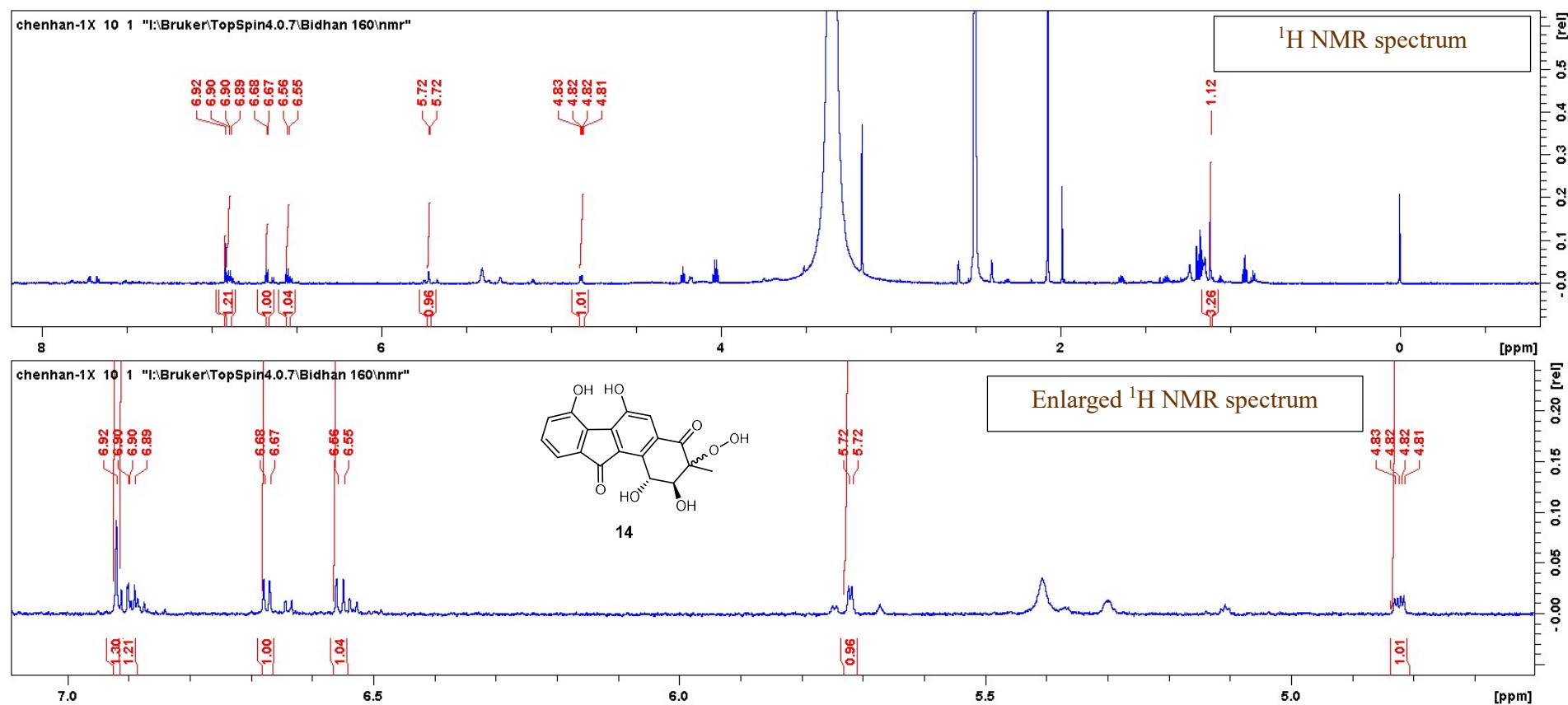
Supplementary Fig. 23. The comparison of UV spectra of 13 and FSTs and LC-HRMS analysis of 13.



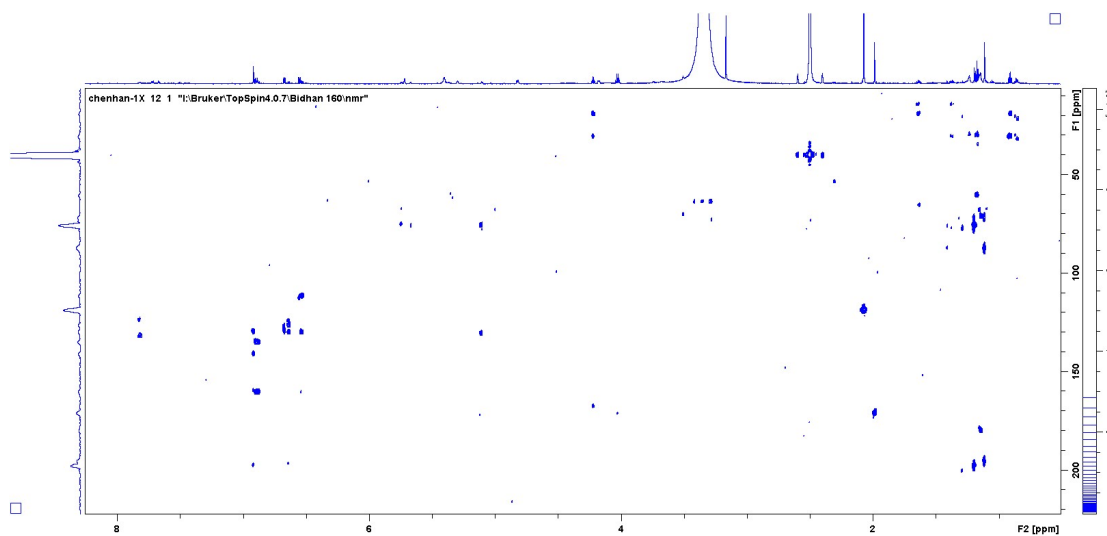
Supplementary Fig. 24. HPLC analysis of reactions with 13. (i) The assay contains 100 μ M 1, 10 μ M FAD and 2 mM NADH at 30 °C for 10 min; (ii) 13 (collected from i) in H₂O; (iii) 13 + 10 μ M FAD; (iv) 13 + 30 % H₂O₂; (v) 13 + 2 mM NADH; (vi) 13 + 10 μ M FAD and 2 mM NADH; (vii) 7 std.; (viii) 3 std.; (ix) 9 std.; (x) 10 std.; (xi) 11 std.; (xii) 12 std. The reaction mixtures of (ii–vi) were incubated at 30 °C for 30 min and the HPLC analysis were performed using a polar column with UV detection at 304 nm.



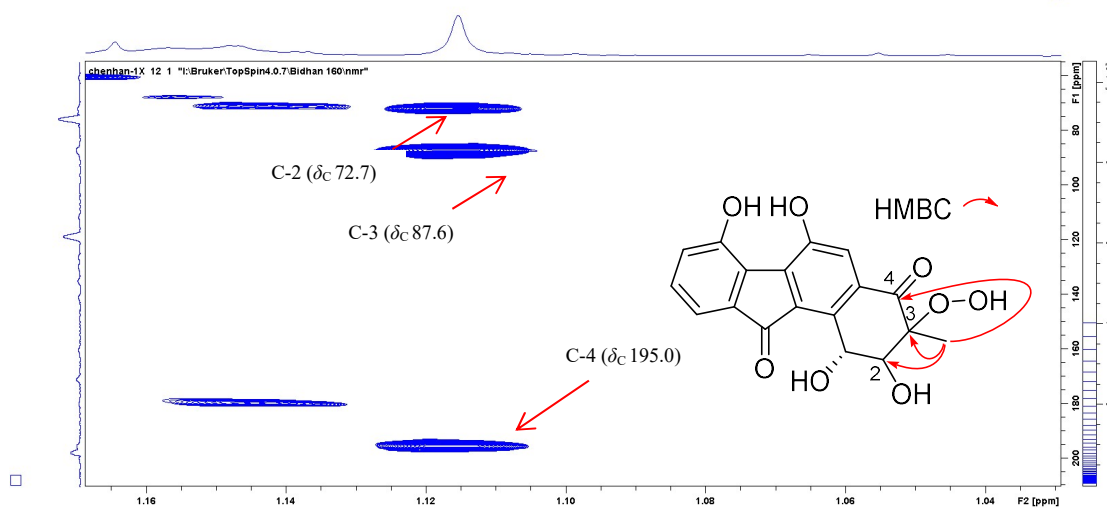
Supplementary Fig. 25. Spectroscopic data for 14. (a) HRESIMS spectrum



Supplementary Fig.25. Spectroscopic data for **14**. (b) The ¹H and enlarged ¹H NMR spectrum of **14** in DMSO-*d*₆.

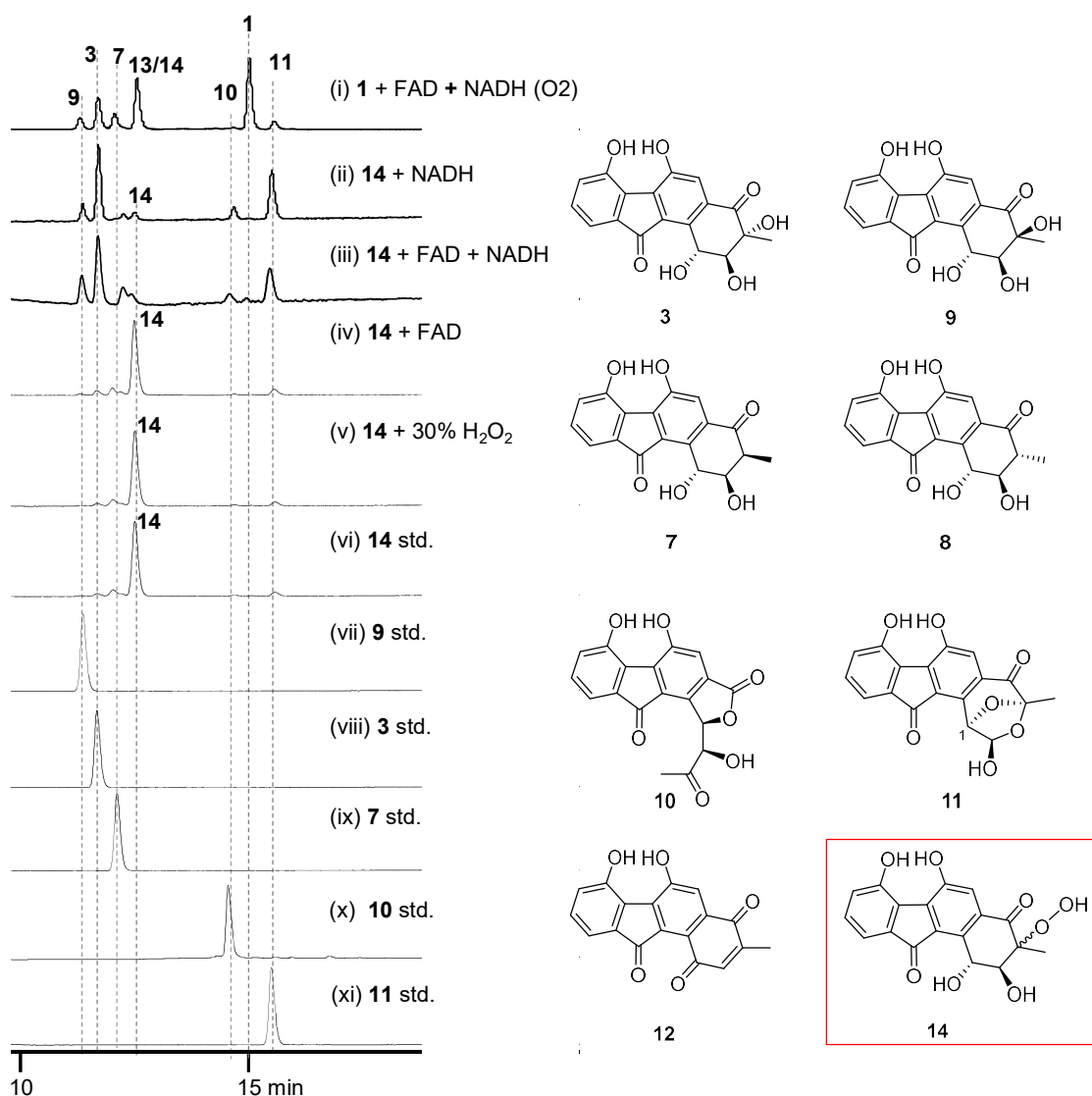


HMBC spectrum

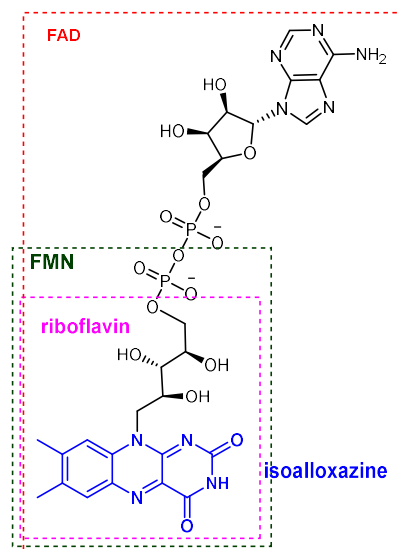
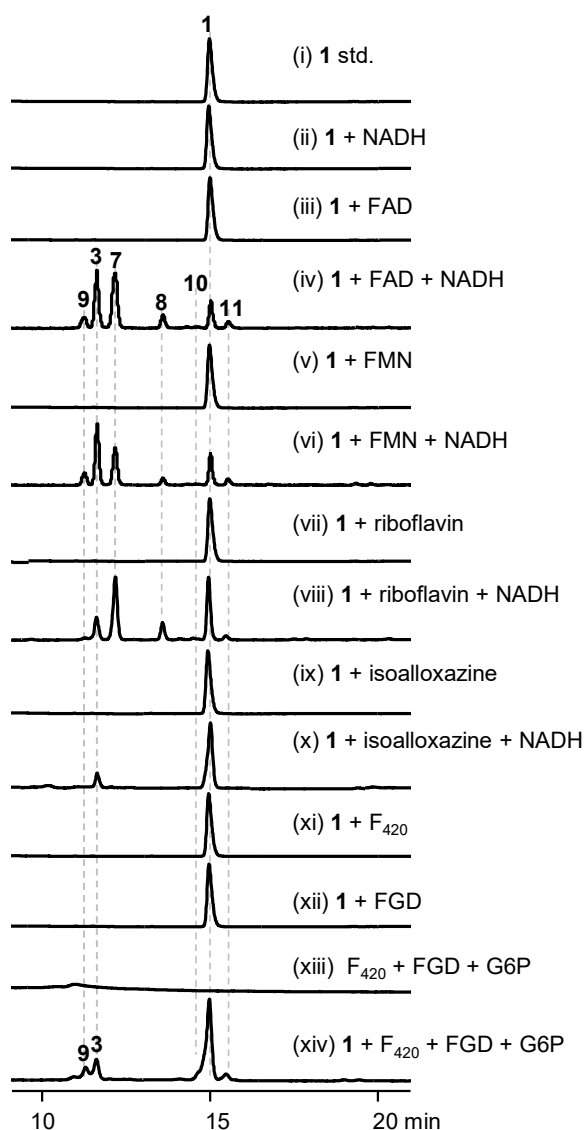


Enlarged HMBC spectrum

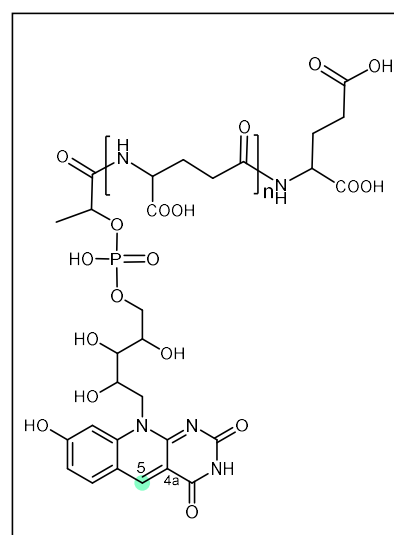
Supplementary Fig.25. Spectroscopic data for 14. (c) The HMBC and enlarged HMBC spectrum of **14** in DMSO- d_6 .



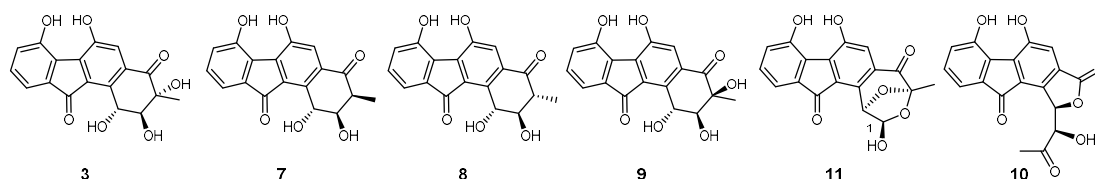
Supplementary Fig. 26. HPLC analysis of reactions with 14. HPLC analysis of reactions with **14** (i) standard assay contains 100 μM **1**, 10 μM FAD and 2 mM NADH in presence of excess O_2 ; (ii) **14** + 2 mM NADH; (iii) 100 μM **14** + 10 μM FAD and 2 mM NADH; (iv) **14** + 10 μM FAD; (v) **14** + 30 % H_2O_2 ; (vi) **14** std.; (vii) **9** std.; (viii) **3** std.; (ix) **7** std.; (x) **10** std.; (xi) **11** std. The reaction mixtures were incubated at 30 $^\circ\text{C}$ for 30 minutes HPLC analysis was performed using a polar column with UV detection at 304 nm.



structures of flavin cofactors



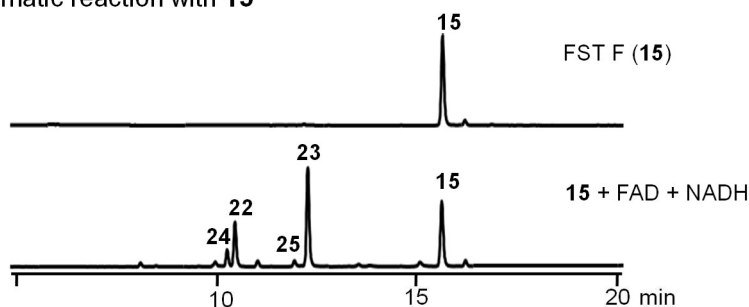
structure of cofactor F420



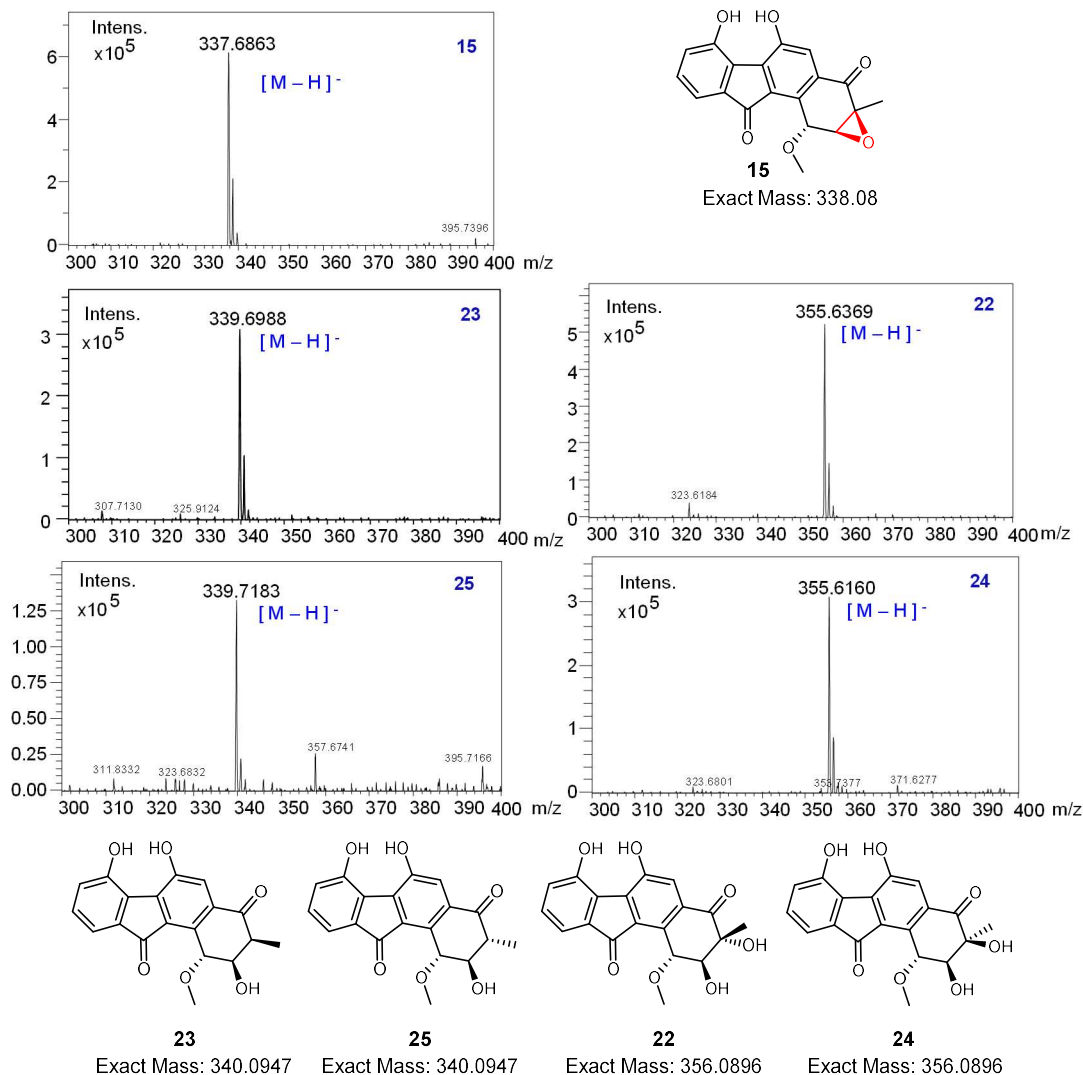
Supplementary Fig. 27. HPLC analysis of reactions 1 with different Flavin cofactors.

HPLC analysis of reactions 1 with different flavin cofactors. (i) 1 standard; (ii) 1 + 10 mM NADH; (iii) 1 + 100 μ M FAD; (iv) 1 + 100 μ M FAD + 10 mM NADH; (v) 1 + 100 μ M FMN; (vi) 1 + 100 μ M FMN + 10 mM NADH; (vii) 1 + 100 μ M riboflavin; (viii) 1 + 100 μ M riboflavin + 10 mM NADH; (ix) 1 + 100 μ M isoalloxazine; (x) 1 + 100 μ M isoalloxazine + 10 mM NADH. The reaction mixtures were incubated at 30 $^{\circ}$ C for 30 min. The next set of reactions were performed using the F₄₂₀ system: (xi) 1 + 200 μ M F₄₂₀; (xii) 1 + 10 μ M F₄₂₀-dependent glucose-6-phosphate dehydrogenase (FGD); (xiii) 200 μ M F₄₂₀ + 2 mM glucose 6-phosphate (G6P) + 10 μ M FGD; (xiv) 1 + 200 μ M F₄₂₀ + 2 mM G6P + 10 μ M FGD. The reaction mixtures were incubated at 30 $^{\circ}$ C for 10 h. HPLC analysis was run using polar column with UV detection at 304 nm.

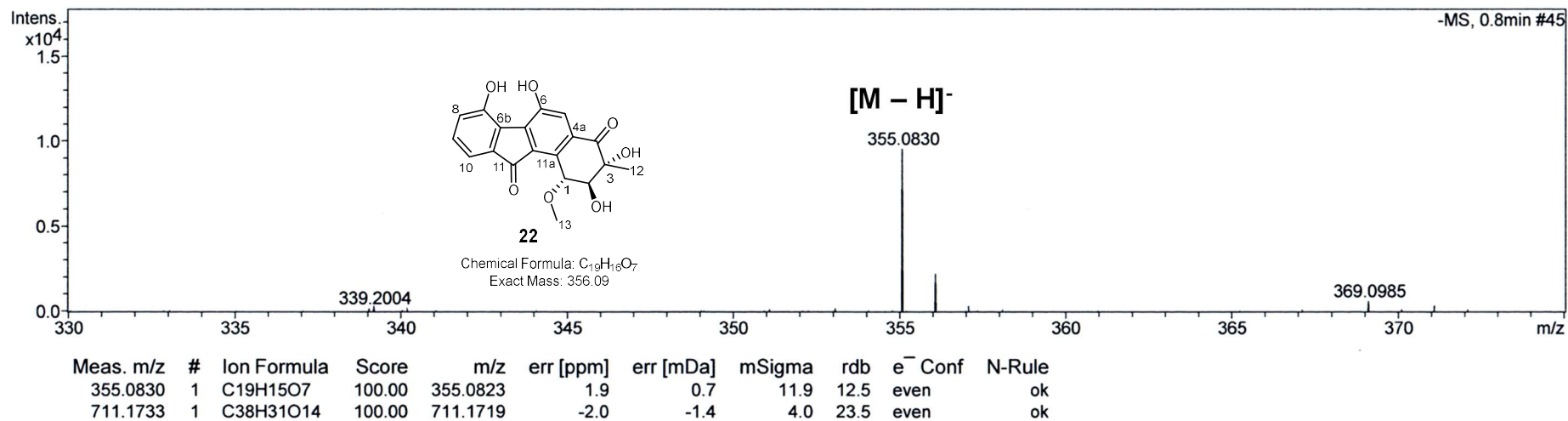
a Non enzymatic reaction with **15**



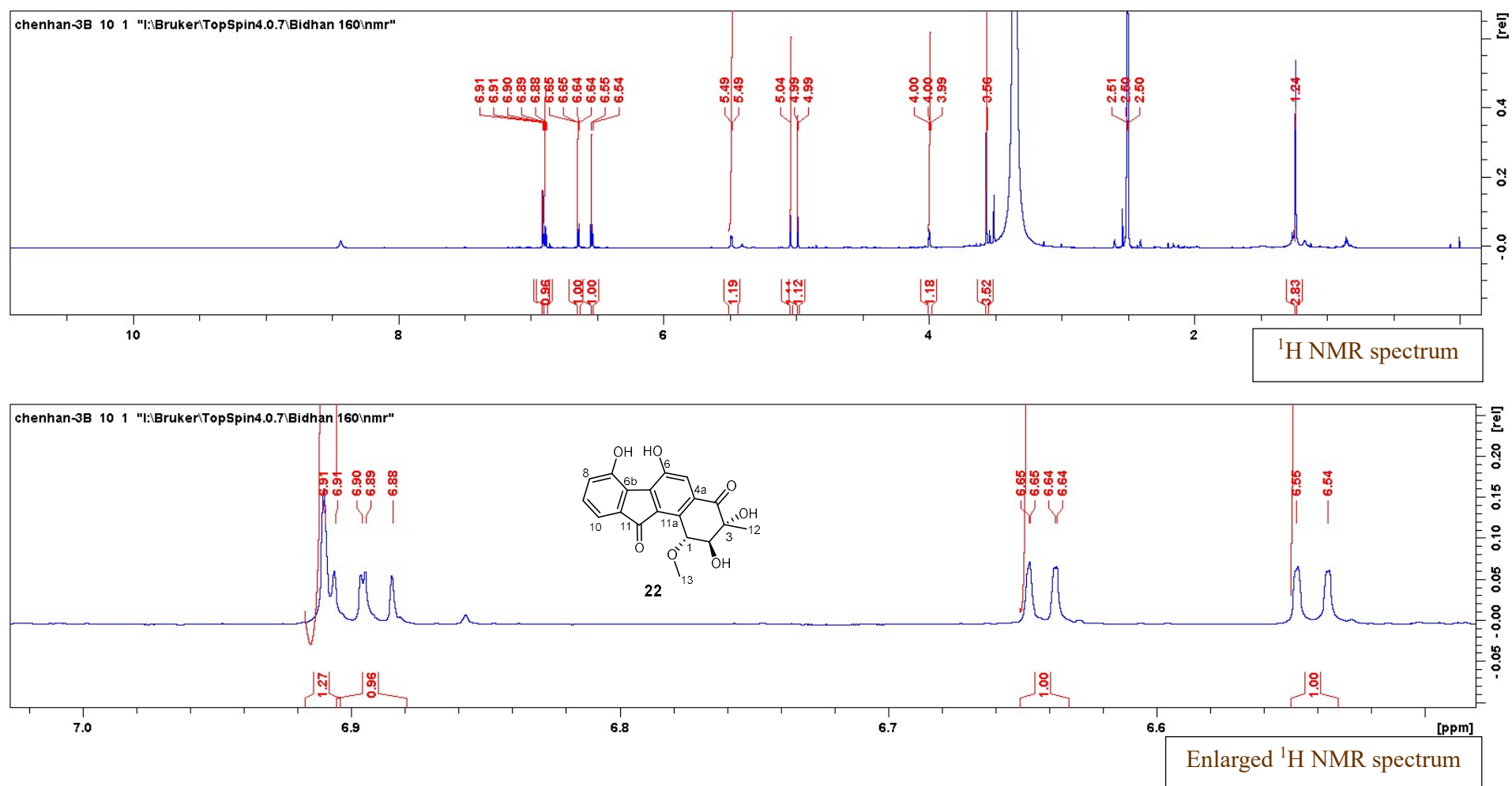
b LC-MS analysis of reaction products



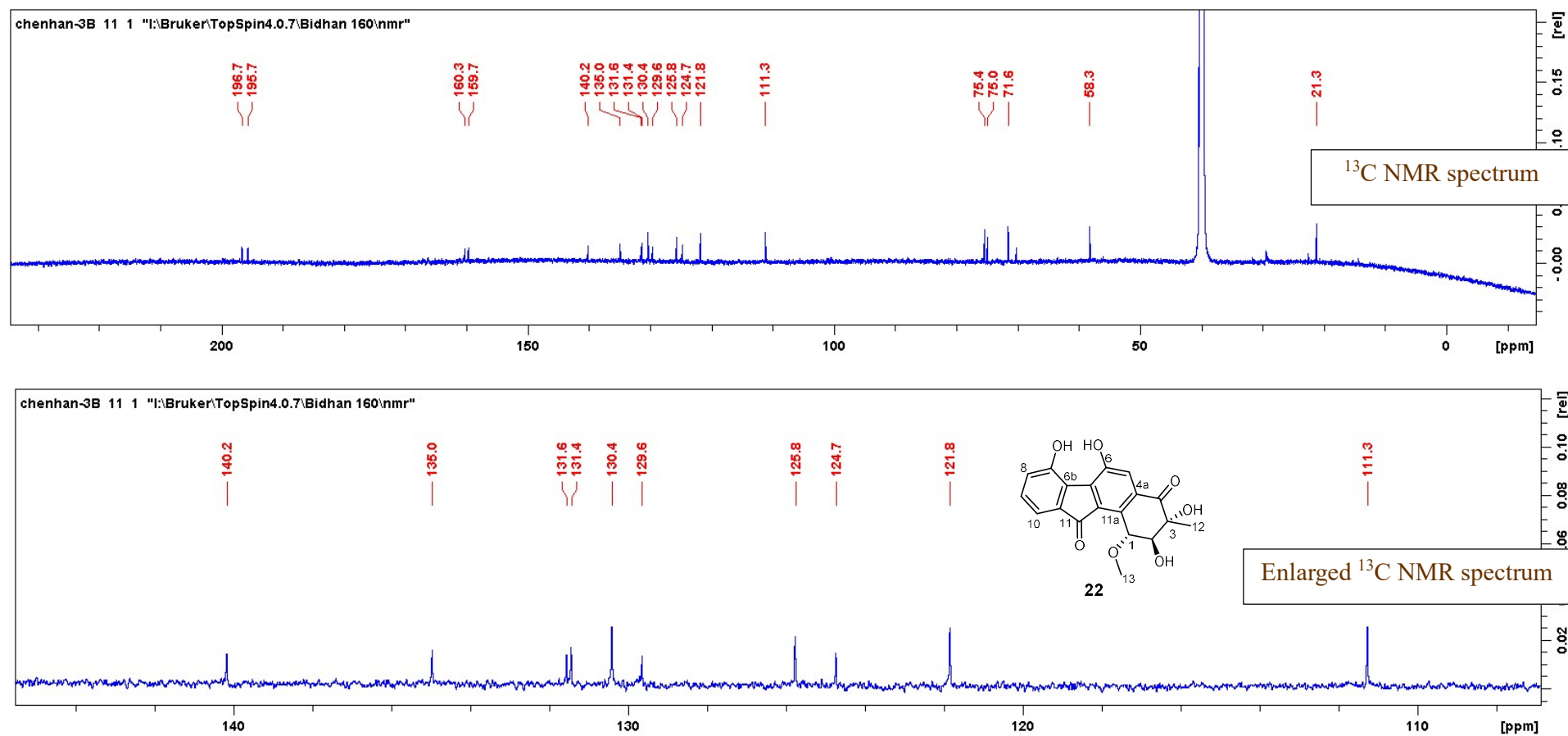
Supplementary Fig. 28. HPLC and LC-MS analysis of FST F (15**) reaction with FAD/NADH.** (a) HPLC analysis of the reaction mixtures containing 100 μ M **15**, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 30 min. (b) LC-MS analysis of 7-methyl- FST F (**15**) reaction products.



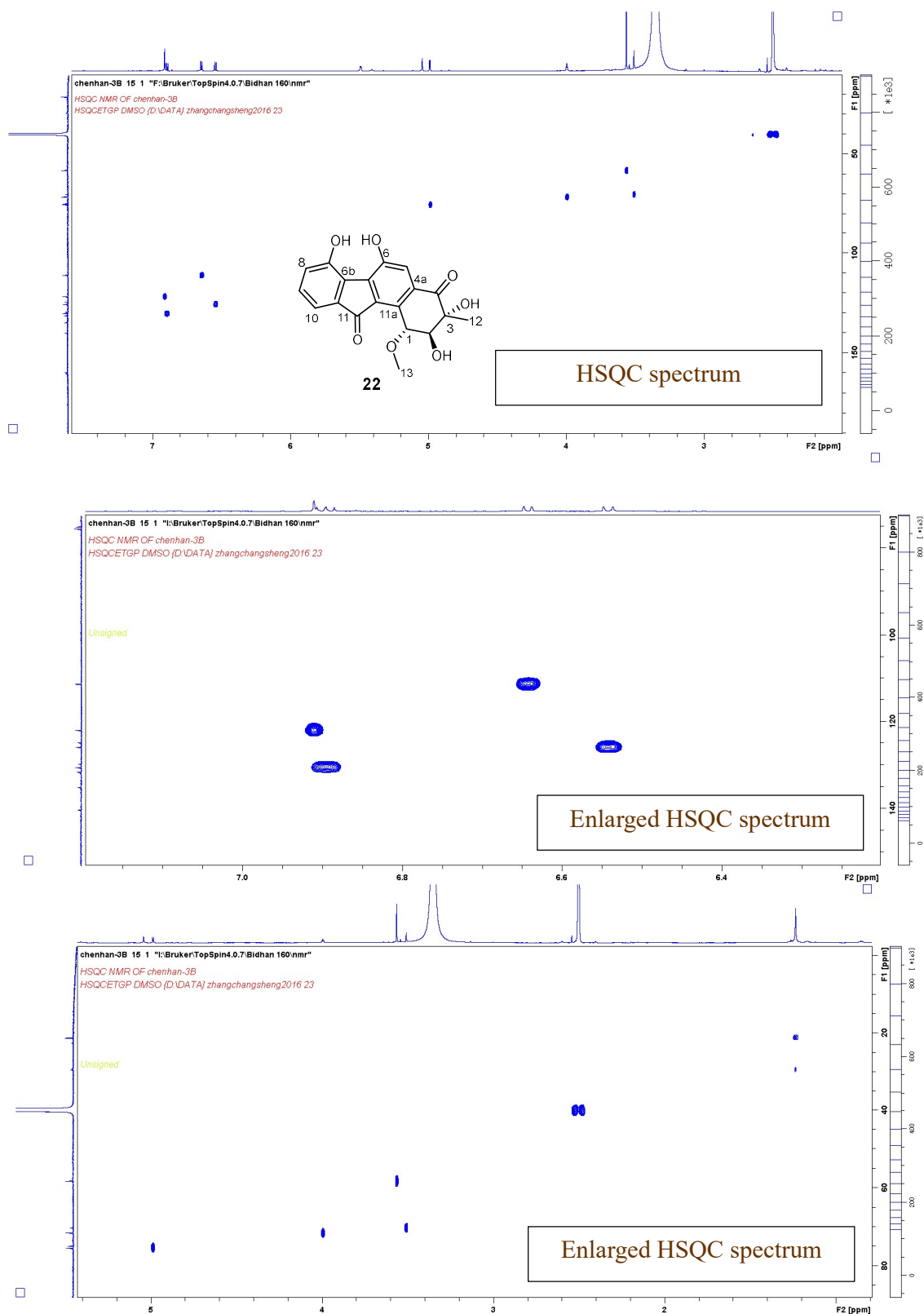
Supplementary Fig. 29. Spectroscopic data for 22. (a) HRESIMS spectrum.



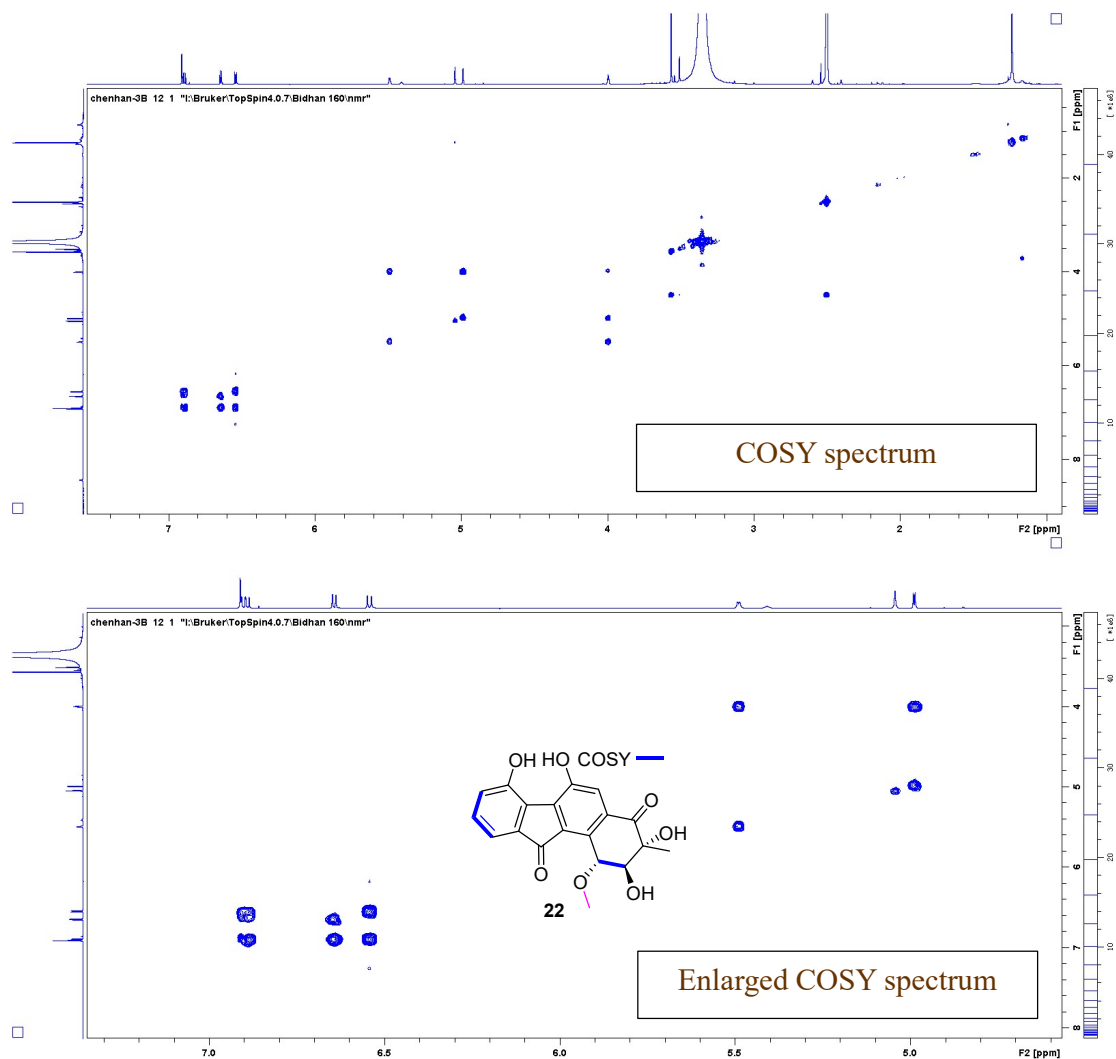
Supplementary Fig. 29. Spectroscopic data for 22. (b) The ¹H and enlarged ¹H NMR spectrum of 22 in DMSO-*d*₆.



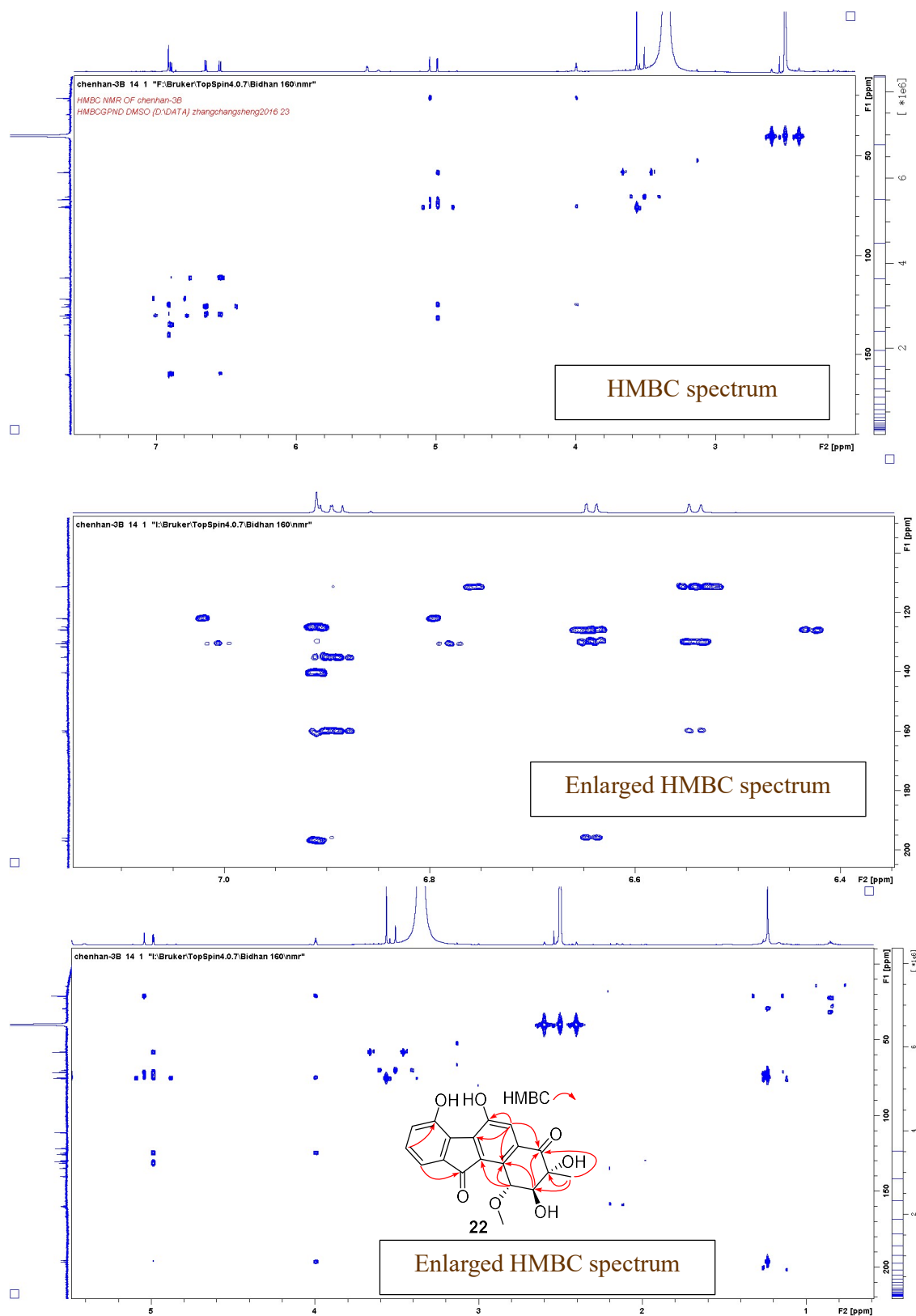
Supplementary Fig. 29. Spectroscopic data for **22**. (c) The ¹³C and enlarged ¹³C NMR spectrum of **22** in DMSO-*d*₆.



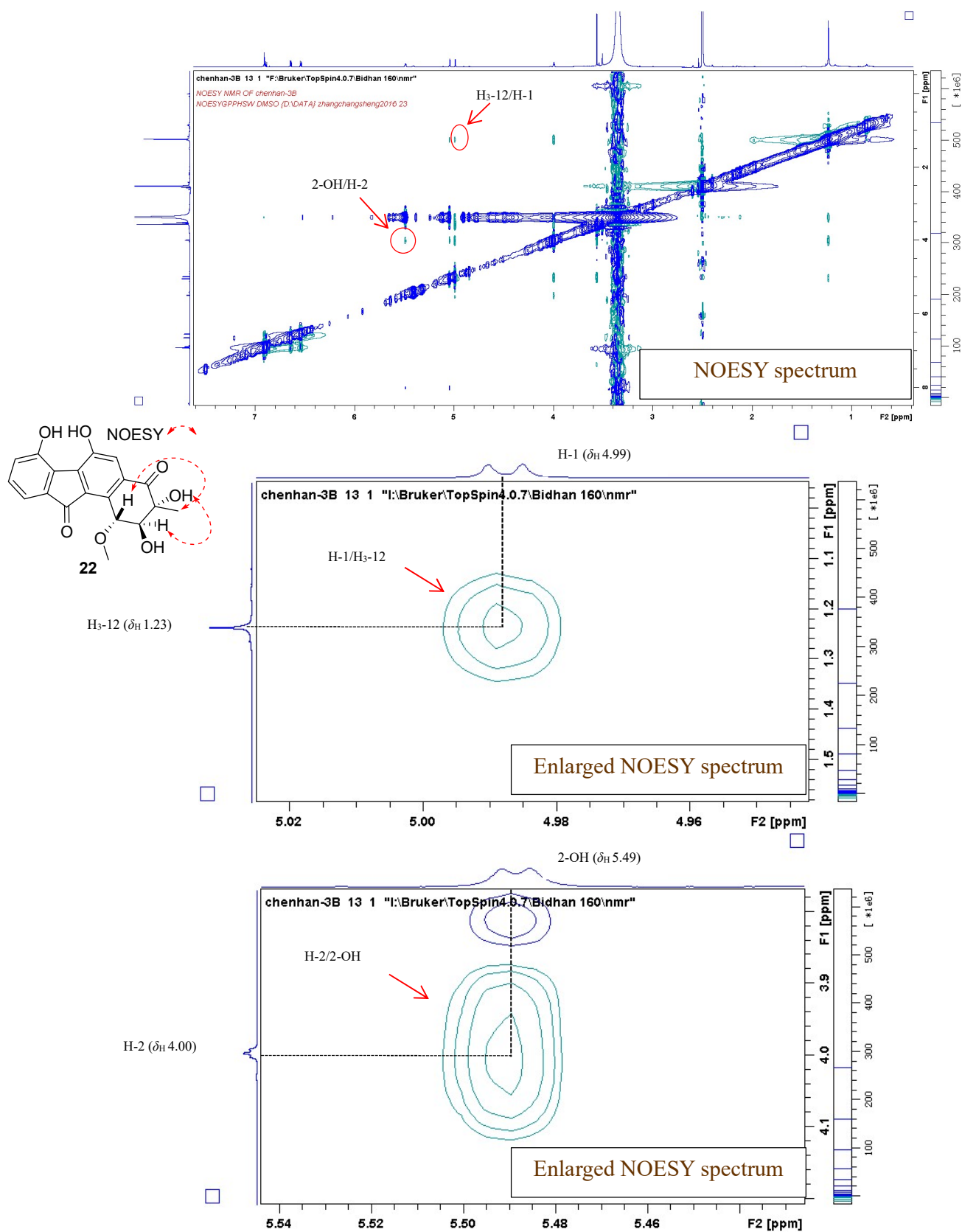
Supplementary Fig. 29. Spectroscopic data for 22. (d) The HSQC and enlarged HSQC spectrum of 22 in DMSO- d_6 .



