Supporting Information

Structure Determination, Functional Characterization and Biosynthetic Implications of Nybomycin Metabolites from a Mining Reclamation Site-Associated *Streptomyces*

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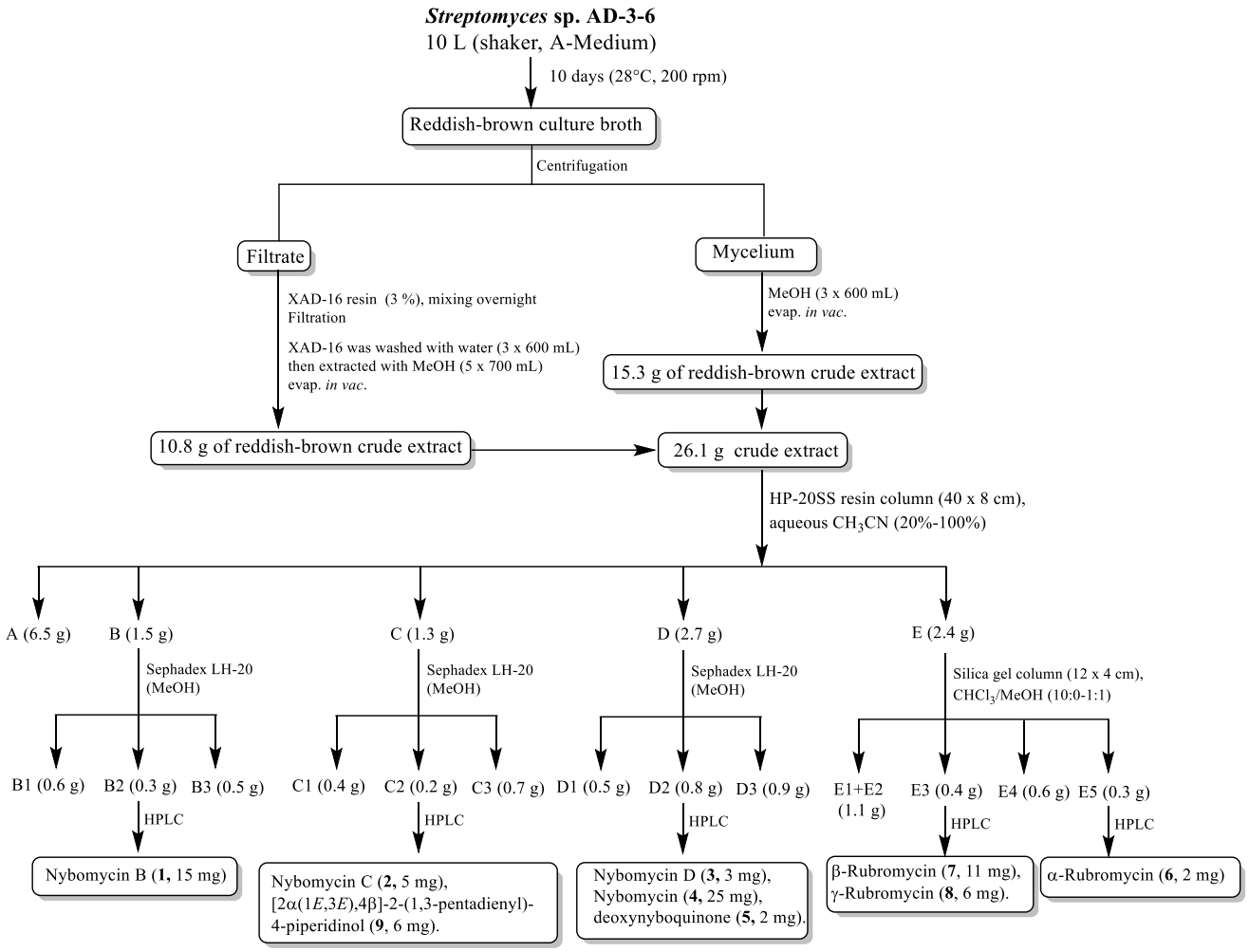

Scheme S1. Work-up scheme of the metabolites produced by *Streptomyces* sp. AD-3-6

Figure S1. Photograph of *Streptomyces* sp. AD-3-6 (7 day growth, M_2-agar).

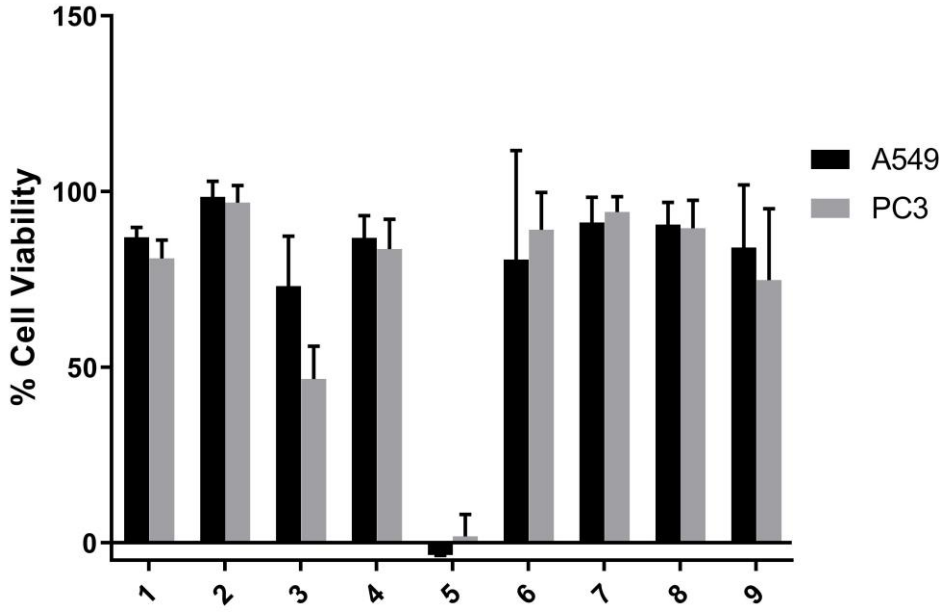

Figure S2. Viability of A549 (non-small cell lung cancer) and PC3 (prostate cancer) human cell lines at 1 μM of compounds **1-9**.

Figure S3. Single dose ETR assay of compounds **1**-**9** at 1 μM concentration. Control 0.1% DMSO showed no effect on regeneration (See manuscript file, figure 4).

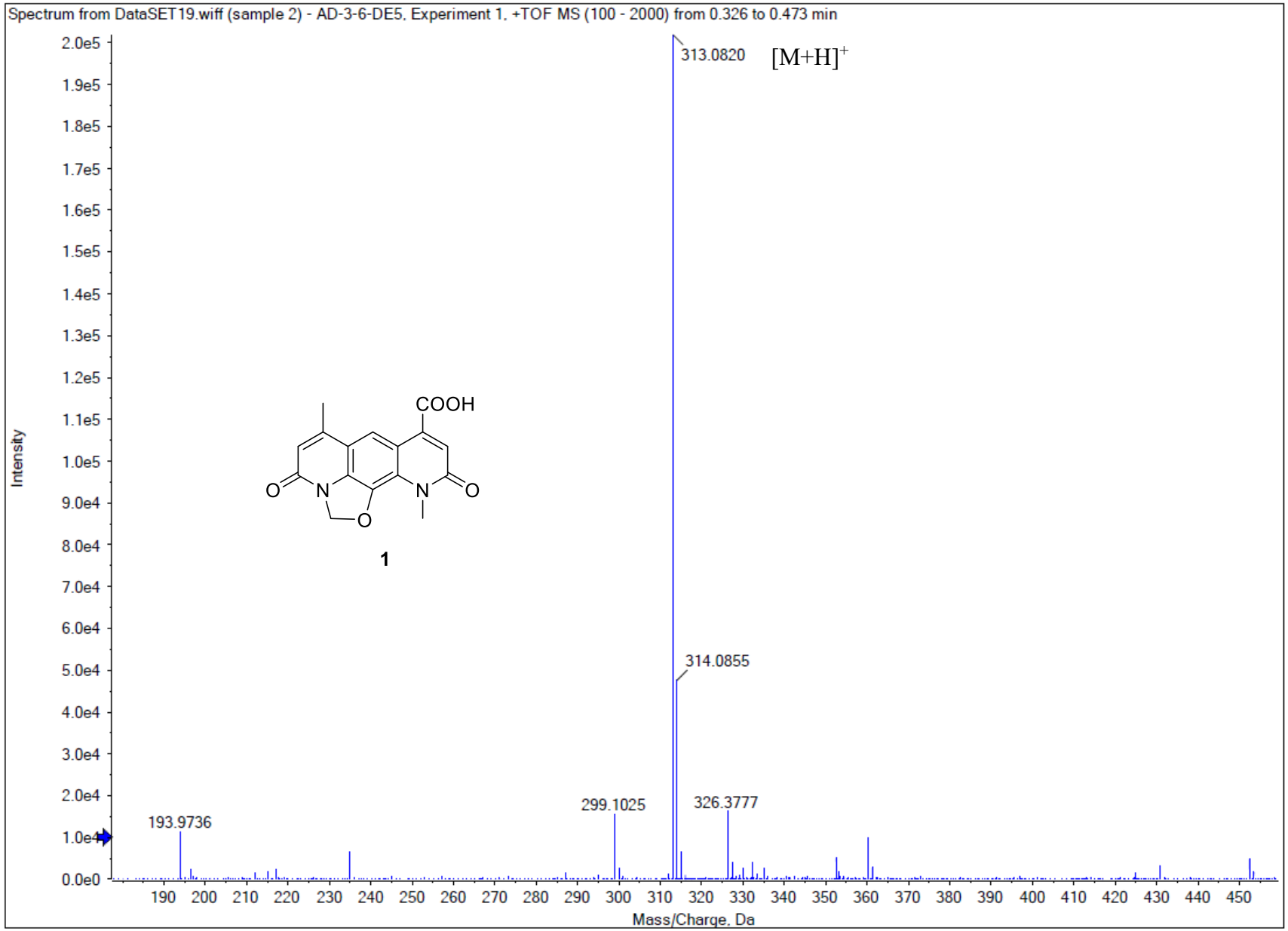

Figure S4. (+)-HRESIMS of nybomycin B (**1**)