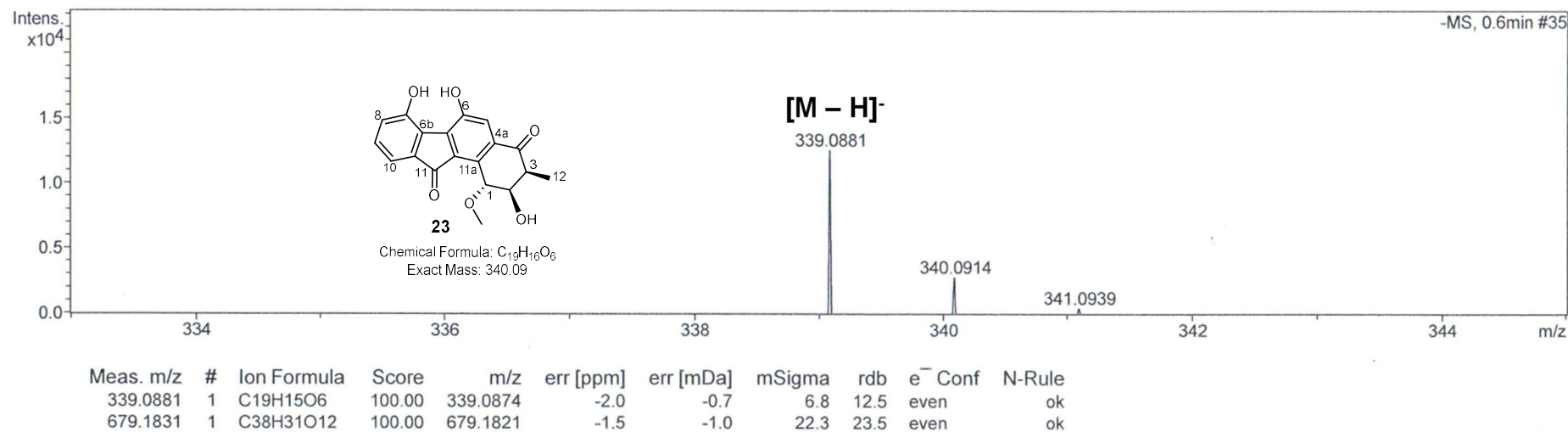
Supplementary Fig. 29. Spectroscopic data for 22. (e) The COSY and enlarged COSY spectrum of **22** in DMSO- d_6 .



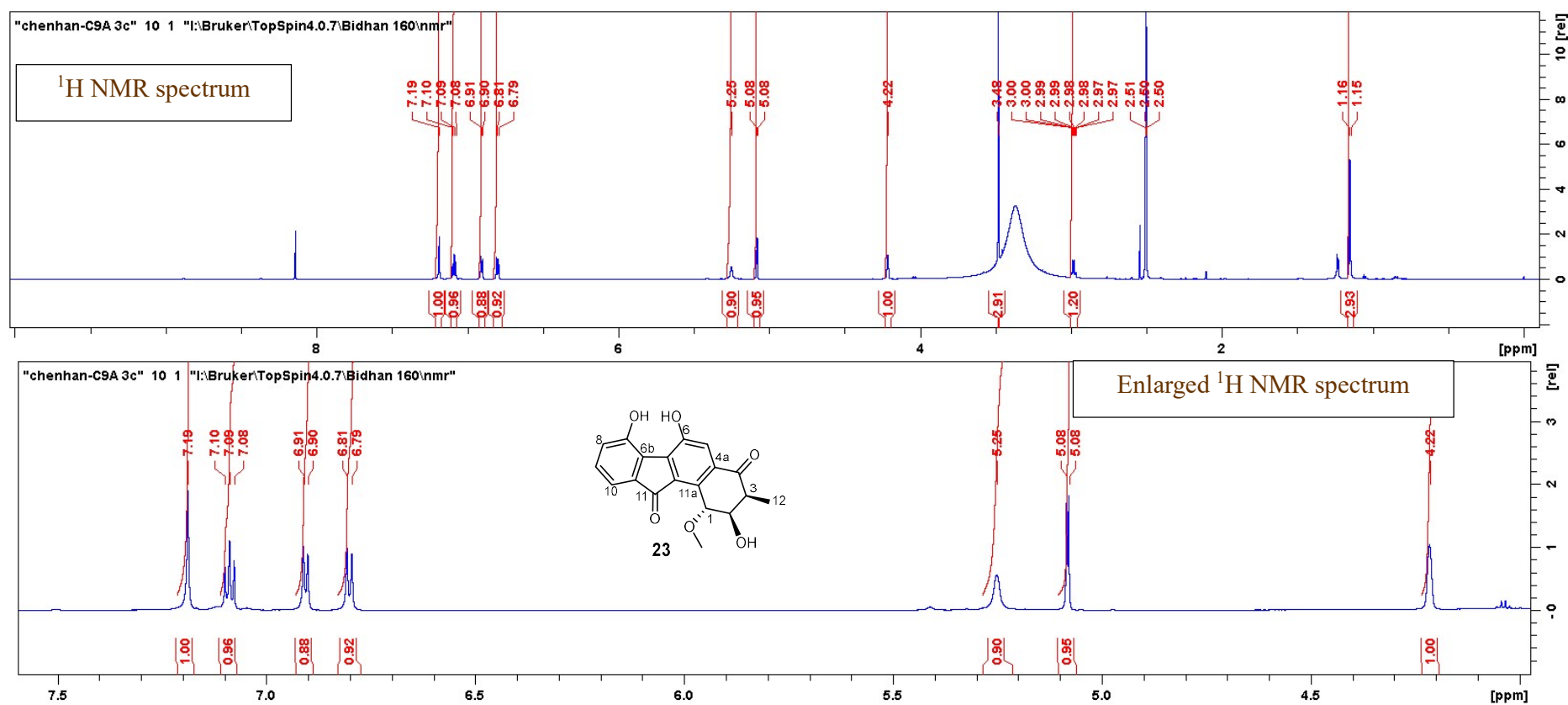
Supplementary Fig. 29. Spectroscopic data for 22. (f) The HMBC and enlarged HMBC spectrum of 22 in DMSO- d_6 .



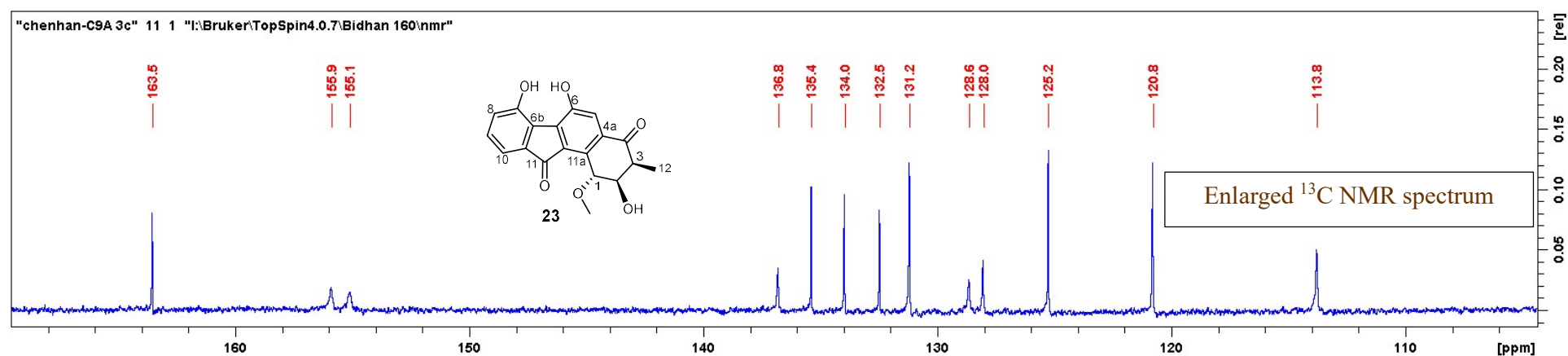
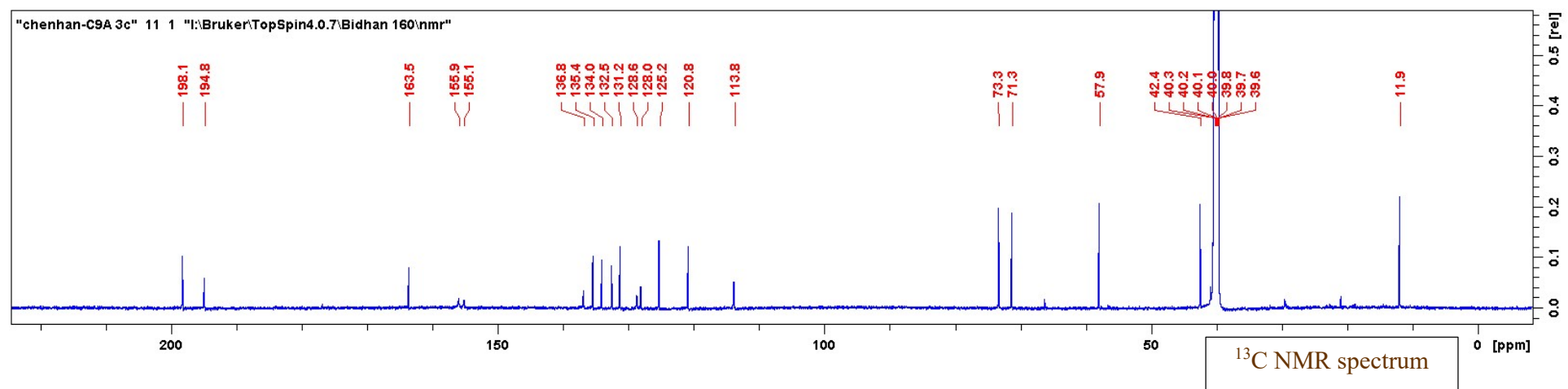
Supplementary Fig. 29. Spectroscopic data for 22. (g) The NOESY and enlarged NOESY spectrum of 22 in DMSO-*d*₆.



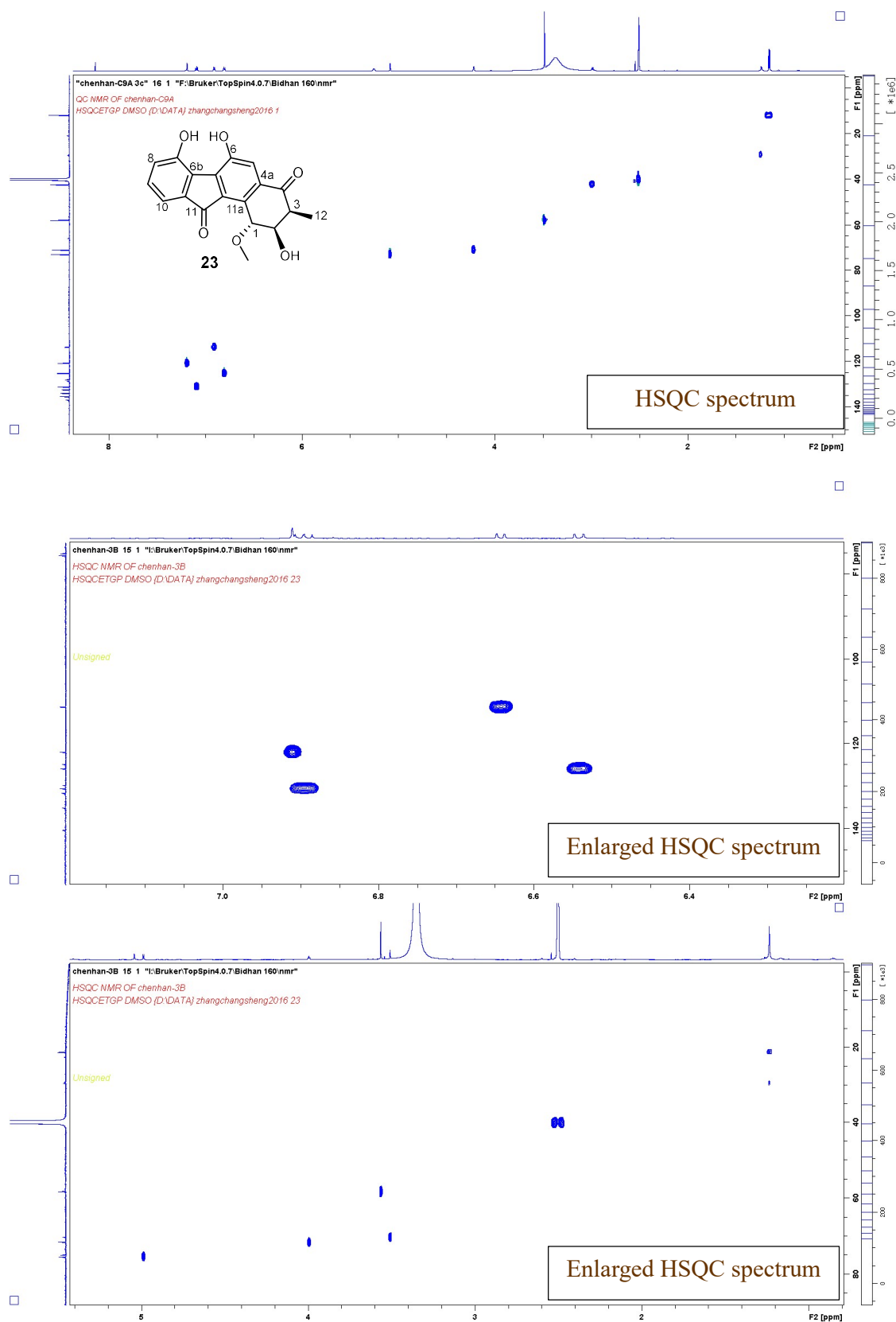
Supplementary Fig. 30. Spectroscopic data for 23. (a) HRESIMS spectrum.



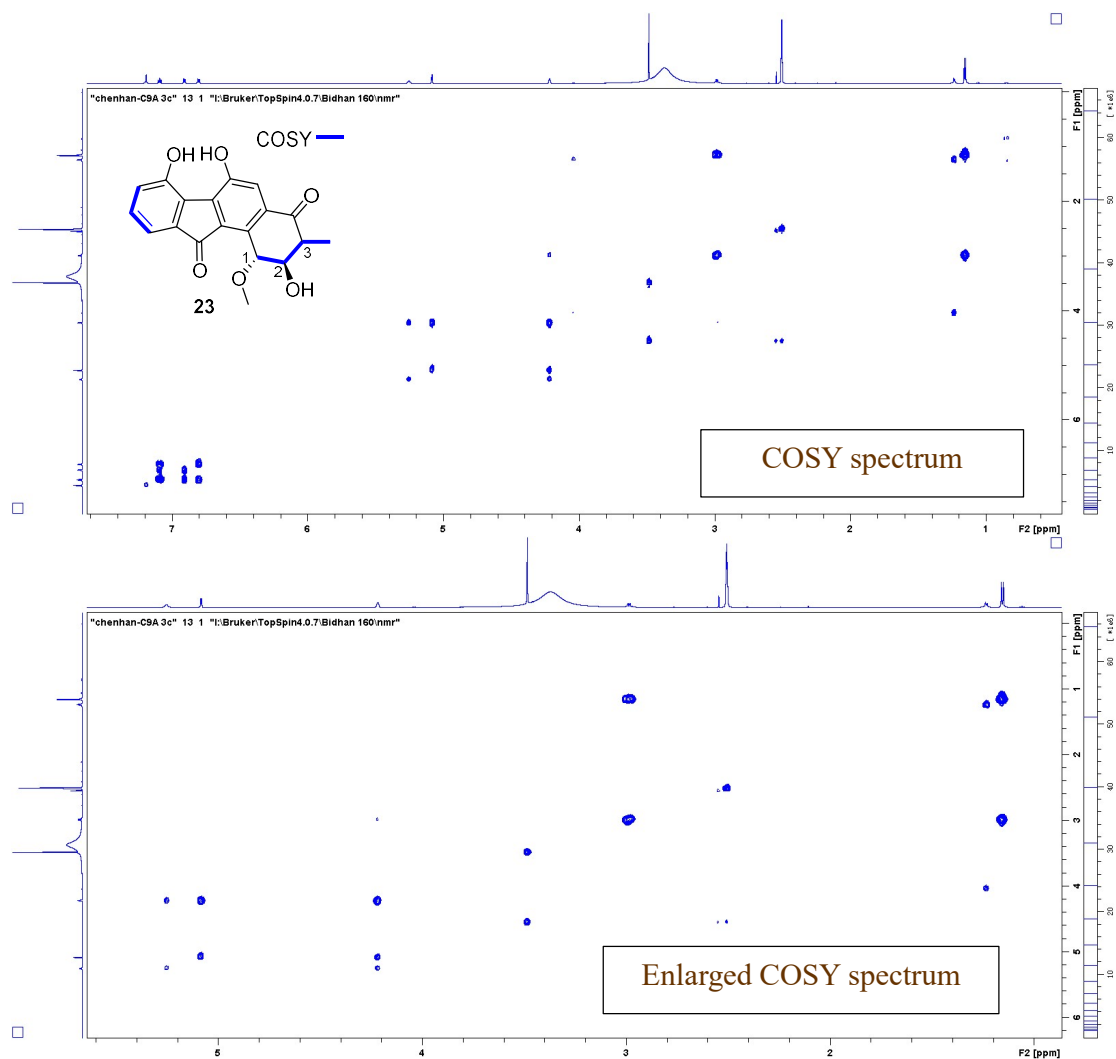
Supplementary Fig. 30. Spectroscopic data for **23**. (b) The ¹H and enlarged ¹H NMR spectrum of **23** in DMSO-*d*₆.



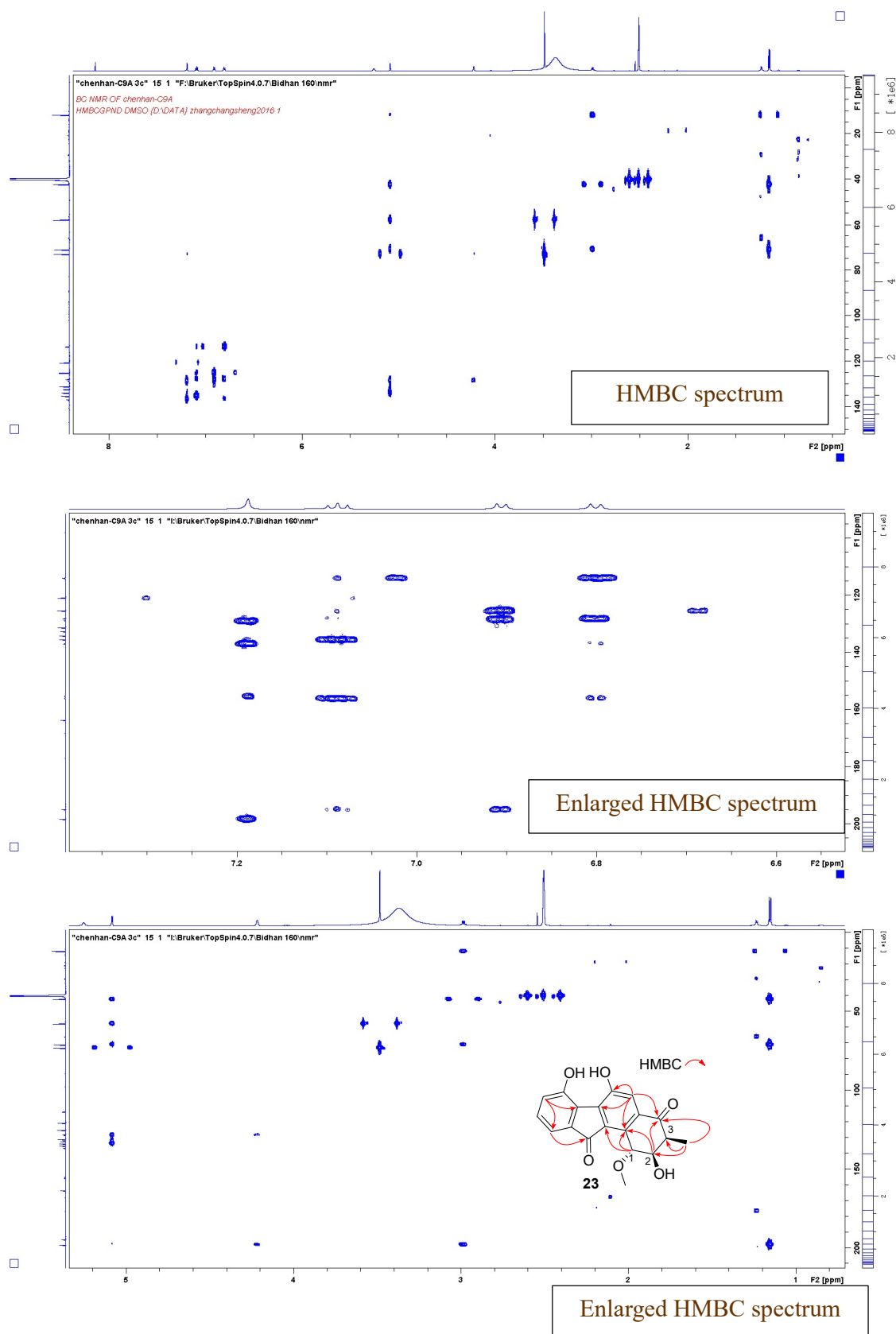
Supplementary Fig. 30. Spectroscopic data for **23**. (c) The ¹³C and enlarged ¹³C NMR spectrum of **23** in DMSO-*d*₆.



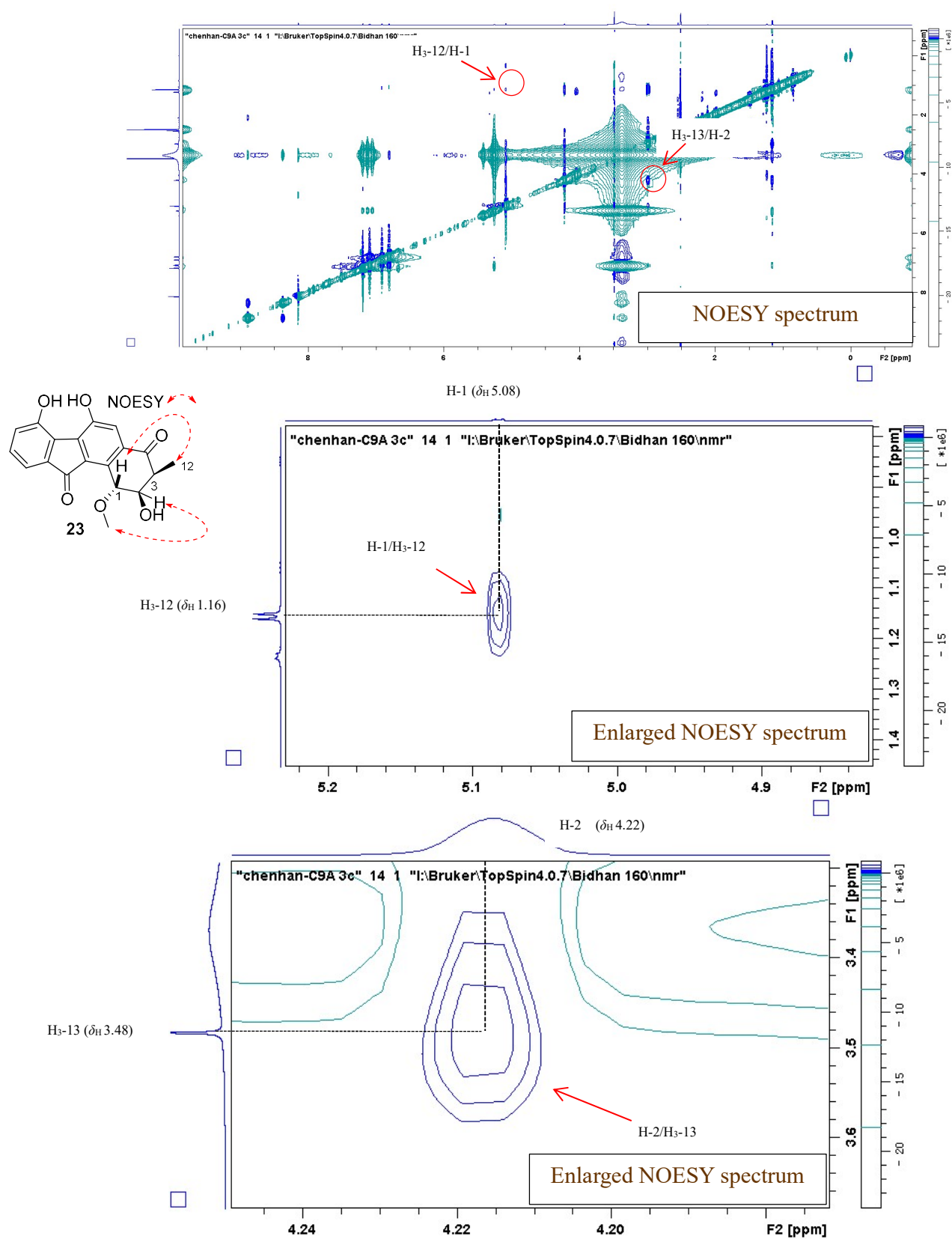
Supplementary Fig. 30. Spectroscopic data for 23. (d) The HSQC and enlarged HSQC spectrum of 23 in DMSO-*d*₆.



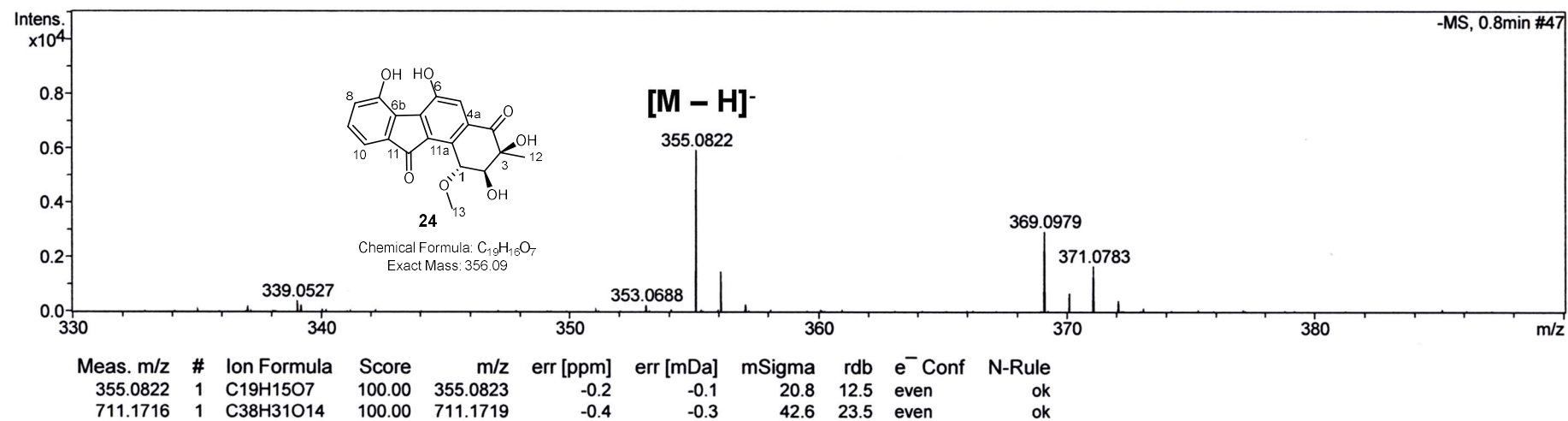
Supplementary Fig. 30. Spectroscopic data for 23. (e) The COSY and enlarged COSY spectrum of **23** in DMSO- d_6 .



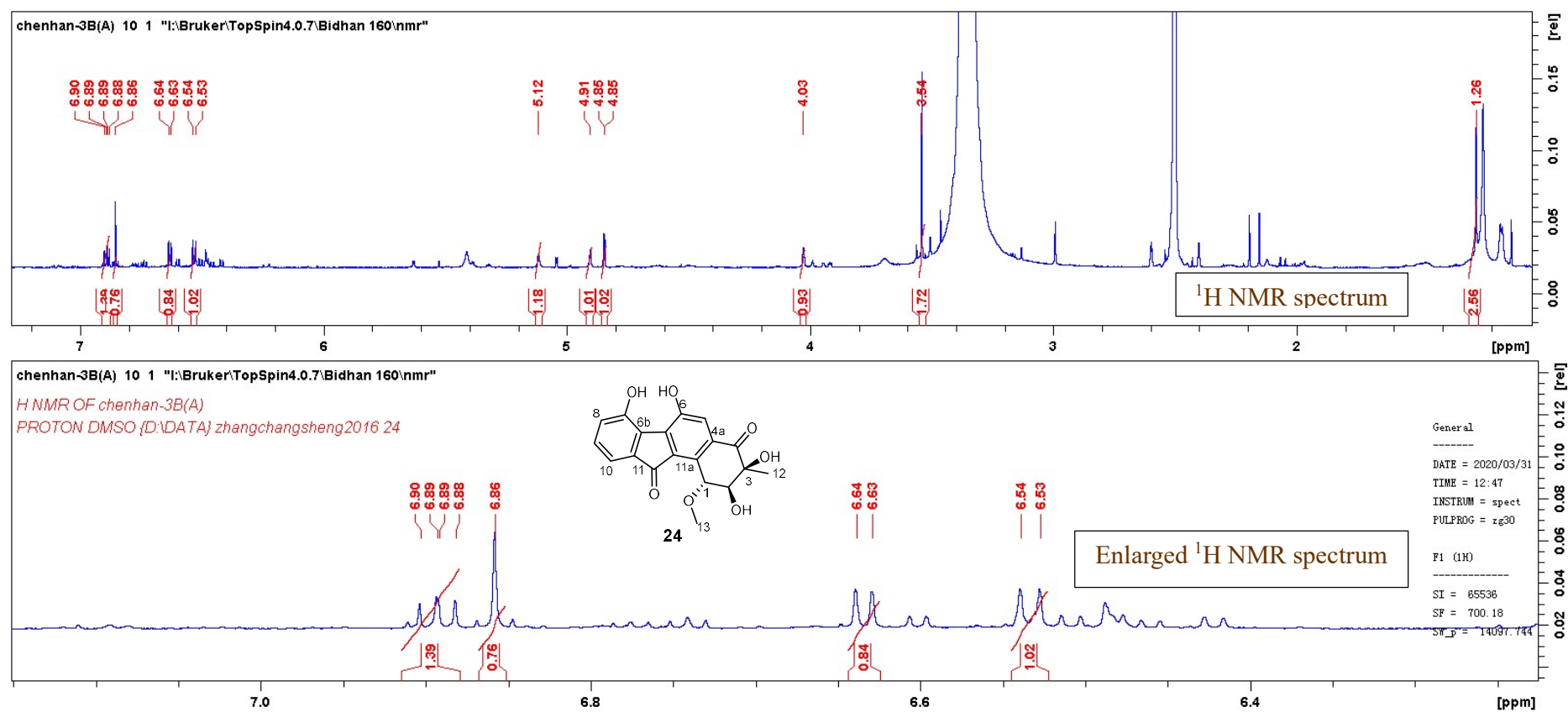
Supplementary Fig. 30. Spectroscopic data for 23. (f) The HMBC and enlarged HMBC spectrum of **23** in DMSO- d_6 .



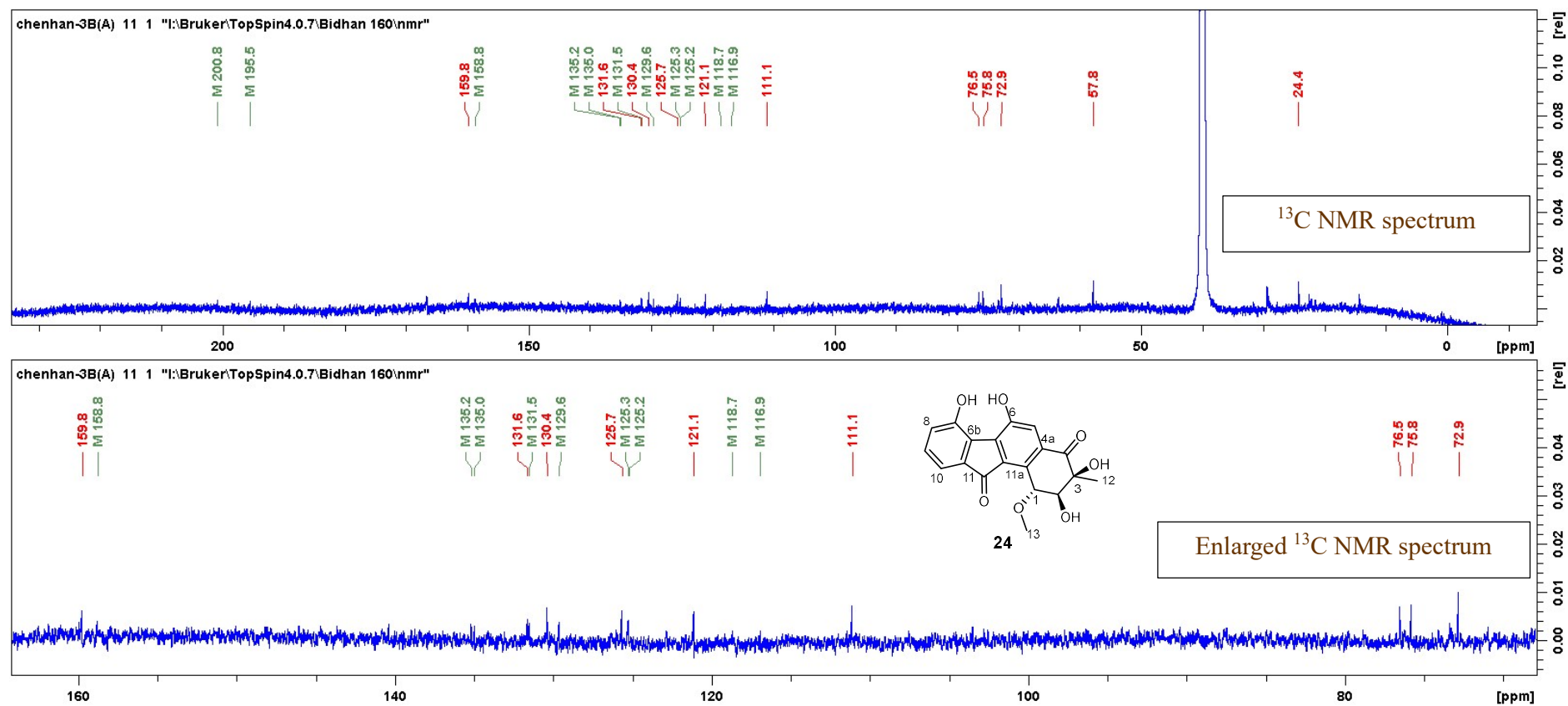
Supplementary Fig. 30. Spectroscopic data for 23. (g) The NOESY and enlarged NOESY spectrum of 23 in DMSO- d_6 .



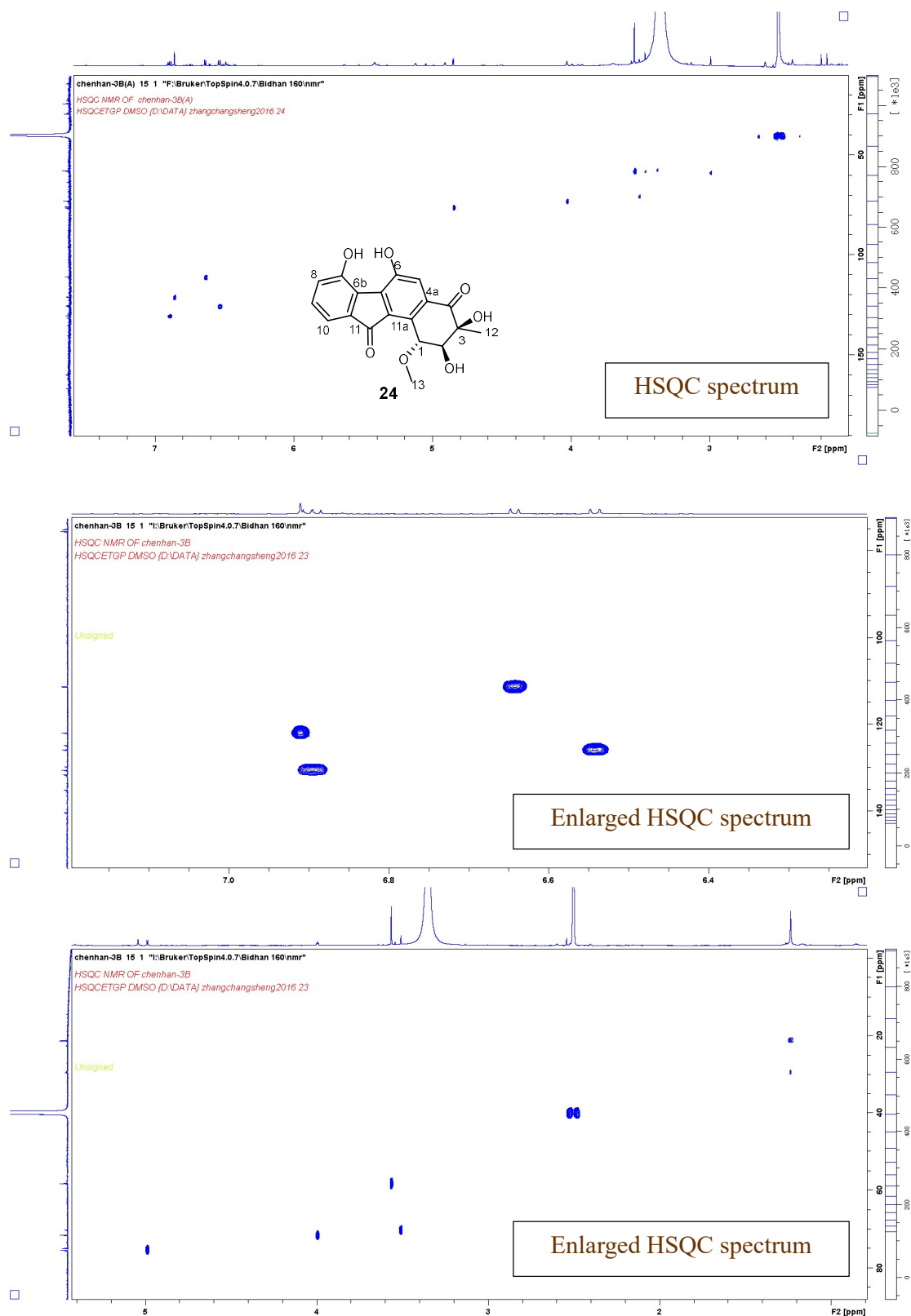
Supplementary Fig. 31. Spectroscopic data for **24**. (a) HRESIMS spectrum.



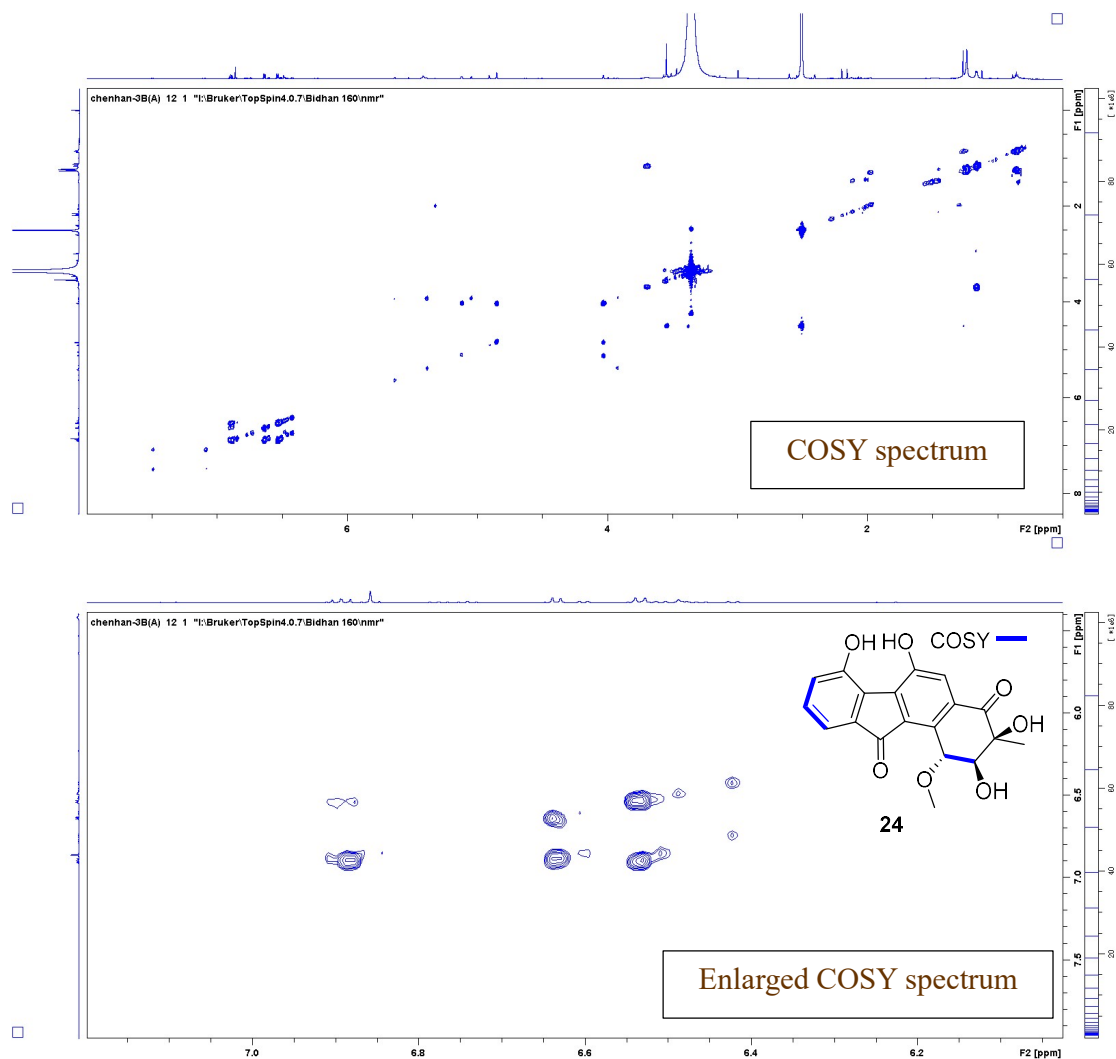
Supplementary Fig. 31. Spectroscopic data for **24**. (b) The ¹H and enlarged ¹H NMR spectrum of **24** in DMSO-*d*₆.



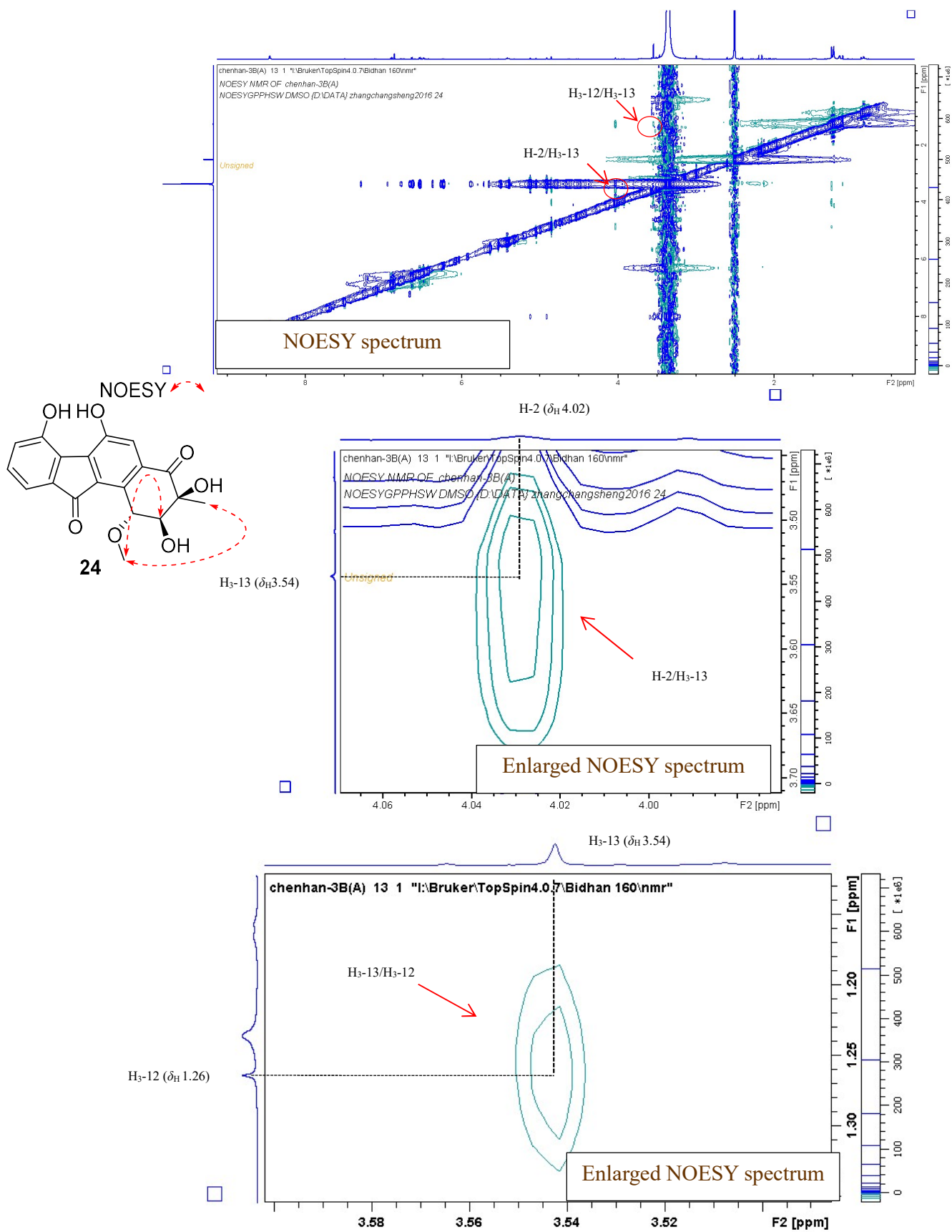
Supplementary Fig. 31. Spectroscopic data for **24**. (c) The ¹³C and enlarged ¹³C NMR spectrum of **24** in DMSO-*d*₆.



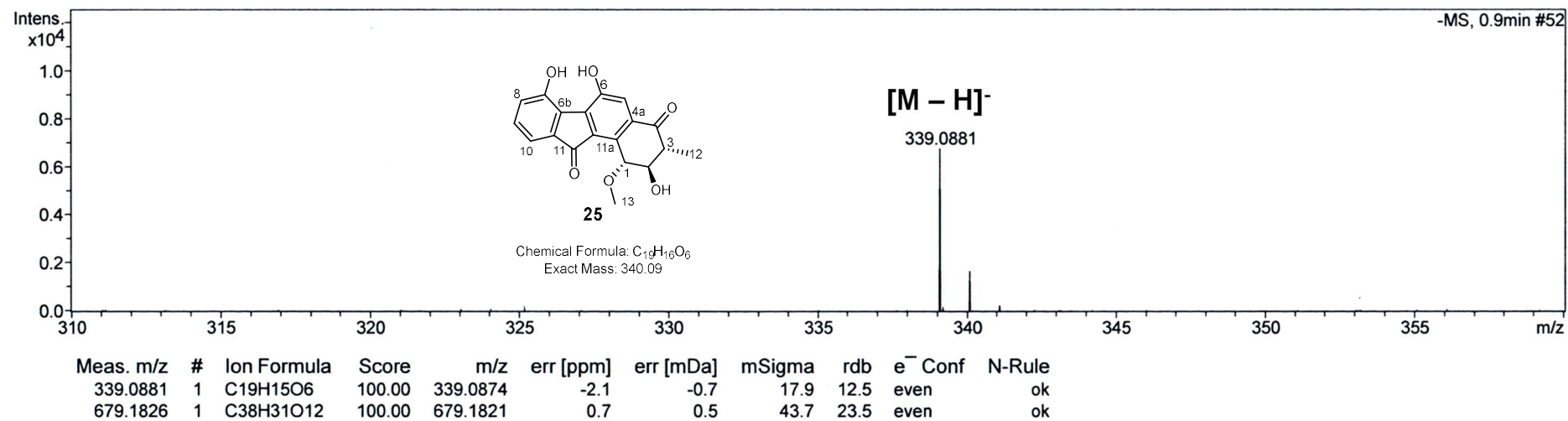
Supplementary Fig. 31. Spectroscopic data for 24. (d) The HSQC and enlarged HSQC spectrum of 24 in DMSO-*d*₆.



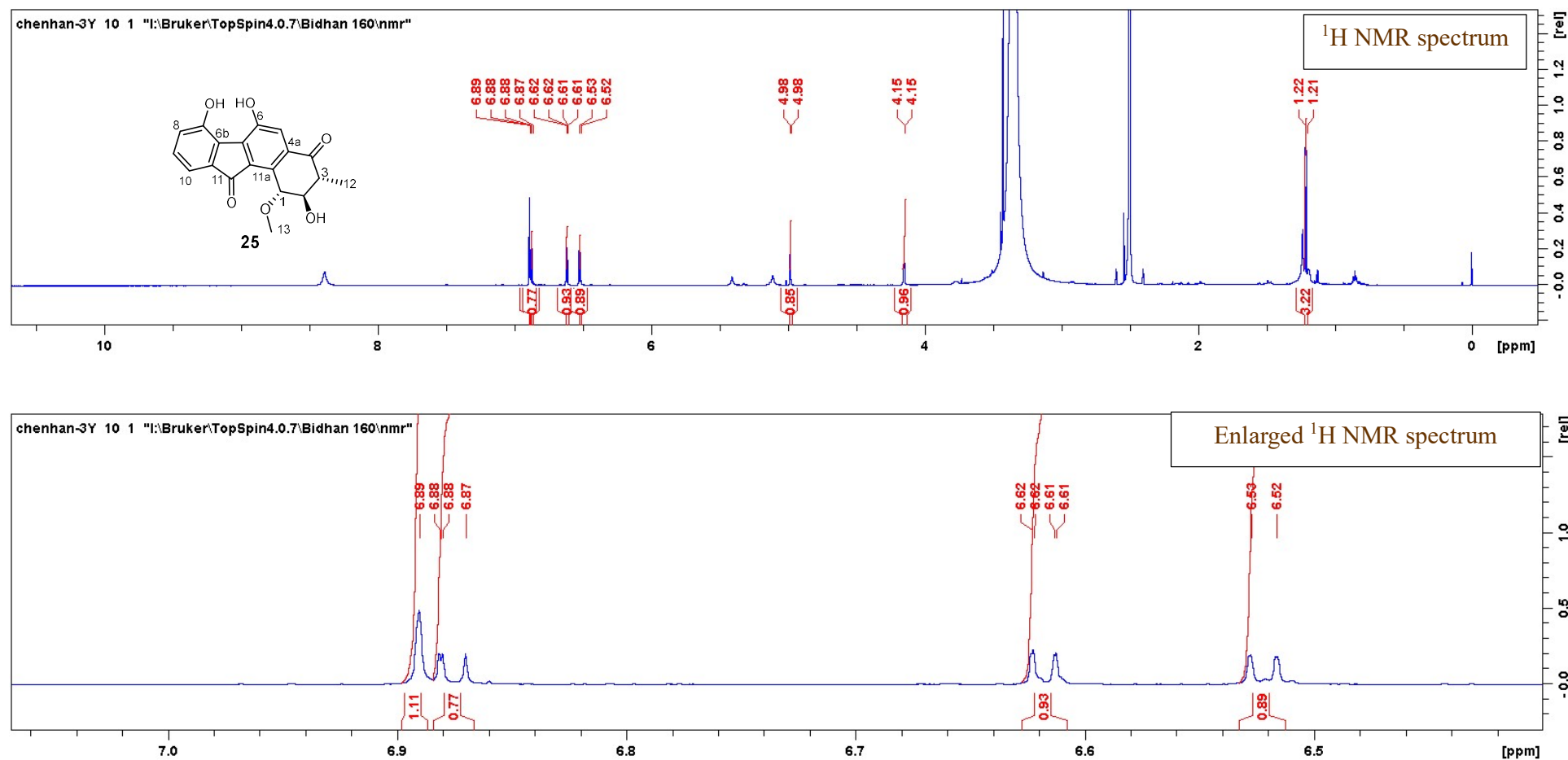
Supplementary Fig. 31. Spectroscopic data for 24. (e) The COSY and enlarged COSY spectrum of **24** in DMSO- d_6 .



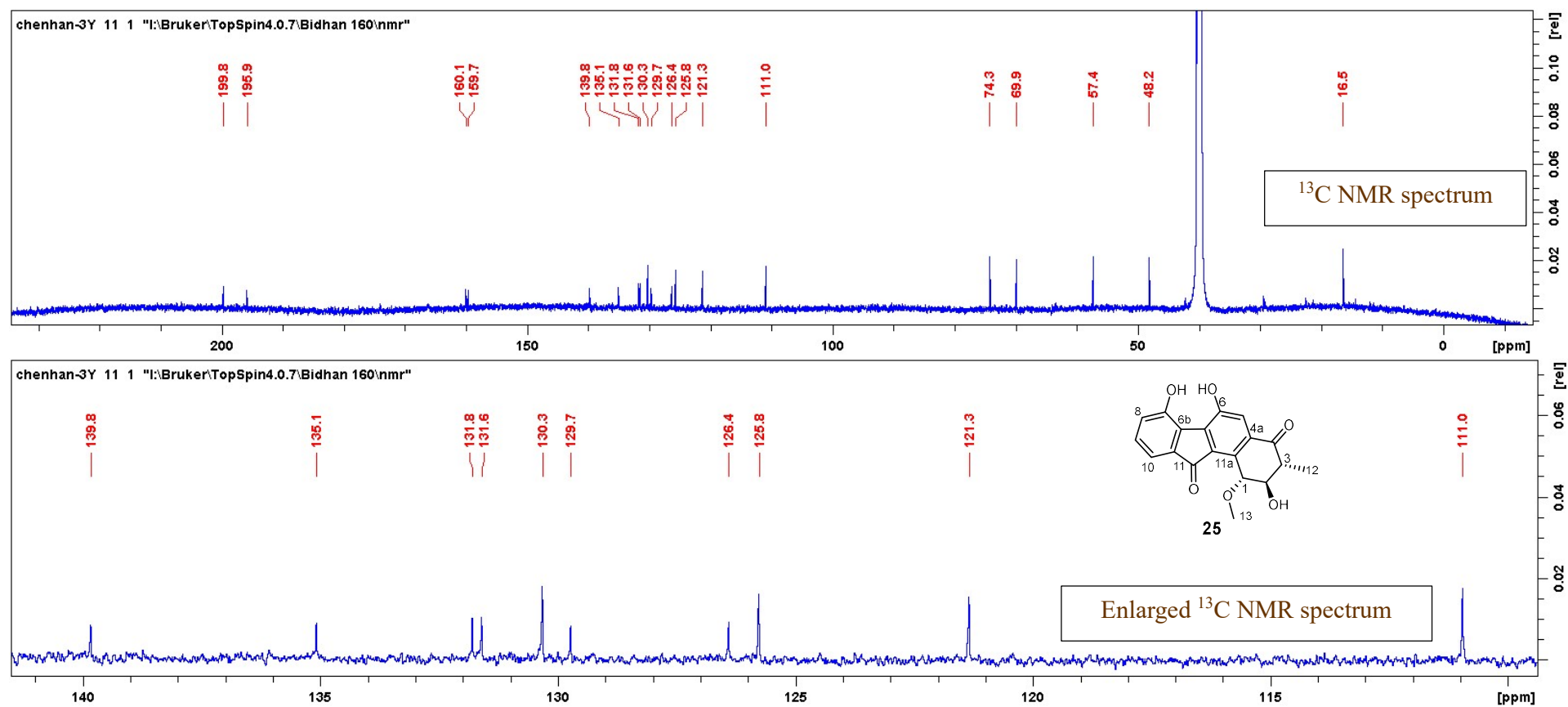
Supplementary Fig. 31. Spectroscopic data for 24. (g) The NOESY and enlarged NOESY spectrum of **24** in DMSO-*d*₆.



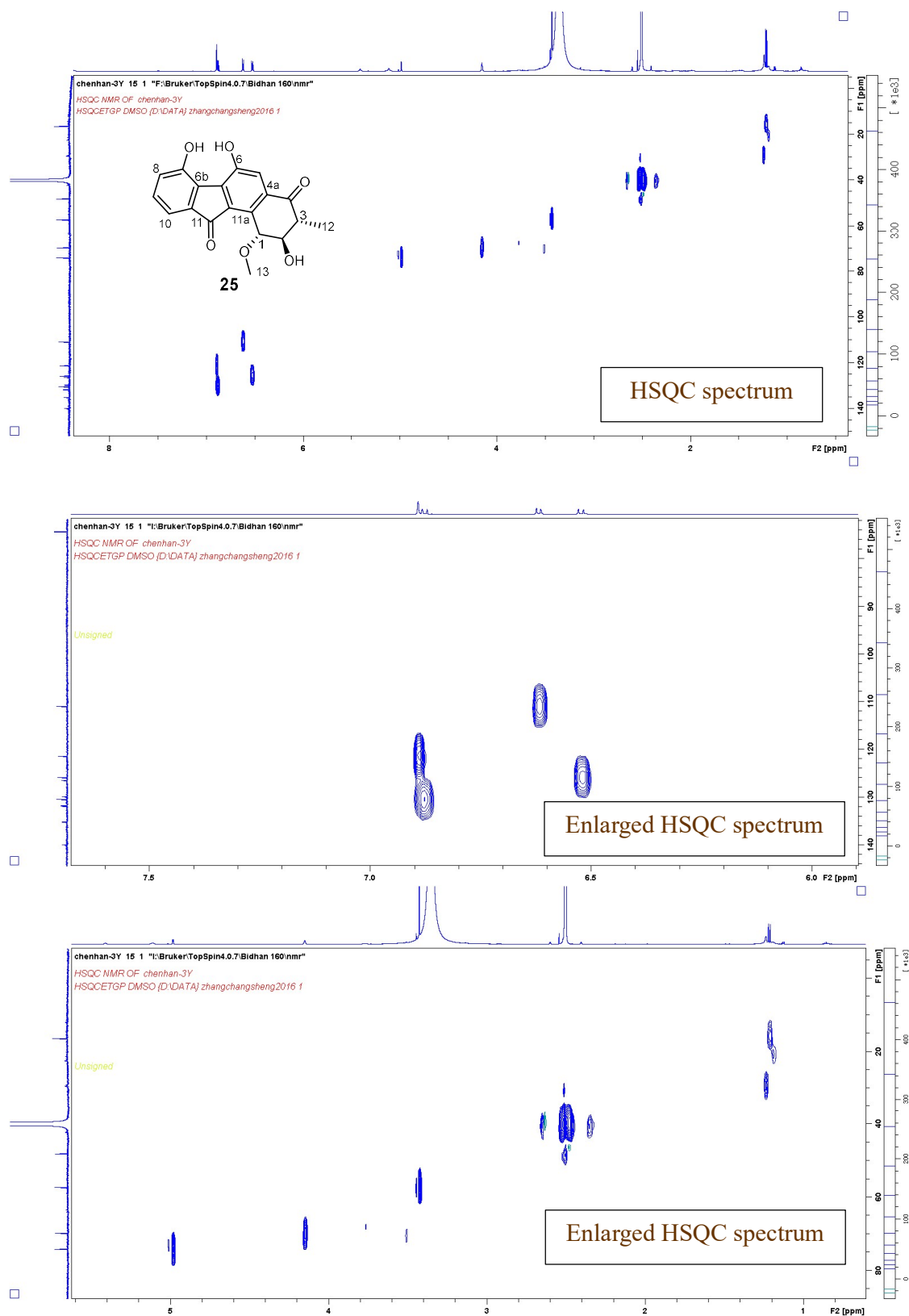
Supplementary Fig.32. Spectroscopic data for 25. (a) HRESIMS spectrum.



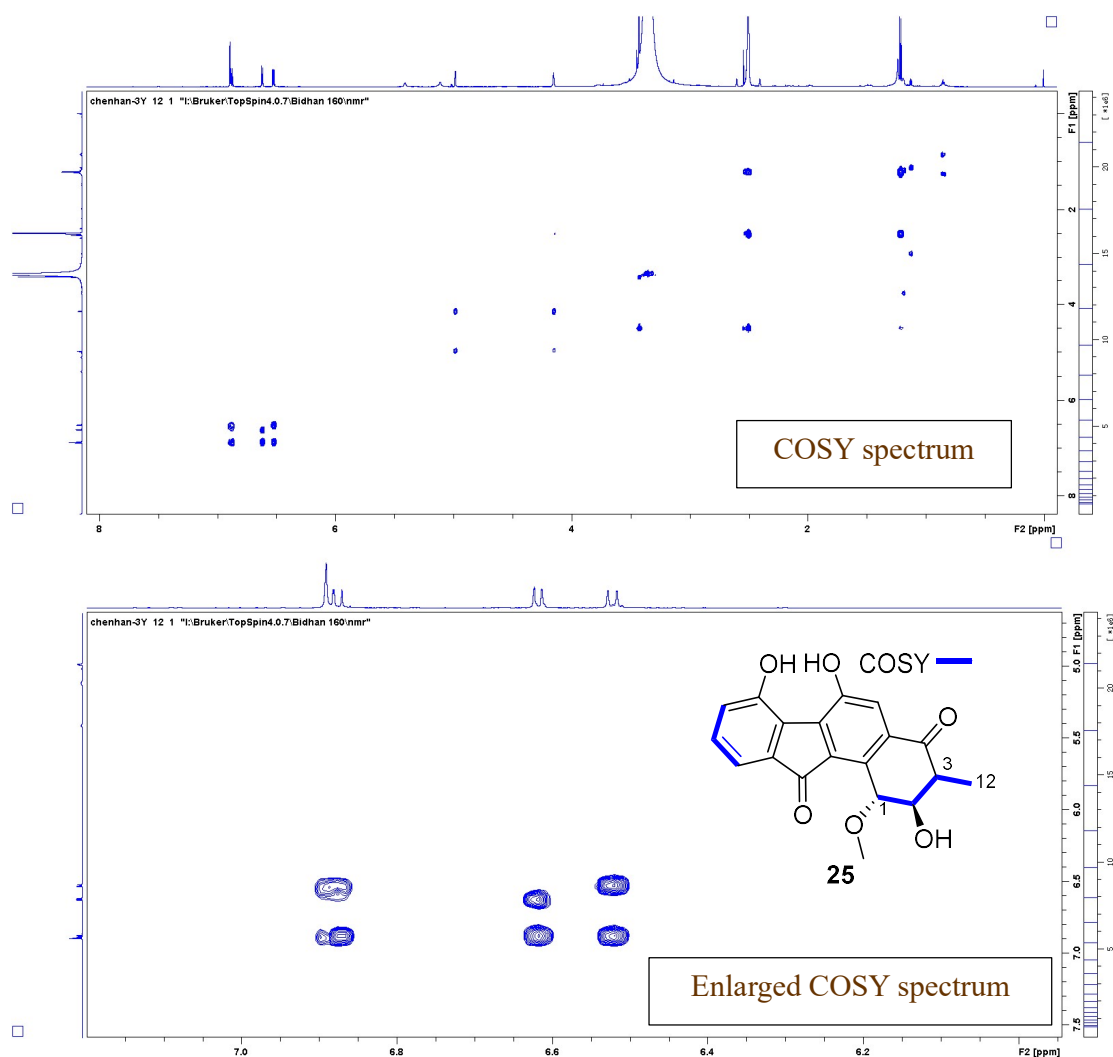
Supplementary Fig.32. Spectroscopic data for 25. (b) The ¹H and enlarged ¹H NMR spectrum of 25 in DMSO-*d*₆.



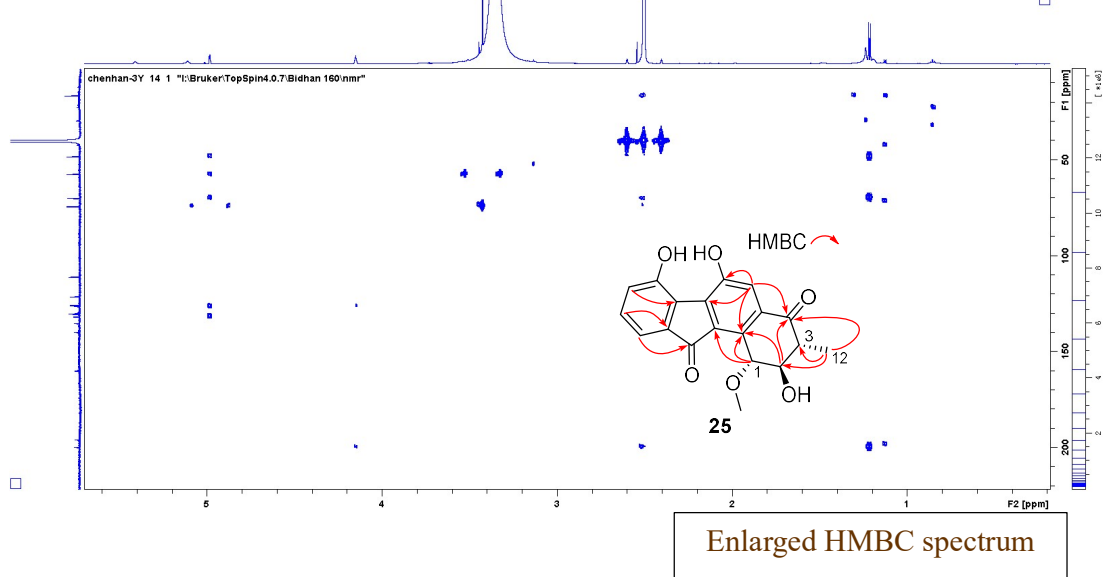
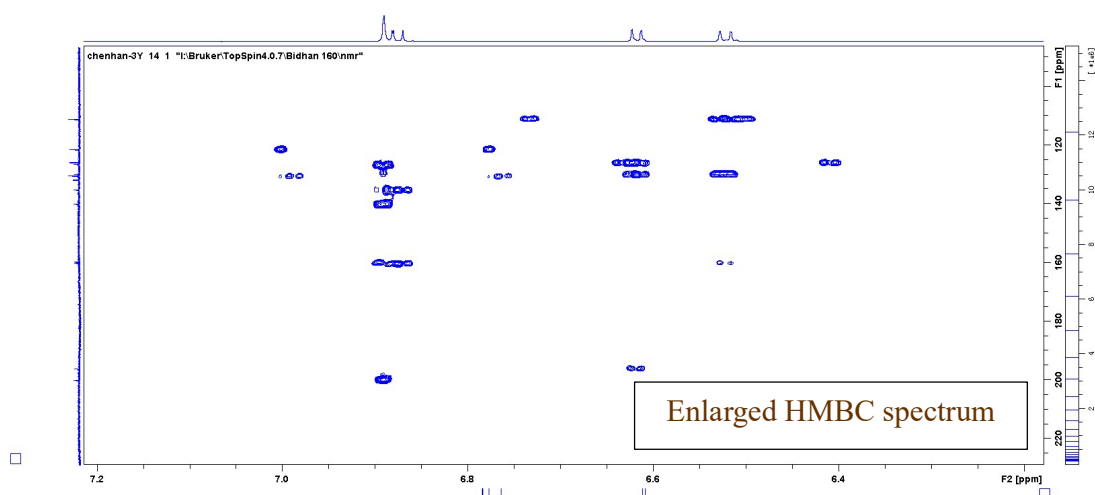
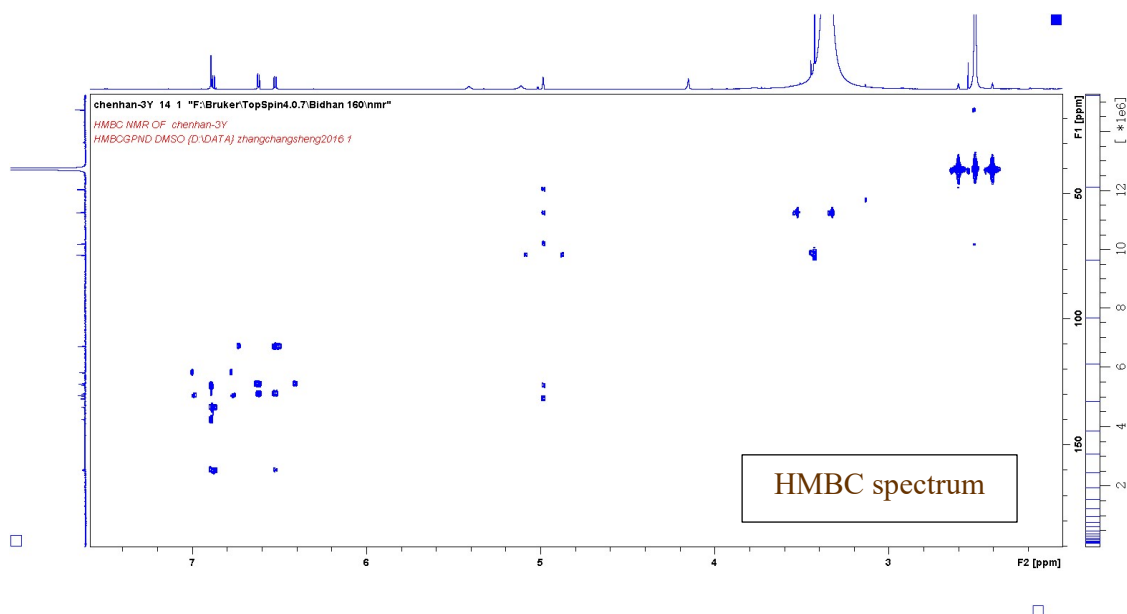
Supplementary Fig.32. Spectroscopic data for **25**. (c) The ¹³C and enlarged ¹³C NMR spectrum of **25** in DMSO-*d*₆.



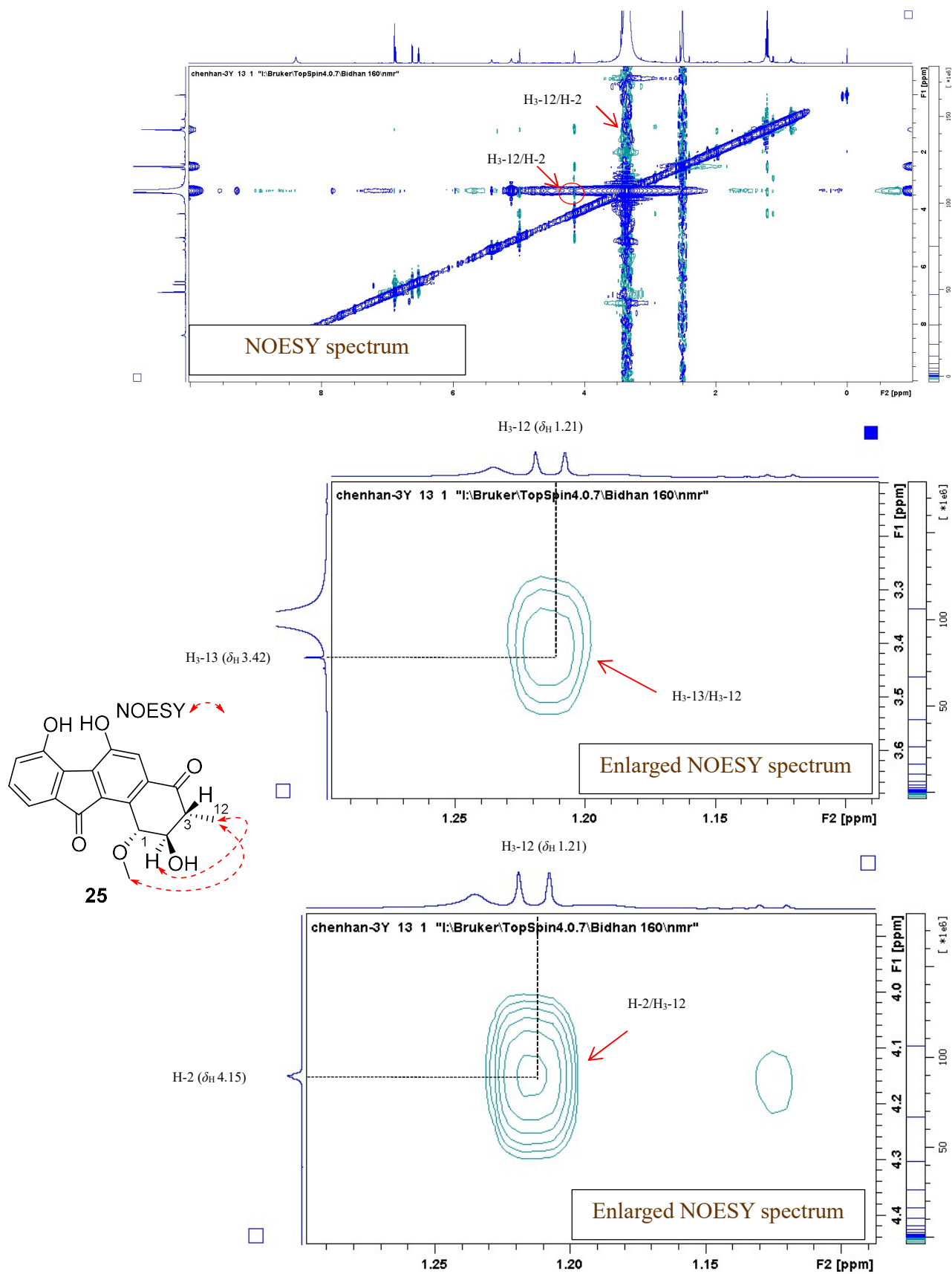
Supplementary Fig.32. Spectroscopic data for 25. (d) The HSQC and enlarged HSQC spectrum of **25** in DMSO-*d*₆.



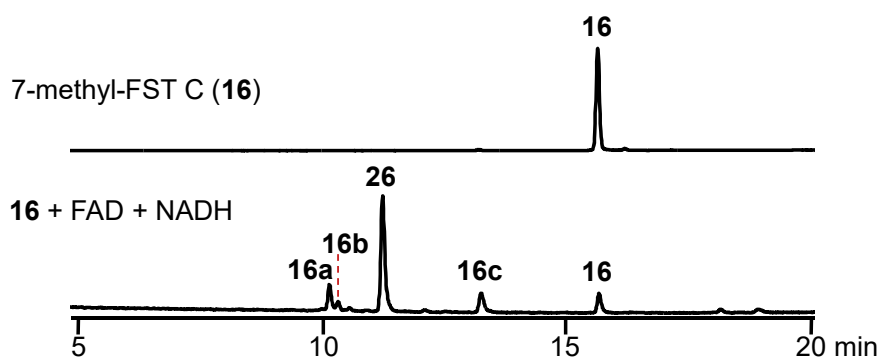
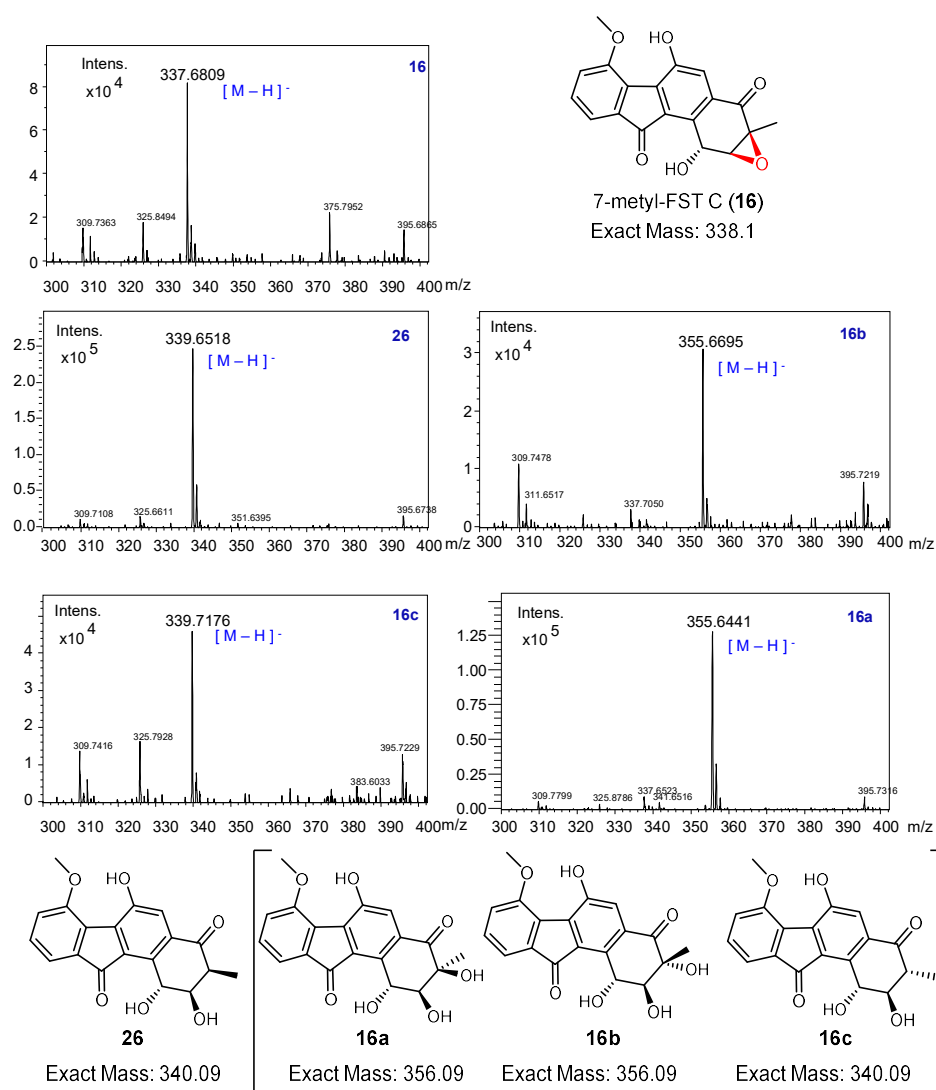
Supplementary Fig.32. Spectroscopic data for 25. (e) The COSY and enlarged COSY spectrum of **25** in DMSO- d_6 .



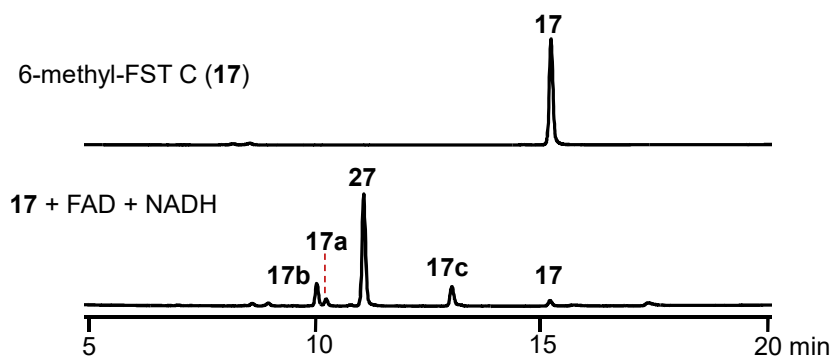
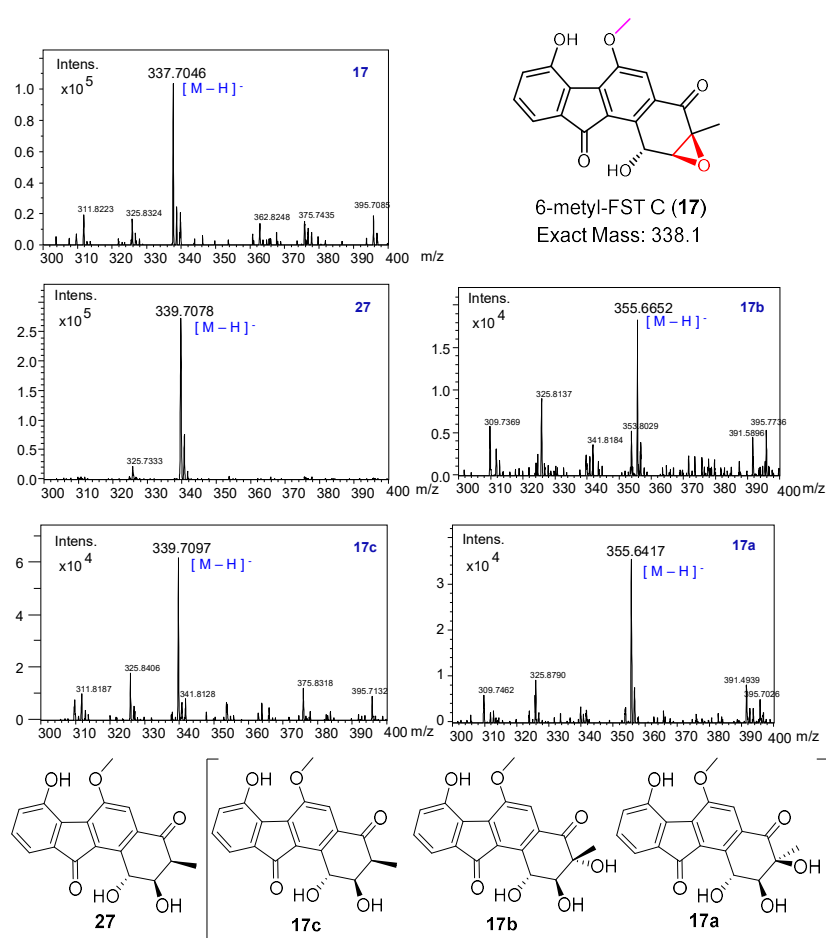
Supplementary Fig.32. Spectroscopic data for 25. (f) The HMBC and enlarged HMBC spectrum of 25 in DMSO- d_6 .



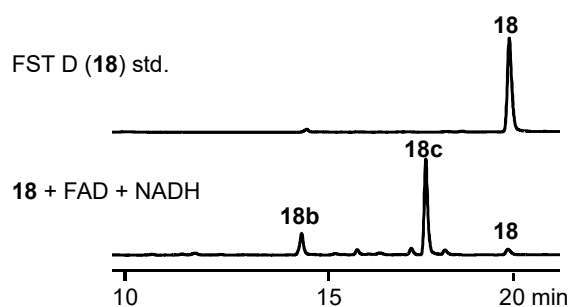
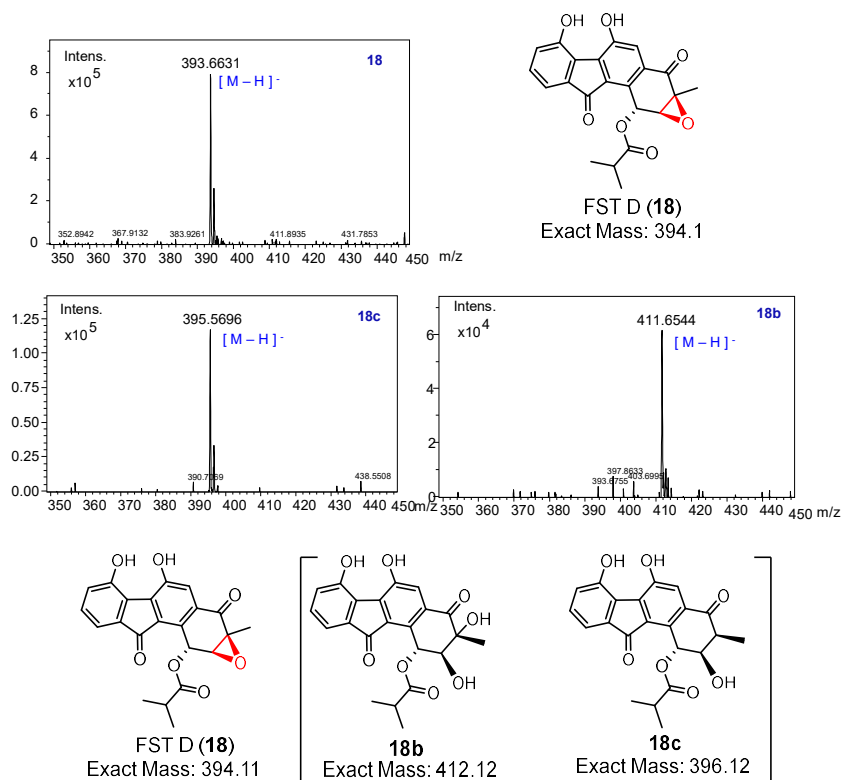
Supplementary Fig.32. Spectroscopic data for 25. (g) The NOESY and enlarged NOESY spectrum of 25 in DMSO-*d*₆.

a**b**

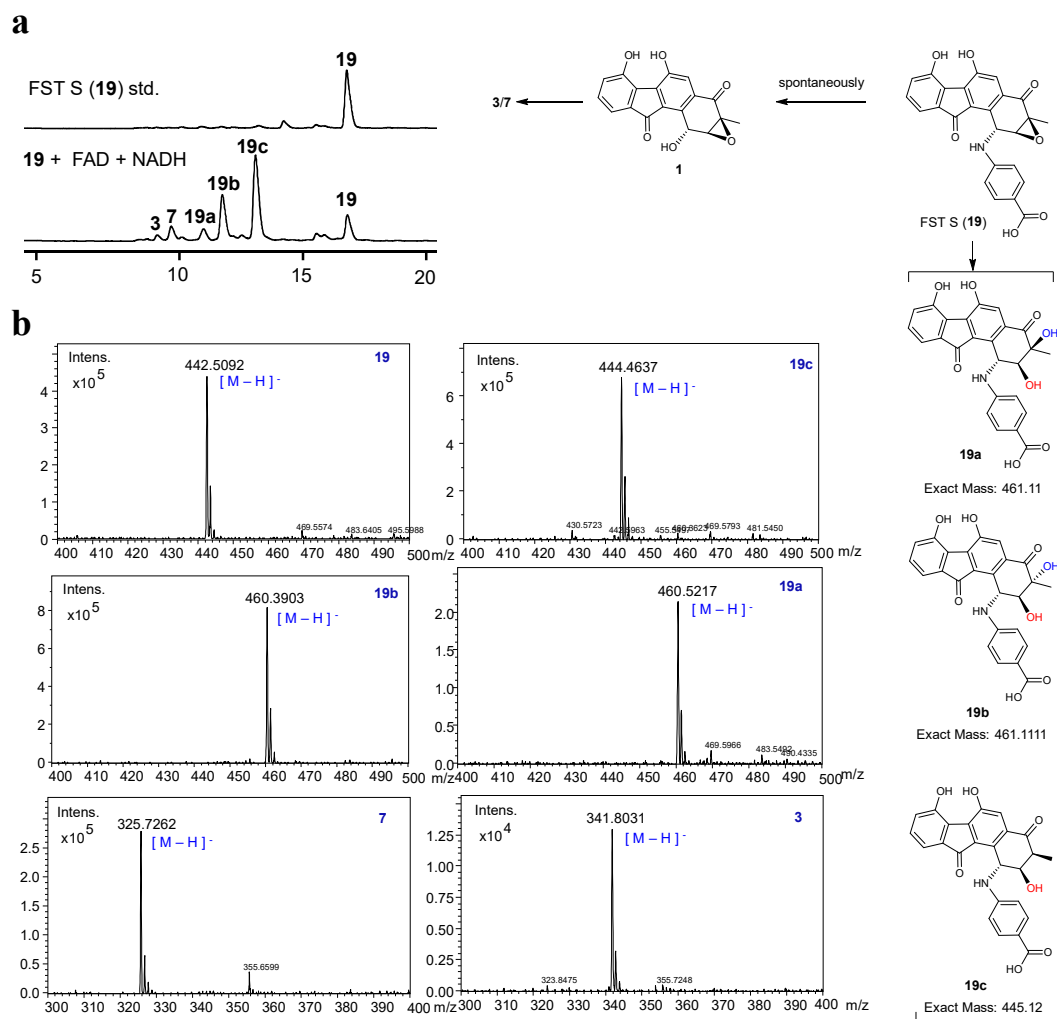
Supplementary Fig. 33. HPLC and LC-MS analysis of 7-methyl-FST C (16) reaction with FAD/NADH. (a) HPLC analysis of 16 standard and a reaction mixture containing 100 μ M 16, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}$ C for 30 min. (b) LC-MS analysis of 7-methyl-FST C (16) reaction products with putative structures.

a**b**

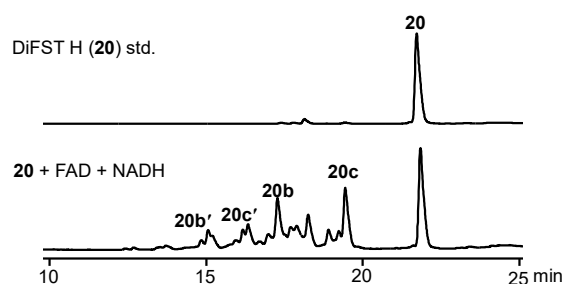
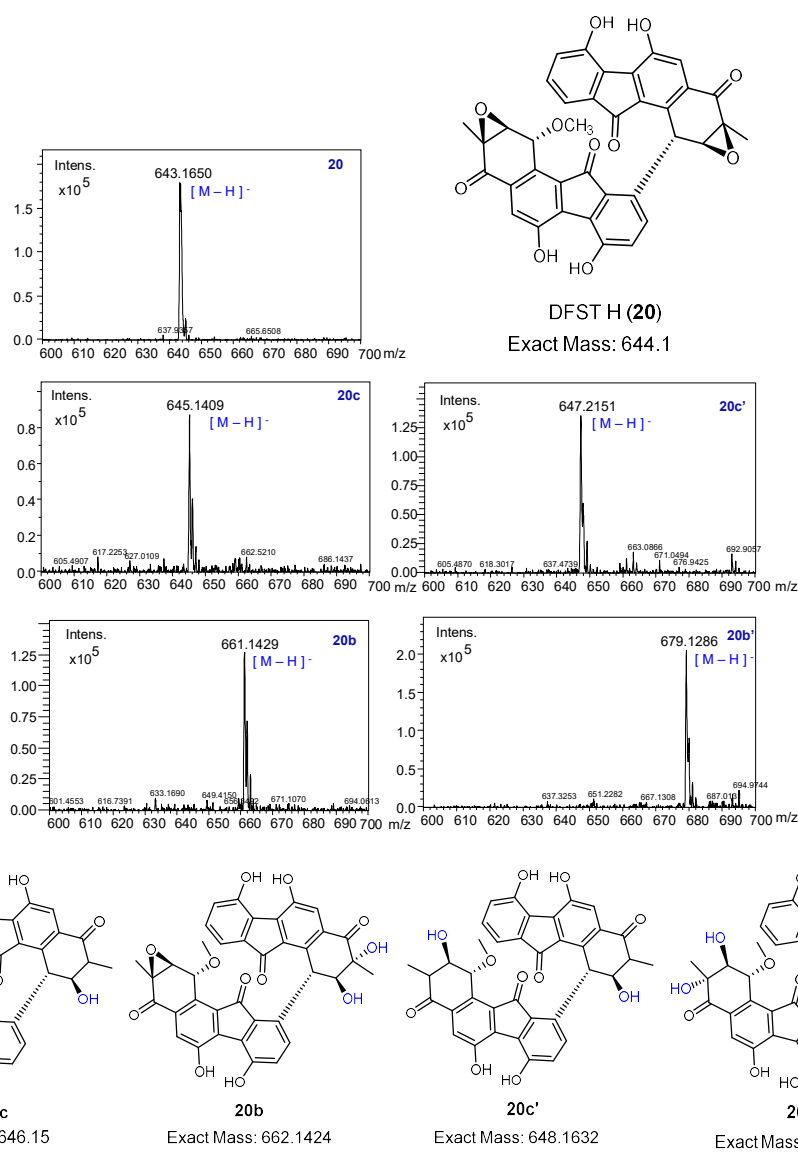
Supplementary Fig. 34. HPLC and LC-MS analysis for 6-methyl-FST C (17) reaction with FAD/NADH. (a) HPLC analysis of 17 standard and a reaction mixture containing 100 μ M 17, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 30 min. (b) LC-MS analysis of 6-methyl-FST C (17) reaction products with putative structures.

a**b**

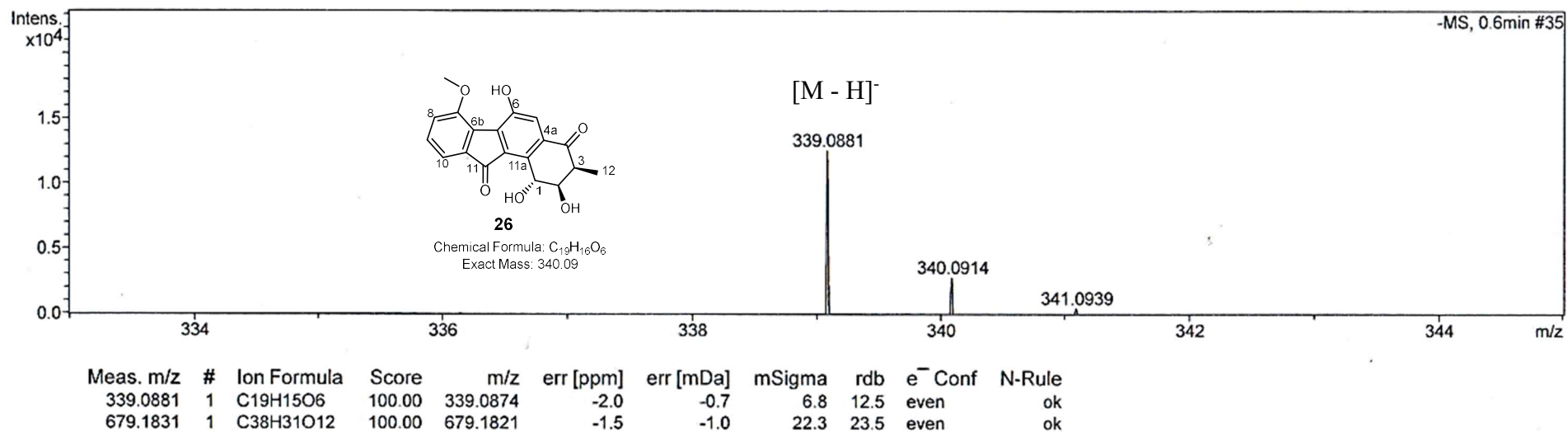
Supplementary Fig. 35. HPLC and LC-MS analysis of FST D (18) reaction with FAD/NADH. (a) HPLC analysis of 17 standard and a reaction mixture containing 100 μ M 18, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 30 min. (b) LC-MS analysis of FST D (18) reaction products with putative structures.