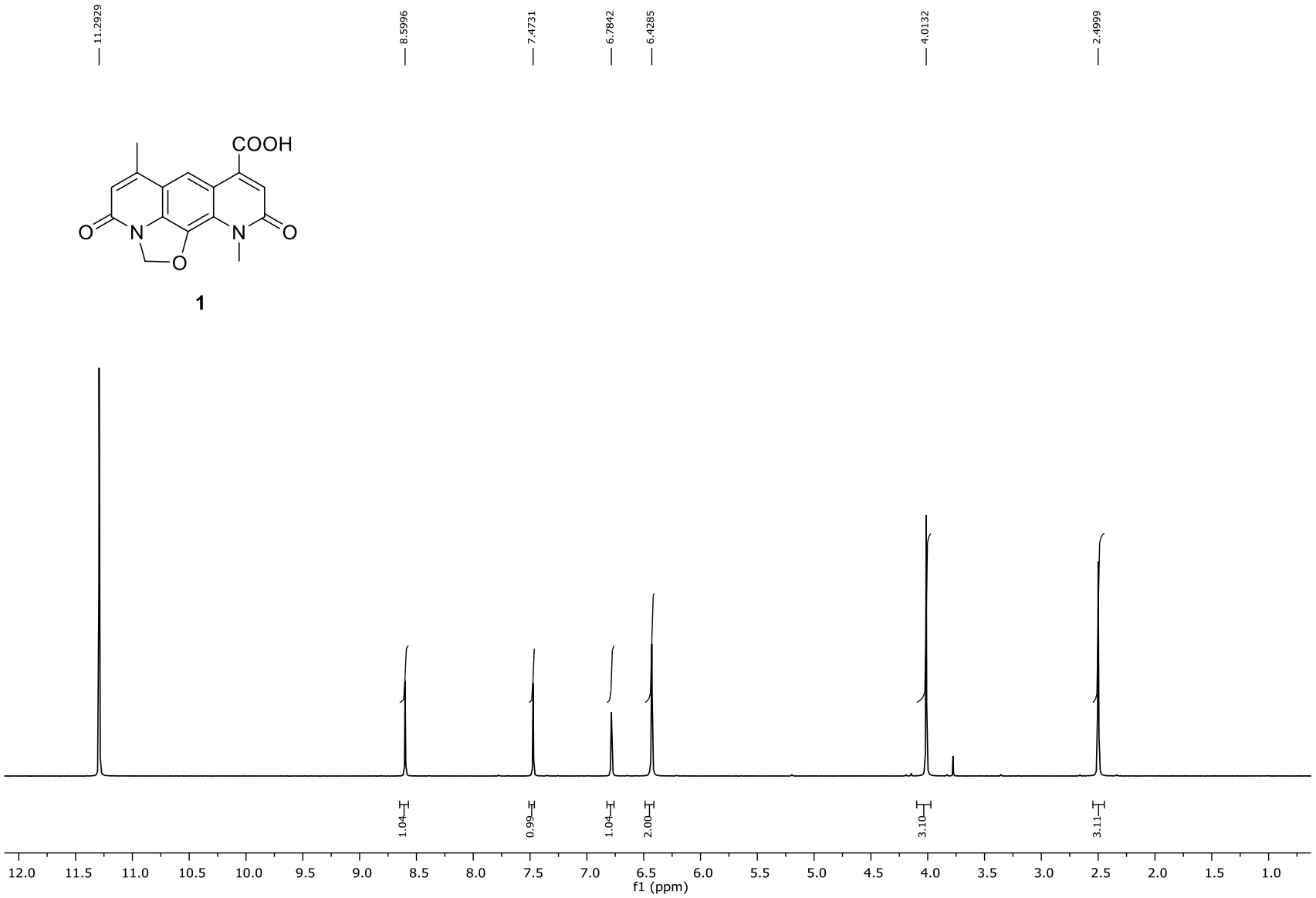

Figure S5. ^{1}H-NMR (CF_3COOD, 400 MHz) of nybomycin B (**1**)

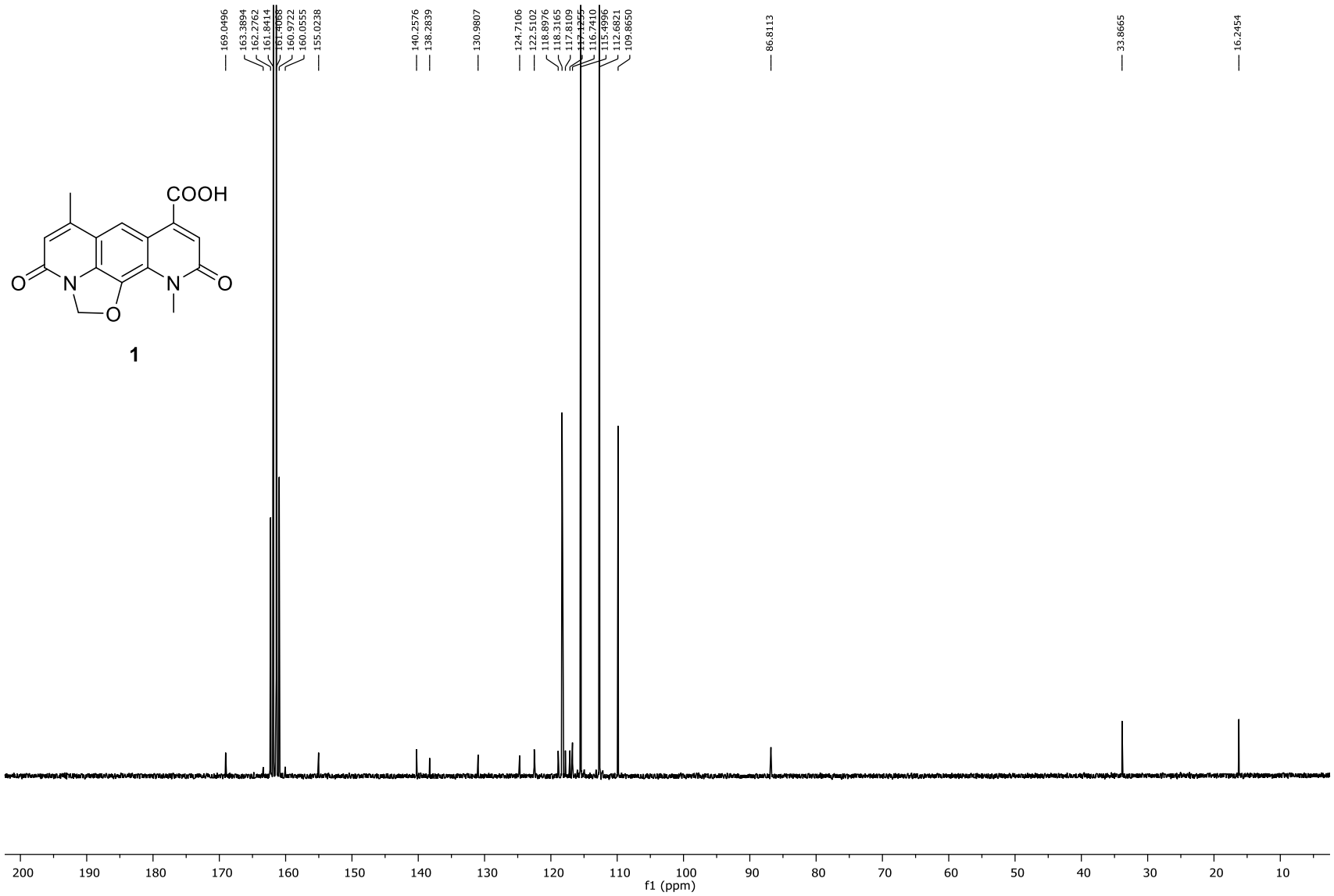

Figure S6. ^{13}C-NMR (CF_3COOD, 100 MHz) of nybomycin B (**1**)

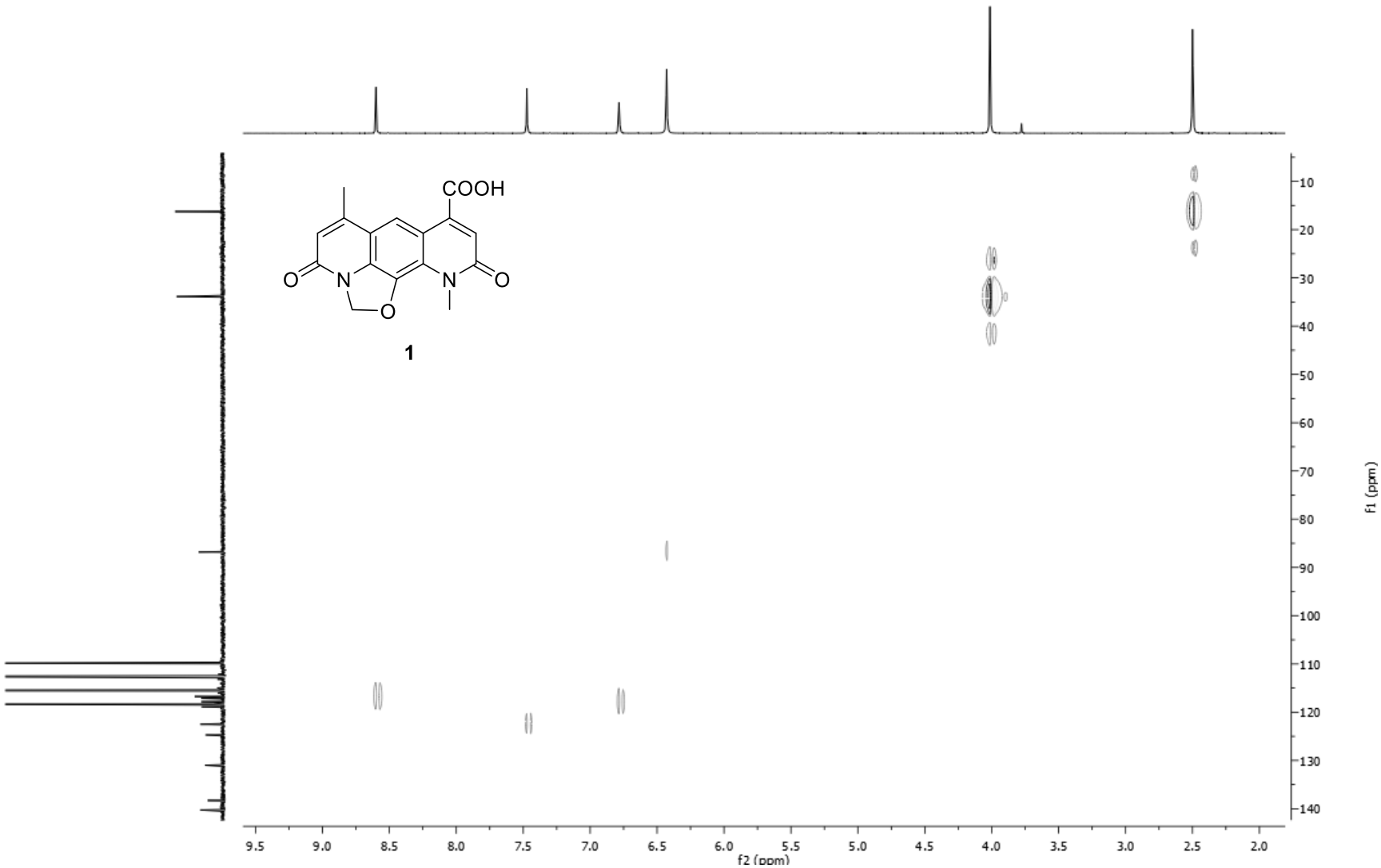

Figure S7. HSQC spectrum (CF_3COOD, 400 MHz) of nybomycin B (**1**)