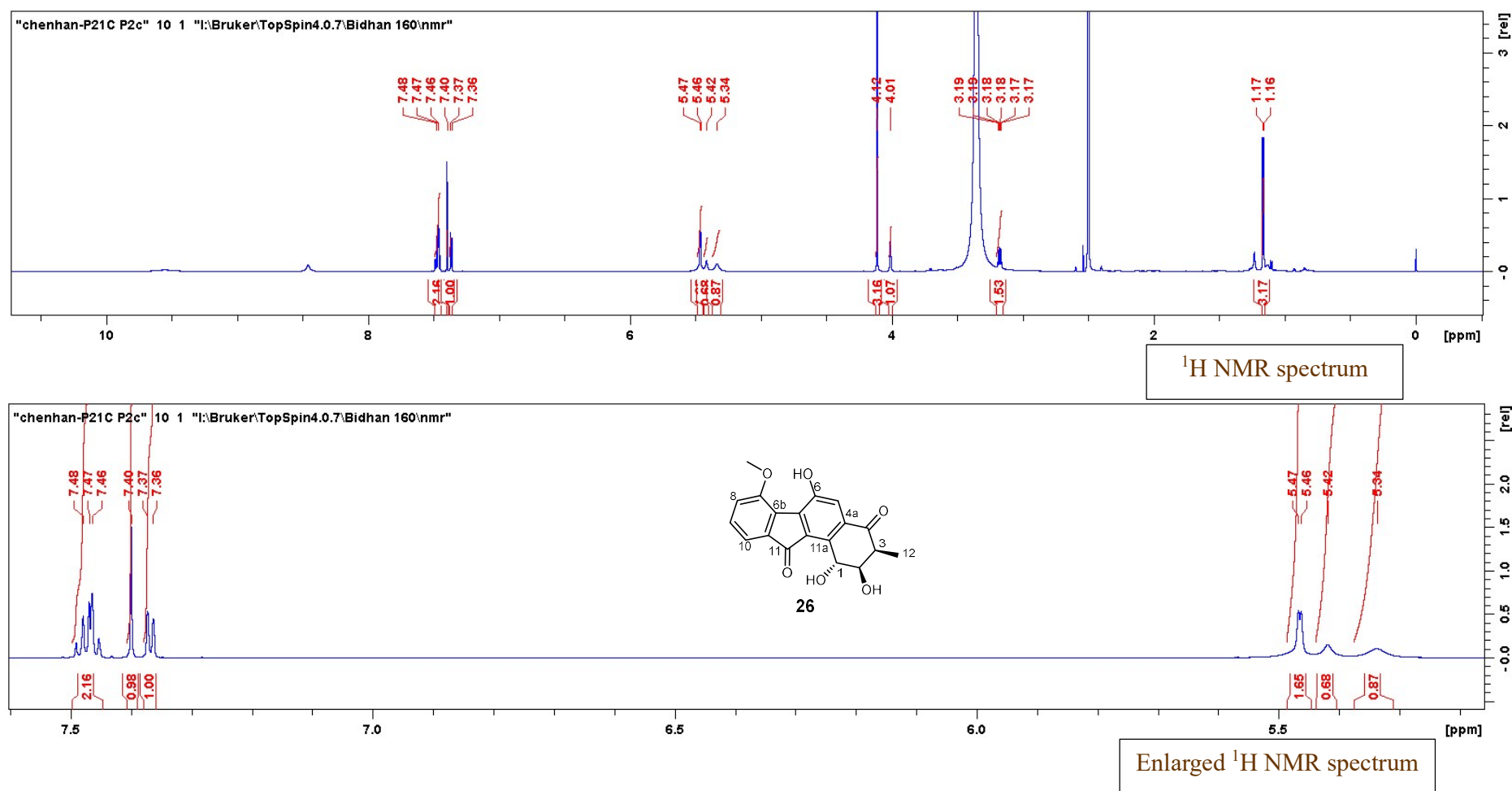
Supplementary Fig. 36. HPLC and LC-MS analysis of FST S (19) reaction with FAD/NADH. (a) HPLC analysis of **19** standard and a reaction mixture containing 100 μ M **19**, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 30 min. (b) LC-MS analysis of FST S (**19**) reaction products with putative structures. The spontaneous conversion of **19** to **1** has been previously reported.

a**b**

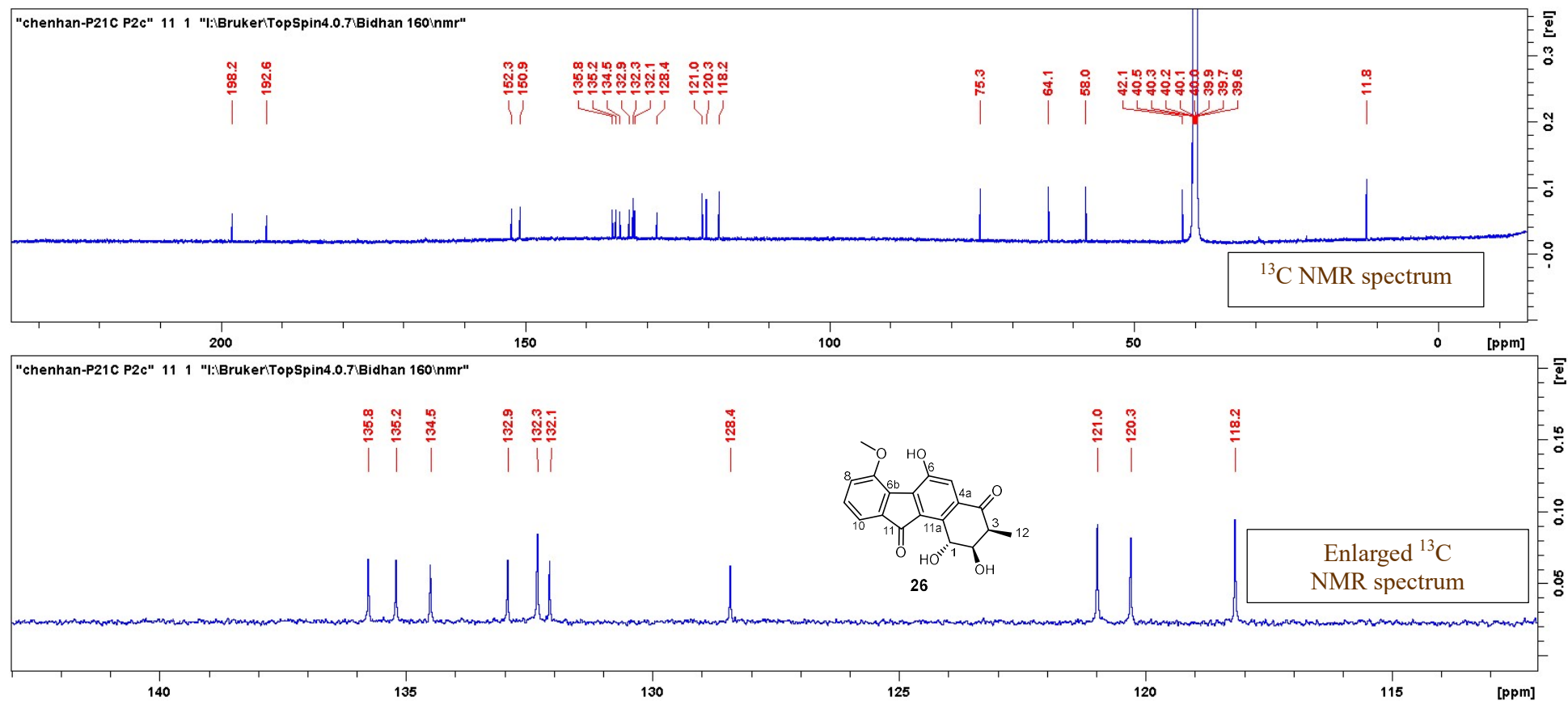
Supplementary Fig. 37. HPLC and LC-MS analysis of DiFST H (20**) reaction with FAD/NADH.** (a) HPLC analysis of **20** standard and a reaction mixture containing 100 μ M **20**, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 30 min. (b) LC-MS analysis of DiFST H (**20**) reaction products with putative structures.



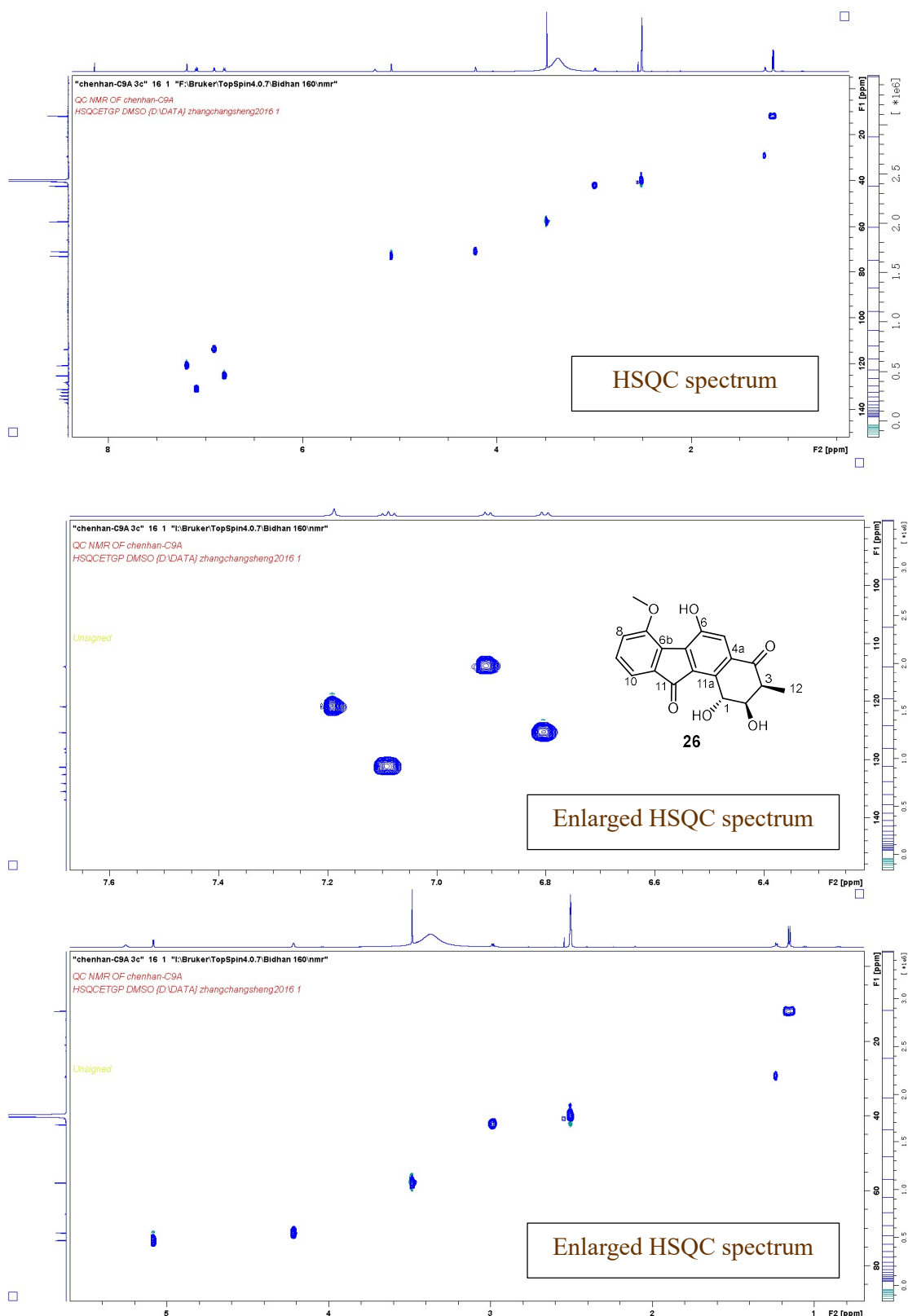
Supplementary Fig. 38. Spectroscopic data for 26. (a) HRESIMS spectrum.



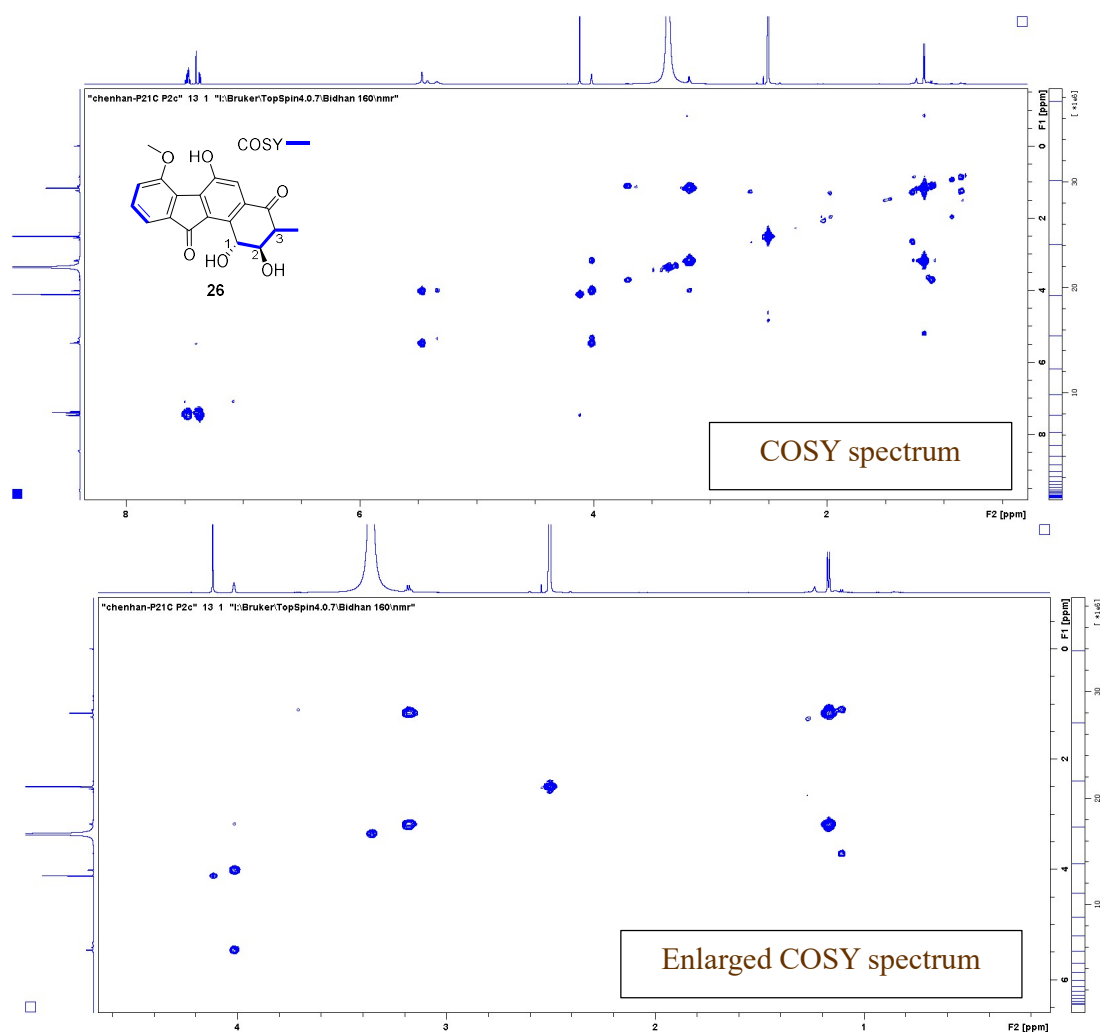
Supplementary Fig. 38. Spectroscopic data for **26**. (b) The ^1H and enlarged ^1H NMR spectrum of **26** in $\text{DMSO}-d_6$.



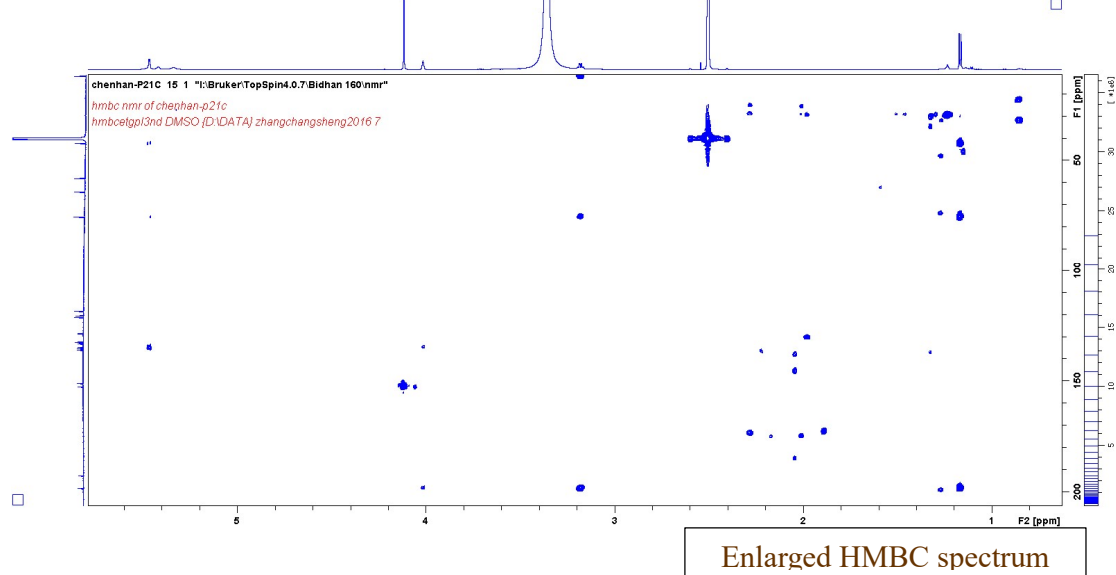
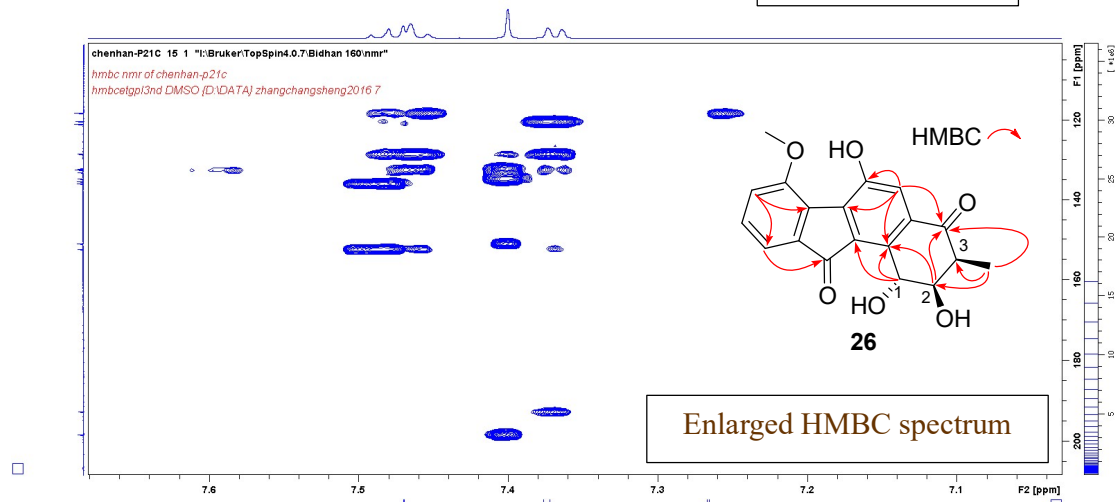
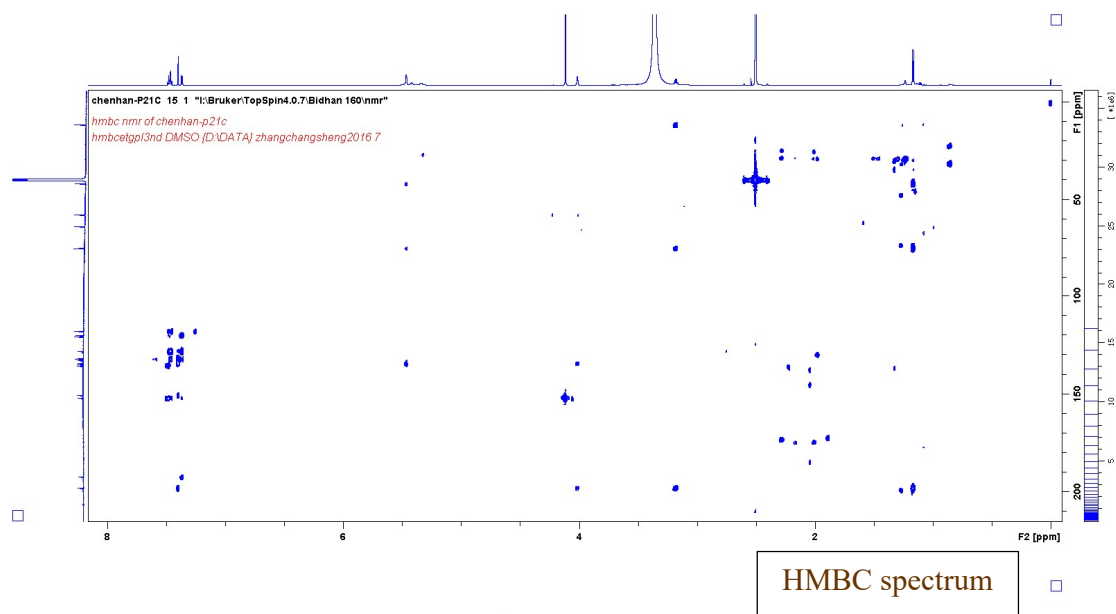
Supplementary Fig. 38. Spectroscopic data for 26. (c) The ^{13}C and enlarged ^{13}C NMR spectrum of 26 in $\text{DMSO-}d_6$.



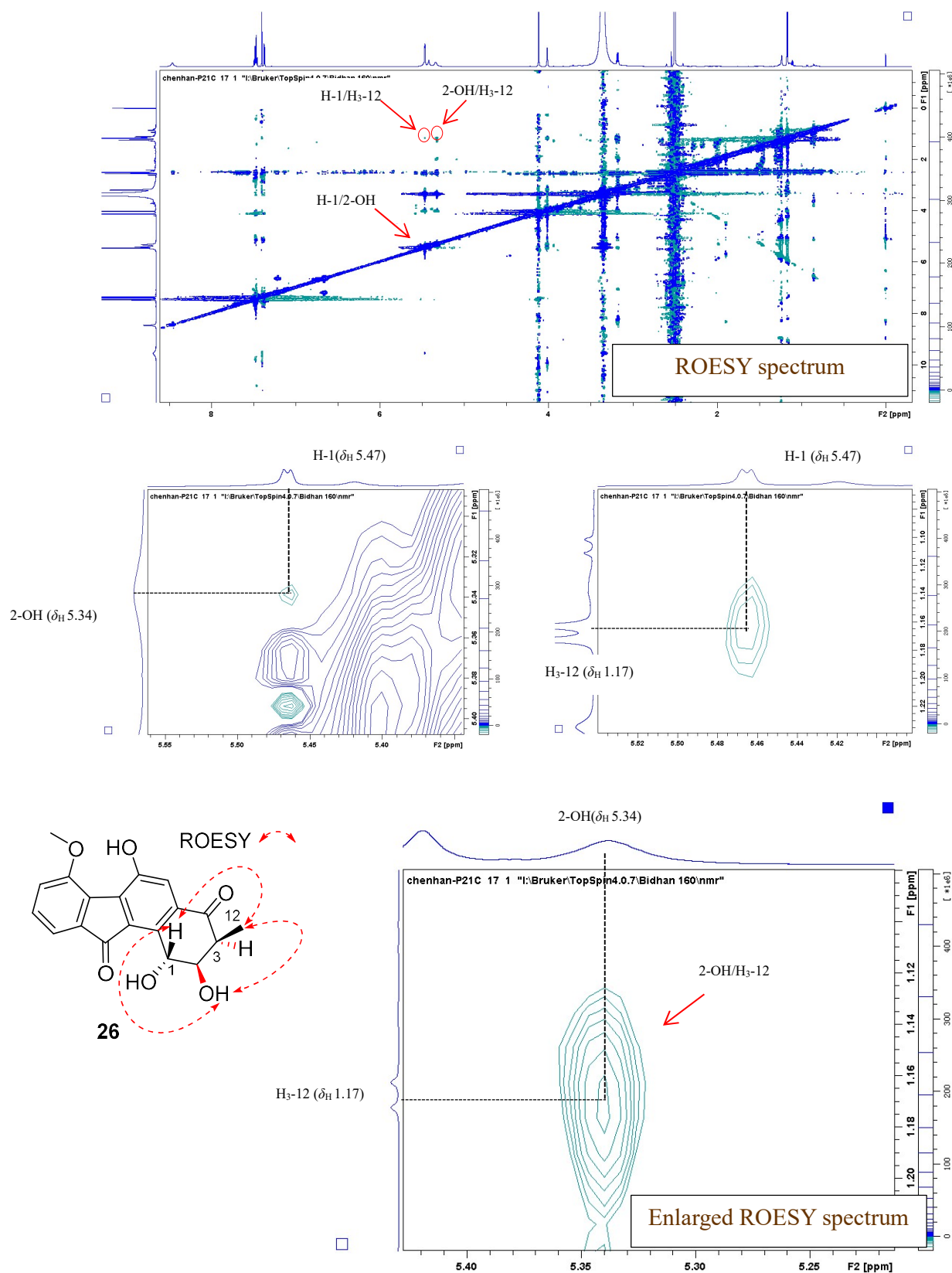
Supplementary Fig. 38. Spectroscopic data for 26. (d) The HSQC and enlarged HSQC spectrum of **26** in DMSO-*d*₆.



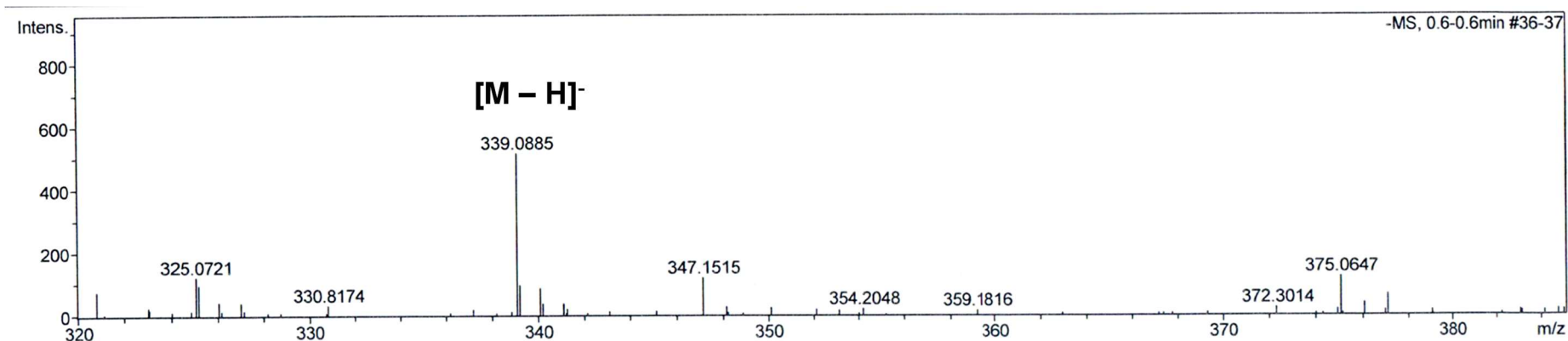
Supplementary Fig. 38. Spectroscopic data for **26** (e) The COSY and enlarged COSY spectrum of **26** in DMSO-*d*₆.



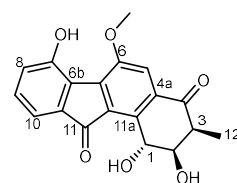
Supplementary Fig. 38. Spectroscopic data for 26. (f) The HMBC and enlarged HMBC spectrum of 26 in DMSO- d_6 .



Supplementary Fig. 38. Spectroscopic data for 26. (g) The ROESY and enlarged ROESY spectrum of 26 in DMSO- d_6 .



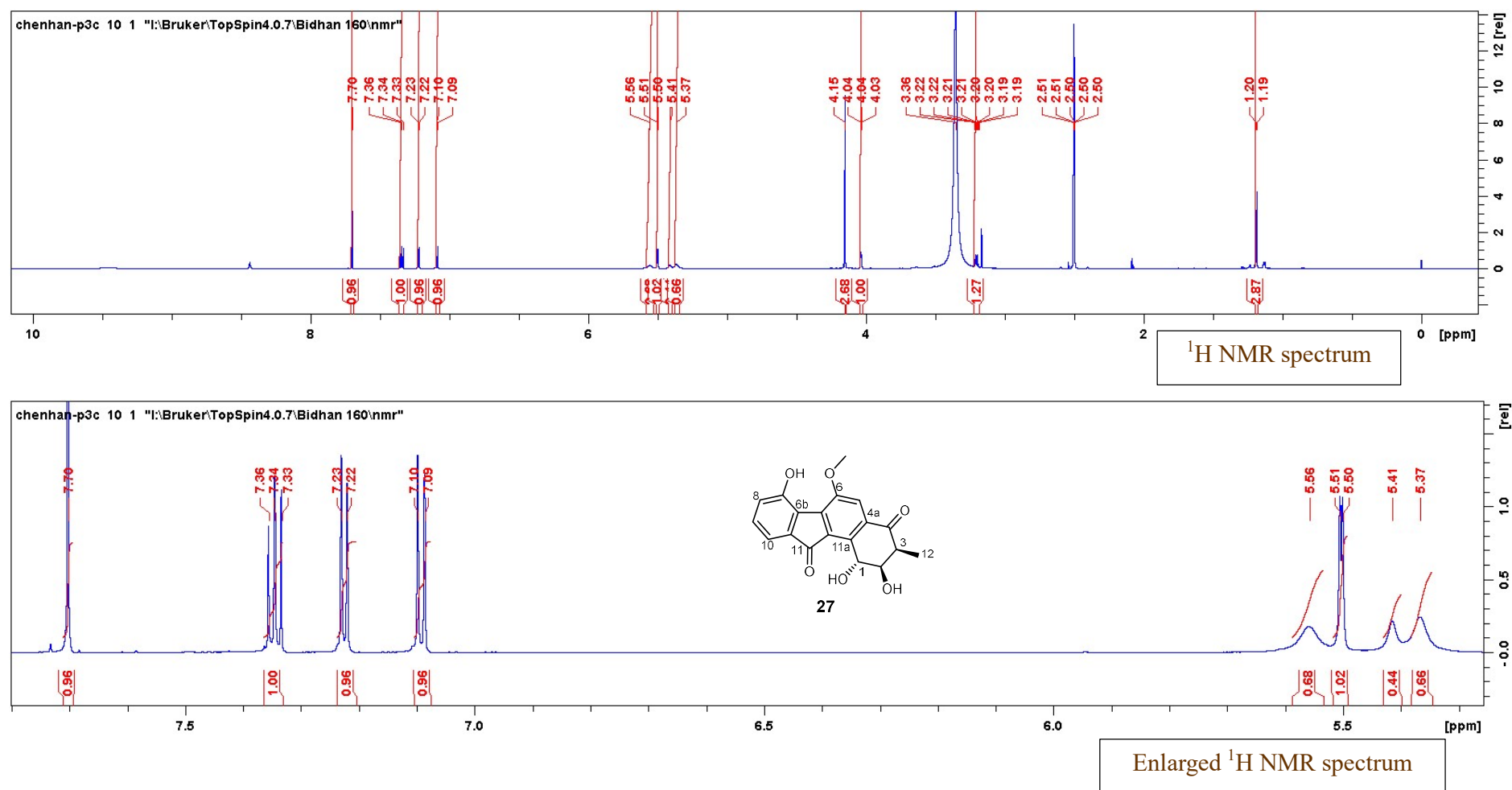
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e ⁻	Conf	N-Rule
339.0885	1	C ₁₉ H ₁₅ O ₆	95.91	339.0874	-3.1	-1.1	25.4	12.5	even		ok
	2	C ₂₀ H ₁₁ N ₄ O ₂	100.00	339.0887	-0.8	-0.3	39.6	17.5	even		ok



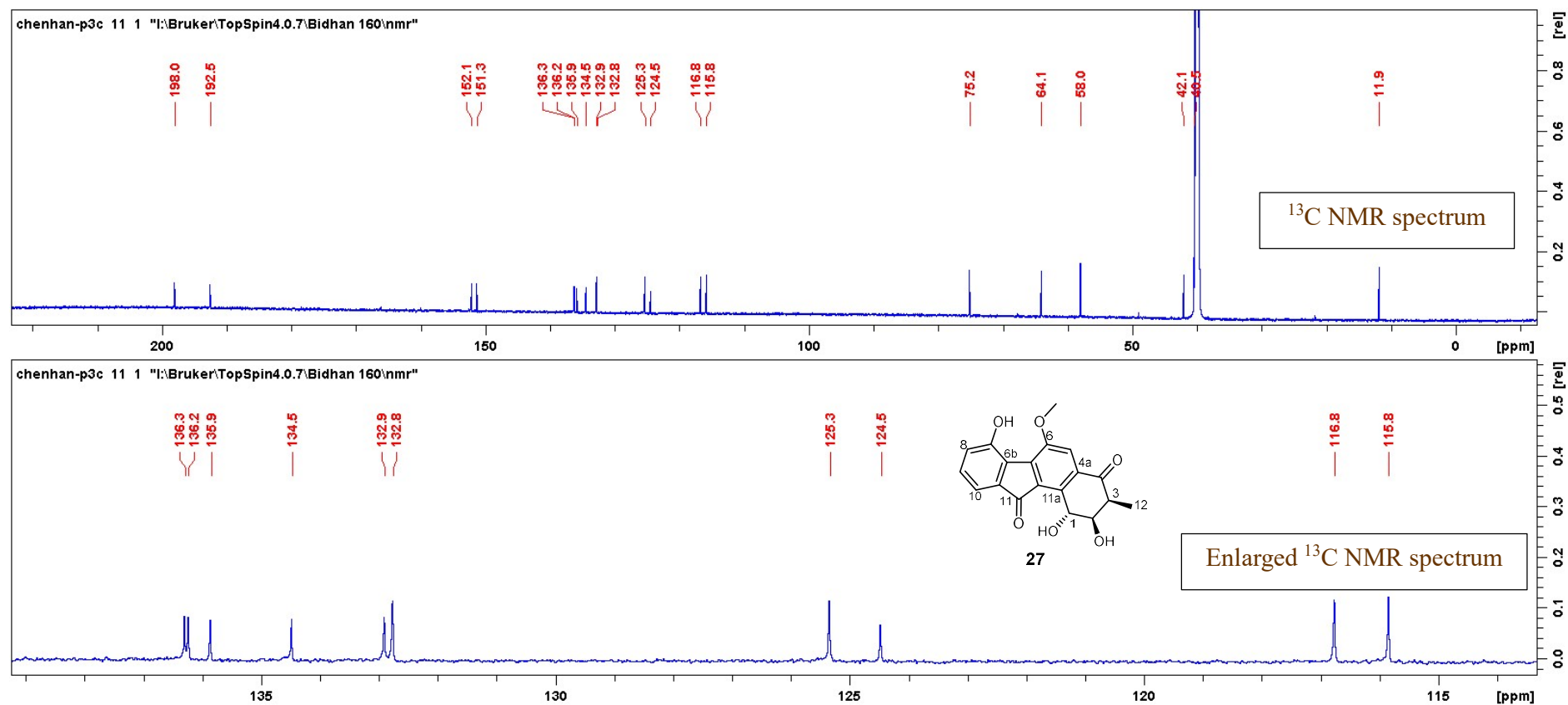
27

Chemical Formula: C₁₉H₁₆O₆
Exact Mass: 340.09

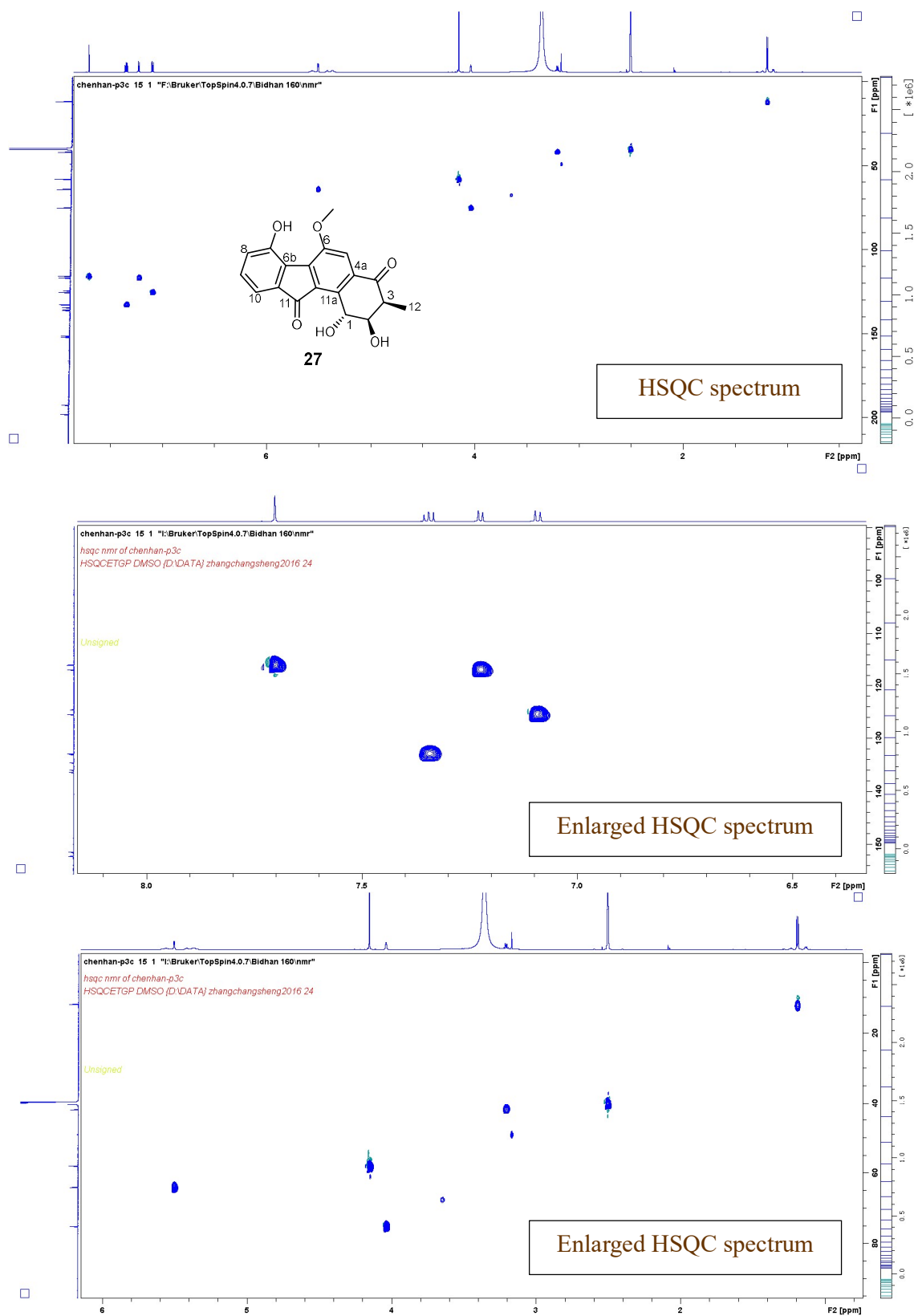
Supplementary Fig. 39. Spectroscopic data for 27. (a) HRESIMS spectrum.



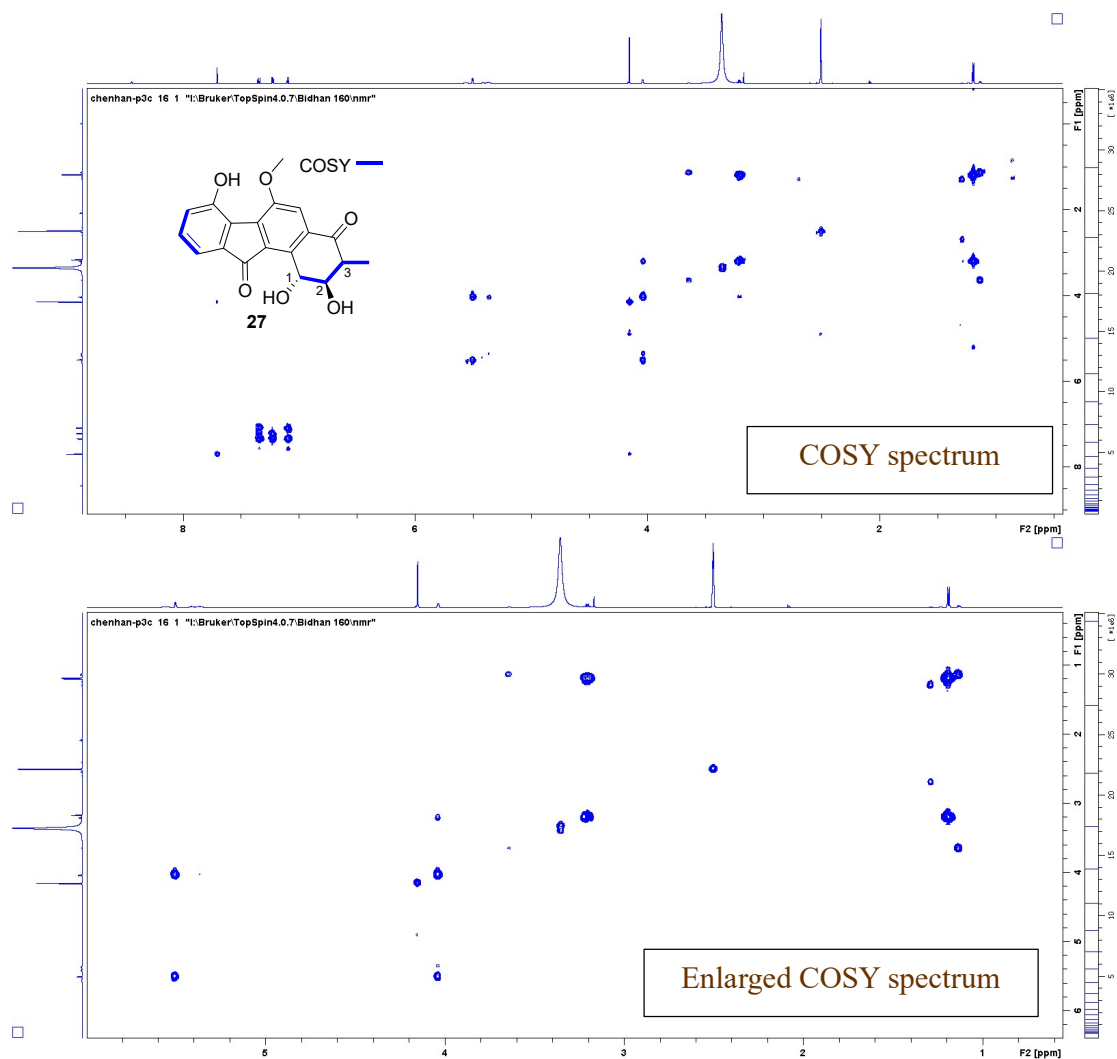
Supplementary Fig. 39. Spectroscopic data for 27. (b) The ^1H and enlarged ^1H NMR spectrum of 27 in $\text{DMSO}-d_6$.



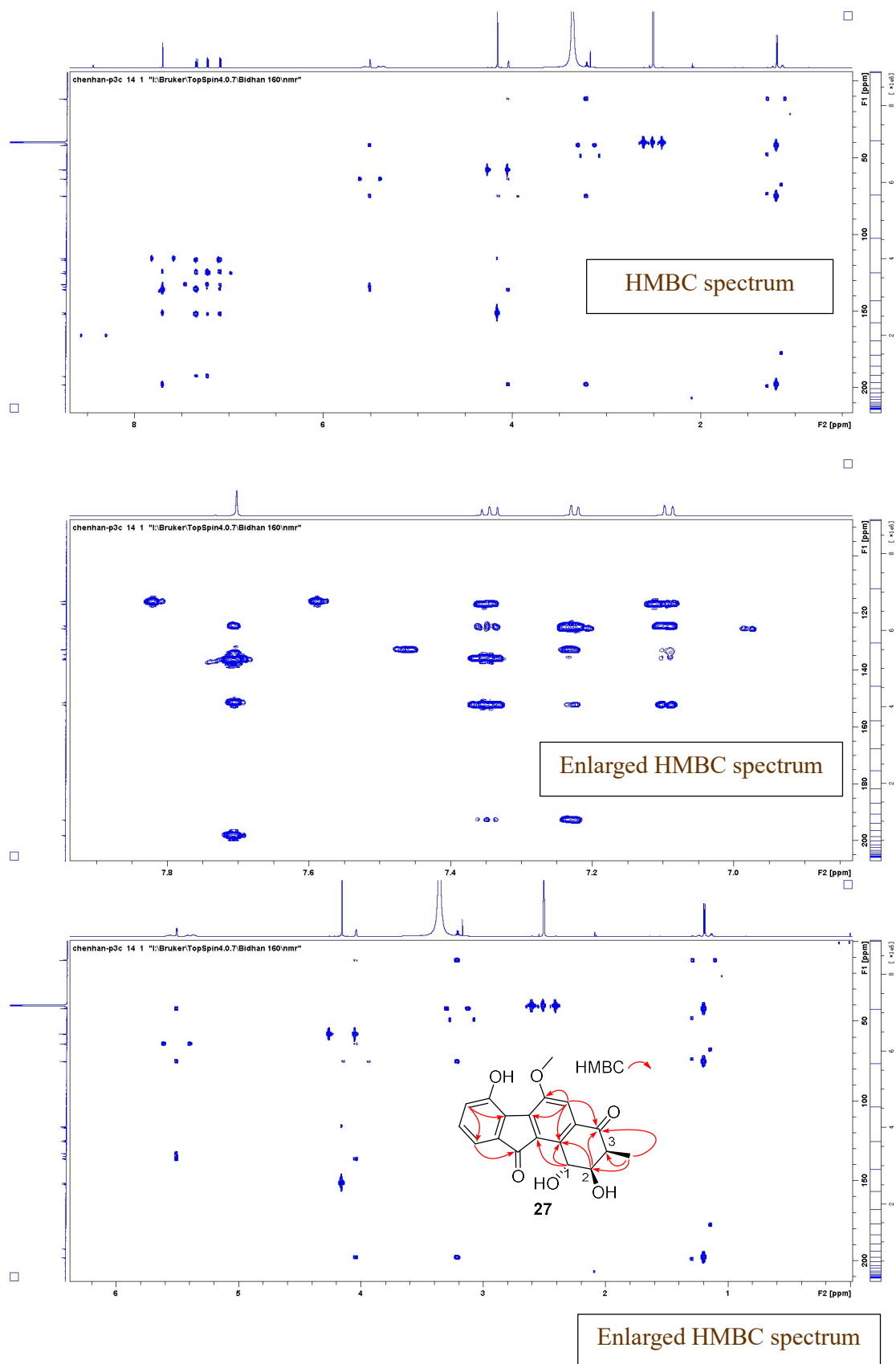
Supplementary Fig. 39. Spectroscopic data for **27**. (c) The ^{13}C and enlarged ^{13}C NMR spectrum of **27** in $\text{DMSO}-d_6$.



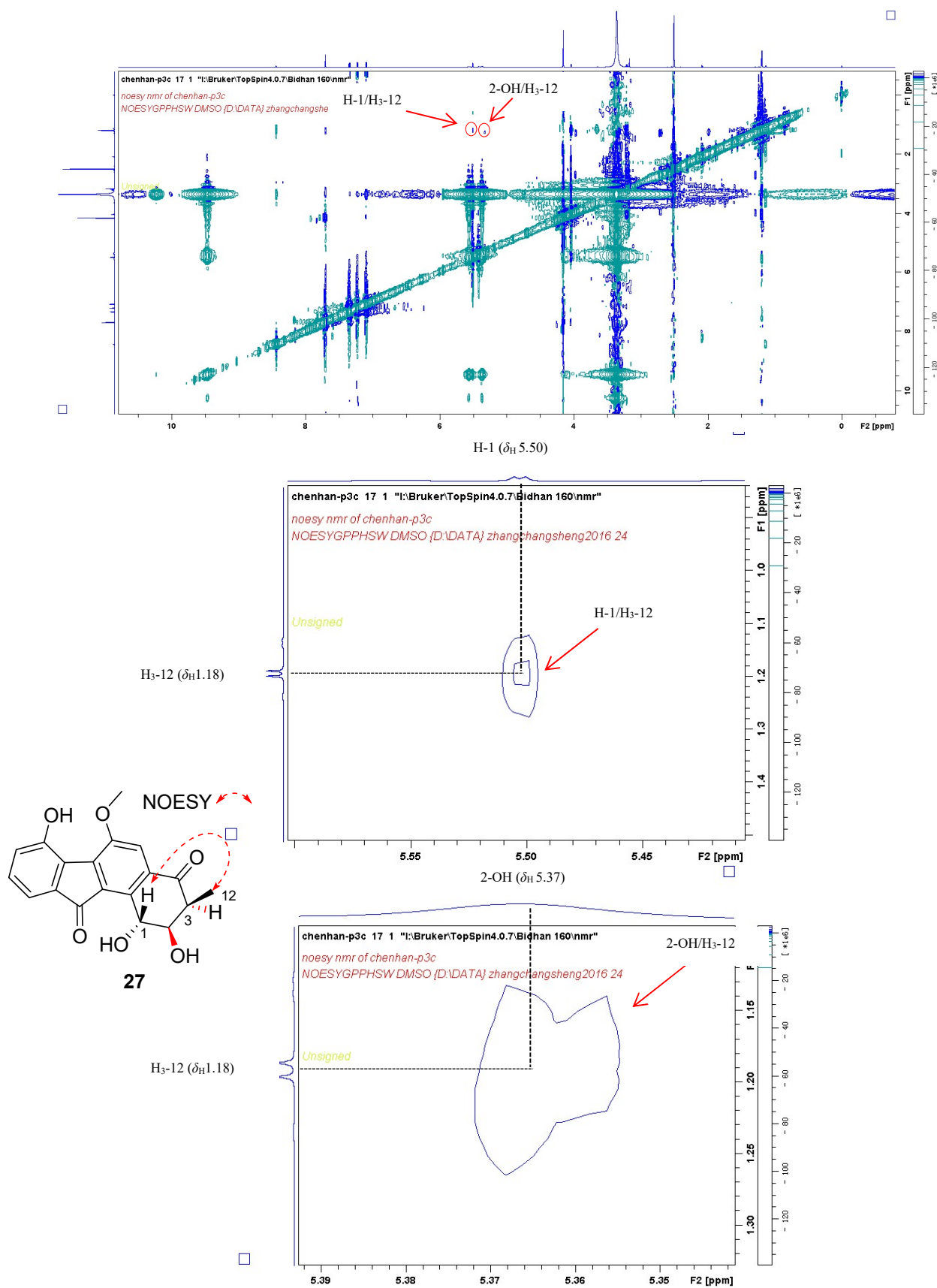
Supplementary Fig. 39. Spectroscopic data for 27. (d) The HSQC and enlarged HSQC spectrum of 27 in DMSO-*d*₆.



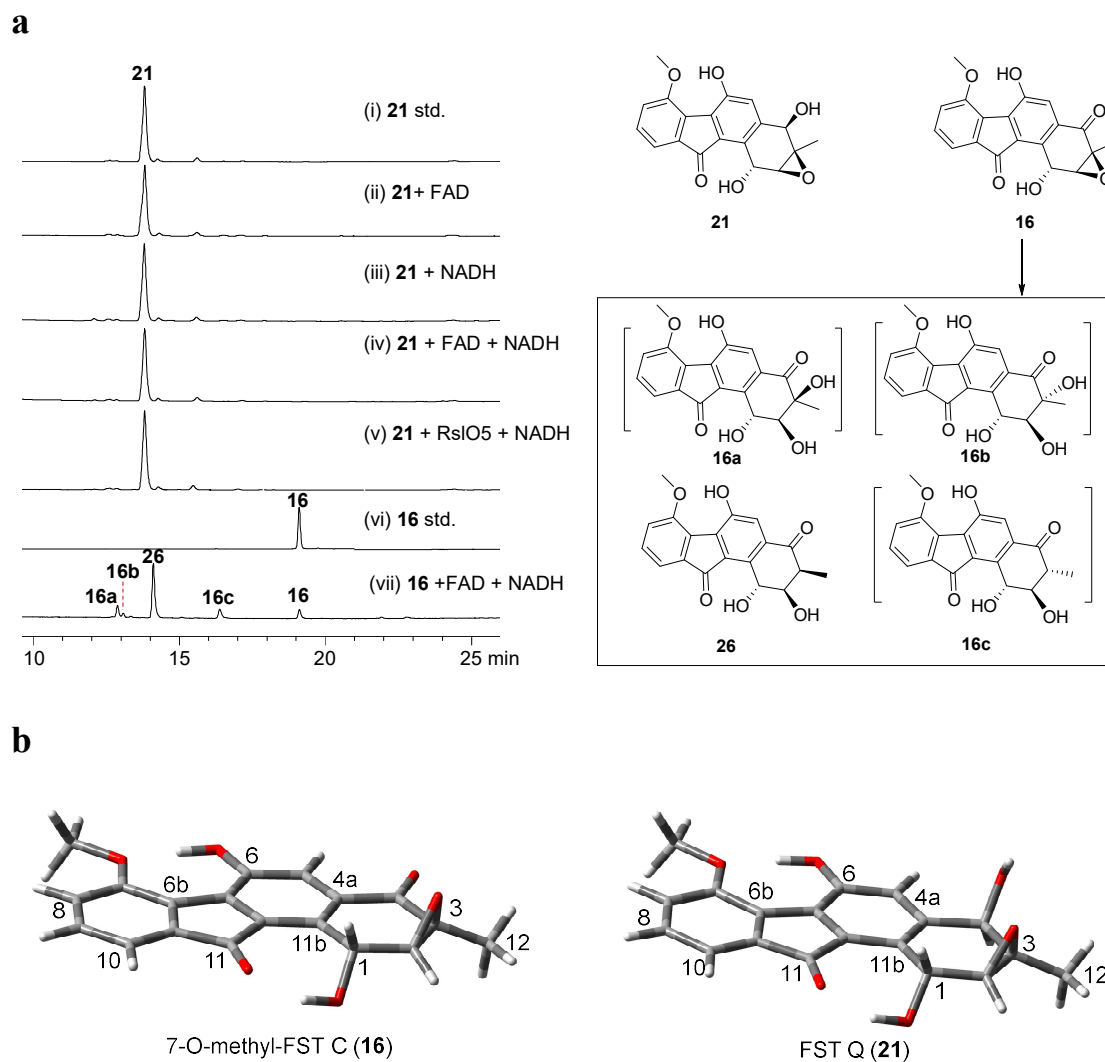
Supplementary Fig. 39. Spectroscopic data for 27. (e) The COSY and enlarged COSY spectrum of 27 in DMSO- d_6 .



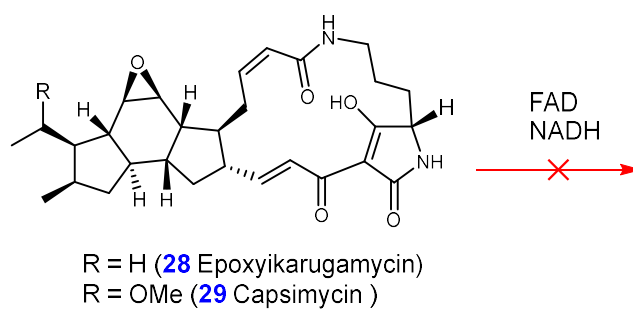
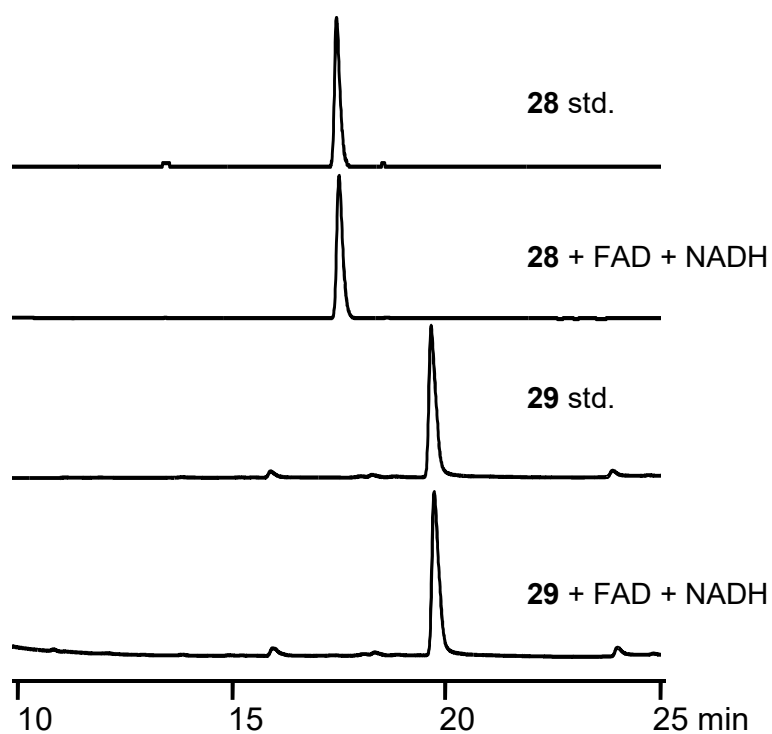
Supplementary Fig. 39. Spectroscopic data for 27. (f) The HMBC and enlarged HMBC spectrum of 27 in DMSO- d_6 .



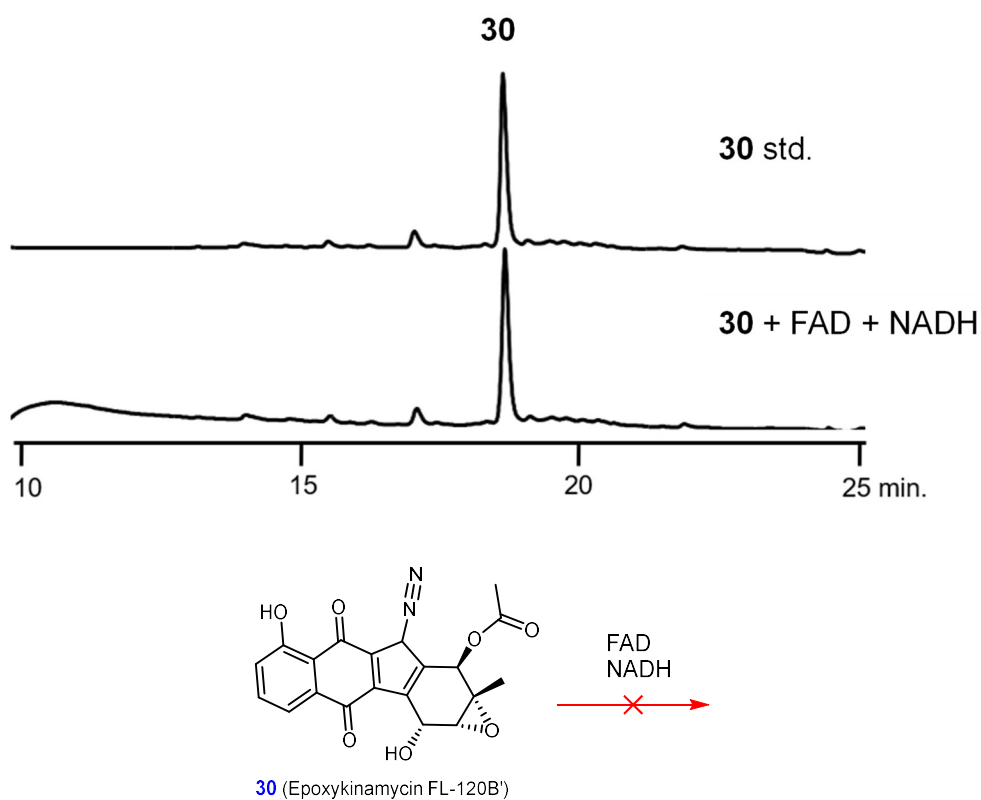
Supplementary Fig. 39. Spectroscopic data for 27. (g) The NOESY and enlarged NOESY spectrum of 27 in DMSO- d_6 .



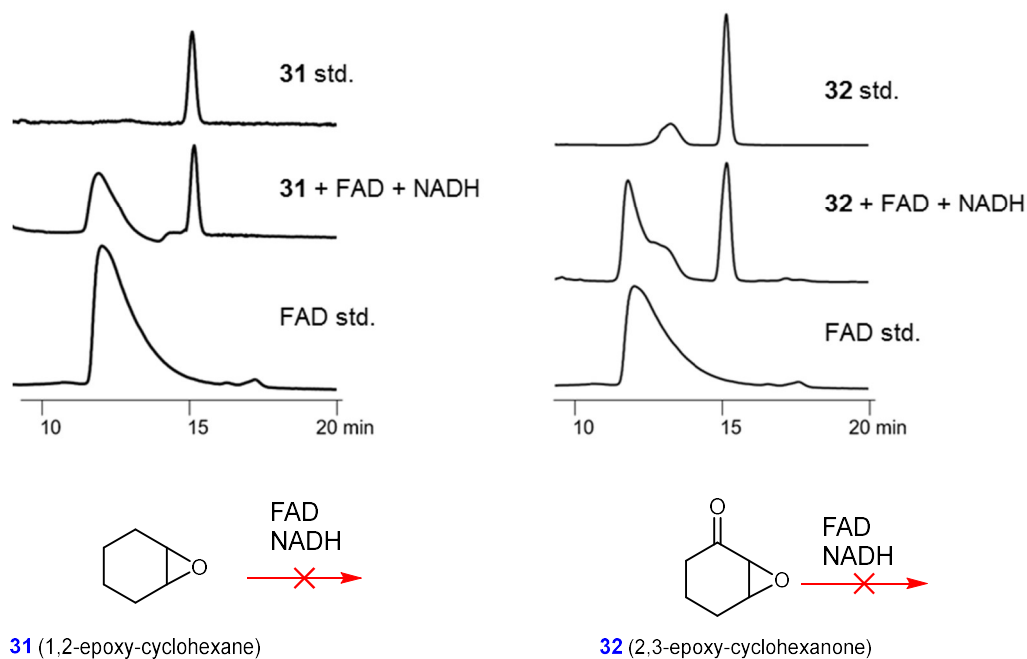
Supplementary Fig. 40. HPLC profile for FST Q (21**) reaction with FAD/NADH . (a)** HPLC analysis of reaction mixtures containing (i) **21** std.; (ii) 100 μ M **21** + 100 μ M FAD; (iii) 100 μ M **21** + 10 mM NADH; (iv) 100 μ M **21** + 100 μ M FAD + 10 mM NADH; (v) 100 μ M **21** + 10 μ M RslO5 + 5mM NADH, (vi) **16** std.; (vii) 100 μ M **16** + 100 μ M FAD + 10 mM NADH. The assays were performed in 50 mM PBS buffer (pH 7) at 30 $^{\circ}$ C for 2 h. HPLC analysis was performed on the Agilent 1260 Infinity series instrument (Agilent Technologies Inc., USA) using a reversed phase C18 column. **(b)** The 3D conformers of **16** and **21**.



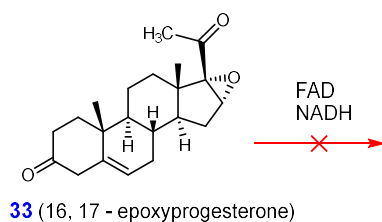
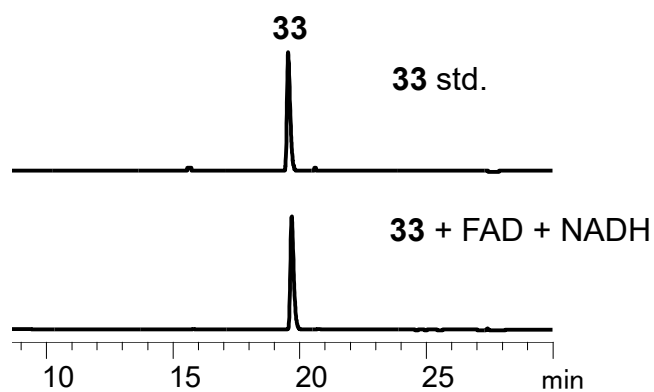
Supplementary Fig. 41. HPLC analysis of FAD/NADH-mediated reactions with epoxyikarugamycin (28) and capsimycin (29). HPLC analysis of reaction mixtures containing 100 μ M **28** (or **29**), 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}$ C for 2 h.



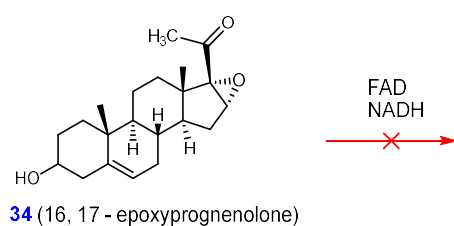
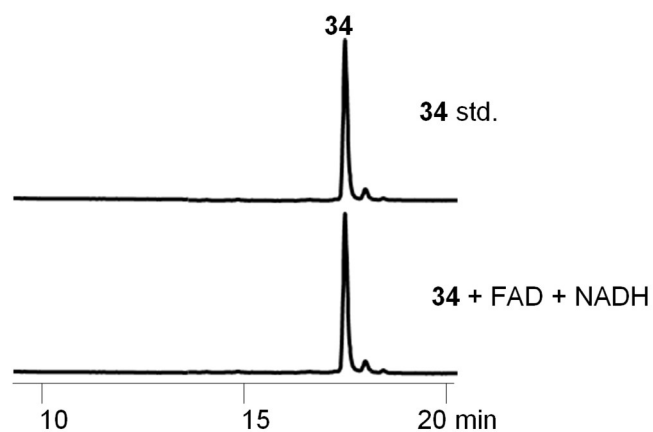
Supplementary Fig. 42. HPLC analysis of FAD/NADH-mediated reaction with epoxykinamycin FL-120B' (30). HPLC analysis of reaction mixture containing 100 μ M 30, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}$ C for 2 h.



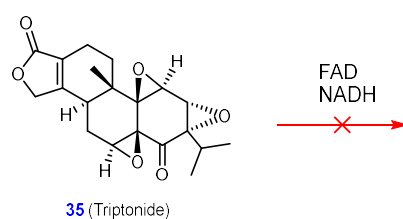
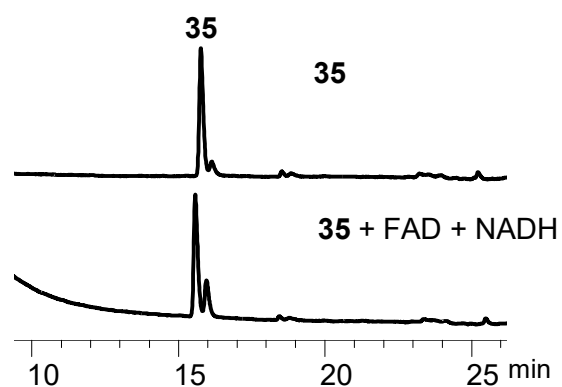
Supplementary Fig. 43. HPLC analysis of FAD/NADH-mediated reaction with 1,2-epoxy-cyclohexane (31) and 2,3-epoxy-cyclohexanone (32). HPLC analysis of reaction mixtures containing 100 μ M 31 (or 32), 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}$ C for 2 h.



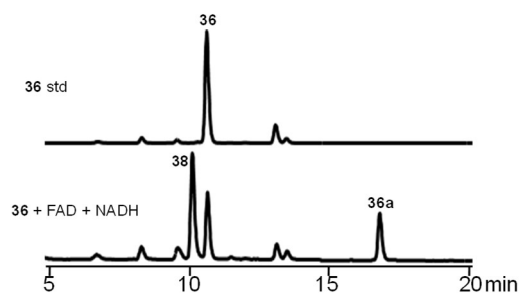
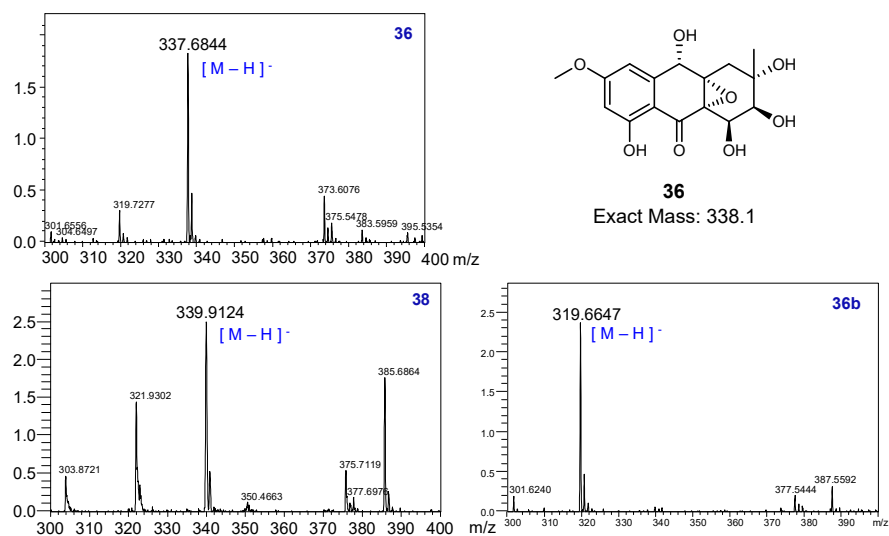
Supplementary Fig. 44. HPLC analysis of FAD/NADH-mediated reaction with 16,17-epoxyprogesterone (33). HPLC analysis of reaction mixture containing 100 μM 33, 100 μM FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}\text{C}$ for 2 h.



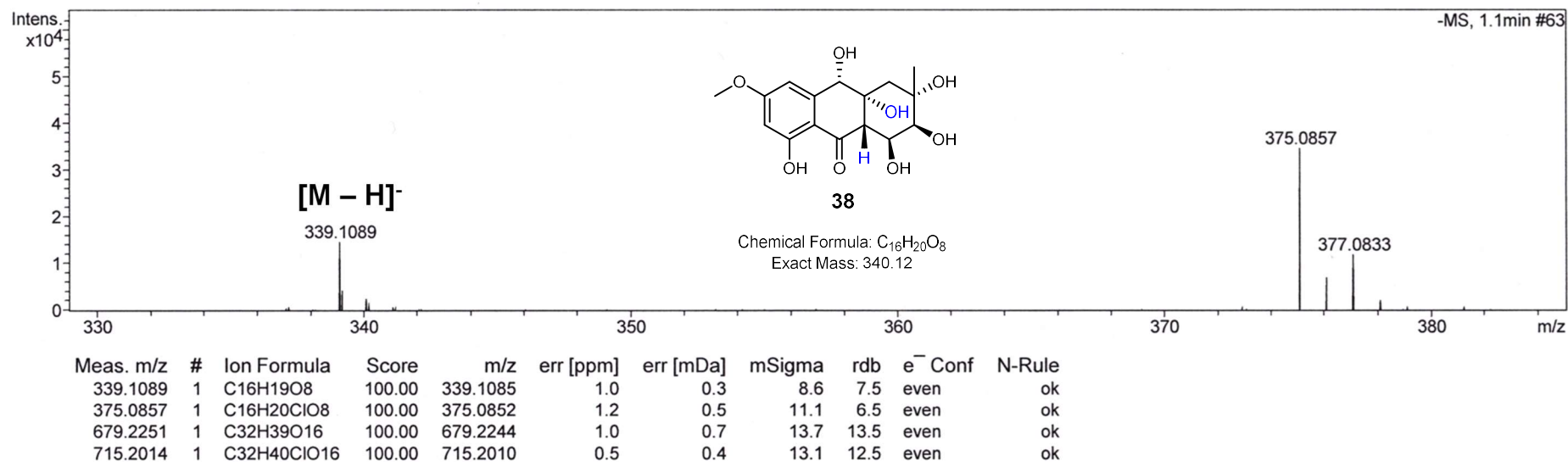
Supplementary Fig. 45. HPLC analysis of FAD/NADH-mediated reaction with 16,17-epoxypregnenolone (34). HPLC analysis of reaction mixture containing 100 μM **34**, 100 μM FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}\text{C}$ for 2 h.



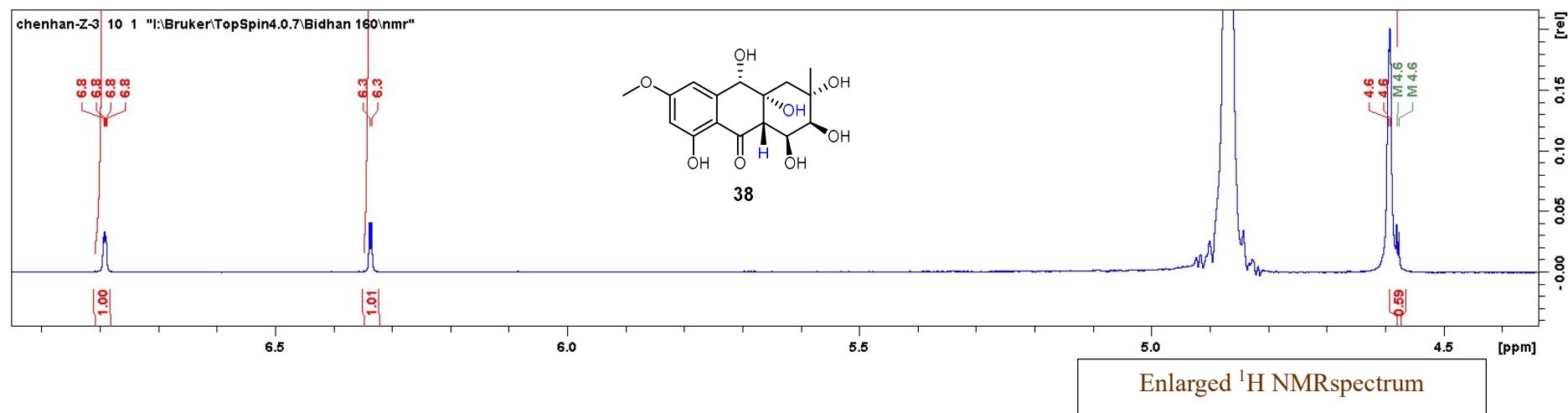
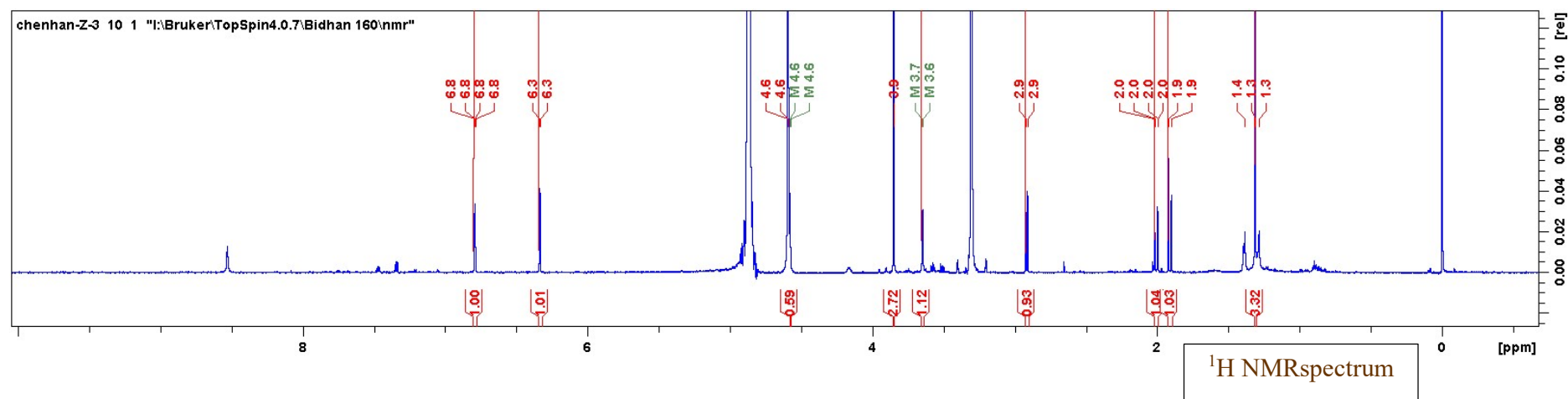
Supplementary Fig. 46. HPLC analysis of FAD/NADH-mediated reaction with triptonide (35). HPLC analysis of the reaction mixture containing 100 μM **35**, 100 μM FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}\text{C}$ for 2 h.

a**b**

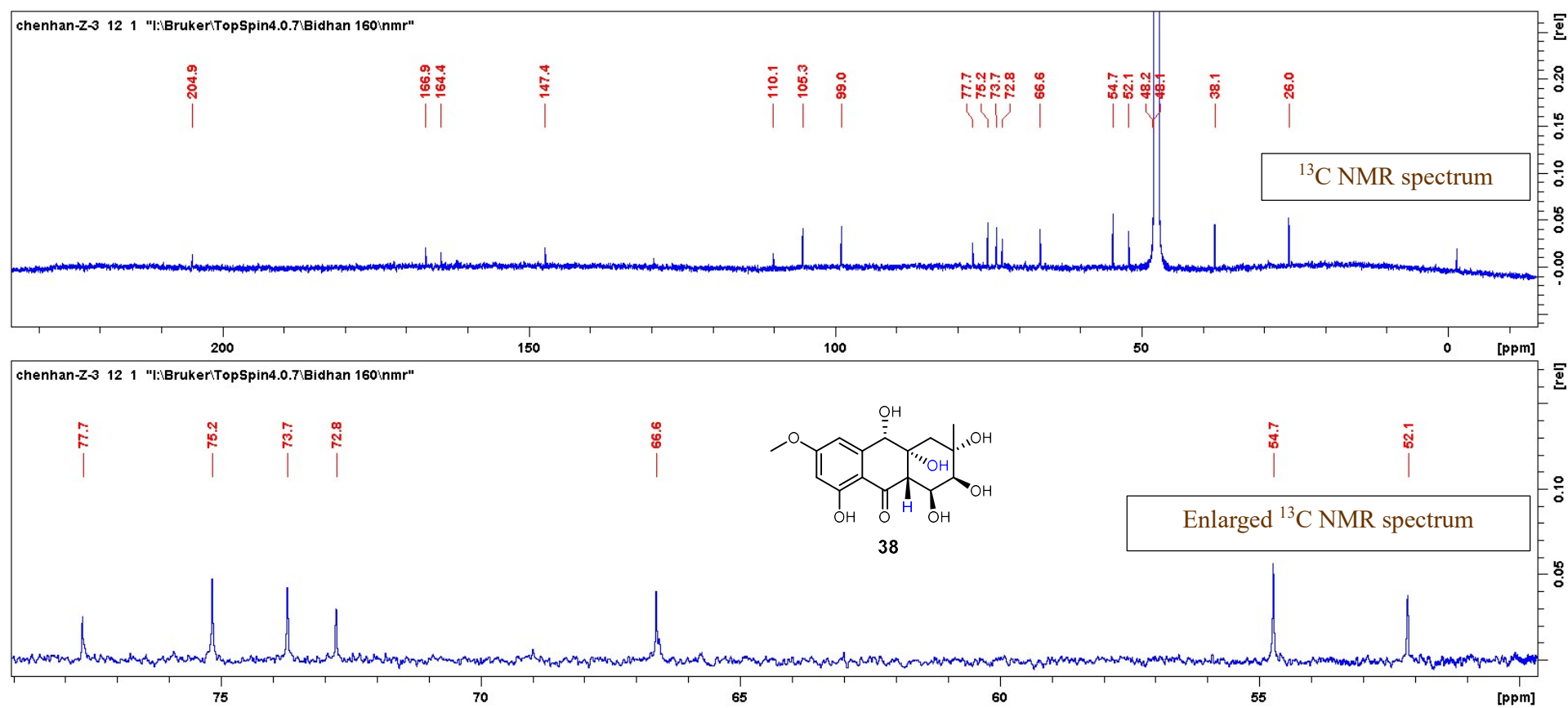
Supplementary Fig. 47. HPLC and LC-MS analysis of the reaction of auxarthrol H (36) with FAD/NADH. (a) HPLC analysis of the reaction mixture containing 100 μ M 36, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}$ C for 30 min. (b) LC-MS analysis of 36 reaction products.



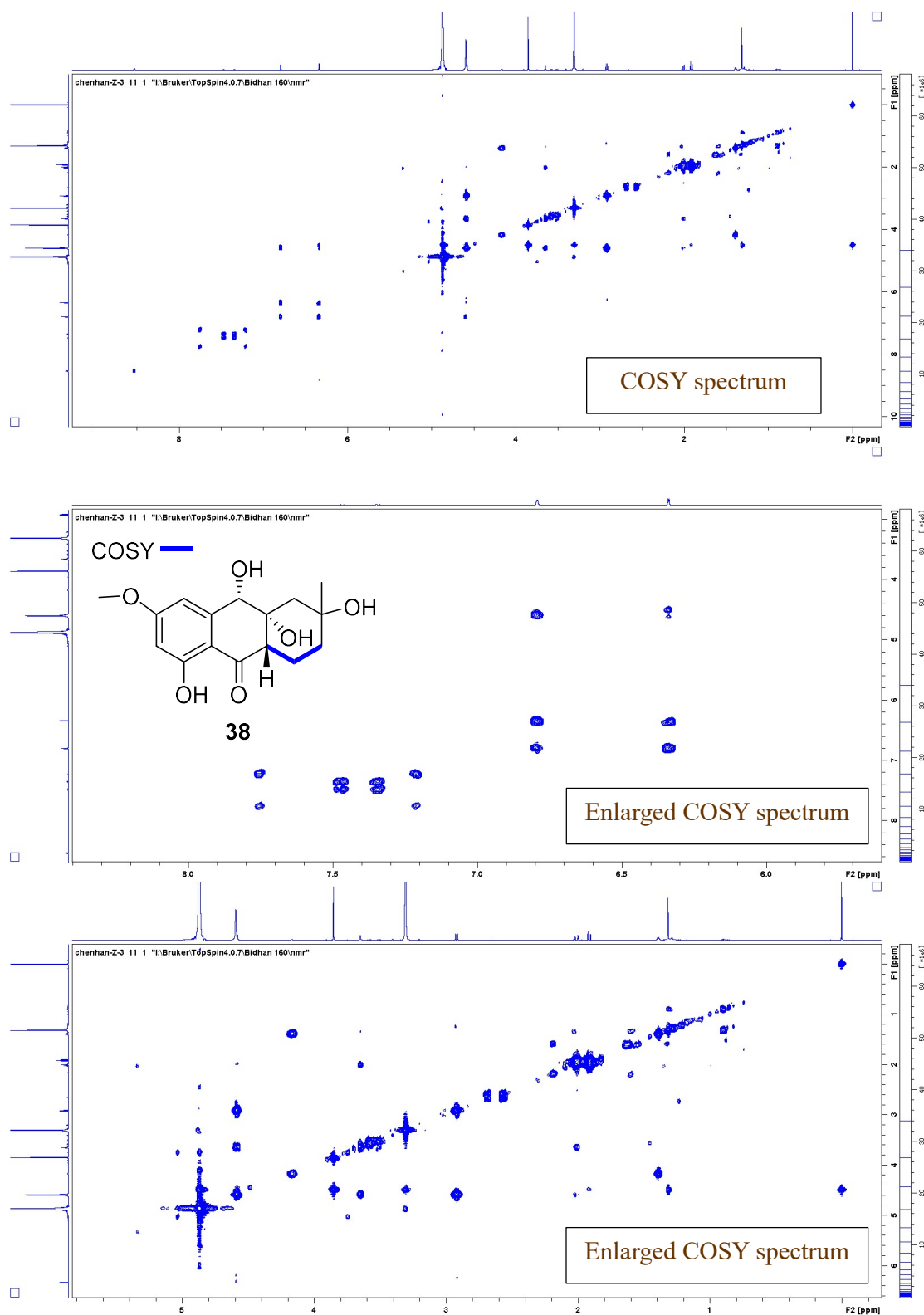
Supplementary Fig. 48. Spectroscopic data for 38. (a) HRESIMS spectrum.



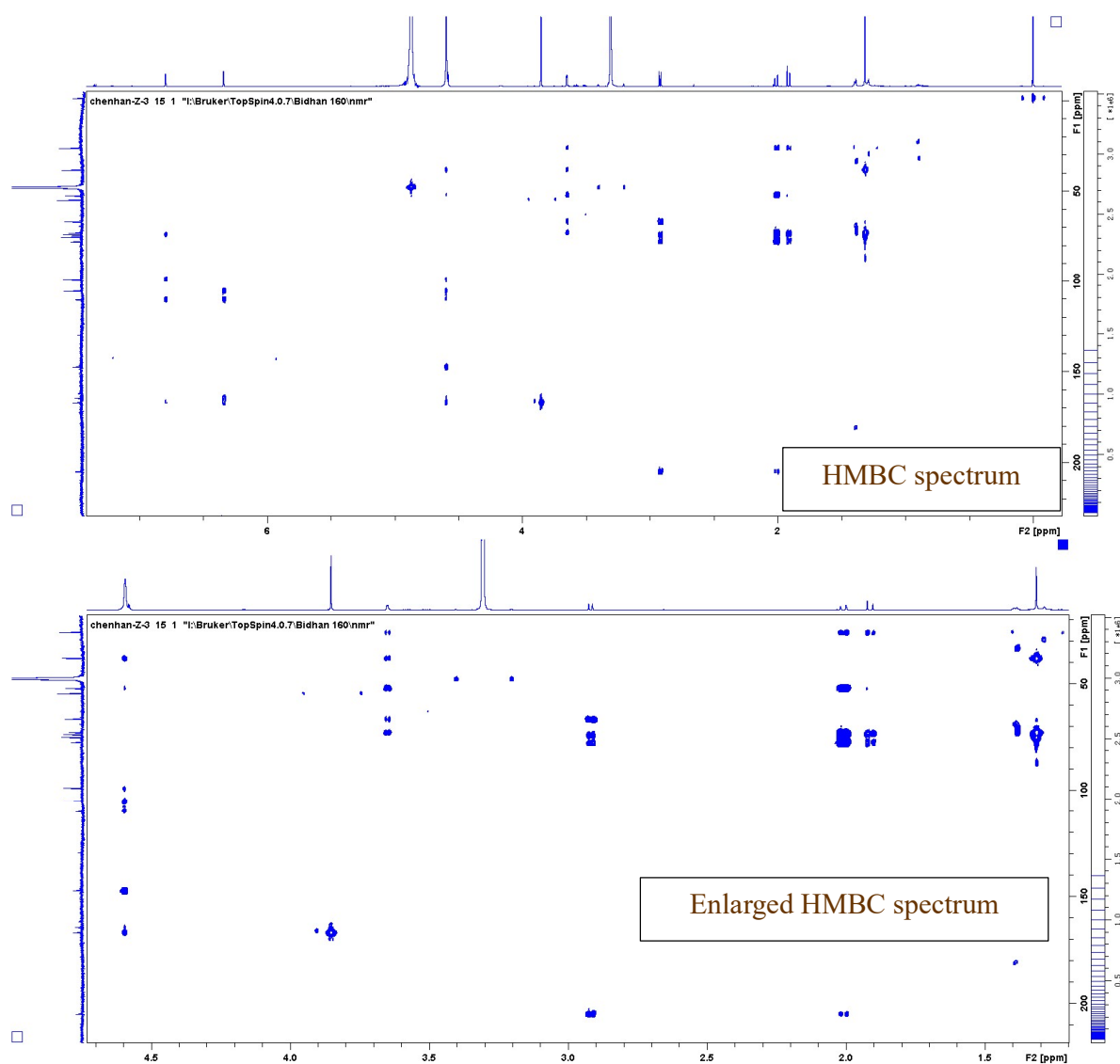
Supplementary Fig. 48. Spectroscopic data for **38**. (b) The ¹H and enlarged ¹H NMR spectrum of **38** in DMSO-*d*₆.



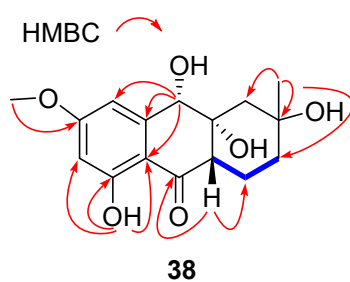
Supplementary Fig. 48. Spectroscopic data for **38**. (c) The ¹³C and enlarged ¹³C NMR spectrum of **38** in DMSO-*d*₆.

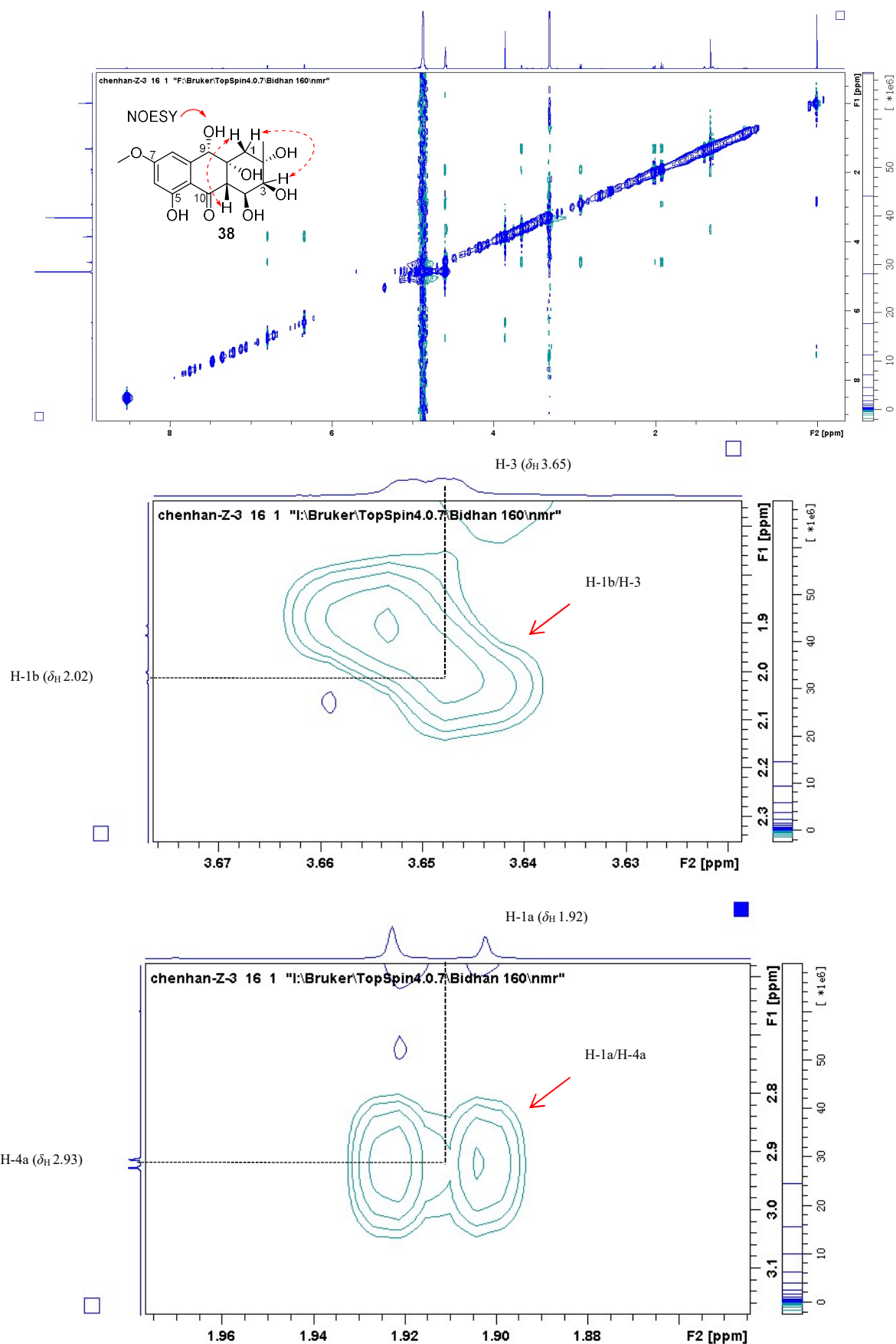


Supplementary Fig. 48. Spectroscopic data for 38. (d) The COSY and enlarged COSY spectrum of 38 in DMSO-*d*₆.