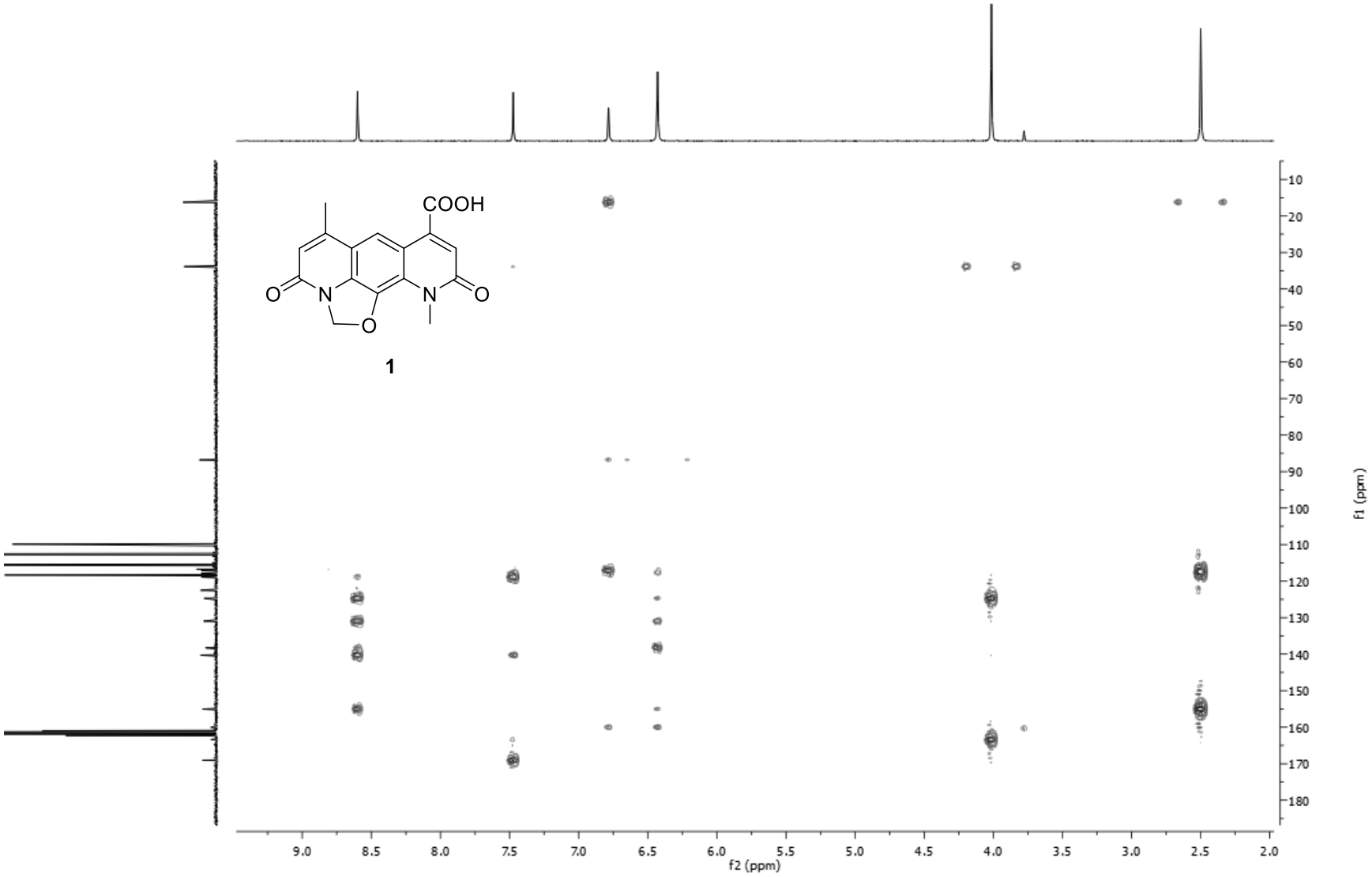

Figure S8. HMBC spectrum (CF_3COOD, 400 MHz) of nybomycin B (**1**)

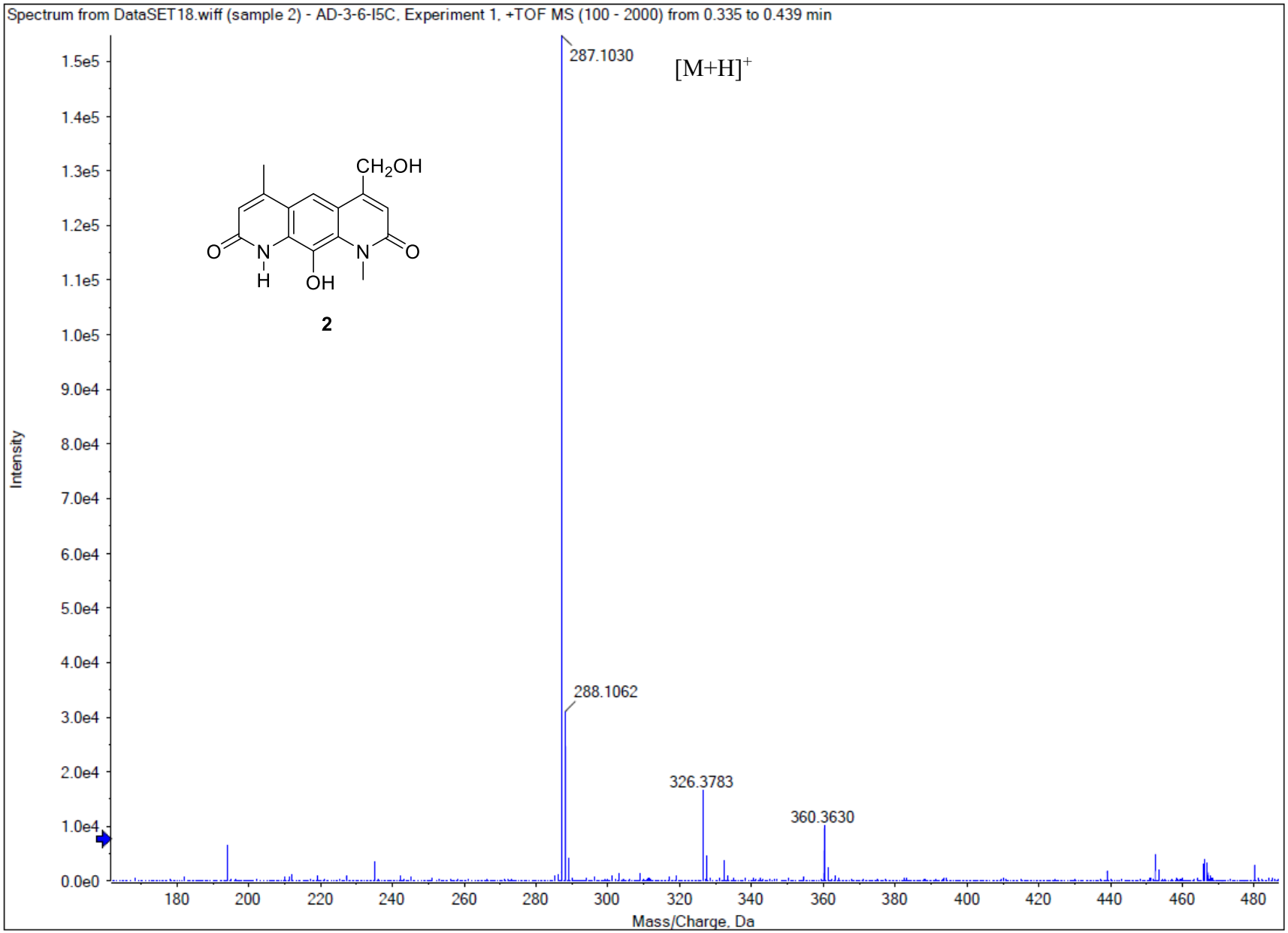

Figure S9. (+)-HRESIMS of nybomycin C (**2**)

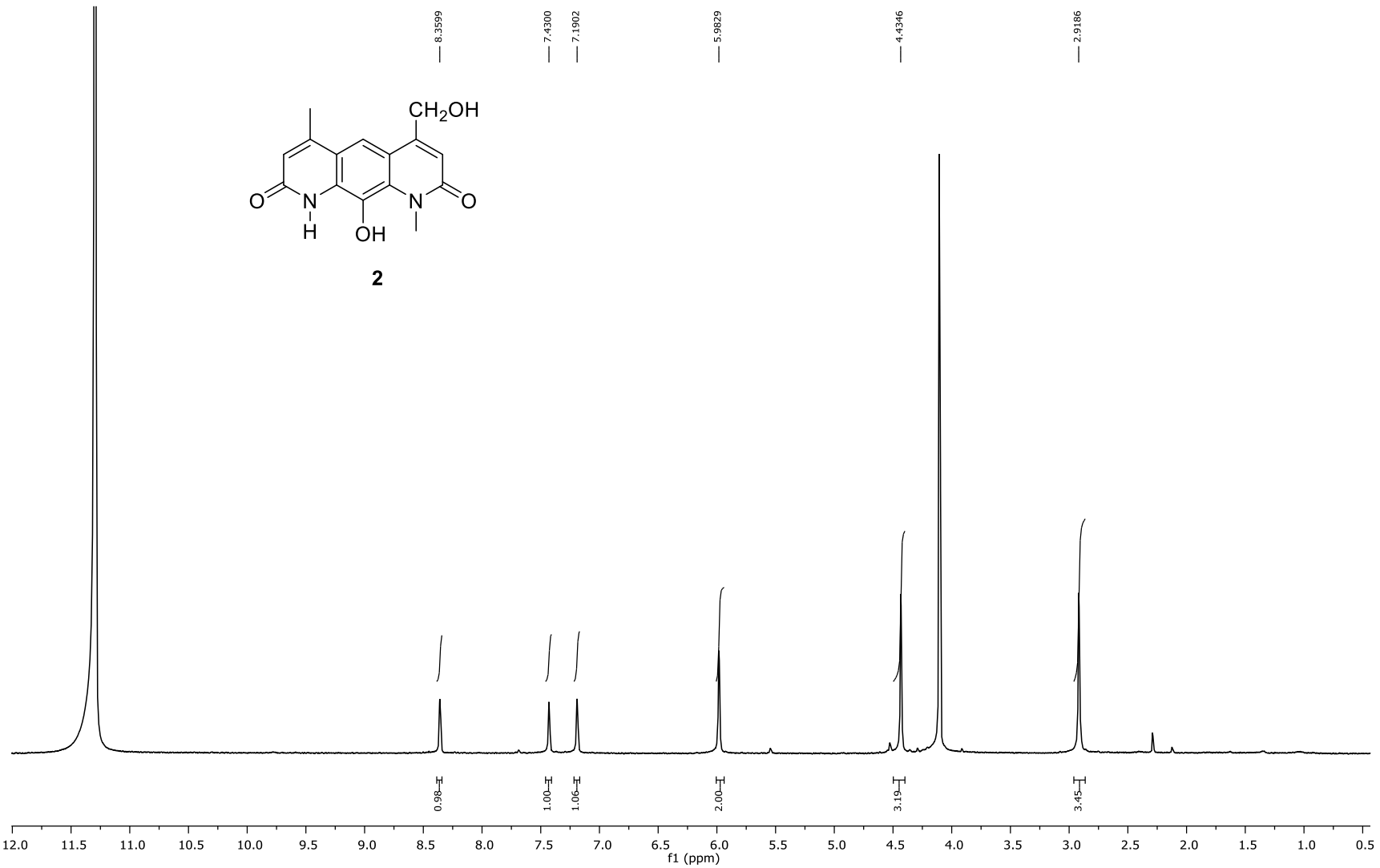

Figure S10. ^{1}H-NMR (CF_3COOD, 400 MHz) of nybomycin C (**2**)

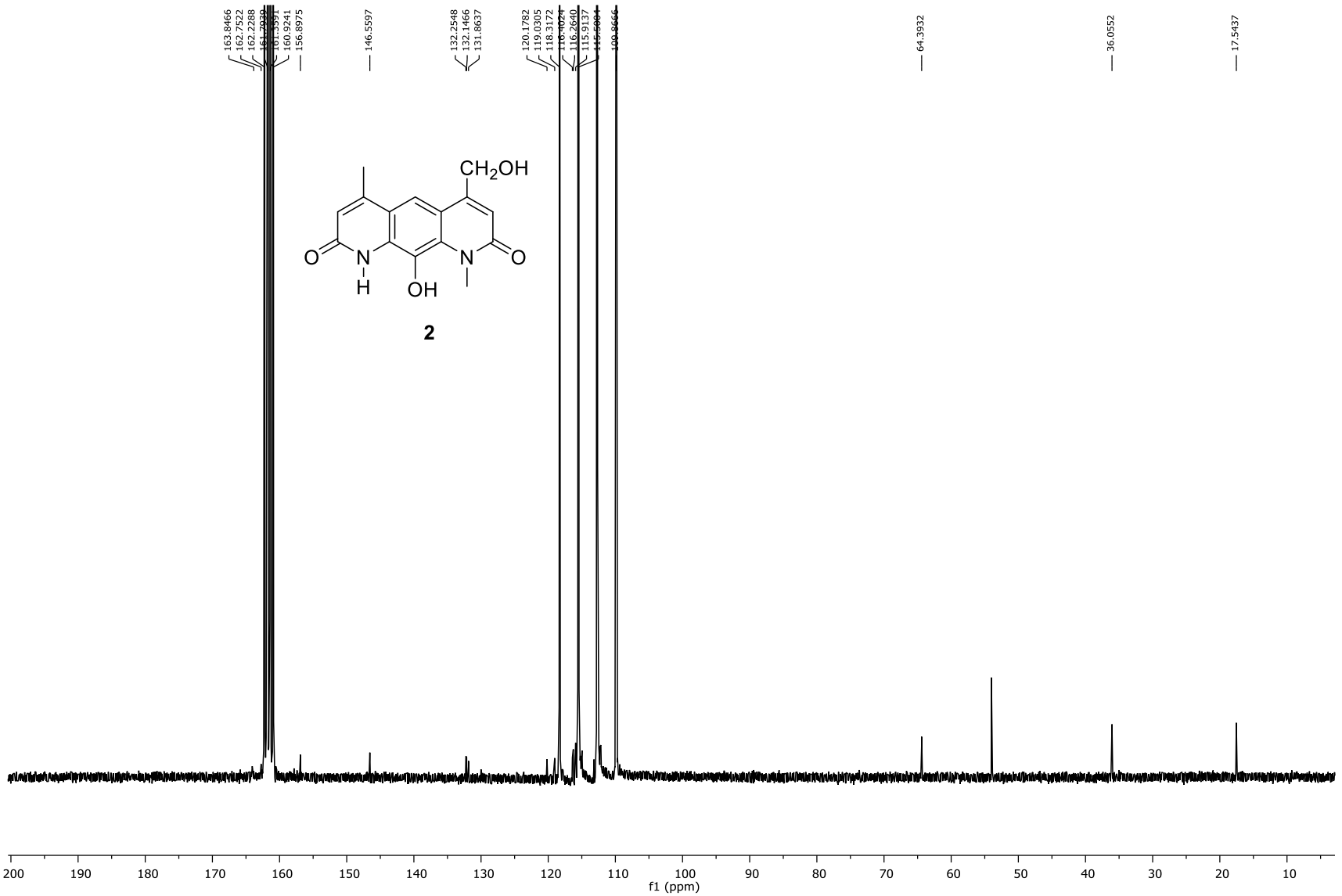

Figure S11. ^{13}C-NMR (CF_3COOD, 100 MHz) of nybomycin C (**2**)

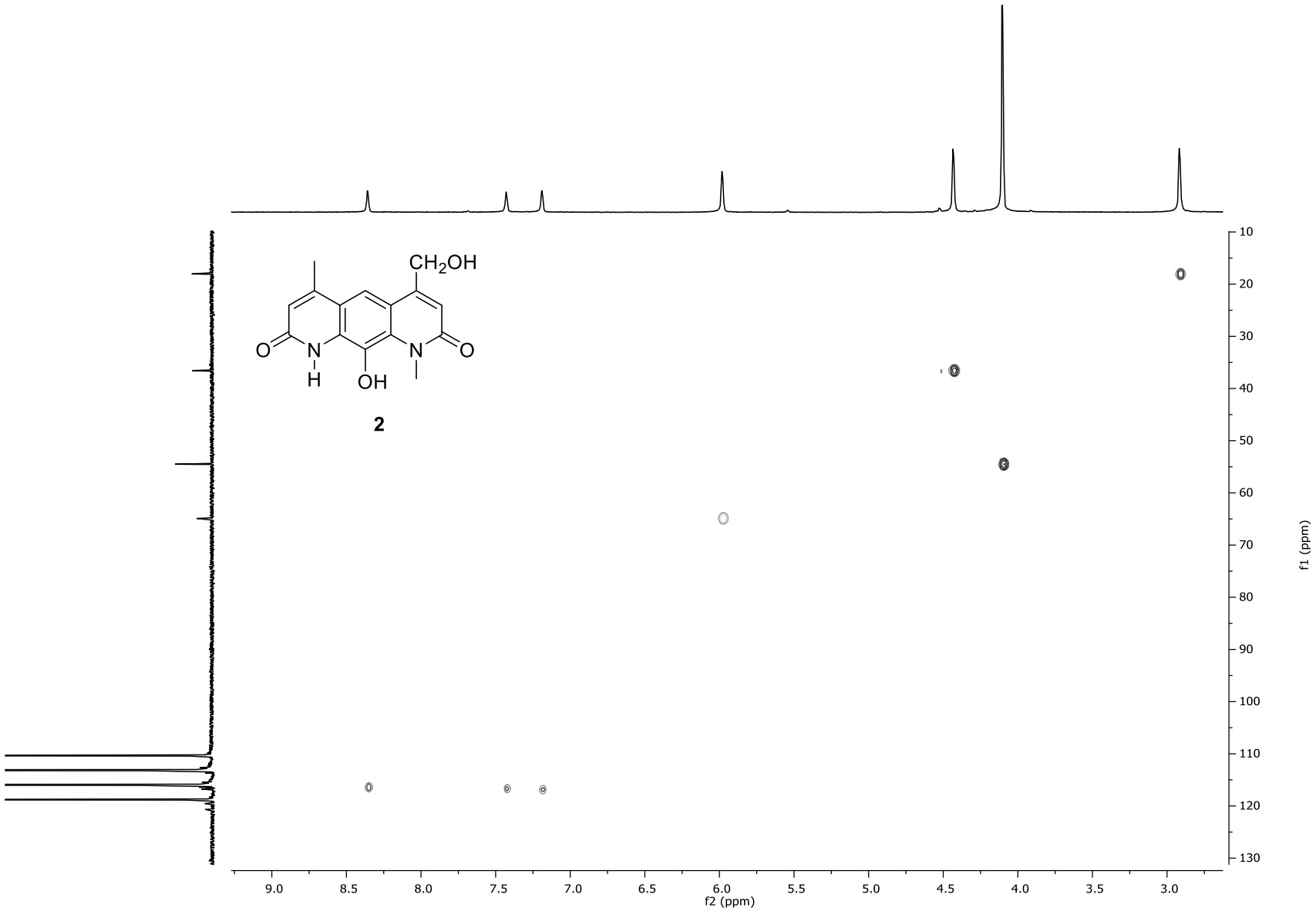

Figure S12. HSQC spectrum (CF_3COOD, 400 MHz) of nybomycin C (**2**)

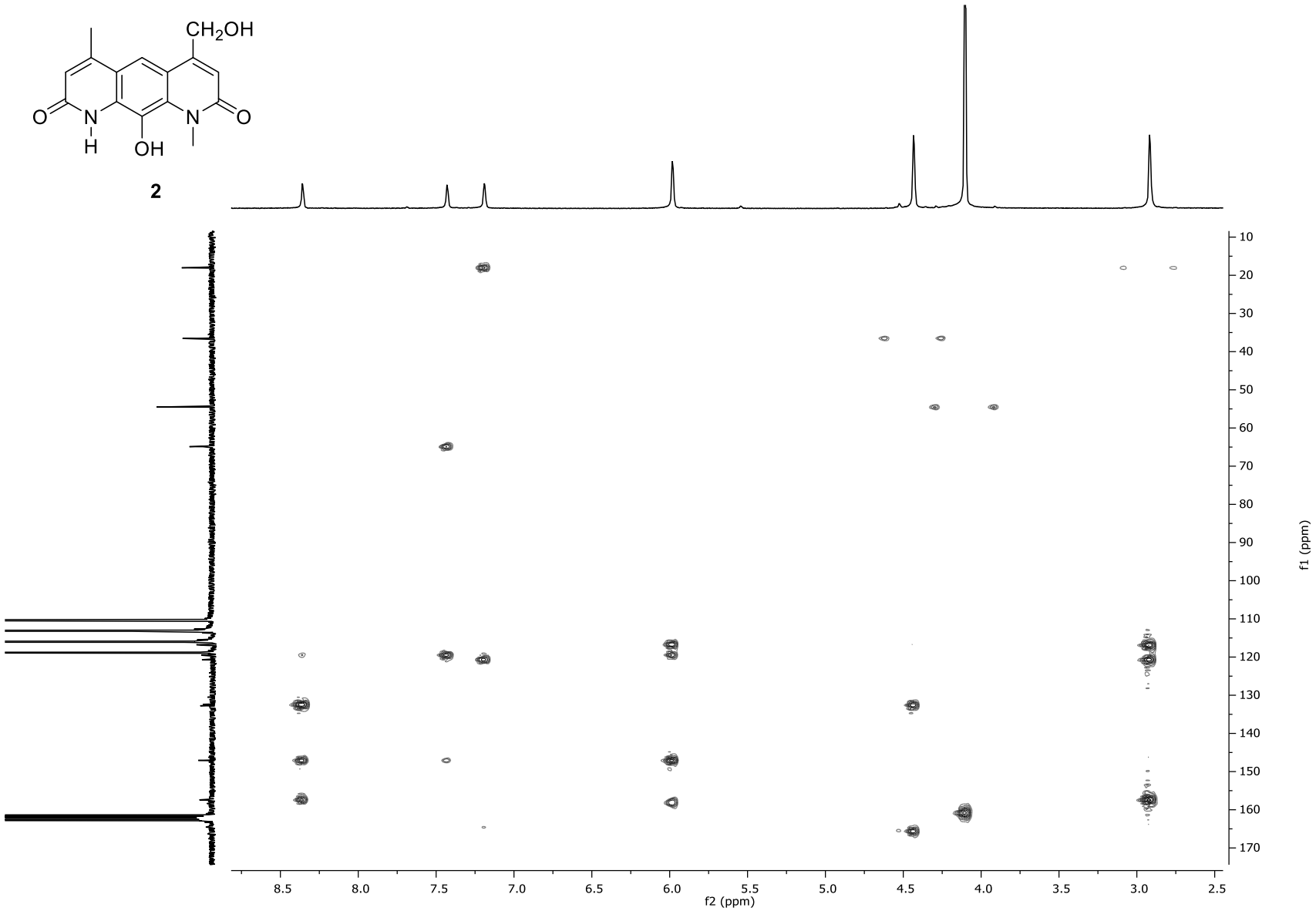

Figure S13. HMBC spectrum (CF_3COOD, 400 MHz) of nybomycin C (**2**)