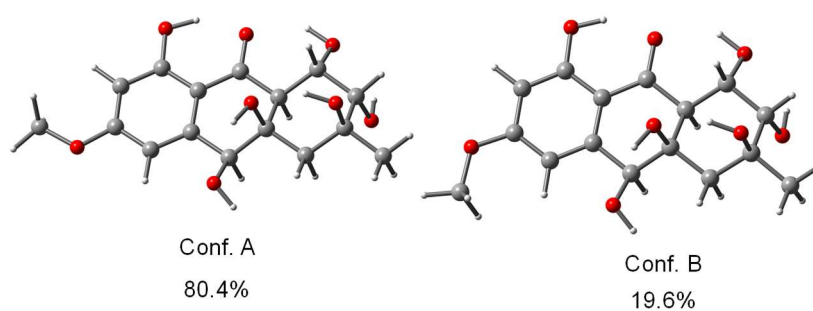
Supplementary Fig. 48. Spectroscopic data for 38. (e) The HMBC and enlarged HMBC spectrum of **38** in DMSO-*d*₆.



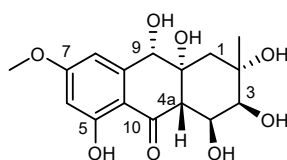
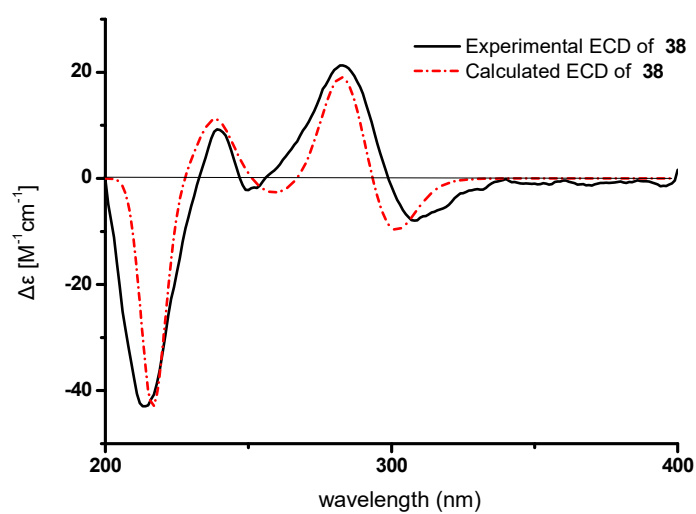


Supplementary Fig. 48. Spectroscopic data for 38. (f) The NOESY and enlarged NOESY spectrum of 38 in DMSO- d_6 .

a

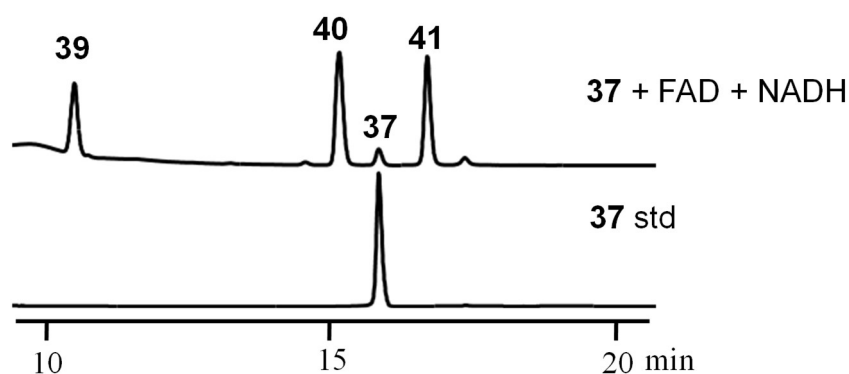
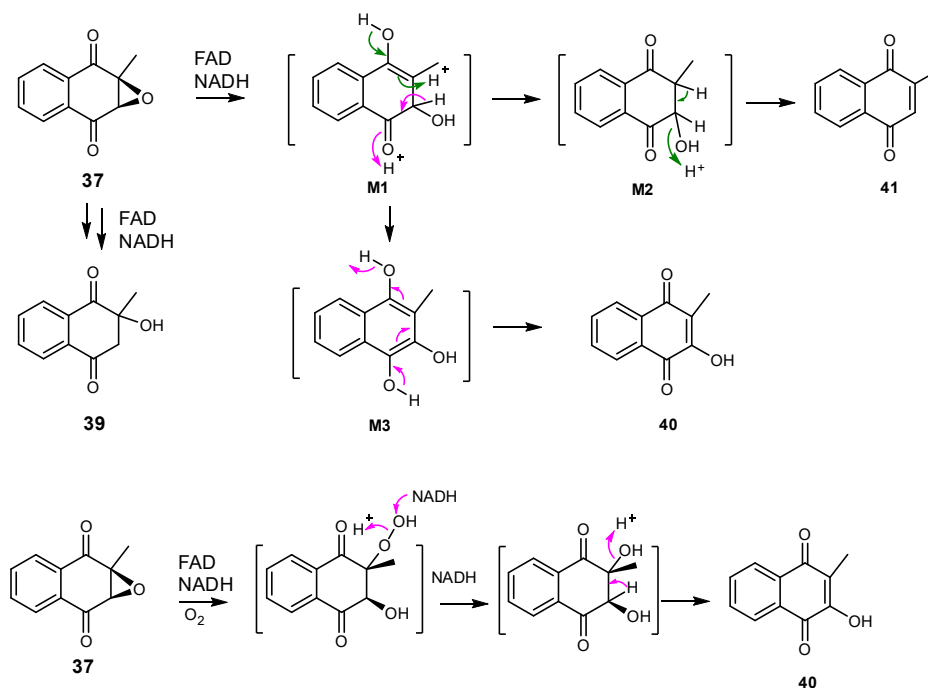


b

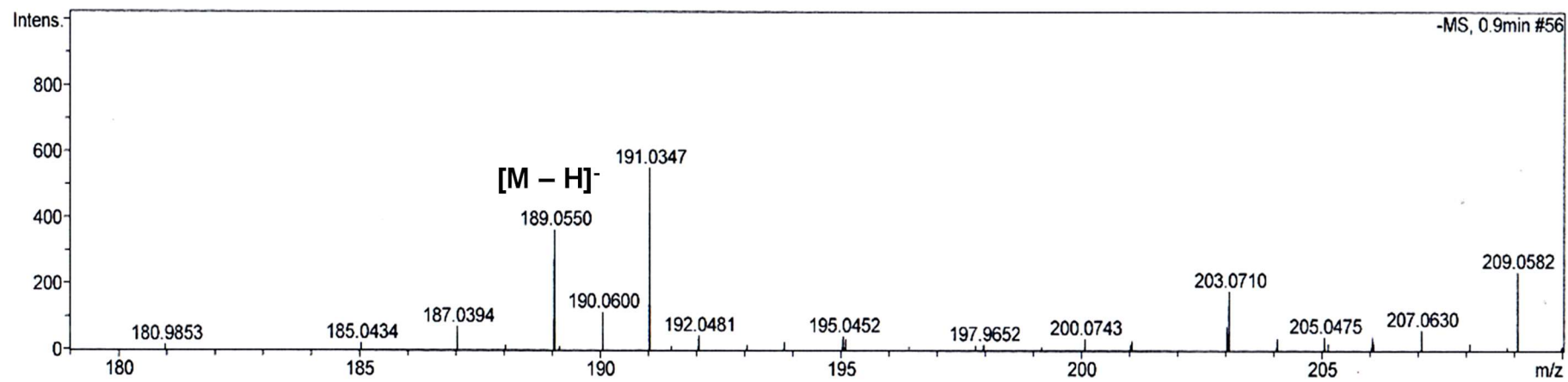


38

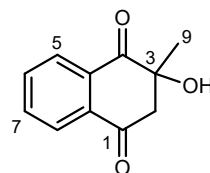
Supplementary Fig. 49. Comparison of experimental and calculated ECD spectra of **38 in MeOH. (a) Structure and population of the low-energy ω B97X/TZVP PCM/MeCN conformers ($> 1\%$) of (2*S*,3*S*,4*S*,4*aS*,9*S*,9*aR*)-**38**. (b) Experimental ECD spectrum of **38** in MeOH compared with the Boltzmann-weighted B3LYP/TZVP PCM/MeCN ECD spectrum of 2*S*,3*S*,4*S*,4*aS*,9*S*,9*aR* computed for the ω B97X/TZVP PCM/MeOH conformers.**

a**b**

Supplementary Fig. 50. HPLC analysis of the reaction of menadione 2,3-epoxide (37) with FAD/NADH. (a) HPLC analysis of the reaction mixture containing 100 μM 37, 100 μM FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 $^{\circ}\text{C}$ for 30 min. (b) The proposed mechanism for the flavin-mediated epoxide ring opening reactions of 37.



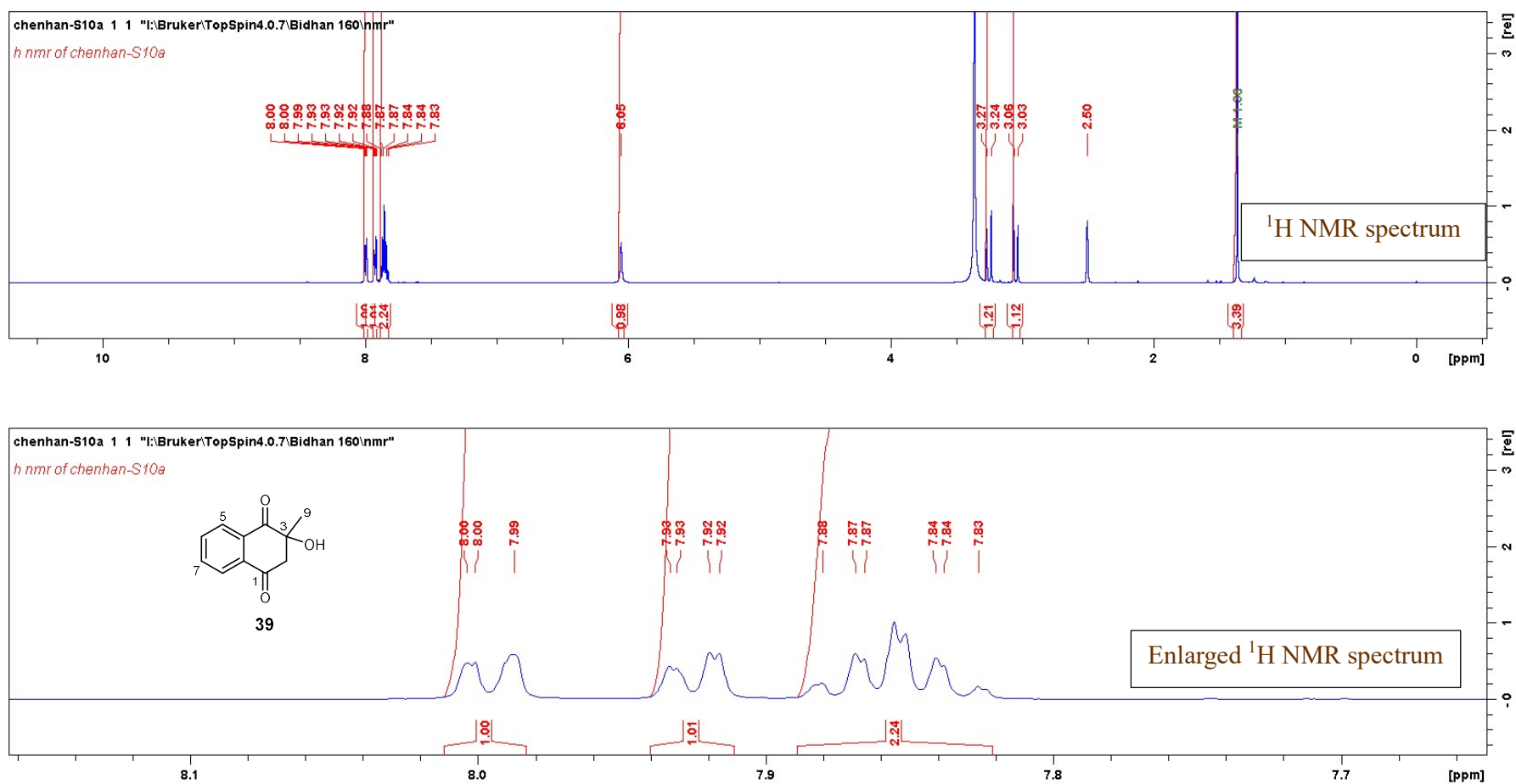
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdB	e ⁻ Conf	N-Rule
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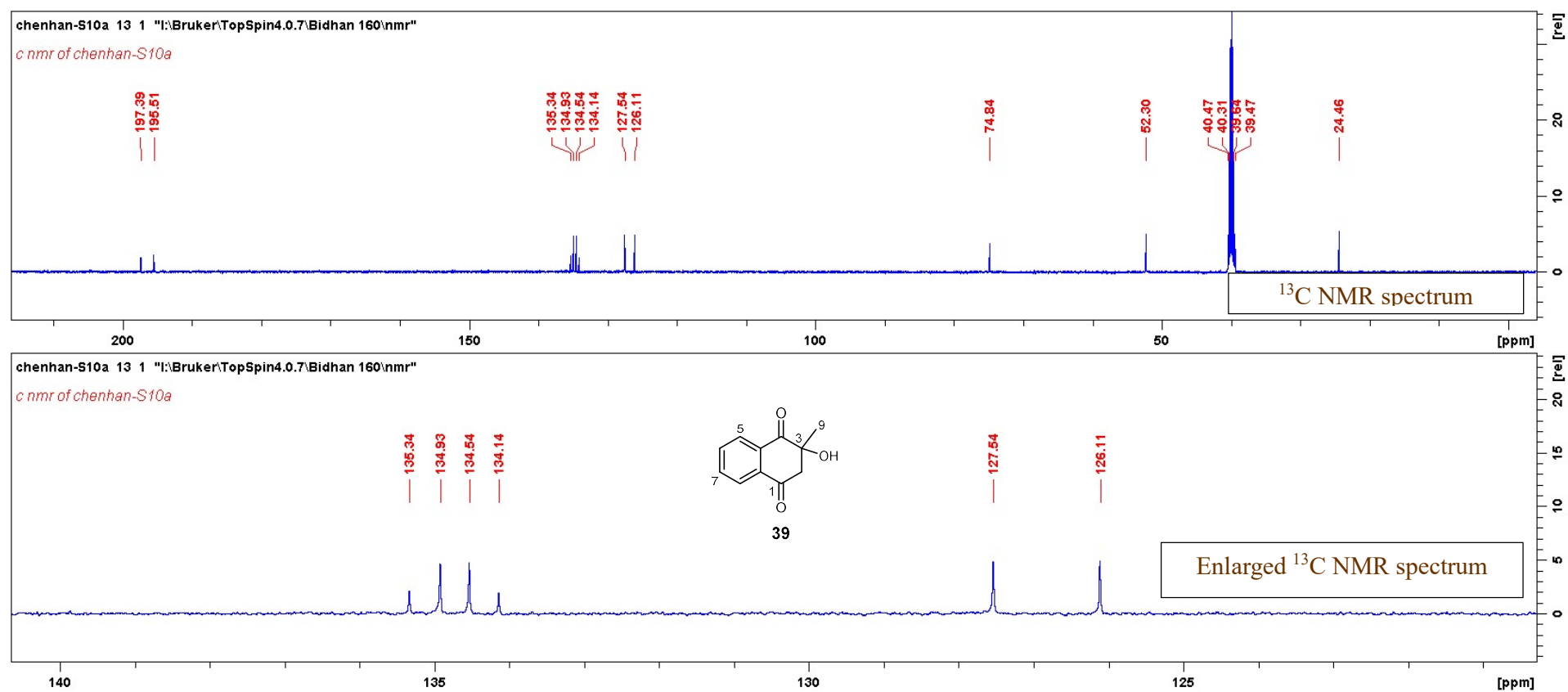
39

Chemical Formula: C₁₁H₁₀O₃
Exact Mass: 190.06

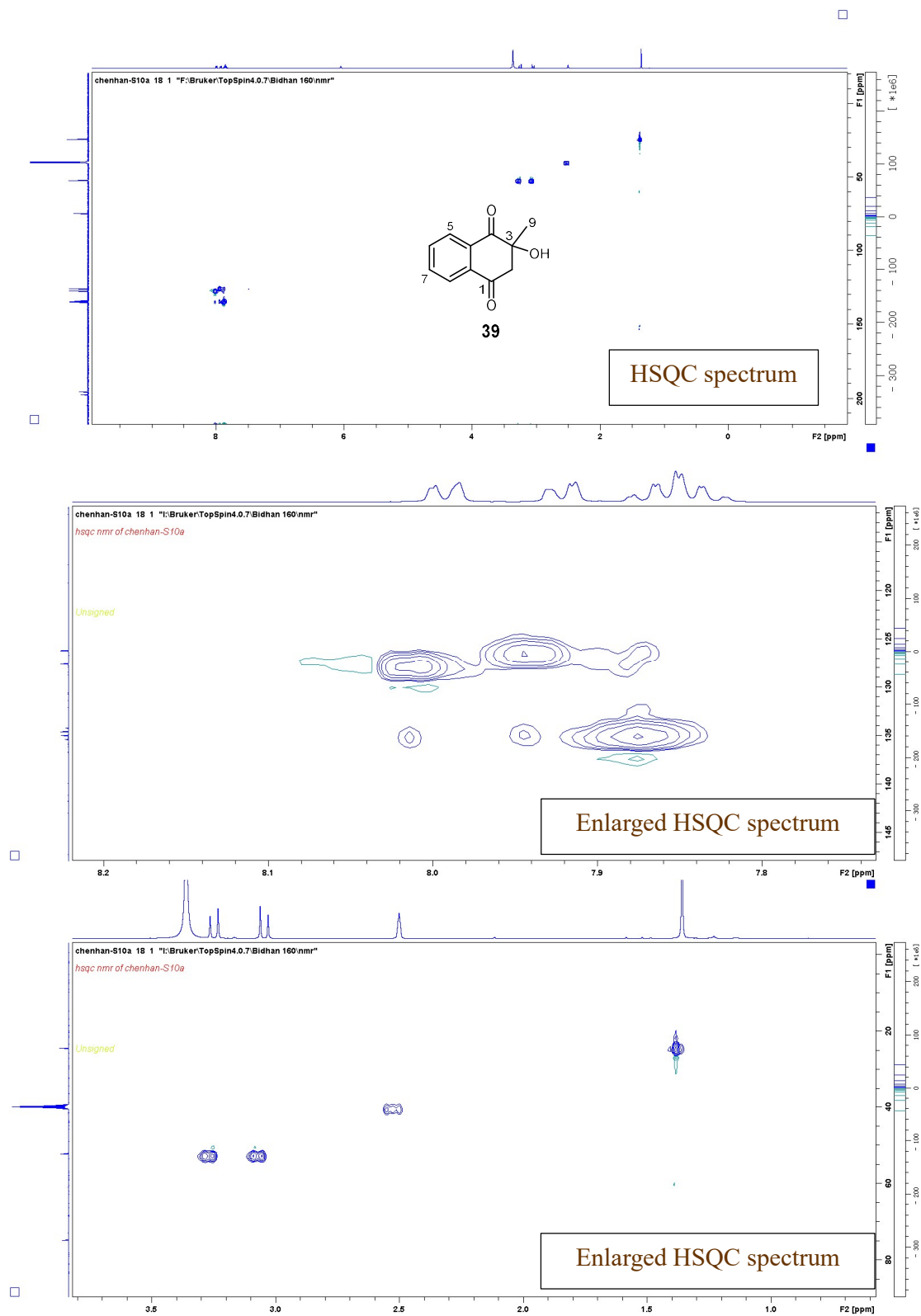
Supplementary Fig. 51. Spectroscopic data for 39. (a) HRESIMS spectrum.



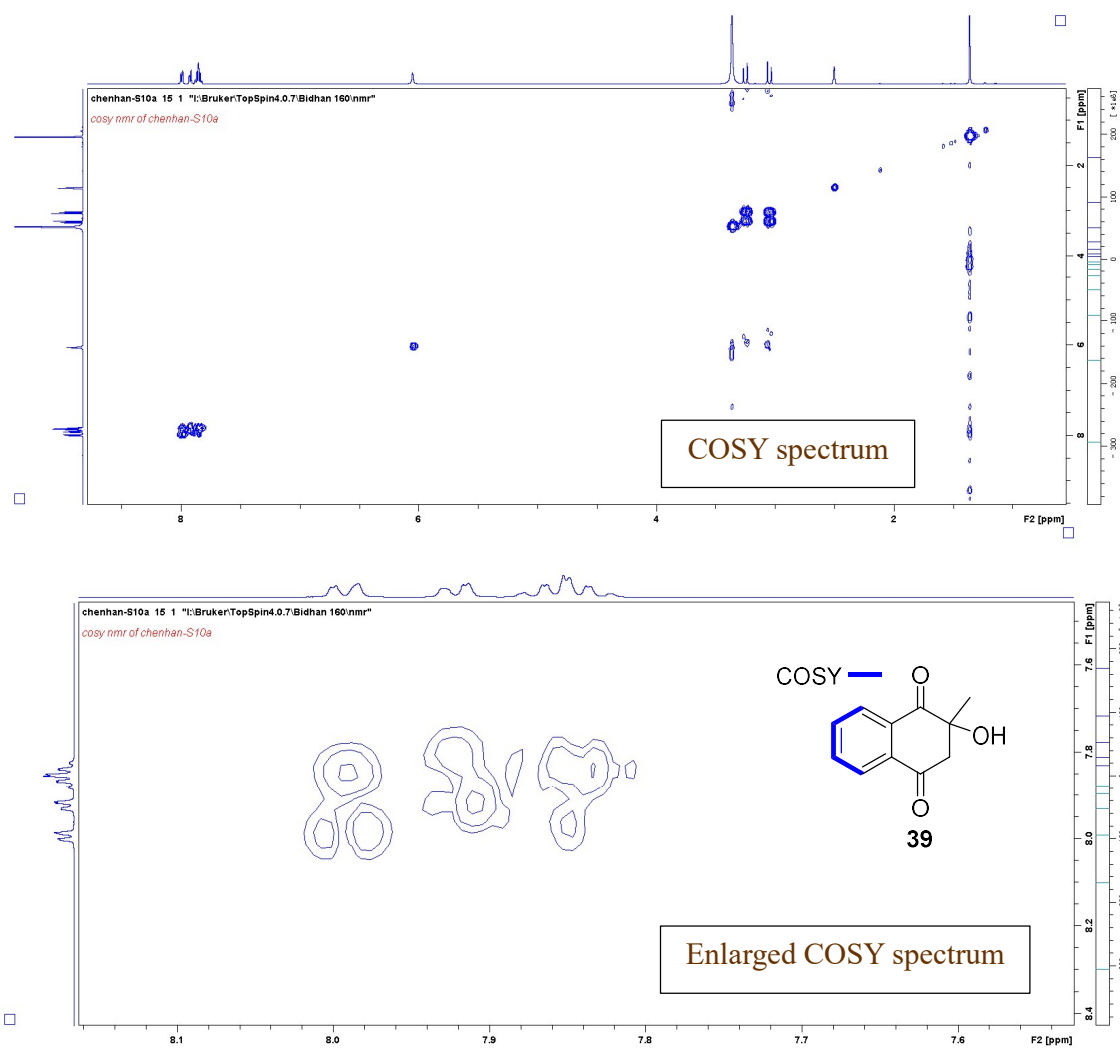
Supplementary Fig. 51. Spectroscopic data for **39**. (b) The ¹H and enlarged ¹H NMR spectrum of **39** in DMSO-*d*₆.



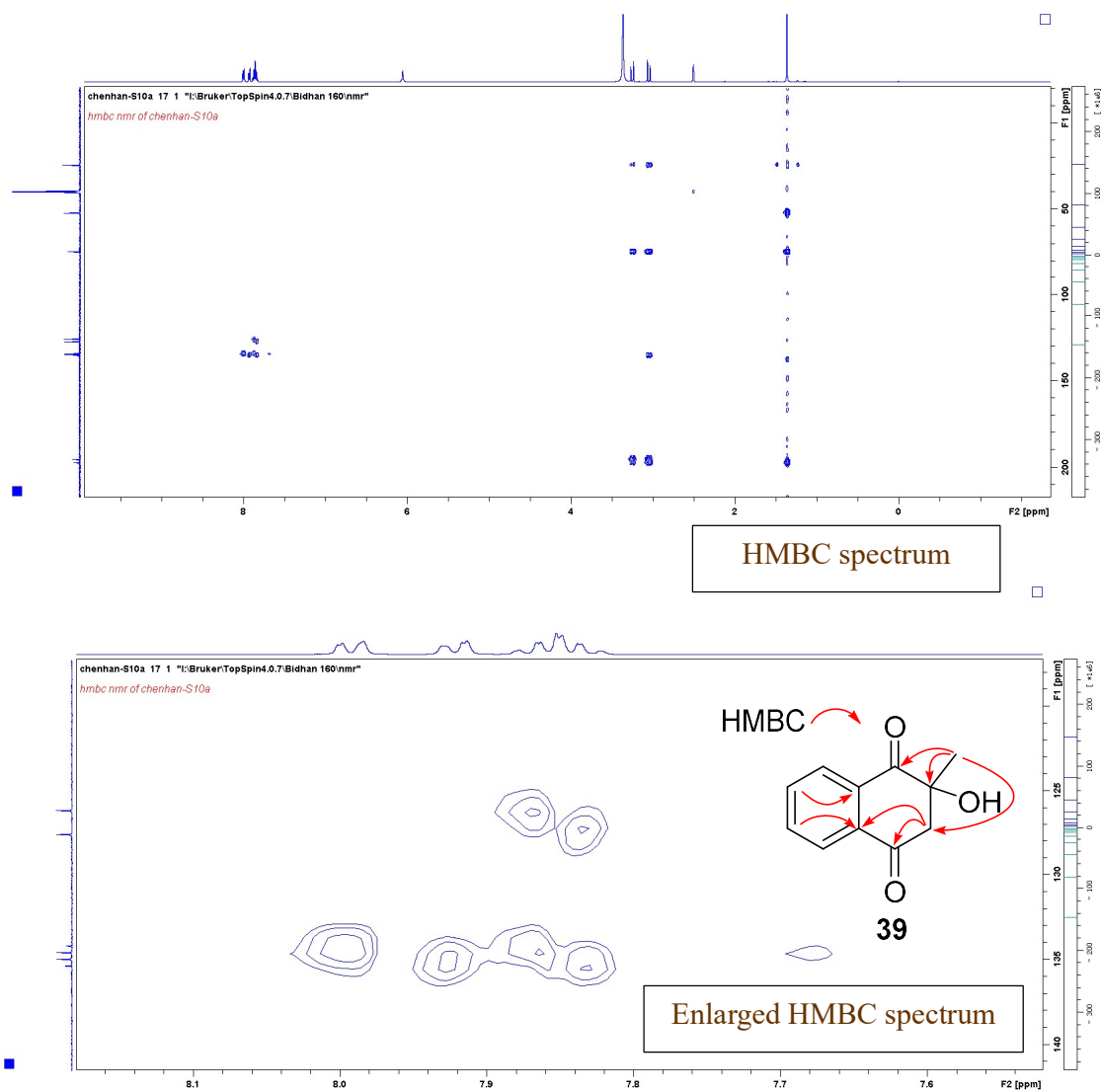
Supplementary Fig. 51. Spectroscopic data for 39. (c) The ¹³C and enlarged ¹³C NMR spectrum of 39 in DMSO-*d*₆.



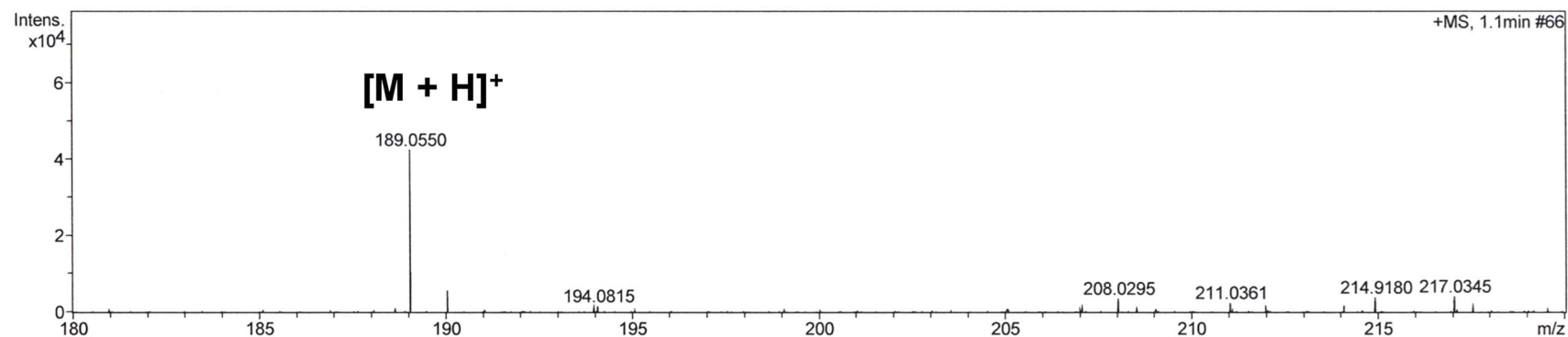
Supplementary Fig. 51. Spectroscopic data for 39. (d) The HSQC and enlarged HSQC spectrum of 39 in DMSO-*d*₆.



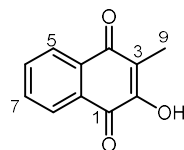
Supplementary Fig. 51. Spectroscopic data for **39. (e) The COSY and enlarged COSY spectrum of **39** in DMSO-*d*₆.**



Supplementary Fig. 51. Spectroscopic data for 39. (f) The HMBC and enlarged HMBC spectrum of **39** in DMSO-*d*₆.



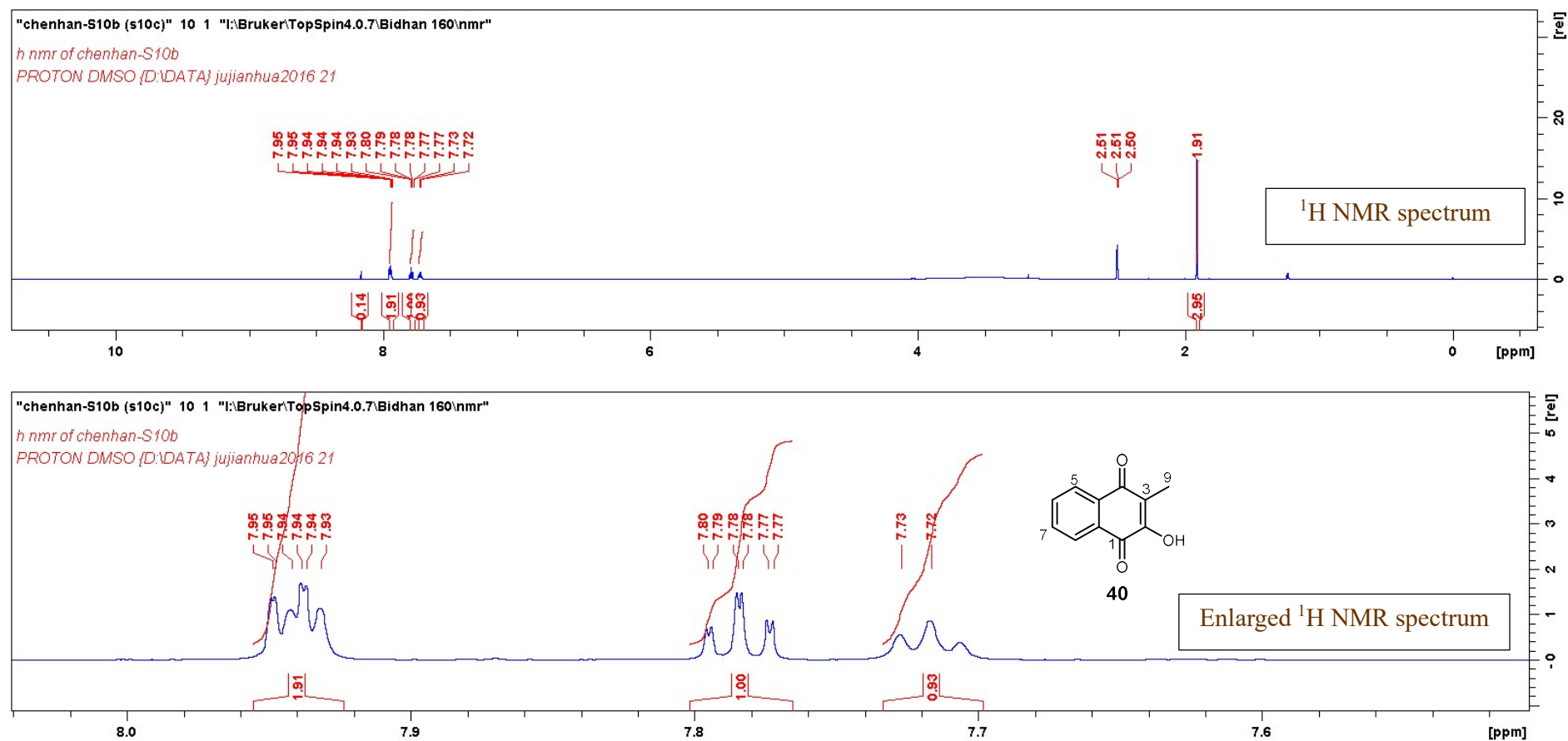
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e ⁻	Conf	N-Rule
189.0550	1	C ₁₁ H ₉ O ₃	100.00	189.0546	1.9	0.4	7.0	7.5	even		ok
211.0361	1	C ₁₁ H ₈ NaO ₃	100.00	211.0366	2.4	0.5	32.6	7.5	even		ok



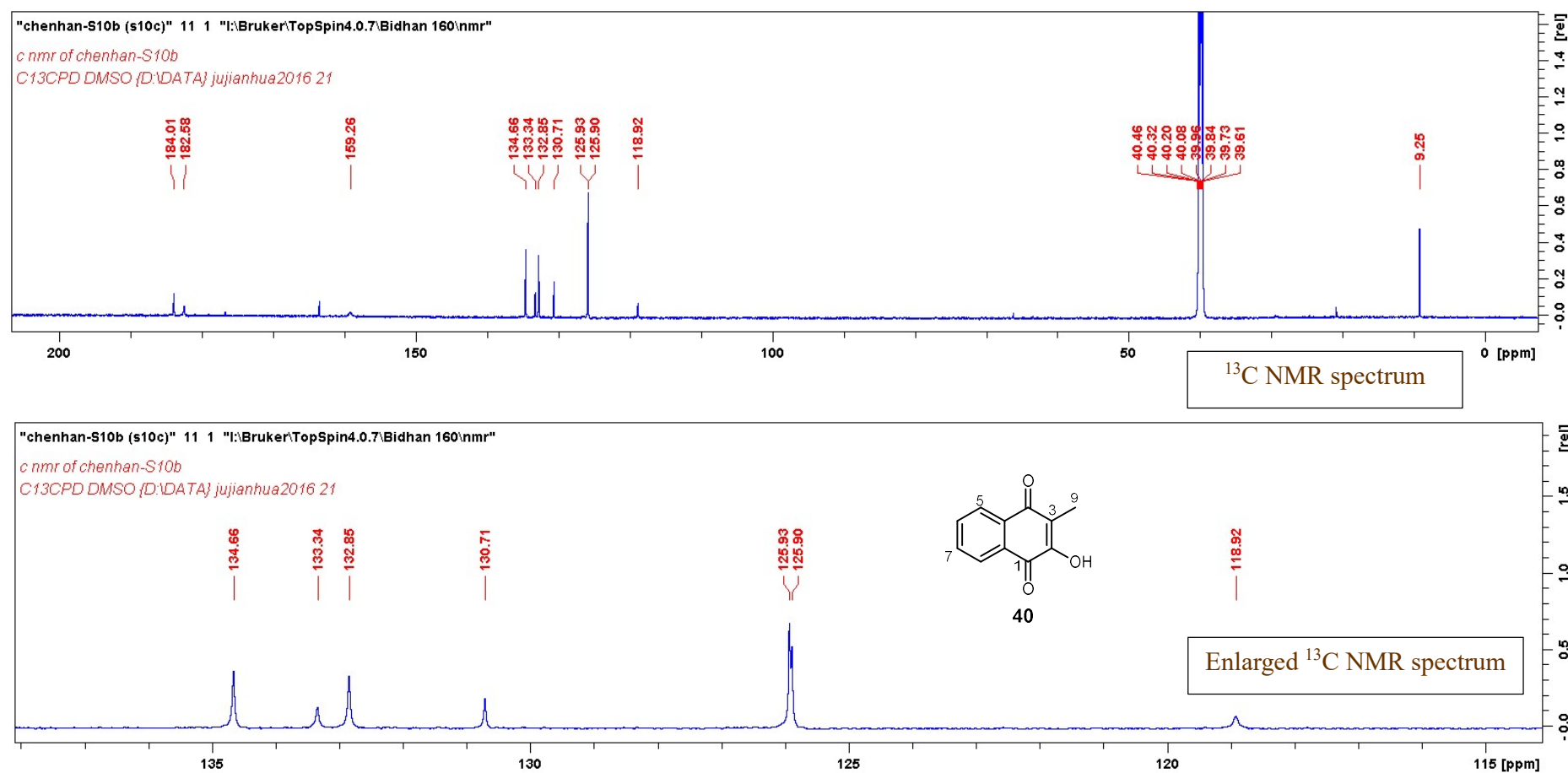
40

Chemical Formula: C₁₁H₈O₃
Exact Mass: 188.05

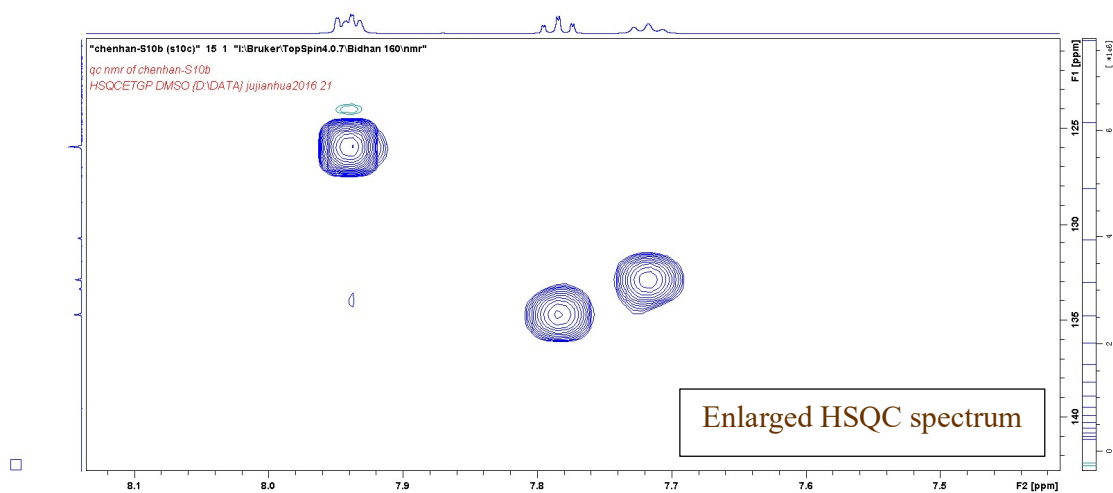
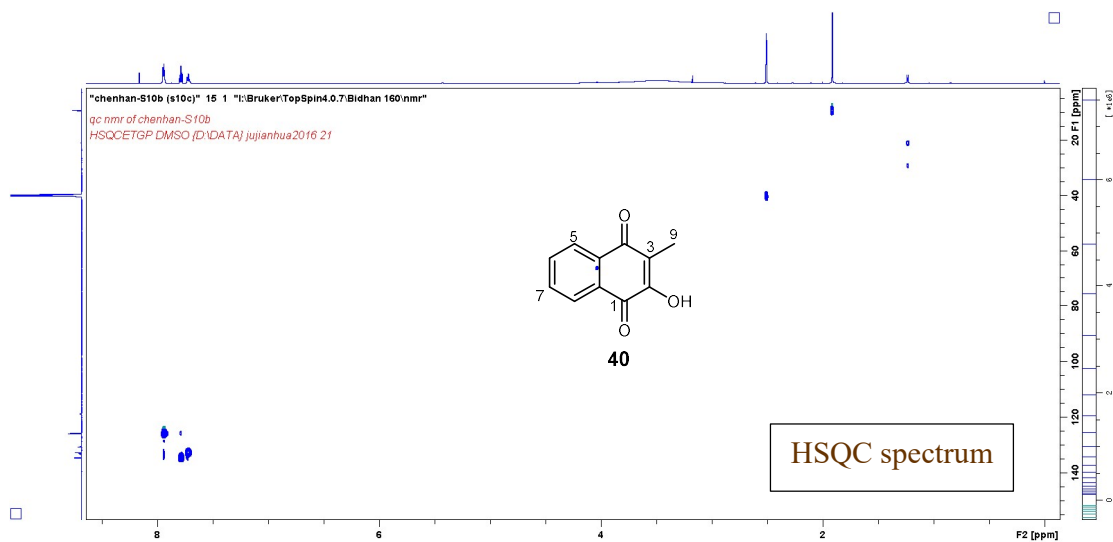
Supplementary Fig. 52 Spectroscopic data for 40. (a) HRESIMS spectrum



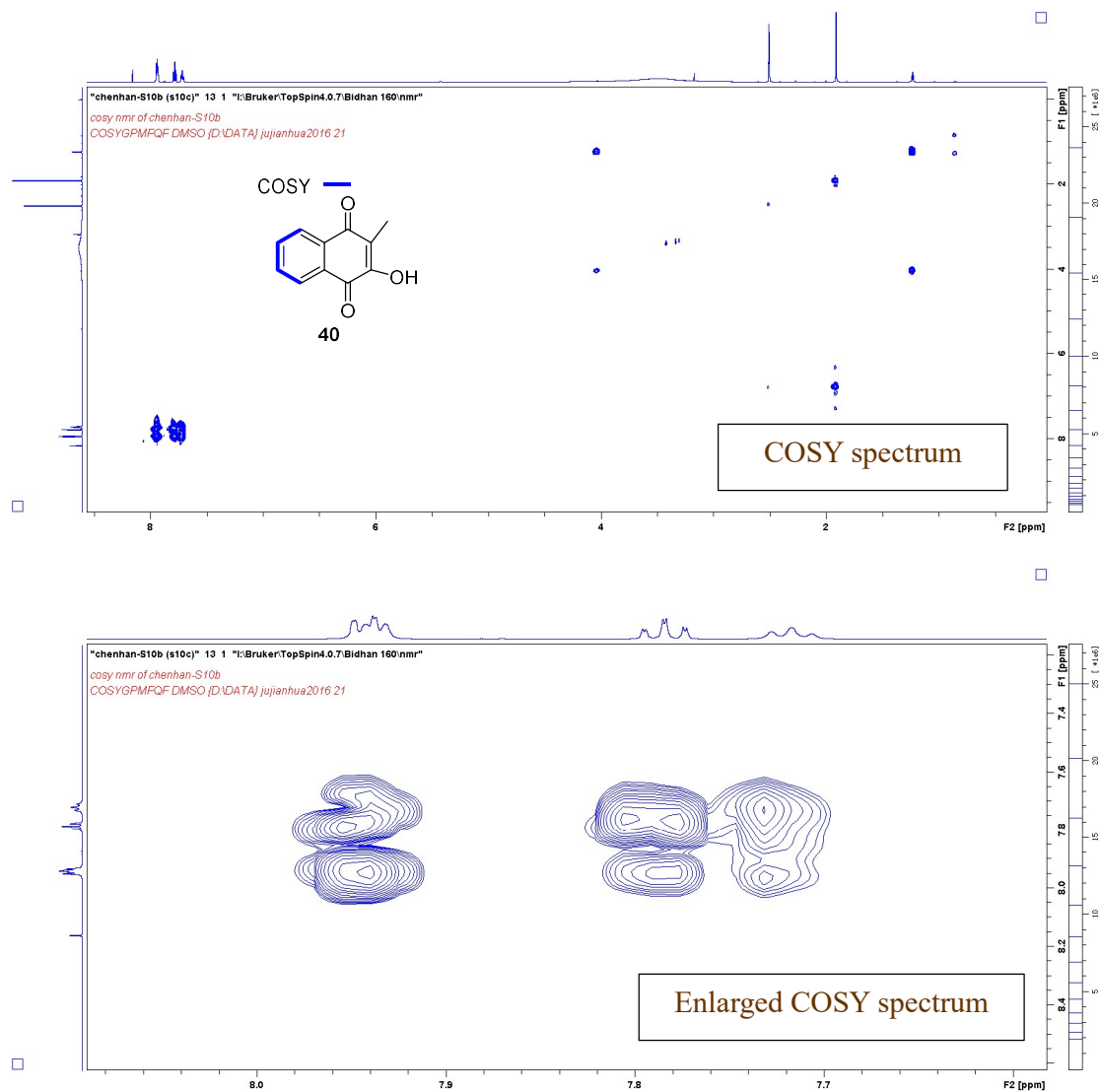
Supplementary Fig. S2 Spectroscopic data for 40. (b) The ¹H and enlarged ¹H NMR spectrum of in 40 in DMSO-*d*₆.