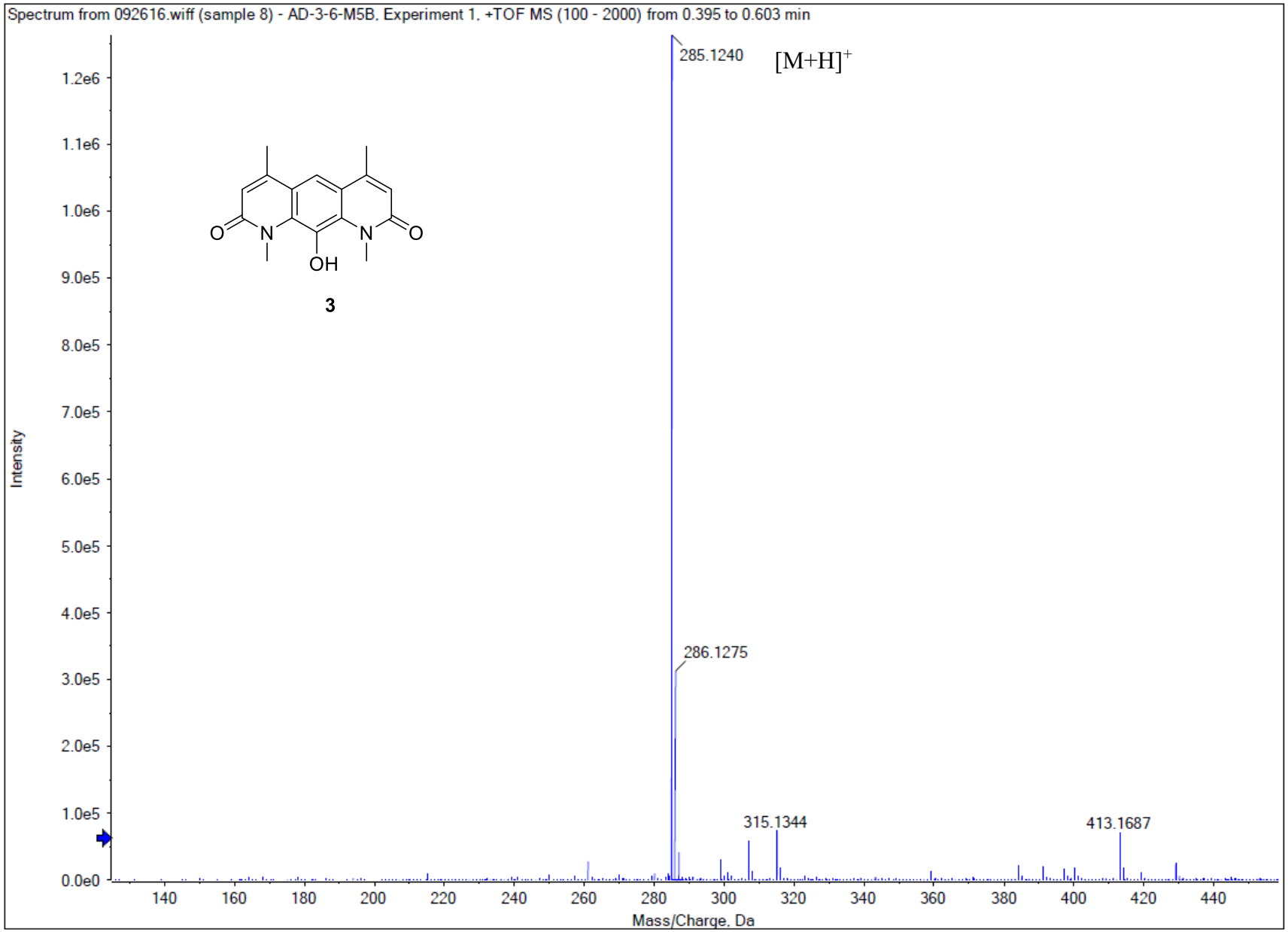

Figure S14. (+)-HRESIMS of nybomycin D (**3**)

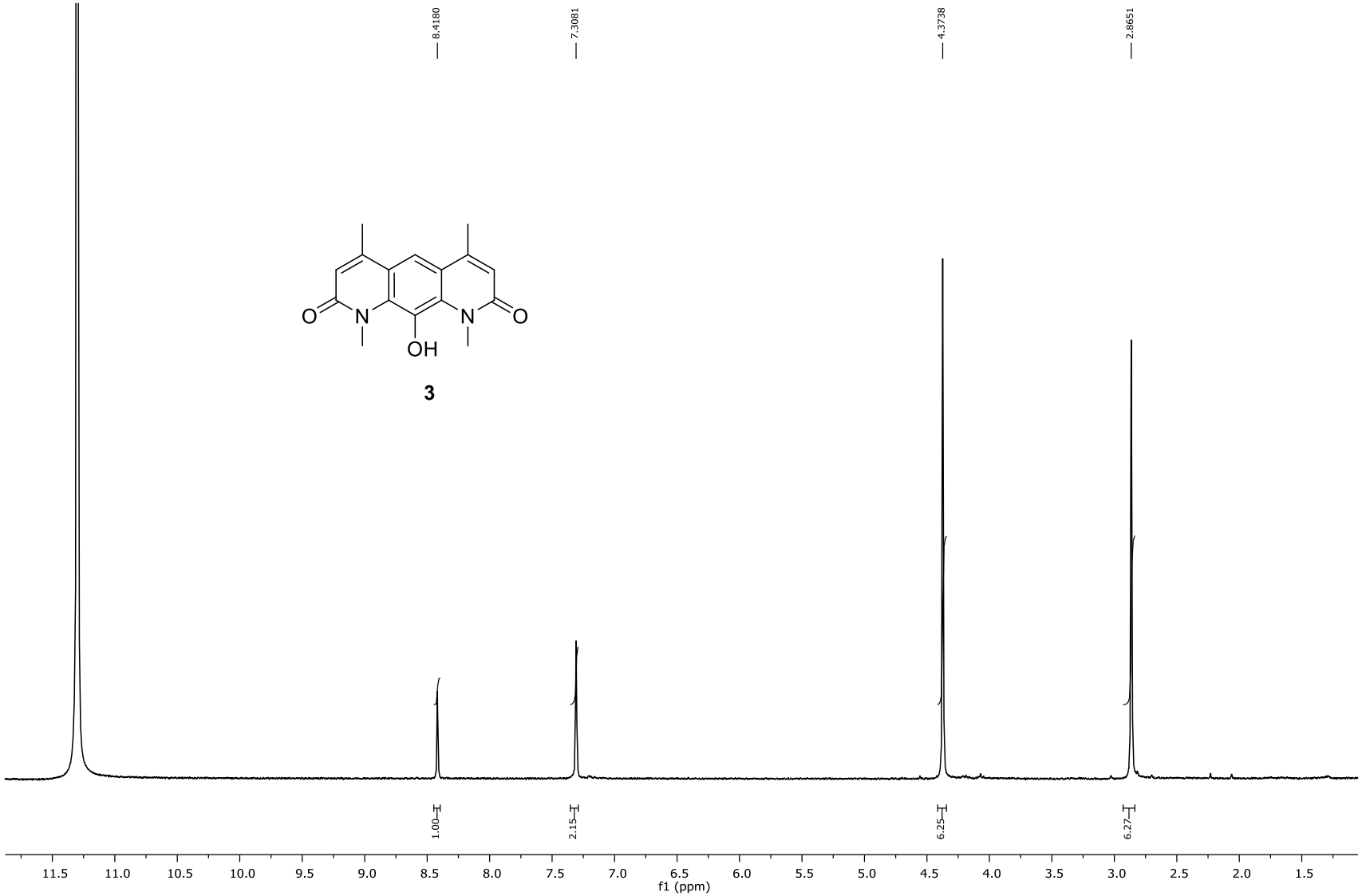

Figure S15. ^{1}H-NMR (CF_3COOD, 400 MHz) of nybomycin D (**3**)

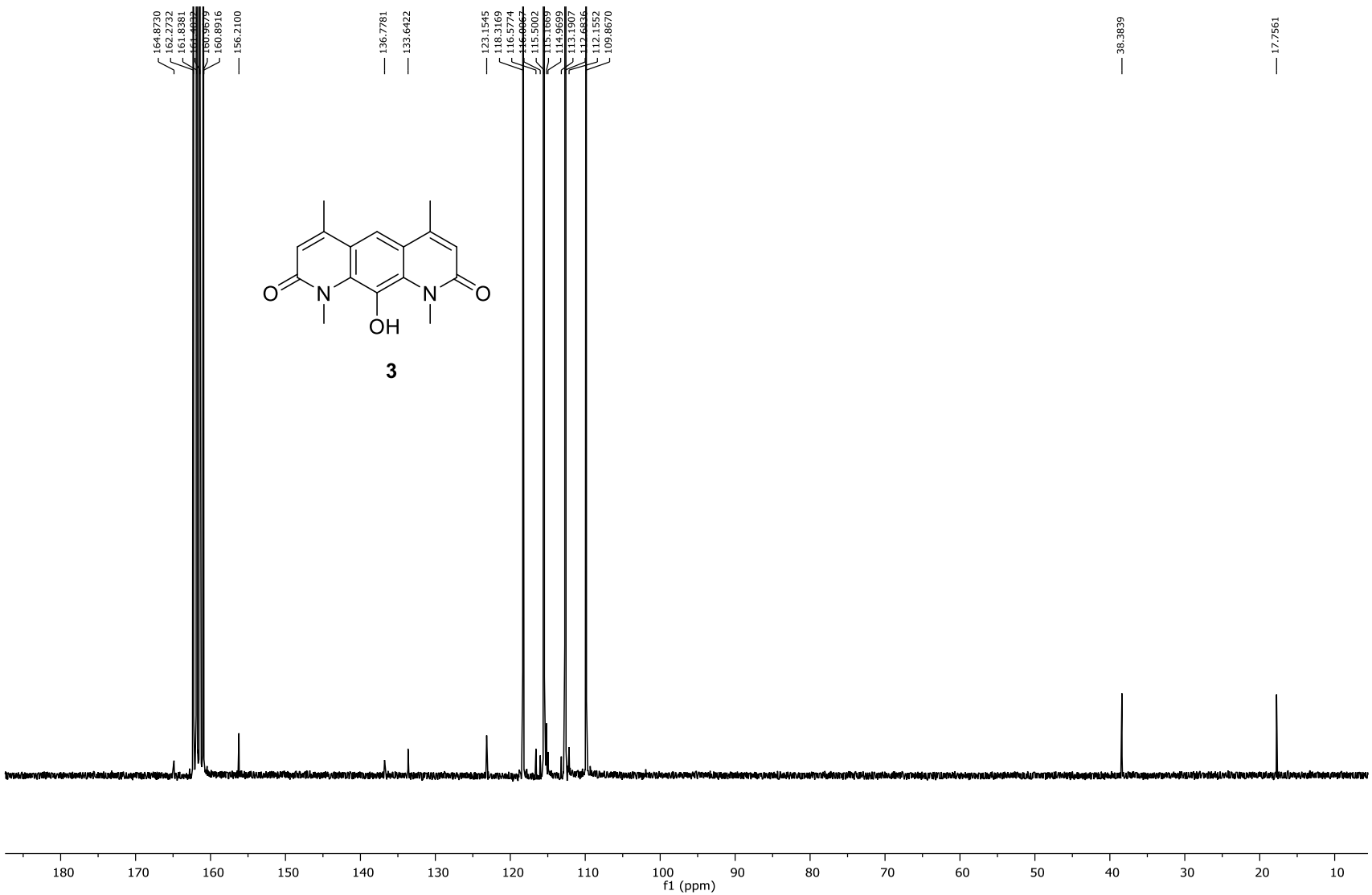

Figure S16. ^{13}C-NMR (CF_3COOD, 100 MHz) of nybomycin D (**3**)

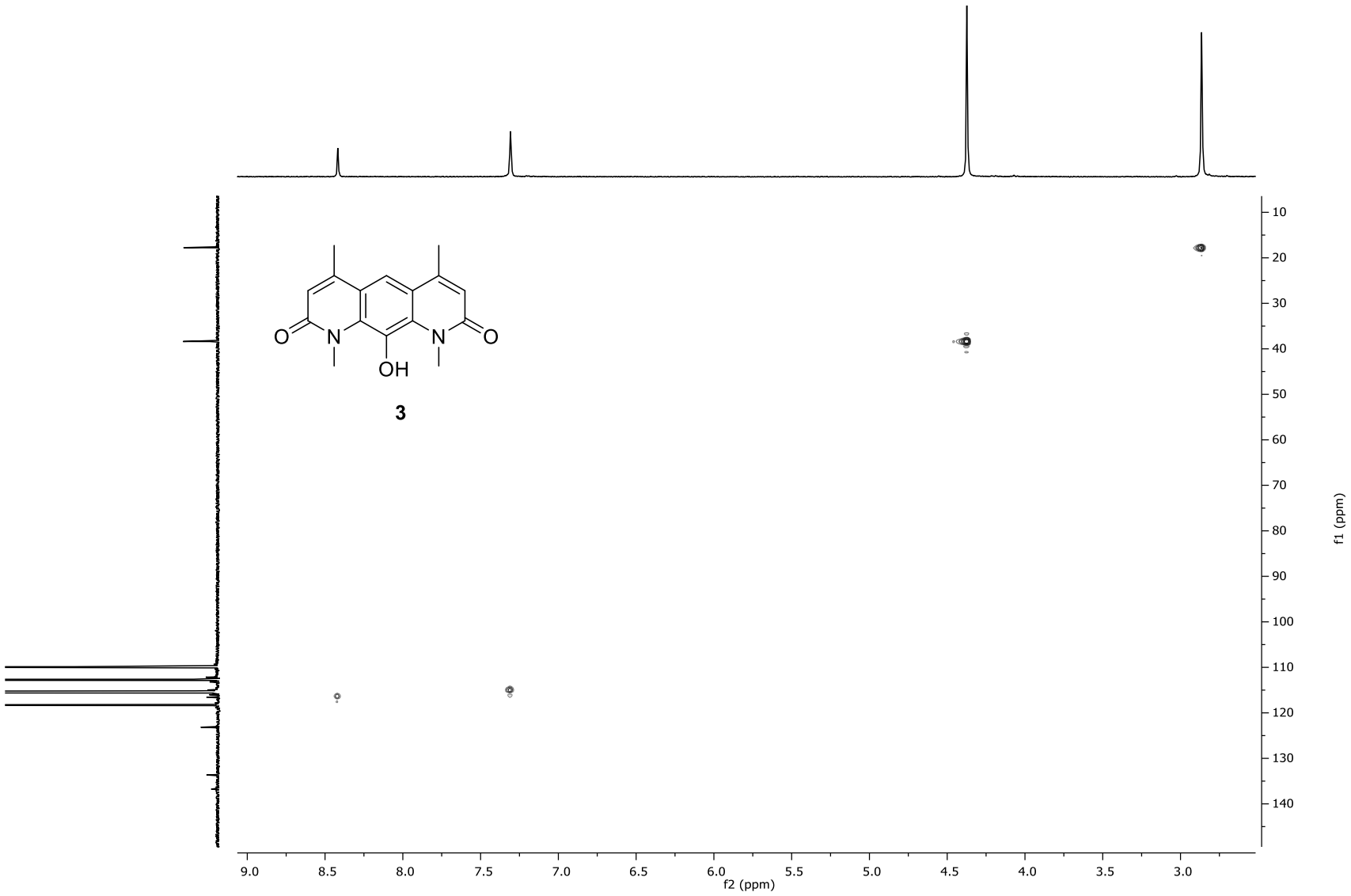

Figure S17. HSQC spectrum (CF_3COOD, 400 MHz) of nybomycin D (**3**)

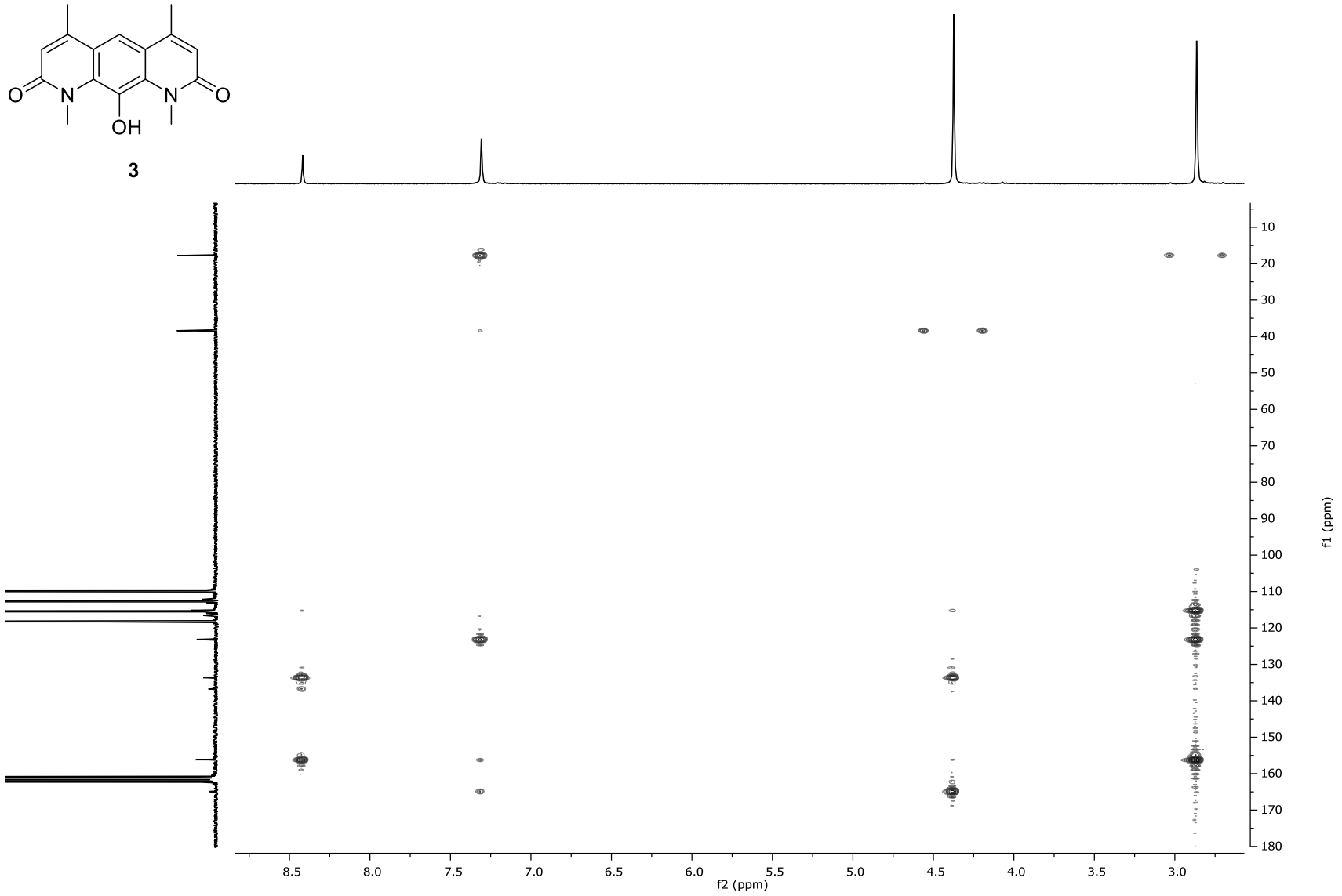

Figure S18. HMBC spectrum (CF_3COOD, 400 MHz) of nybomycin D (**3**)