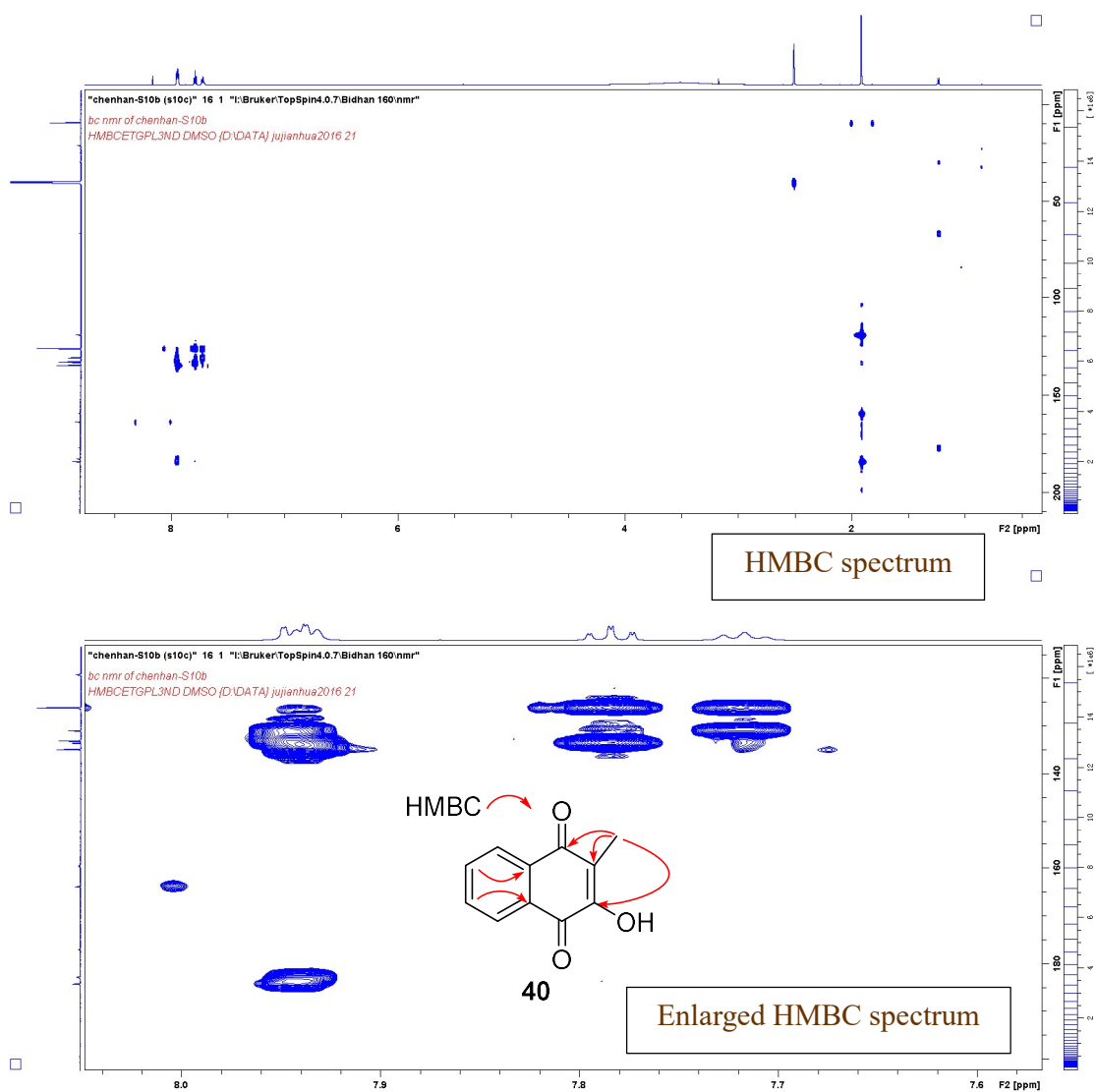
Supplementary Fig. 52 Spectroscopic data for 40. (c) The ¹³C and enlarged ¹³C NMR spectrum of 40 in DMSO-*d*₆.



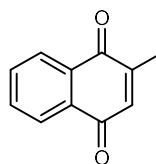
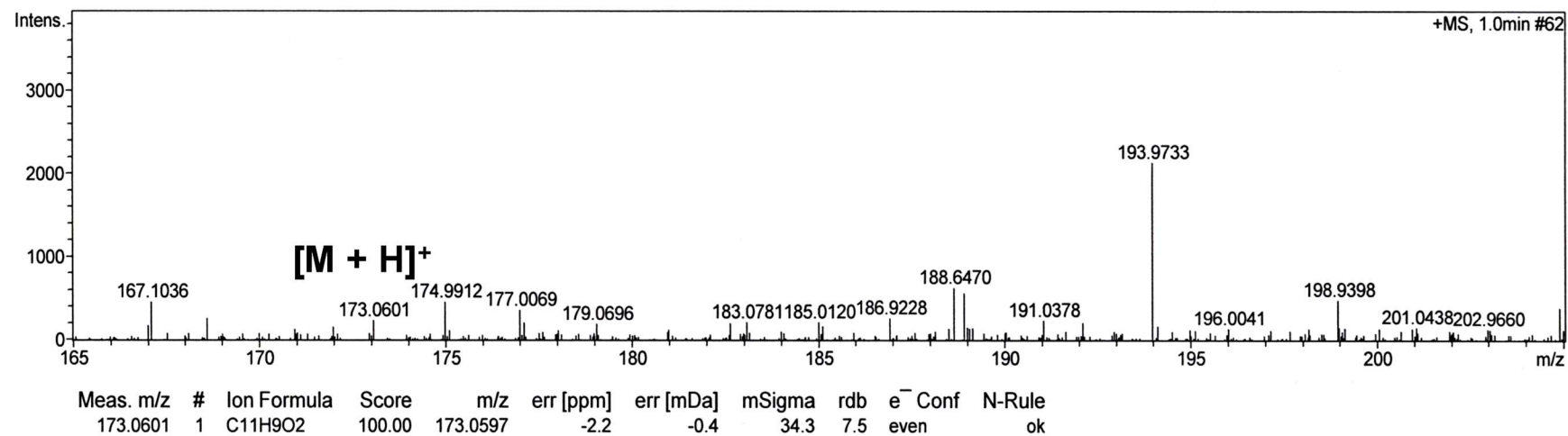
Supplementary Fig. 52 Spectroscopic data for 40. (d) The HSQC and enlarged HSQC spectrum of 40 in DMSO-*d*₆.



Supplementary Fig. 52 Spectroscopic data for **40**. (e) The COSY and enlarged COSY spectrum of **40** in DMSO-*d*₆.



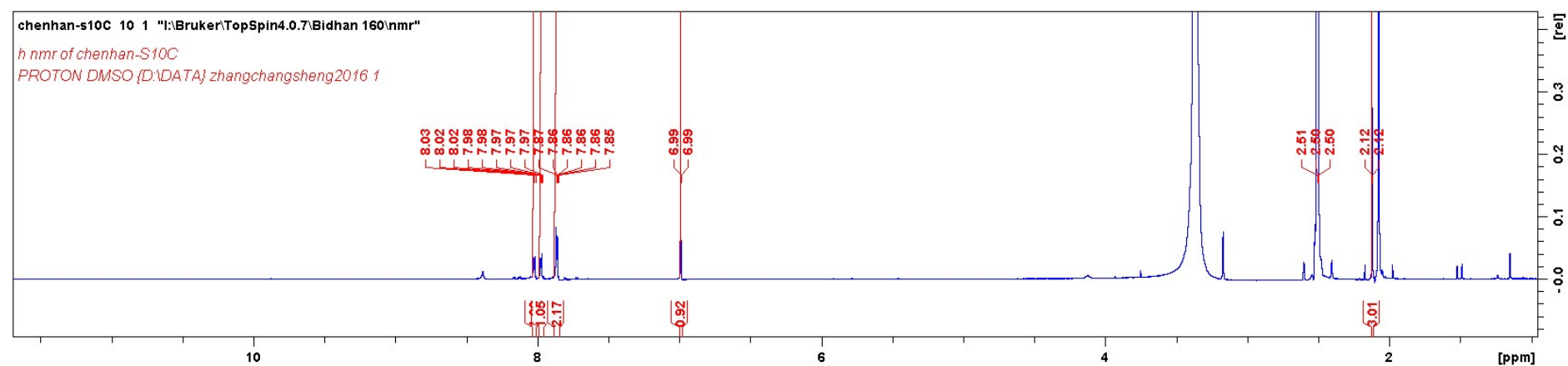
Supplementary Fig. S2 Spectroscopic data for 40. (f) The HMBC and enlarged HMBC spectrum of **40** in $\text{DMSO}-d_6$.



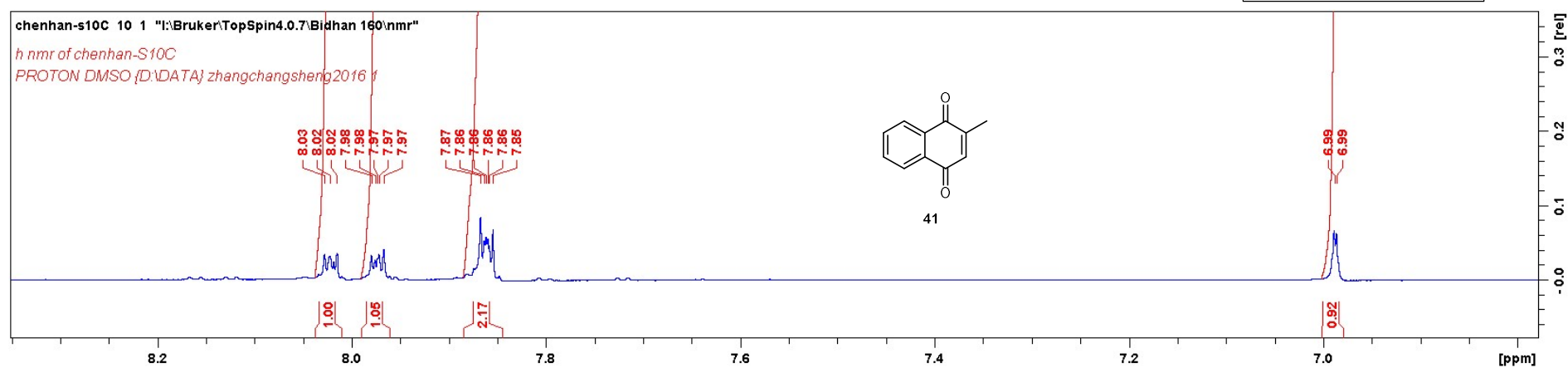
41

Chemical Formula: C₁₁H₈O₂
Exact Mass: 172.05

Supplementary Fig. 53 Spectroscopic data for 41. (a) HRESIMS spectrum

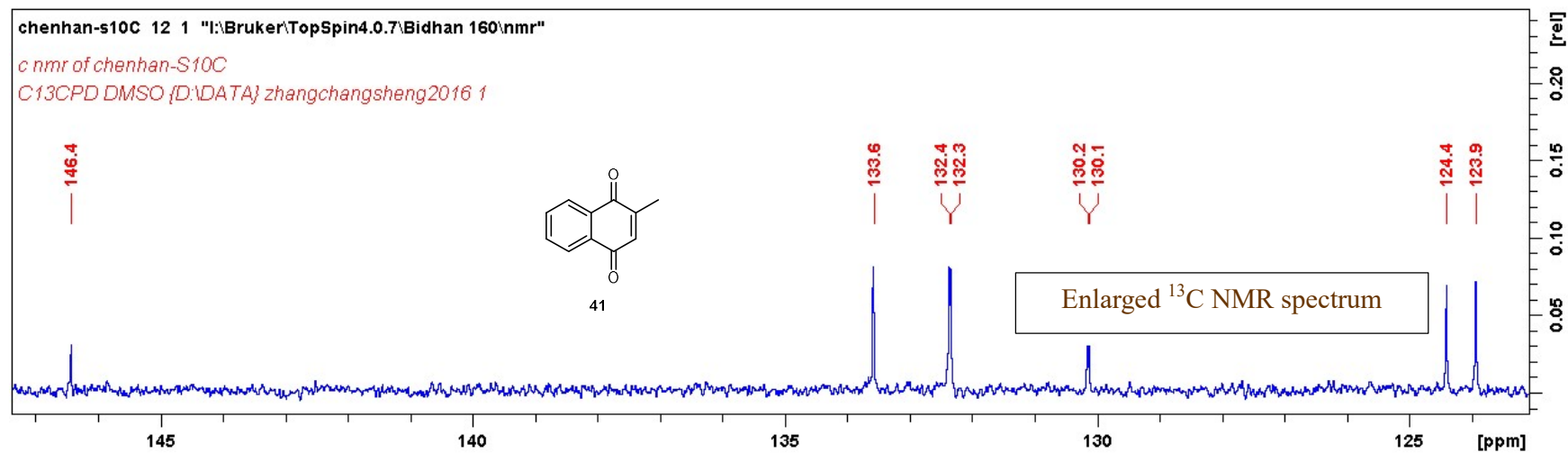
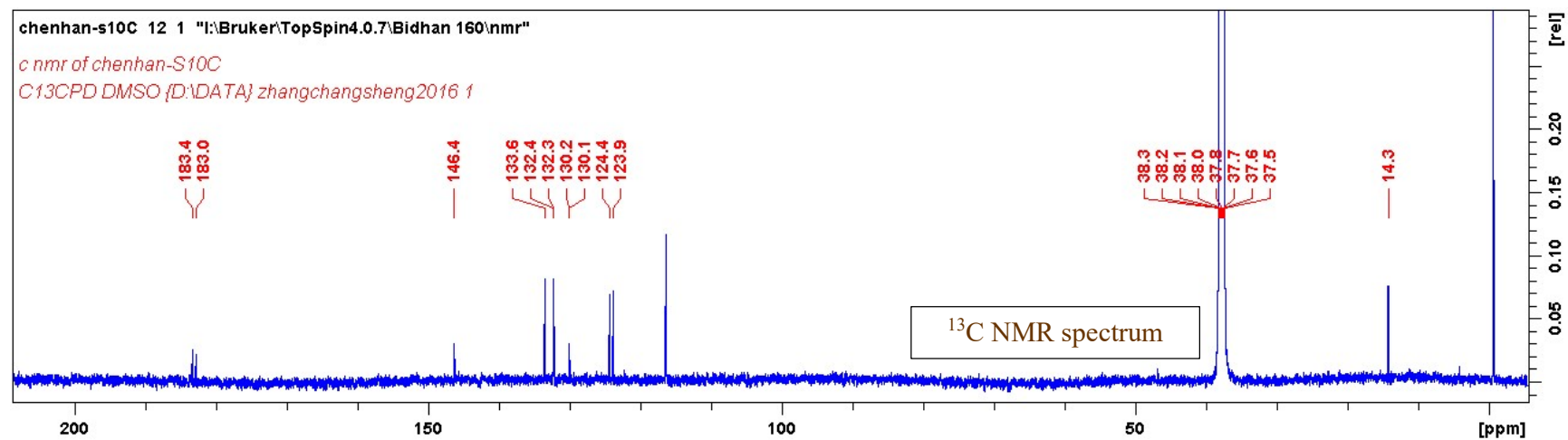


¹H NMR spectrum

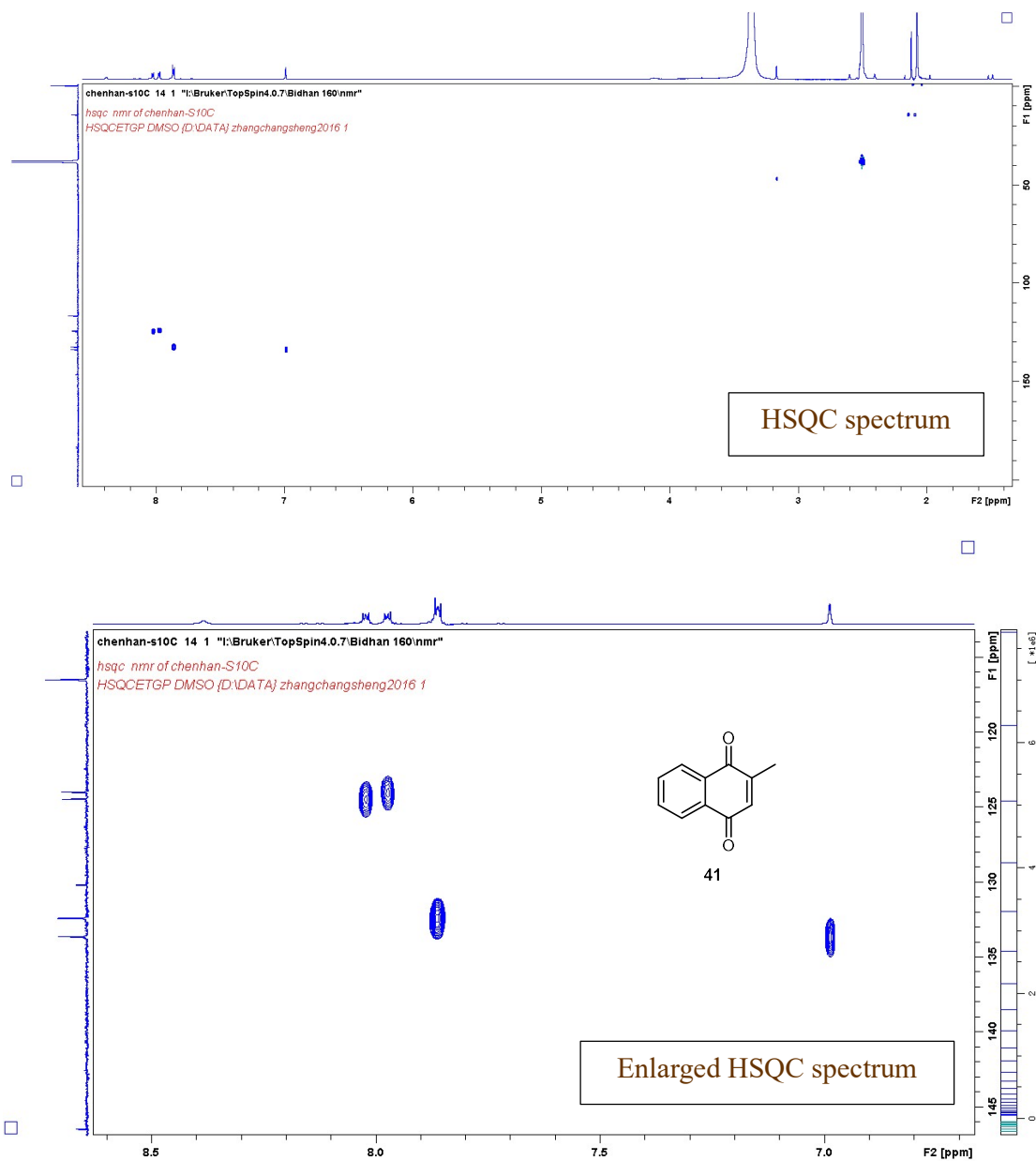


Enlarged ¹H NMR spectrum

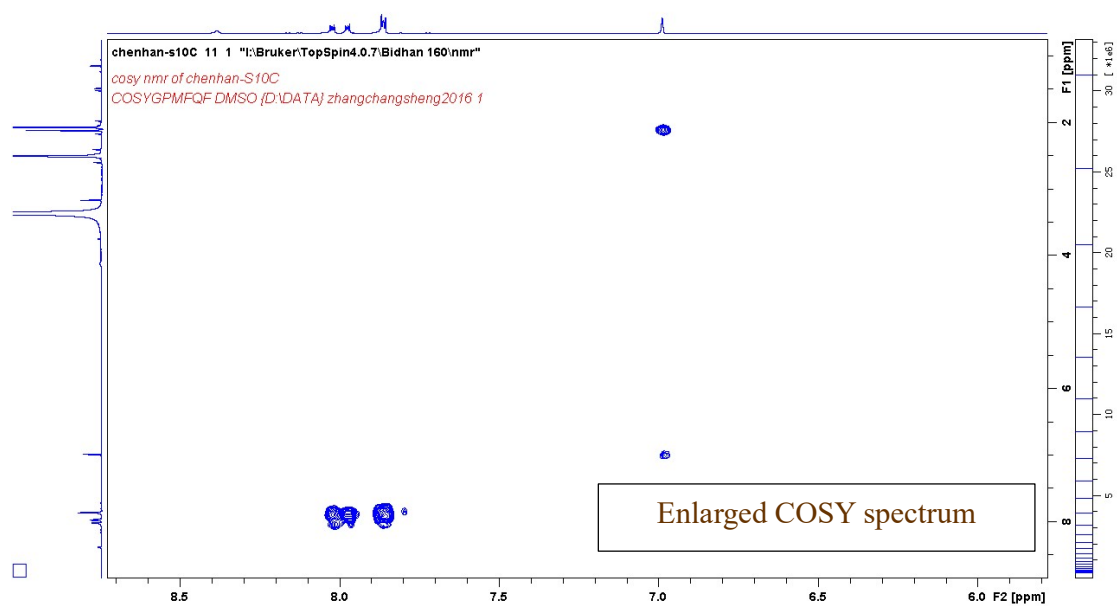
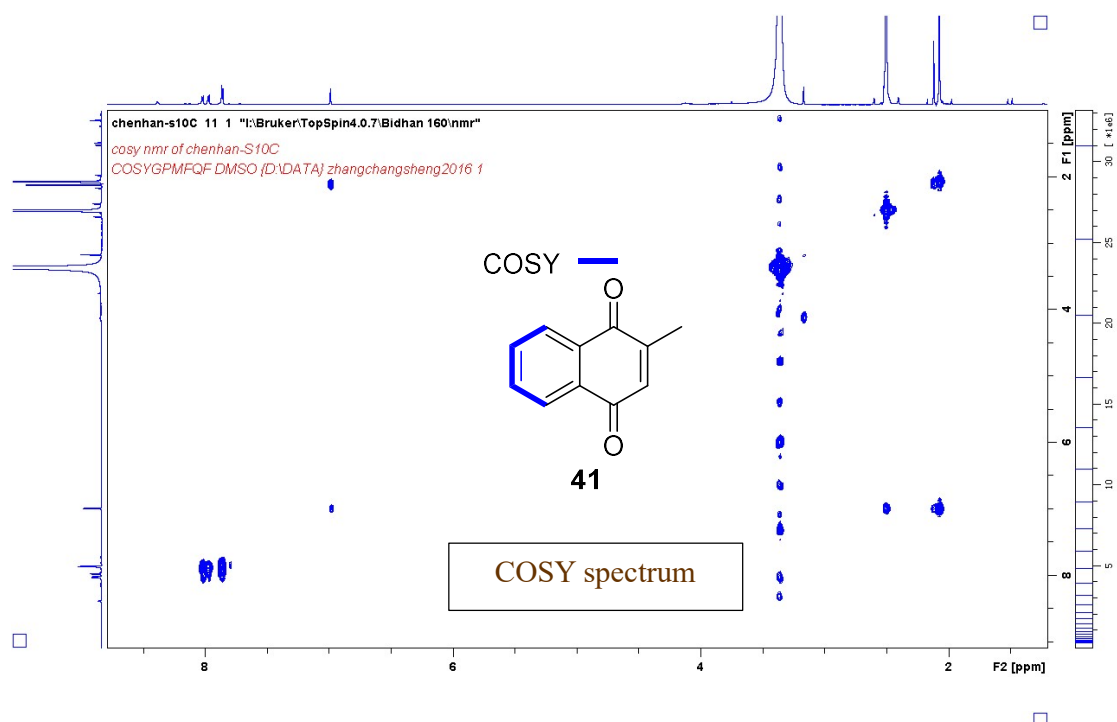
Supplementary Fig. 53 Spectroscopic data for **41**. (b) The ¹H and enlarged ¹H NMR spectrum of **41** in DMSO-*d*₆.



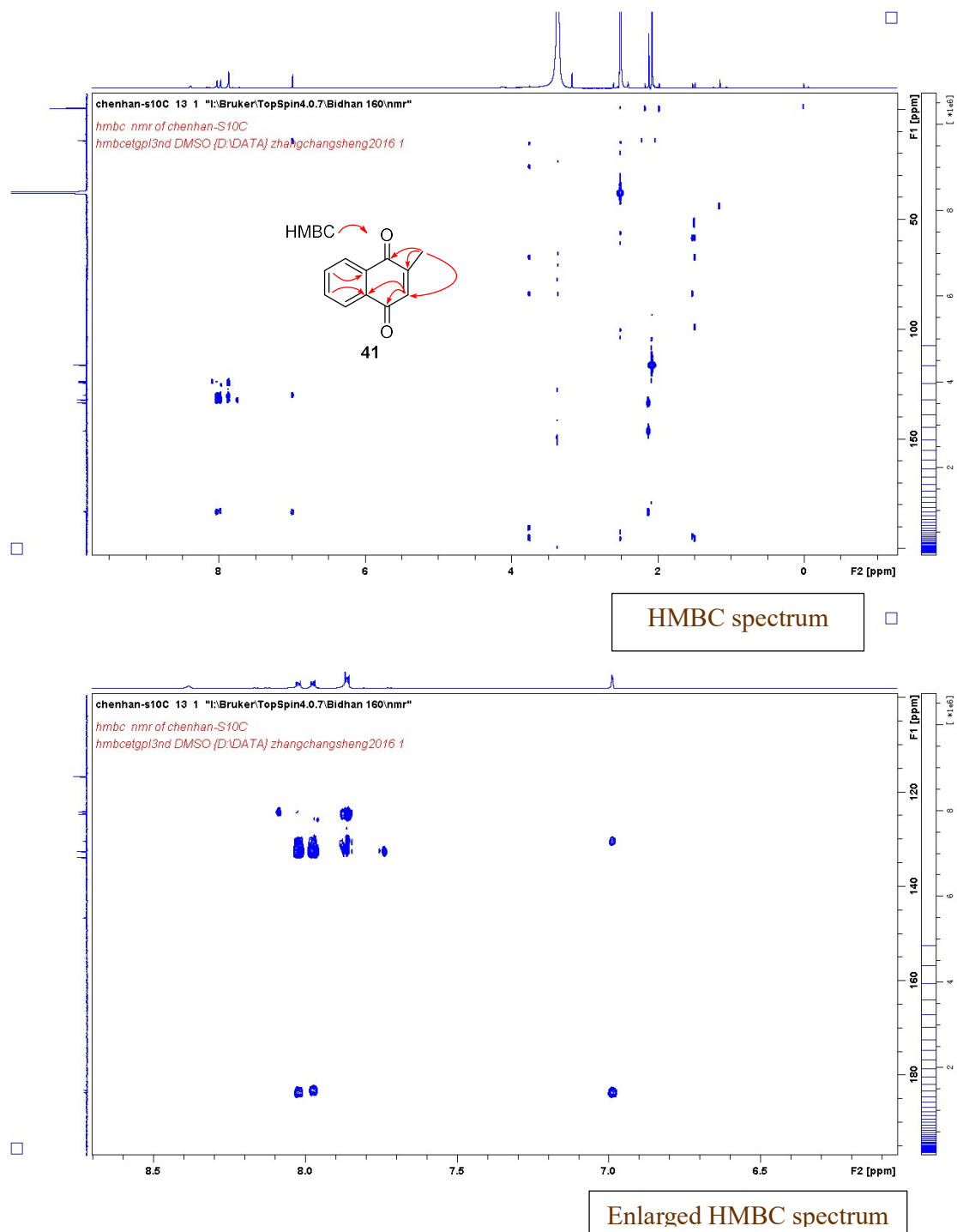
Supplementary Fig. 53 Spectroscopic data for **41**. (c) The ¹³C and enlarged ¹³C NMR spectrum of **41** in DMSO-*d*₆.



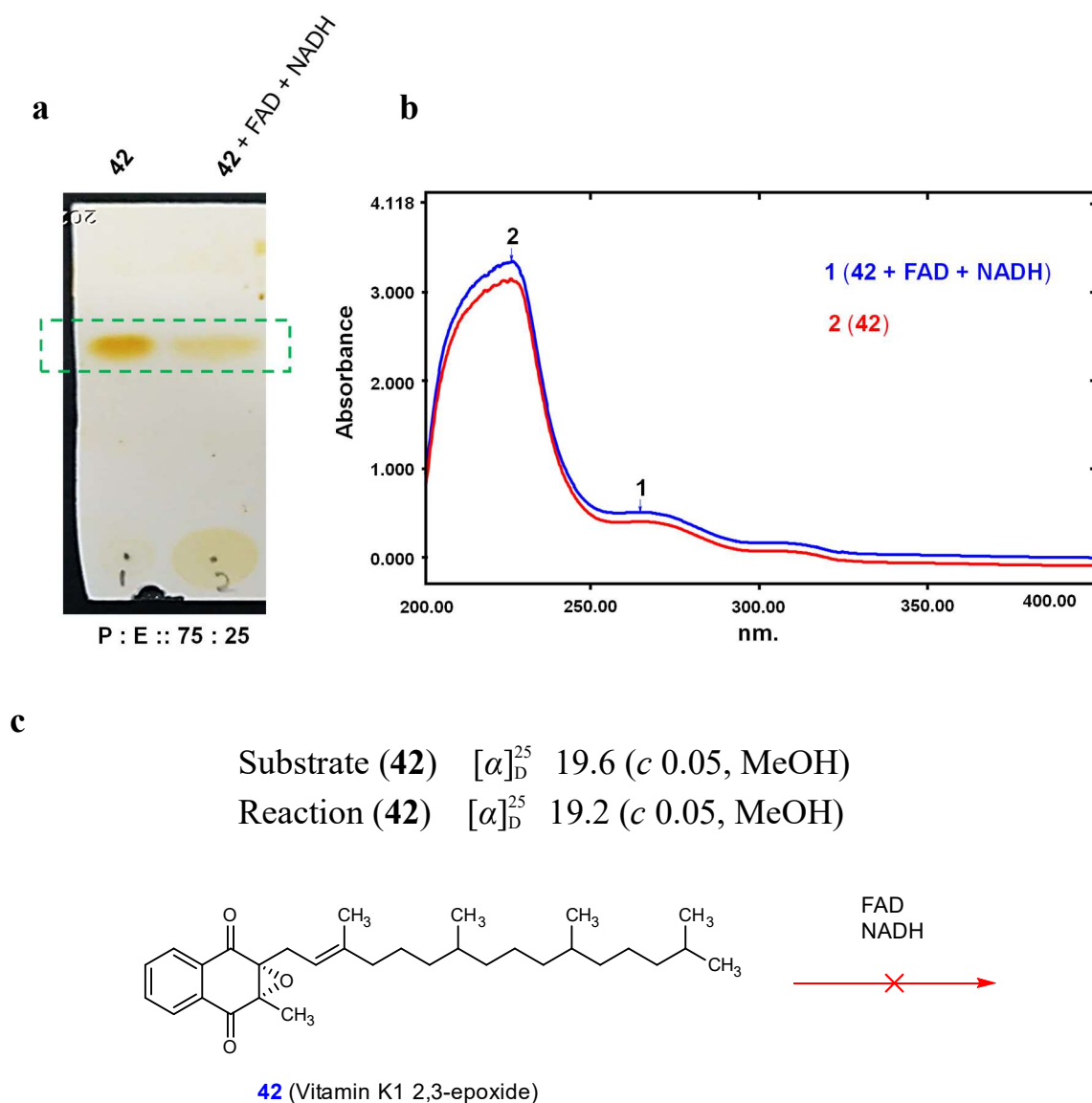
Supplementary Fig. 53 Spectroscopic data for 41. (d) The HSQC and enlarged HSQC spectrum of **41** in DMSO- d_6 .



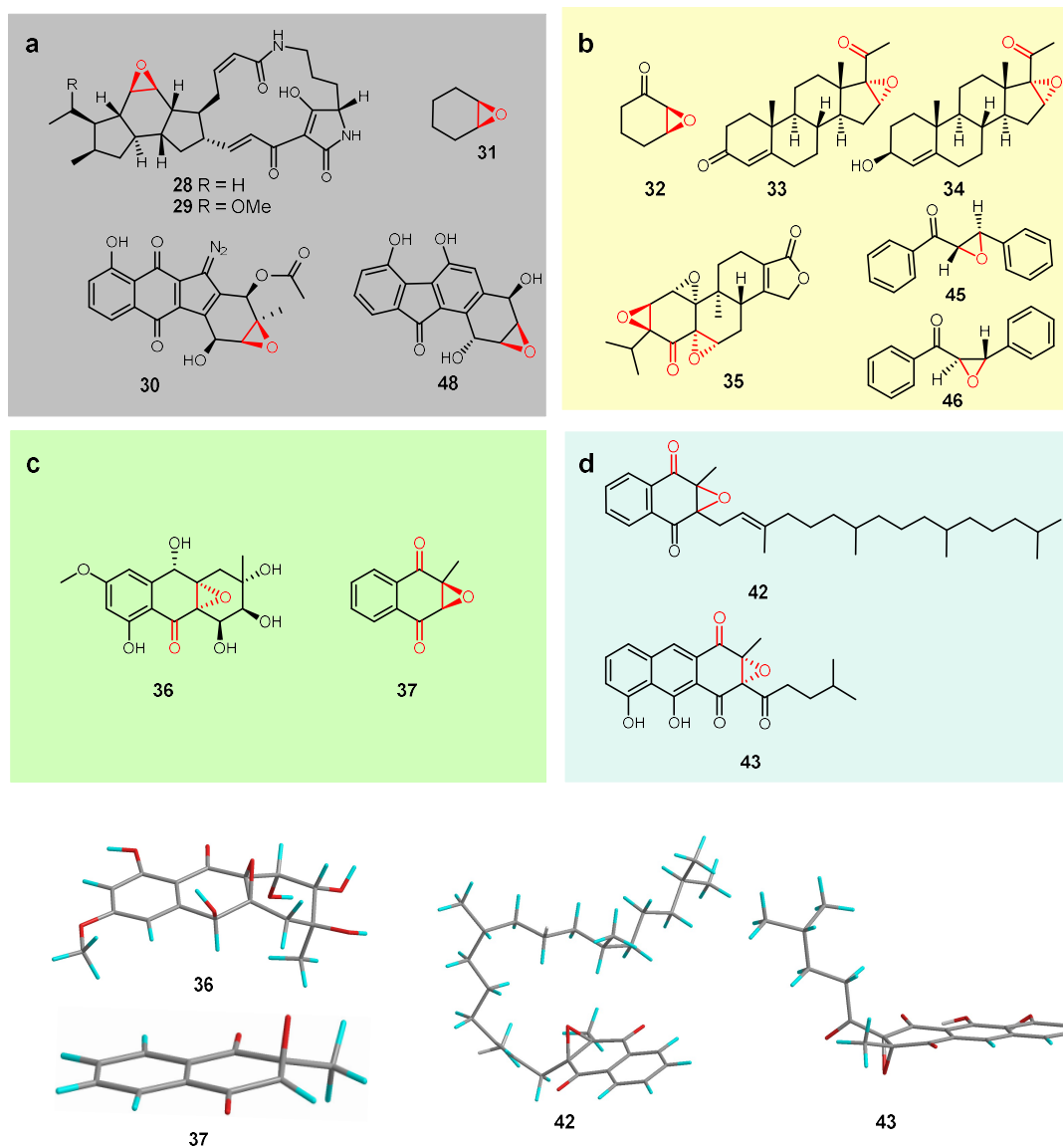
Supplementary Fig. 53 Spectroscopic data for **41**. (e) The COSY and enlarged COSY spectrum of **41** in DMSO- d_6 .



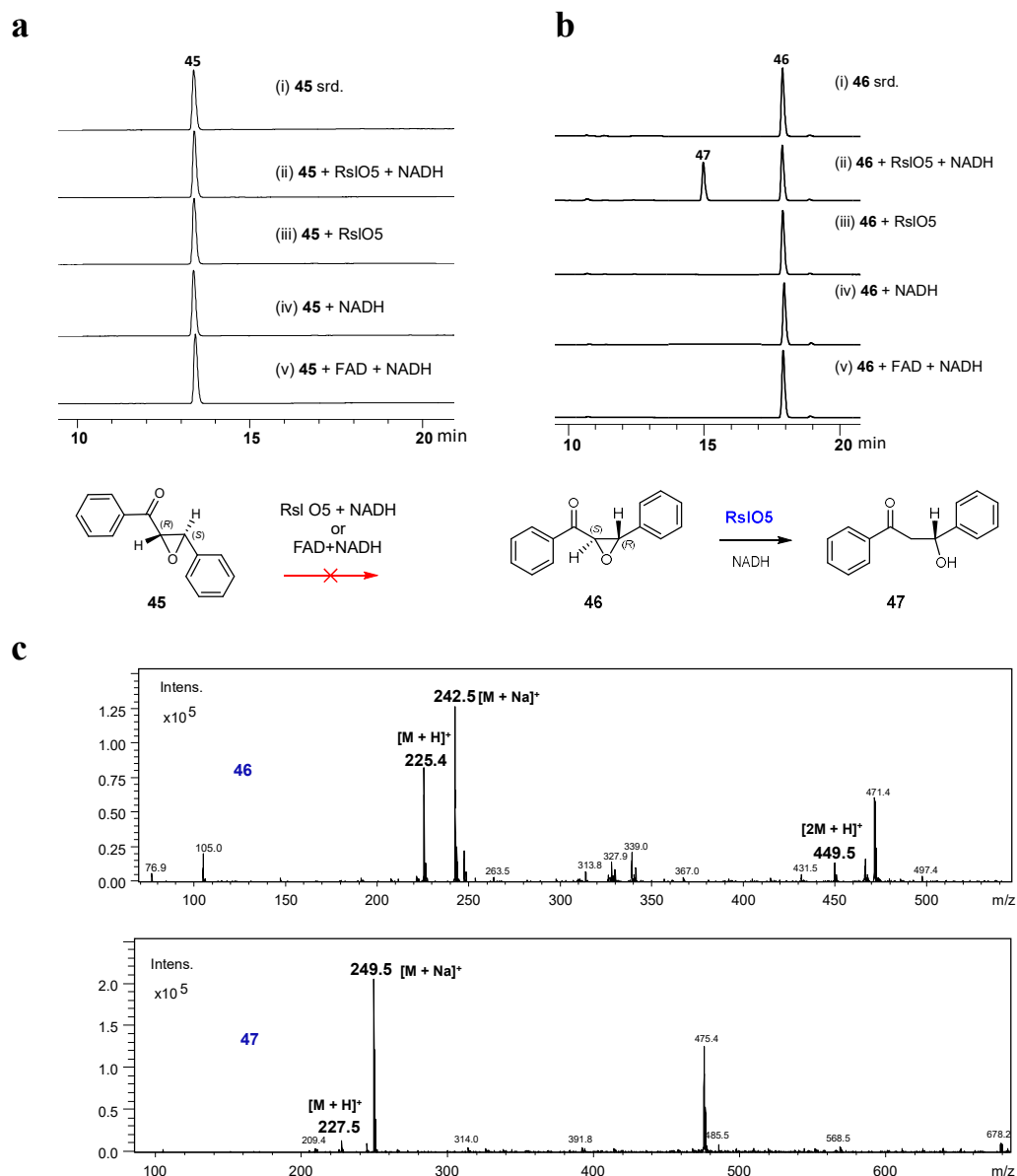
Supplementary Fig. 53 Spectroscopic data for 41. (f) The HMBC and enlarged HMBC spectrum of **41** in DMSO-*d*₆.



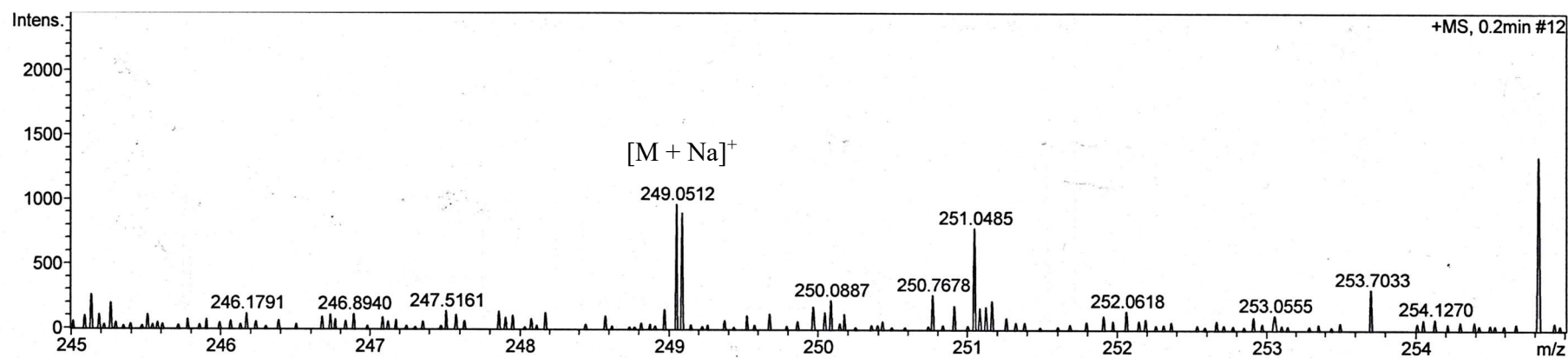
Supplementary Fig. 54. TLC profile for the reaction, UV spectra, and Optical rotation of vitamin K1 2,3-epoxide (42**) with FAD/NADH.** (a) TLC analysis of the reaction mixtures containing 100 μ M **42**, 100 μ M FAD and 10 mM NADH in 50 mM PBS buffer (pH 7). (b) UV spectra of 100 μ M **42** (substrate) and 100 μ M **42** (after reaction with FAD and NADH, isolated from preparative TLC). (c) Optical rotation data of **42** (substrate) and 100 μ M **42** (after reaction). The reaction mixtures were incubated at 30 $^{\circ}$ C for 2 h. After ethyl acetate extraction, the crude extract was dissolved in MeOH. TLC condition: petroleum ether (P): ethyl acetate (E) (75:25).



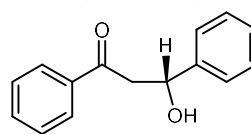
Supplementary Fig. 55. Structure features of substrated for FAD/NADH-mediated epoxide ring opening reactions. Features of substrates used for the FAD/NADH-mediated epoxide ring opening reaction. **(a)** A neighboring carbonyl group to the epoxide is absent. **(b)** An aromatic ring nearby is absent. **(c)** Substrates featuring both carbonyl and aromatic ring. **(d).** Substrates featuring both carbonyl and aromatic ring and presence of a long side chain next to the epoxide.