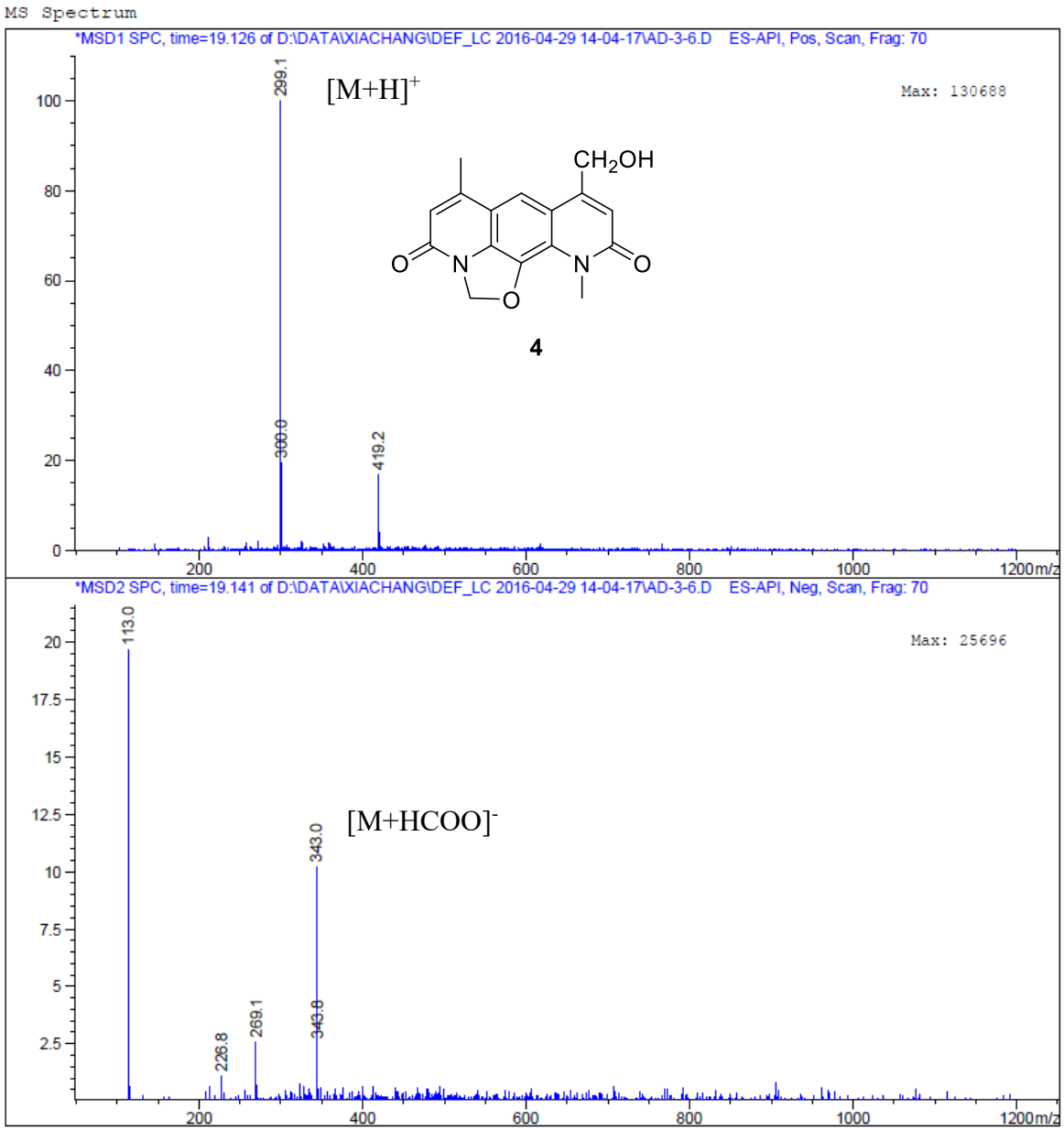

Figure S19. (±)-ESI-MS of nybomycin (**4**)

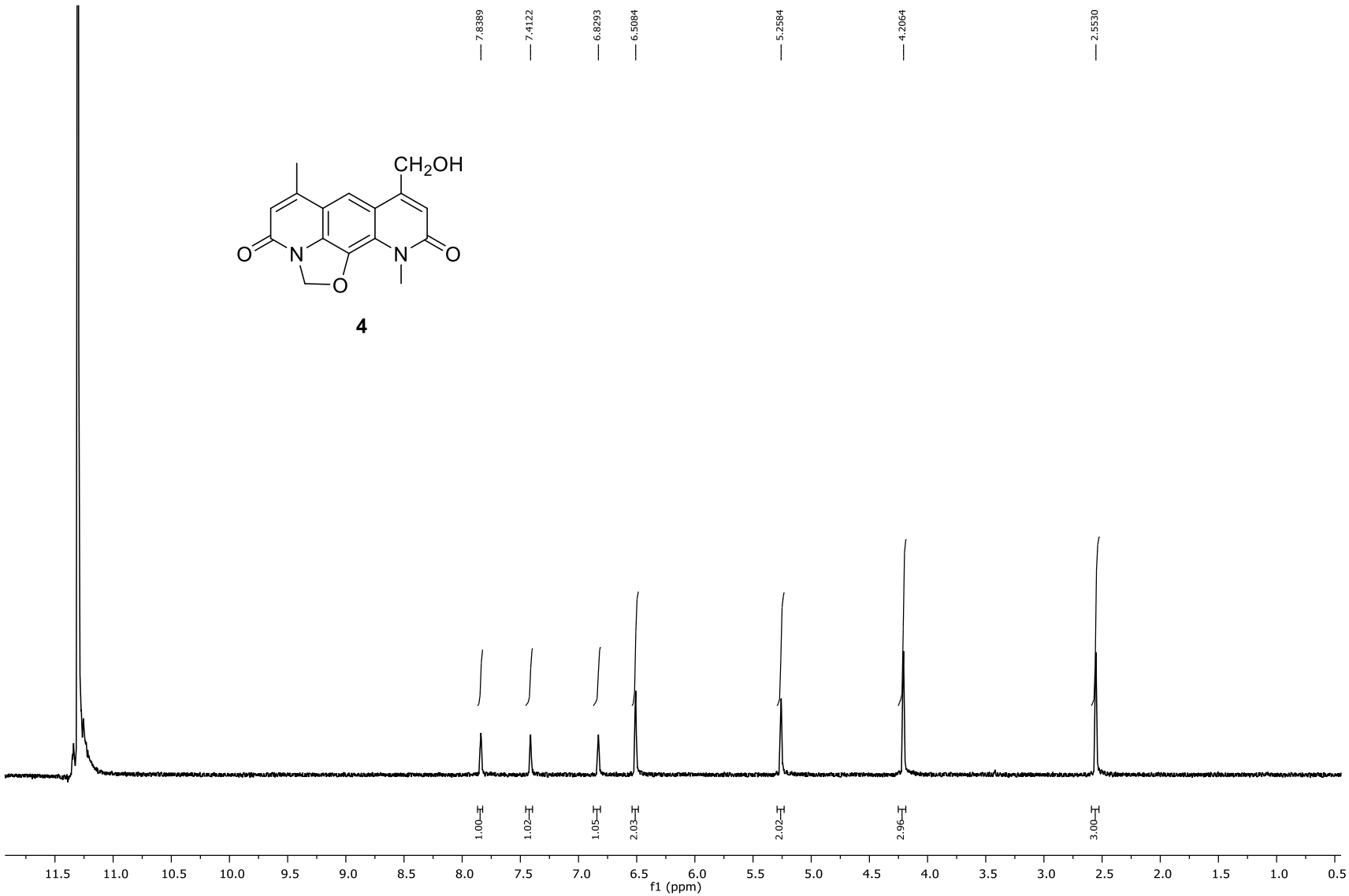

Figure S20. ^{1}H-NMR (CF_3COOD, 400 MHz) of nybomycin (**4**)

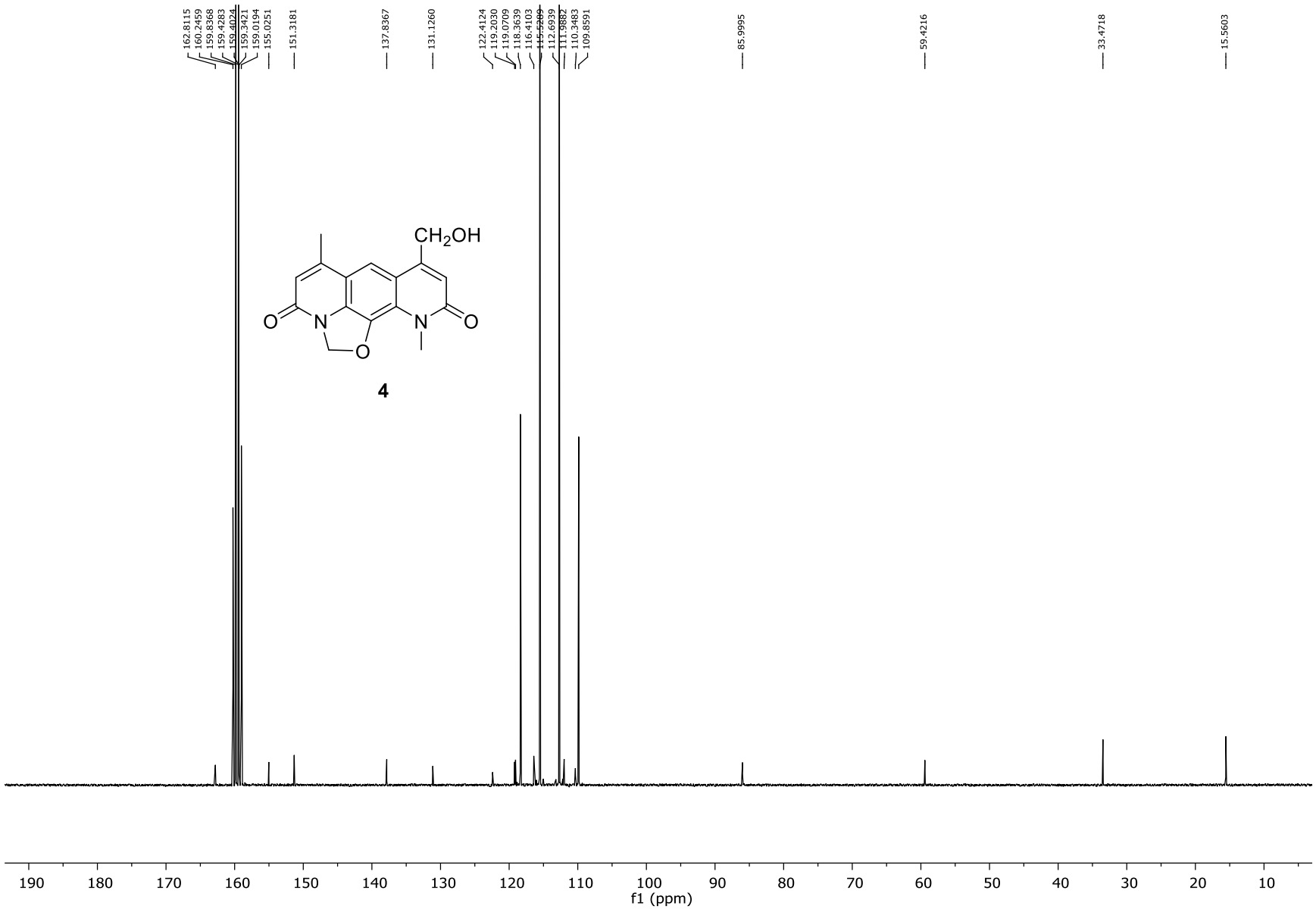

Figure S21. ^{13}C-NMR (CF_3COOD, 100 MHz) of nybomycin (**4**)