Supplementary Fig. 56. HPLC analysis of the reactions of *trans*-1,3-diphenyl-2,3-epoxypropan-1-one (45**) and chalcone α,β -epoxide (**46**) reaction with RslO5. (a) HPLC analysis of reaction mixture containing (i) **45** std.; (ii) 100 μ M **45** + 10 μ M RslO5 and 5 mM NADH; (iii) **45** + RslO5; (iv) **45** + NADH; (v) **45** + FAD + NADH; in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 2 h. (b) HPLC analysis of reaction mixture containing (i) **46** std.; (ii) 100 μ M **46** + 10 μ M RslO5 and 5 mM NADH; (iii) **46** + RslO5; (iv) **46** + NADH; (v) **46** + FAD + NADH; in 50 mM PBS buffer (pH 7). The reaction mixtures were incubated at 30 °C for 2 h. (c) LC-ESI-MS of **46** and **47**.**



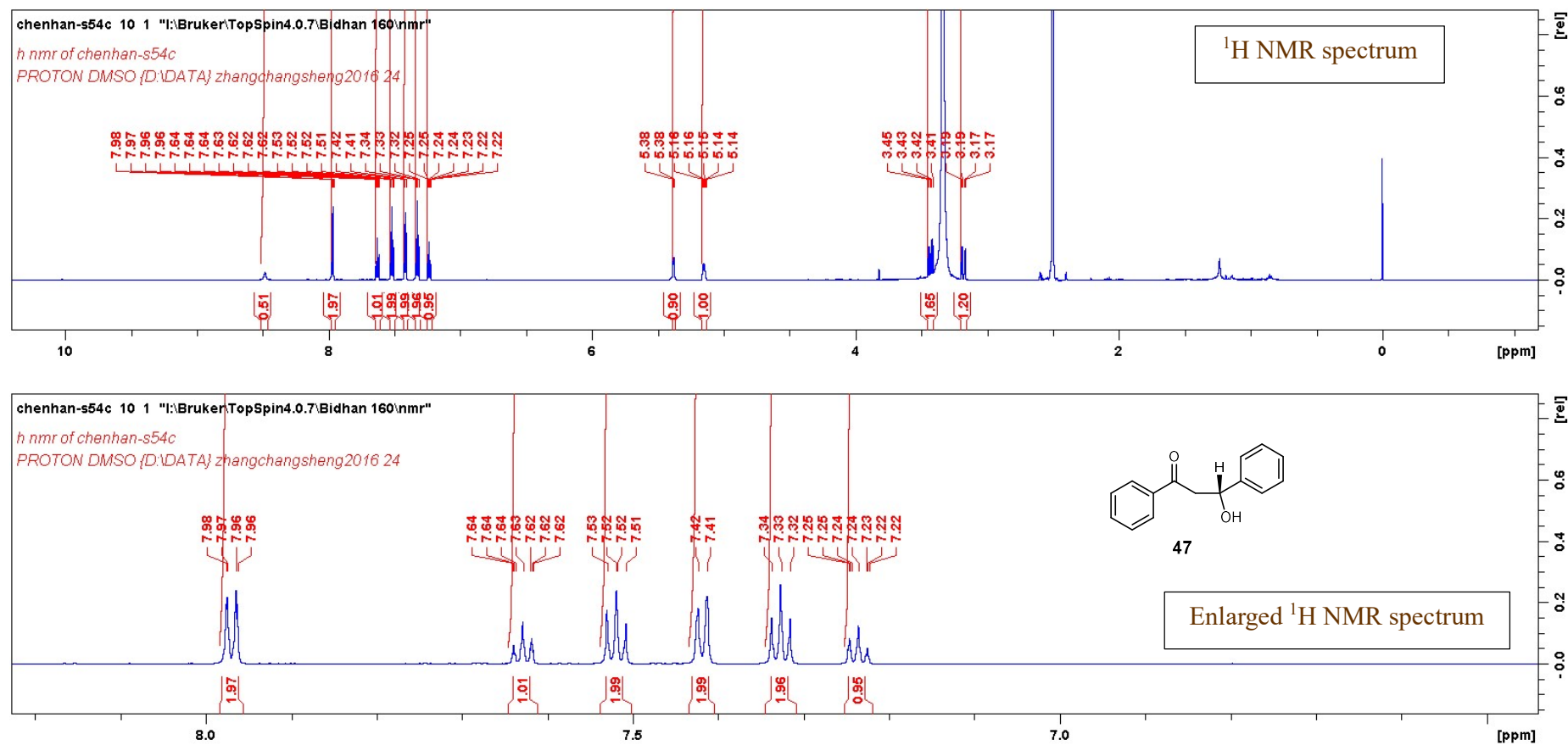
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e ⁻ Conf	N-Rule
249.0894	1	C15H14NaO2	-1.#J	249.0886	-3.3	-0.8	n.a.	8.5	even	ok



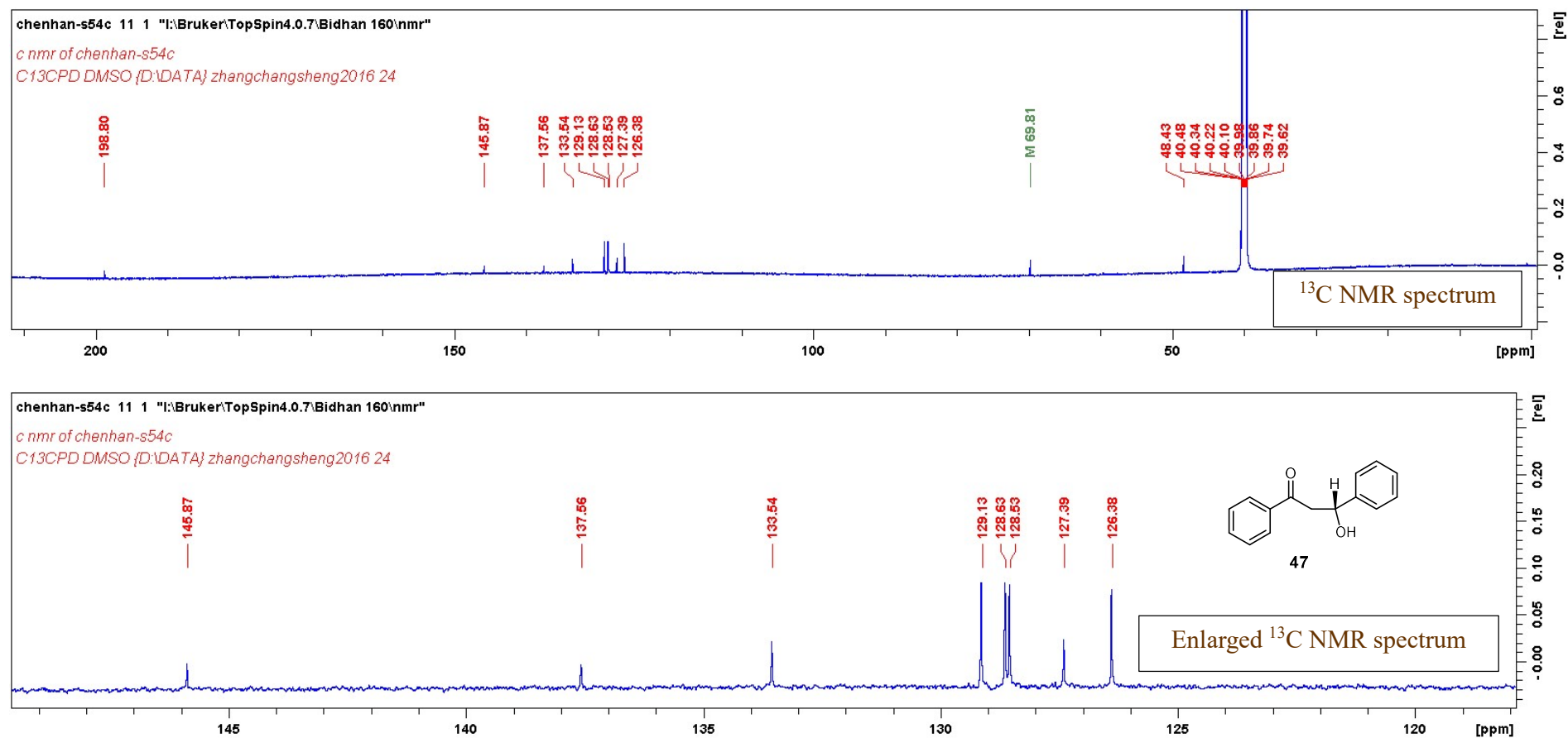
47

Chemical Formula: C₁₅H₁₄O₂
Exact Mass: 226.10

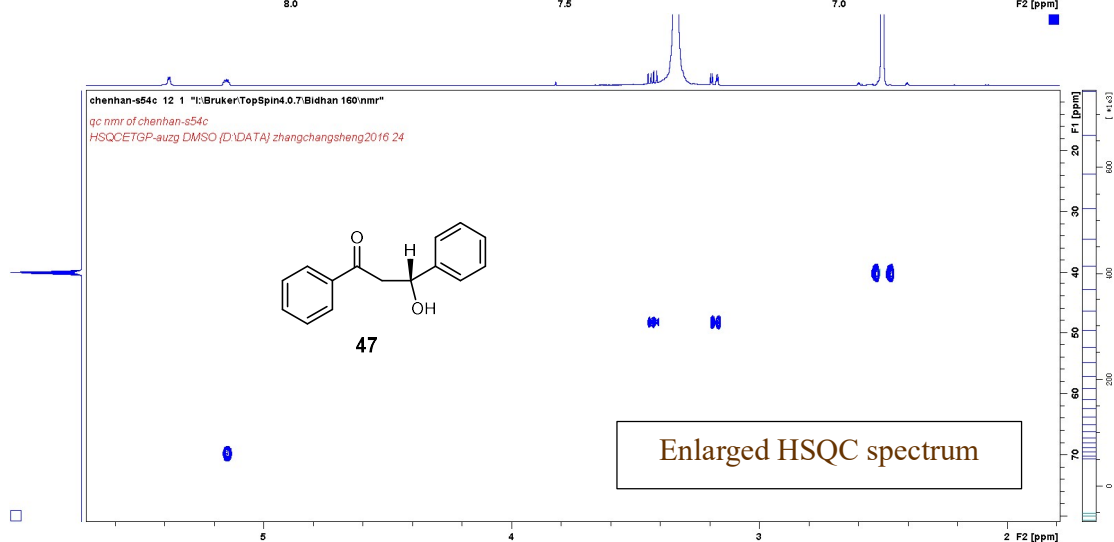
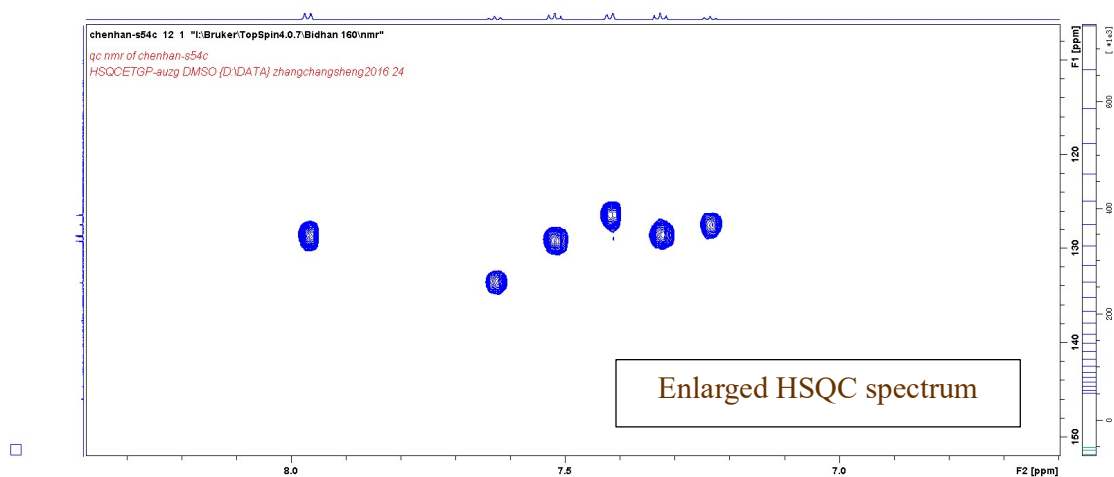
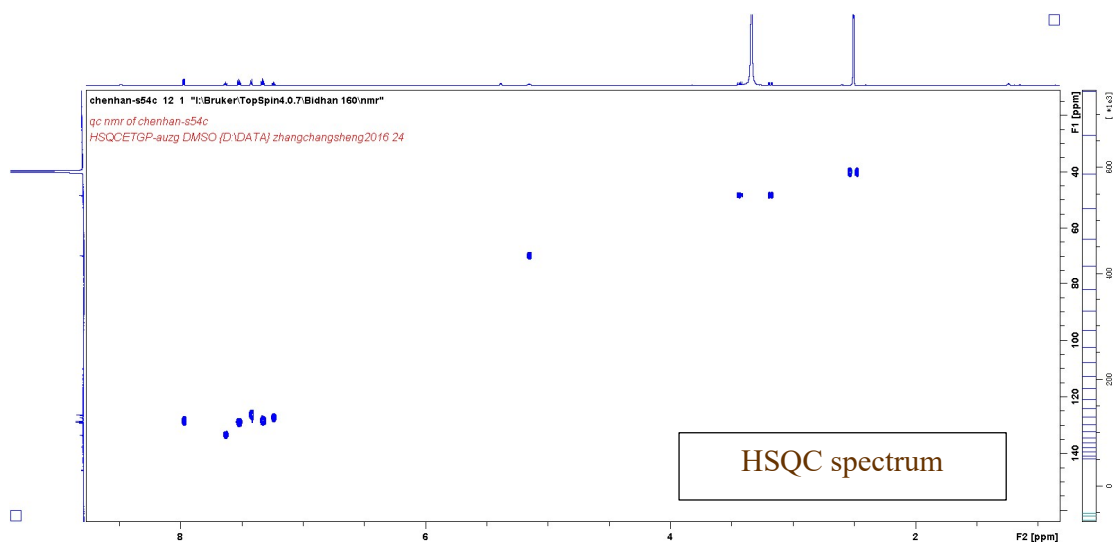
Supplementary Fig. 57. Spectroscopic data for 47. (a) HRESIMS spectrum.



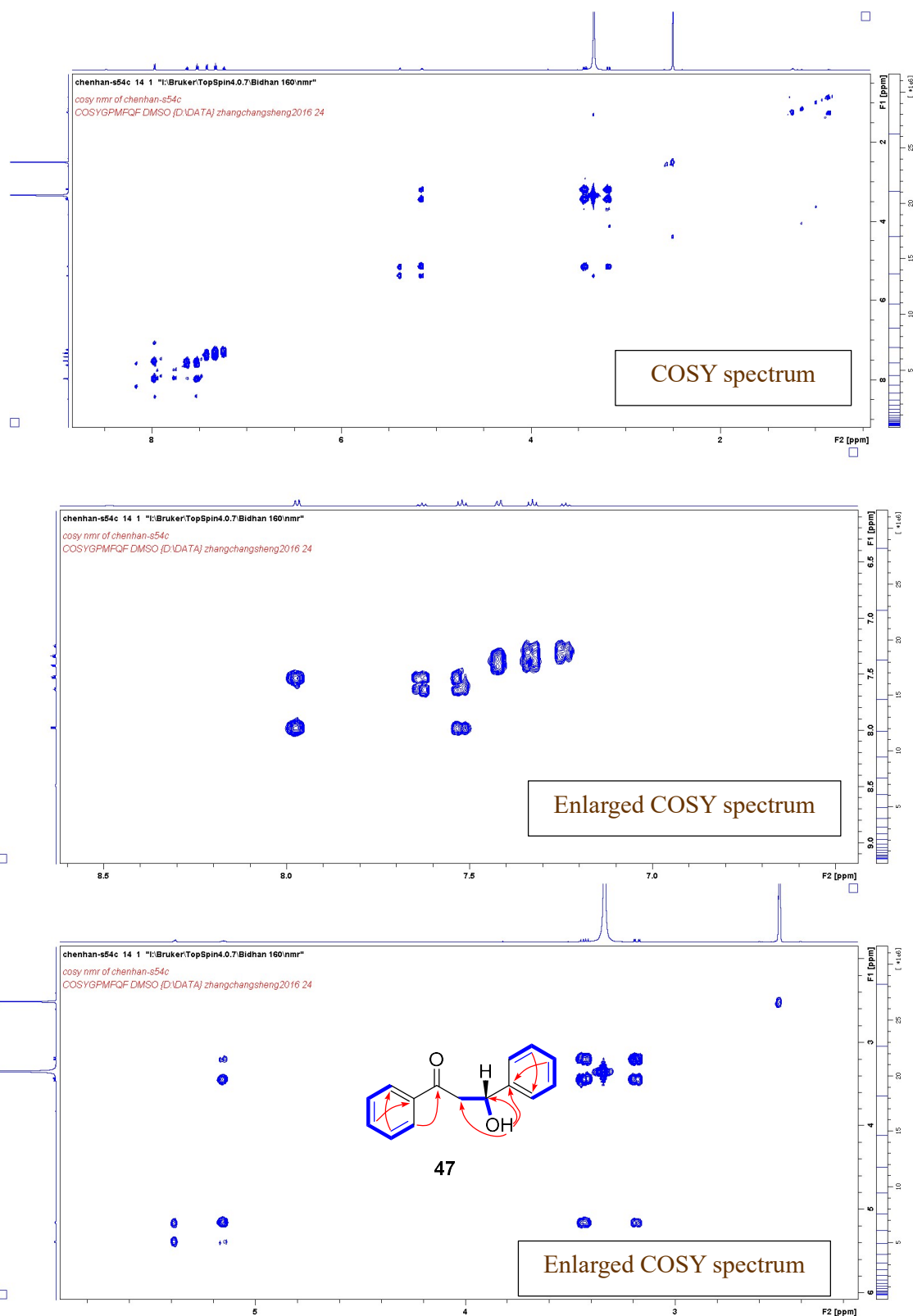
Supplementary Fig. S7. Spectroscopic data for 47. (b) The ^1H and enlarged ^1H NMR spectrum of **47** in $\text{DMSO}-d_6$.



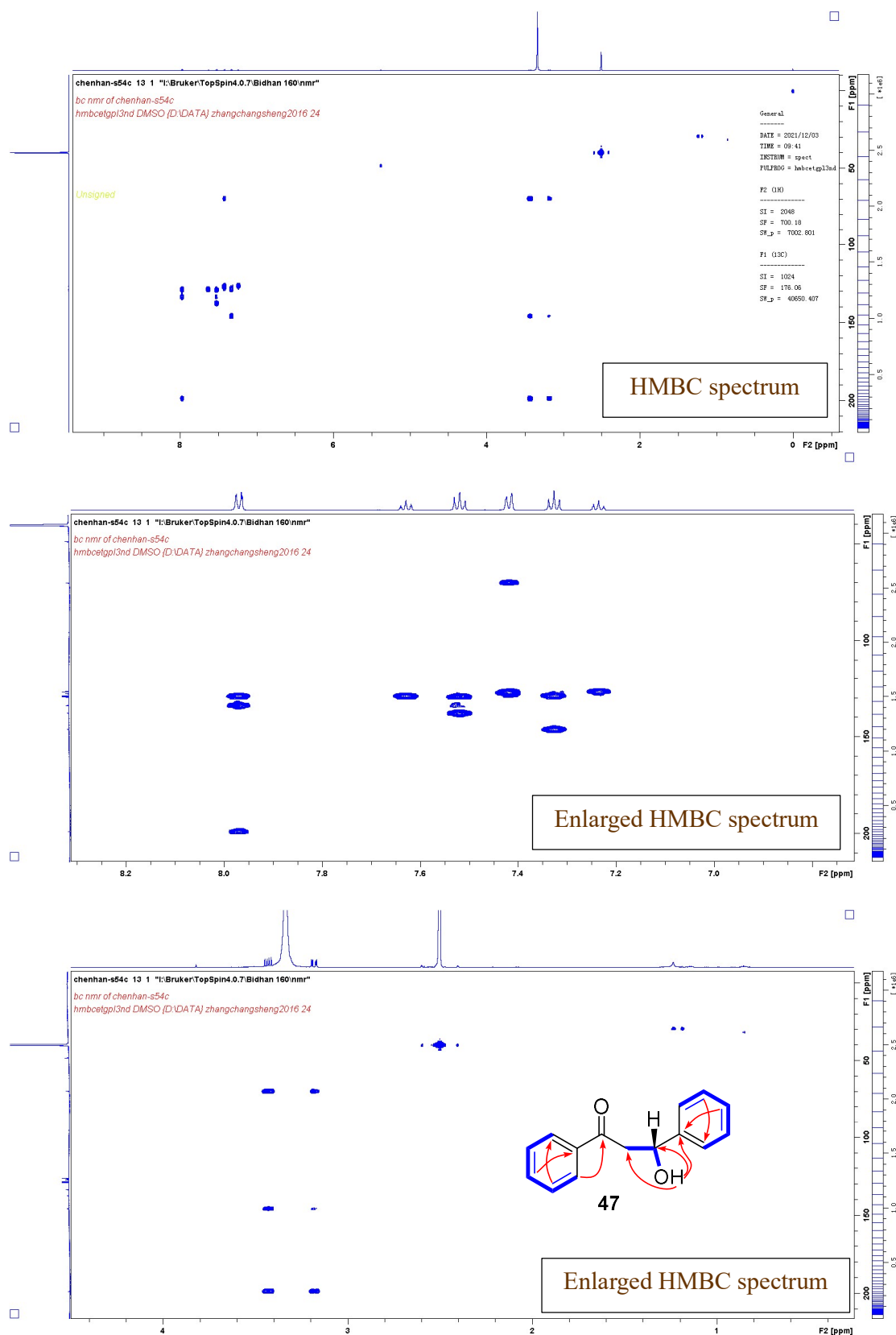
Supplementary Fig. S57. Spectroscopic data for 47. (c) The ¹³C and enlarged ¹³C NMR spectrum of 47 in DMSO-*d*₆.



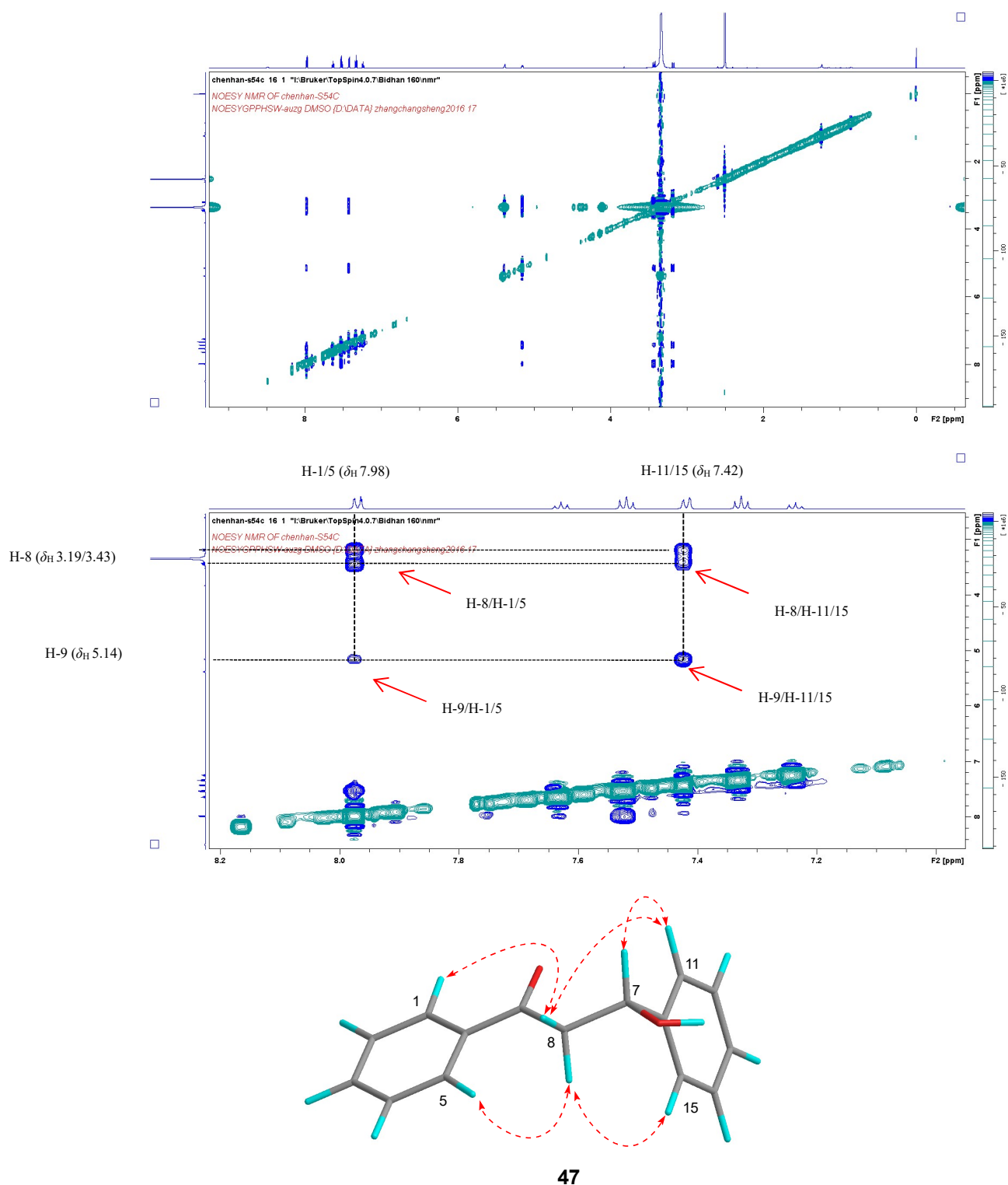
Supplementary Fig. S7. Spectroscopic data for 47. (d) The HSQC and enlarged HSQC spectrum of 47 in DMSO- d_6 .



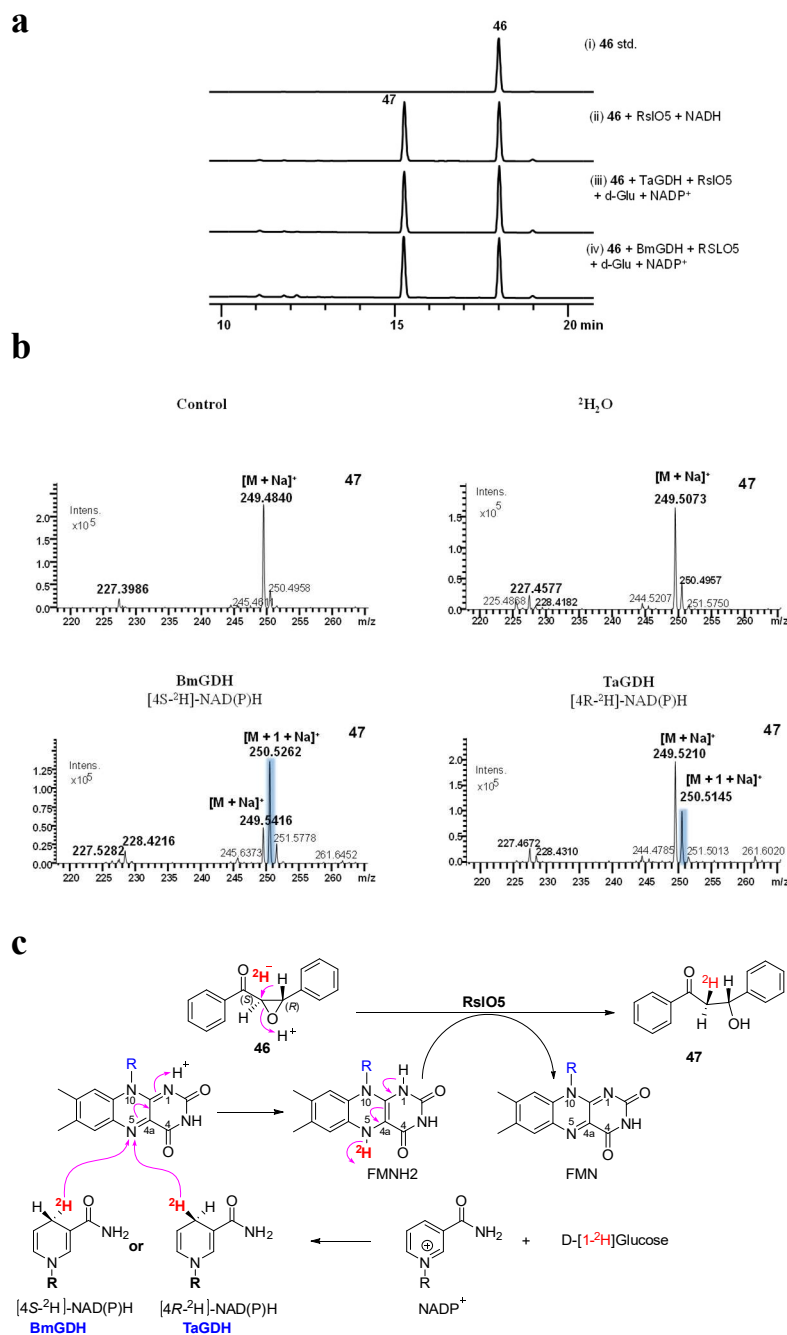
Supplementary Fig. S7. Spectroscopic data for **47. (e) The COSY and enlarged COSY spectrum of **47** in DMSO-*d*₆.**



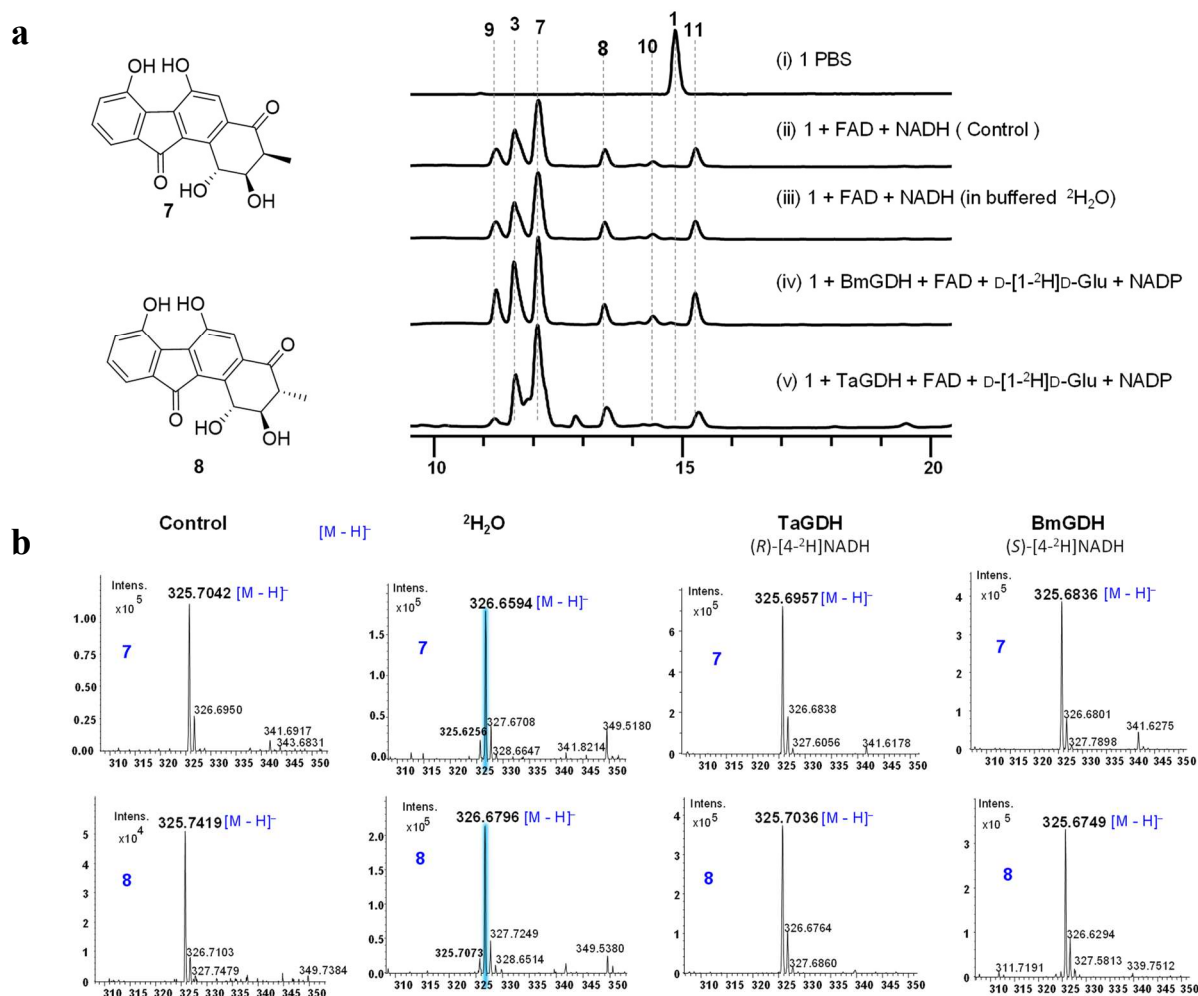
Supplementary Fig. S7. Spectroscopic data for 47. (f) The HMBC and enlarged HMBC spectrum of 47 in DMSO-*d*₆.



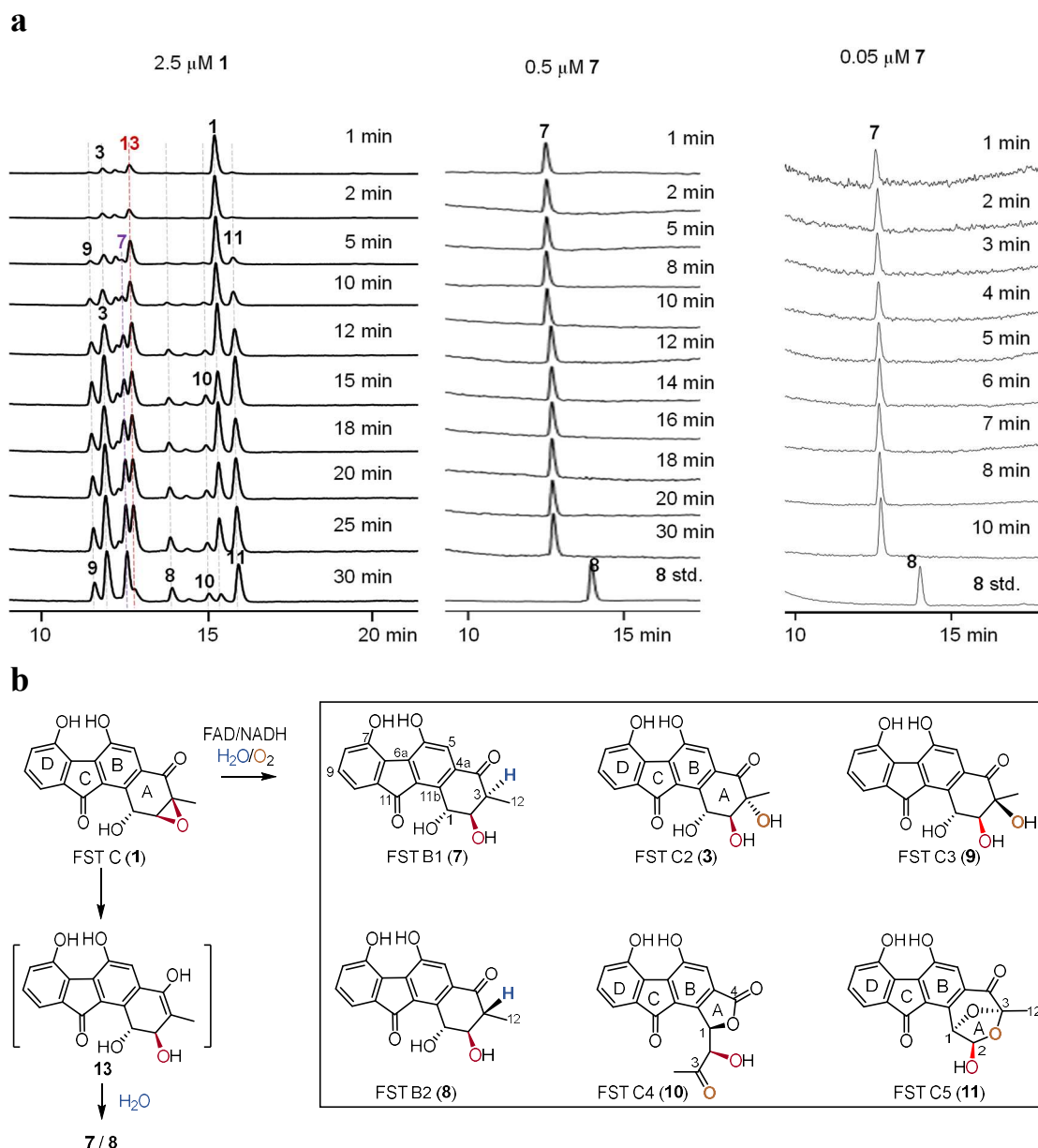
Supplementary Fig. 57. Spectroscopic data for 47. (g) The NOESY and enlarged NOESY spectrum of 47 in DMSO- d_6 .



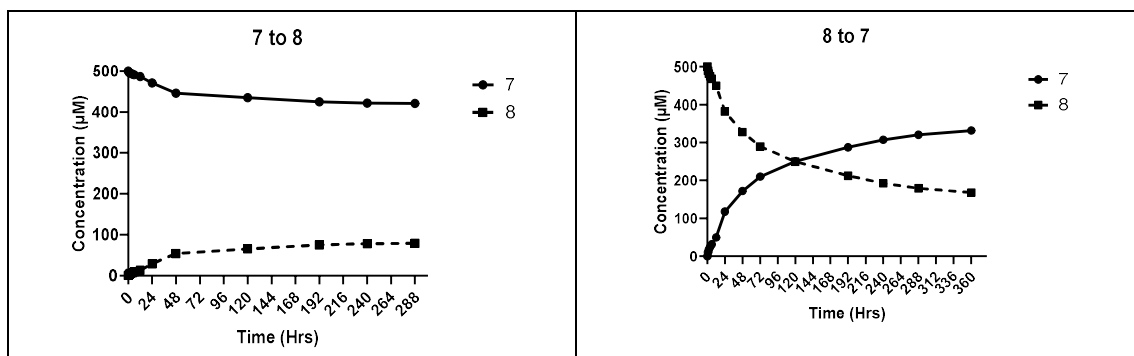
Supplementary Fig. 58. HPLC and LC-HRMS analysis of the reactions of 46 with RslO5 in coupling with GDHs. (a) HPLC analysis of RslO5 reactions. (i) The standard compound 46; (ii) 46 + RslO5 + NADH reaction in PBS (pH 7) or PBS- $^2\text{H}_2\text{O}$; (iii) 46 + RslO5 + TaGDH + 2.5 mM NADP⁺, and D-[1- ^2H]glucose; iv) 46 + RslO5 + BmGDH + 2.5 mM NADP⁺, and D-[1- ^2H]glucose. (b) LC-HRMS analysis for the product 47 in 50 mM PBS buffer (pH 7.0), or 50 mM PBS buffer (pH 7) prepared with $^2\text{H}_2\text{O}$, [4S- ^2H]-NAD(P)H, [4R- ^2H]-NAD(P)H. (c) Schematic representation of a proposed reaction mechanism of RslO5 catalysis.



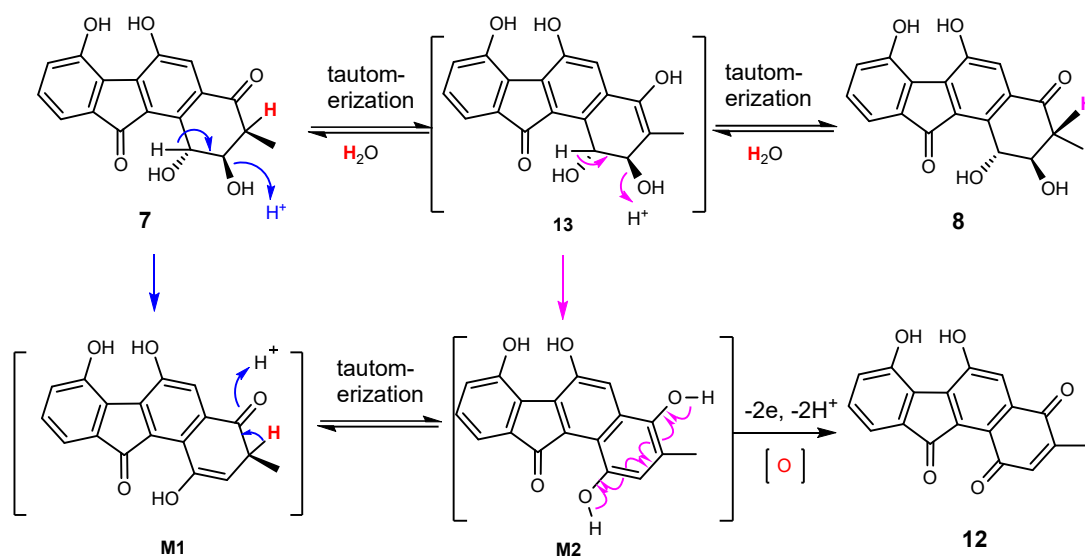
Supplementary Fig. 59. HPLC and LC-HRMS analysis of the reactions of 1 with FAD/NAD(P)H in the coupled reactions with GDHs. (a) HPLC analysis of reactions of 1 and FAD coupled with GDH system. (i) 1 std.; (ii) 100 μM 1 + 100 μM FAD + 2 mM NADH in 50 mM PBS buffer (pH 7); (iii) 100 μM 1 + 100 μM FAD + 2 mM NADH in 50 mM PBS buffer (pH 7) prepared with $^2\text{H}_2\text{O}$; (iv) 100 μM 1 + 100 μM FAD + 10 μM BmGDH + 2.5 mM NADP, and 100 mM D-[1- ^2H]glucose; (v) 100 μM 1 + 100 μM FAD + 10 μM TaGDH + 2.5 mM NADP, and 100 mM D-[1- ^2H]glucose. (b) LC-HRMS analysis for the product 7 and 8 in 50 mM PBS buffer (pH 7), or 50 mM PBS buffer (pH 7.0) prepared with $^2\text{H}_2\text{O}$, (R)-[4- ^2H]NADH, (S)-[4- ^2H]NADH.



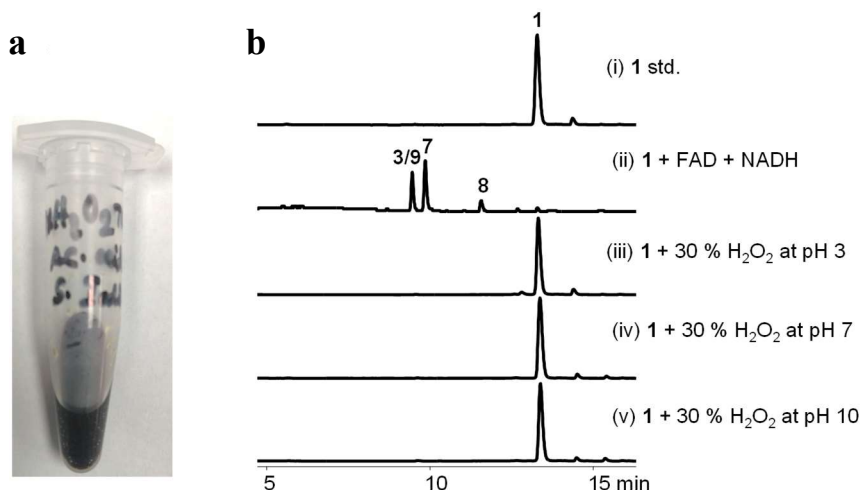
Supplementary Fig. 60. The time course assay of reduced concentration of 1 and 7 with FAD and NADH. (a) HPLC analysis of the time course assays of 1 or 7 with FAD and NADH. The standard assay of 1 contains 2.5 μM 1, 10 μM FAD, 2 mM NADH in 50 mM PBS buffer (pH 7), with incubation at 30 $^{\circ}\text{C}$ and sampling at 1, 2, 5, 10, 12, 15, 18, 20, 25 and 30 min. The assay of 7 contains 0.5 μM 7, 10 μM FAD, 2 mM NADH in 50 mM PBS buffer (pH 7), with incubation at 30 $^{\circ}\text{C}$ and sampling at 1, 2, 5, 8, 10, 12, 14, 16, 18, 20 and 30 min; or the assay contains 0.05 μM 7, 10 μM FAD, 2 mM NADH in 50 mM PBS buffer (pH 7), with incubation at 30 $^{\circ}\text{C}$ and sampling at 1, 2, 3, 4, 5, 6, 7, 8 and 10 min. HPLC analysis was performed on the Agilent 1260 Infinity series instrument, using a polar column (Comixsep[®], P/N FMG-BPF5-EONU, Polar BiPFP 5u, 250 $\times$ 4.6 mm, China) with UV detection at 304 nm. (b) A schematic representation of the reactions. It was clearly demonstrated that the putative intermediate 13 in reactions of 1 and FAD/NADH should appear before the formation of 7/8, and under the assay conditions, 7 kept unchanged and the formation of 13 from 7 was not observed in a short time.



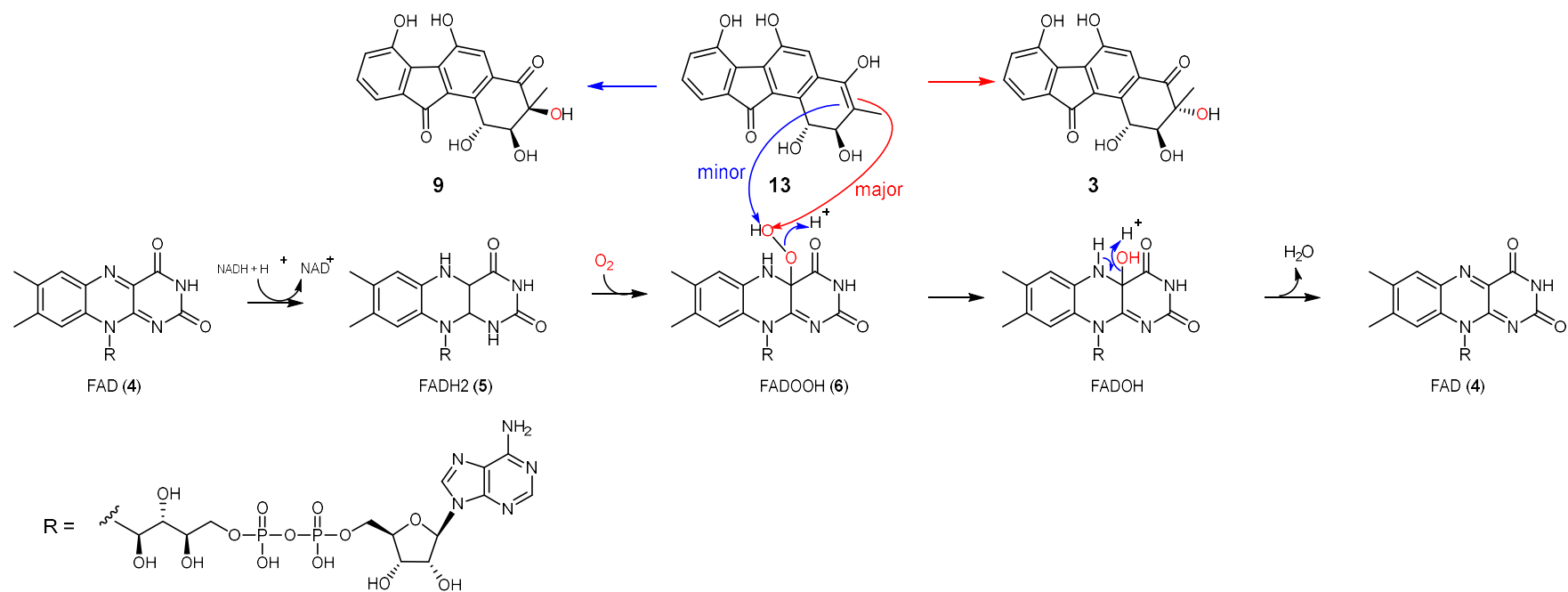
Supplementary Fig. 61. Determination of the equilibrium of the spontaneous conversion of 7 to 8 (or 8 to 7). The spontaneous assays were performed in 1 mL of 50 mM PBS buffer (pH 7.0) containing 500 μM 7 and incubated at 30 °C. 50 μL of samples were taken and stopped by addition of 50 μL of MeOH after 0, 1, 2, 4, 6, 12, 24, 48, 120, 192, 240, 288 h. The apparent $[7]_{eq}/[8]_{eq}$ was calculated as $(421.7/78.9) = 5.4$.



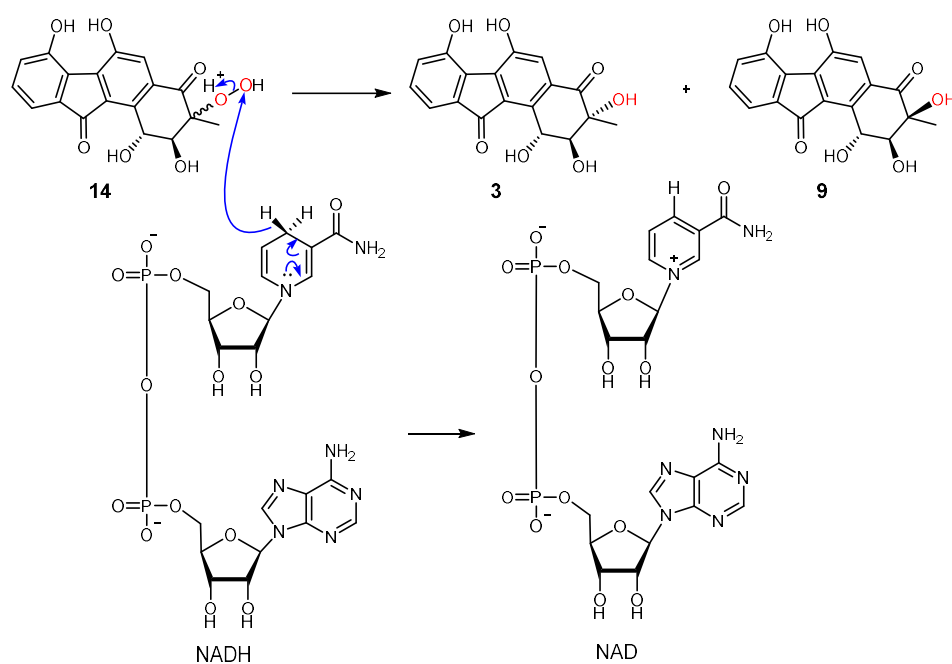
Supplementary Fig. S62. The proposed mechanism for the formation of 12 from 13 and 7.



Supplementary Fig. 63. HPLC analysis of reactions of 1 with H₂O₂. (a) The purity of H₂O₂ was checked by chemical method containing 100 μ L H₂O₂ solution with equal volume of glacial acetic acid containing about 0.1 g of sodium iodide. A brown color indicates the high concentration of peroxide. (b) HPLC analysis of reactions of **1**. (i) **1** std.; (ii) standard assay containing 100 μ M **1**, 100 μ M FAD and 10 mM NADH; (iii-v) **1** + 30 % H₂O₂ in buffers with pH 3, pH 7 and pH 10. The buffers include citric acid/Na₂HPO₄ buffer (50 mM, pH 3); PBS buffer (50 mM, pH 7); borax/NaOH buffer (50 mM, pH 10). The reaction mixtures were incubated at 30 °C for 2 h. HPLC was run using a reversed phase C18 column.



Supplementary Fig. 64. The proposed mechanism for the formation of 3 and 9 from 13.



Supplementary Fig. 65. The proposed mechanism for the formation of **3** and **9** from **14**.

Supplementary References

1. Zhang W, *et al.* Fluostatins I-K from the South China Sea-derived micromonospora rosaria SCSIO N160. *J Nat Prod* **75**, 1937-1943 (2012).
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4. Zhu Y, *et al.* Insights into Caerulomycin A Biosynthesis: A Two-Component Monooxygenase CrmH-Catalyzed Oxime Formation. *J Am Chem Soc* **135**, 18750-18753 (2013).
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