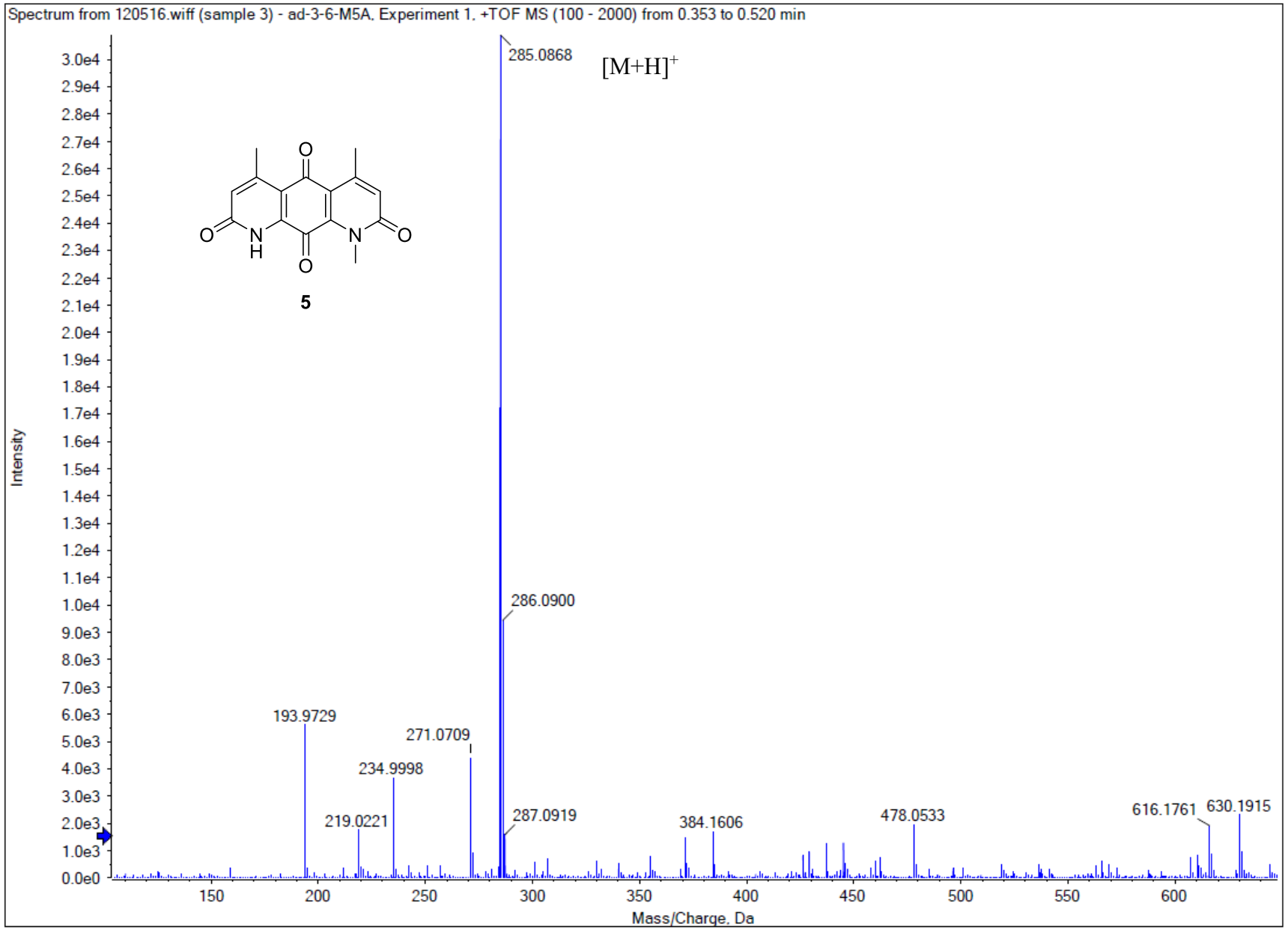

Figure S22. (+)-HRESIMS of deoxynyboquinone (**5**)

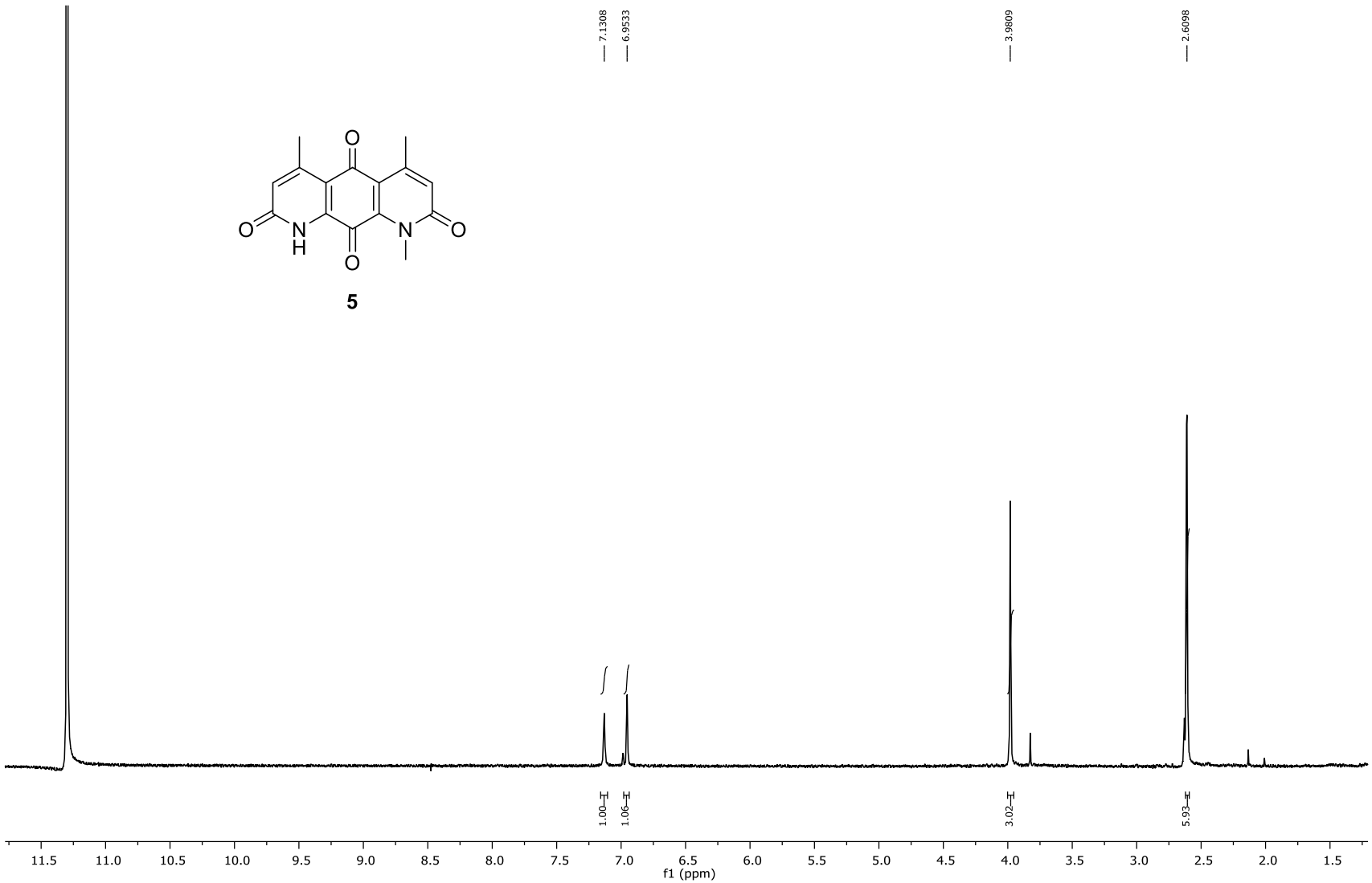

Figure S23. ^{1}H-NMR (CF_3COOD, 400 MHz) of deoxynyboquinone (**5**)

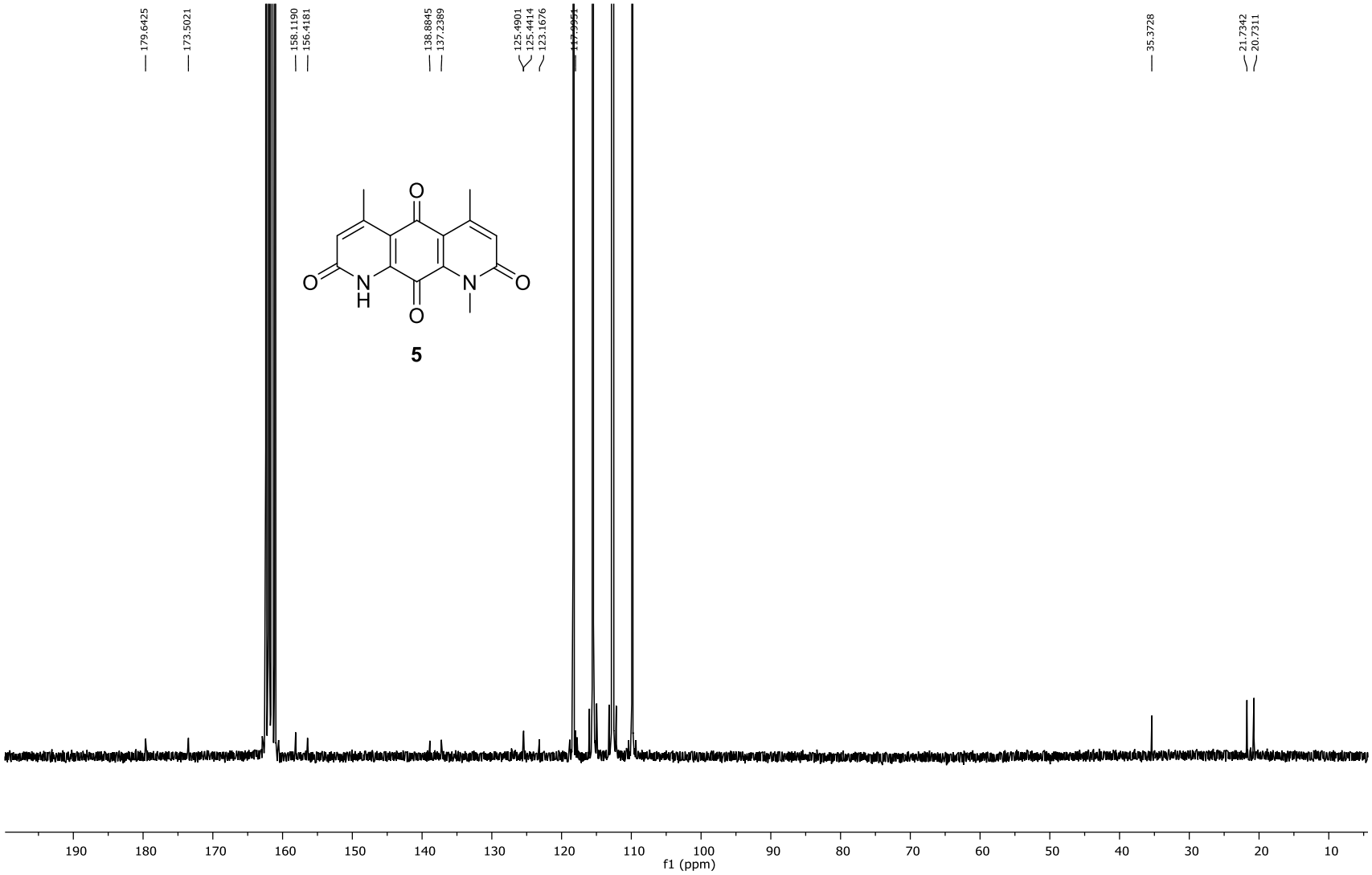

Figure S24. ^{13}C-NMR (CF_3COOD, 100 MHz) of deoxynyboquinone (**5**)

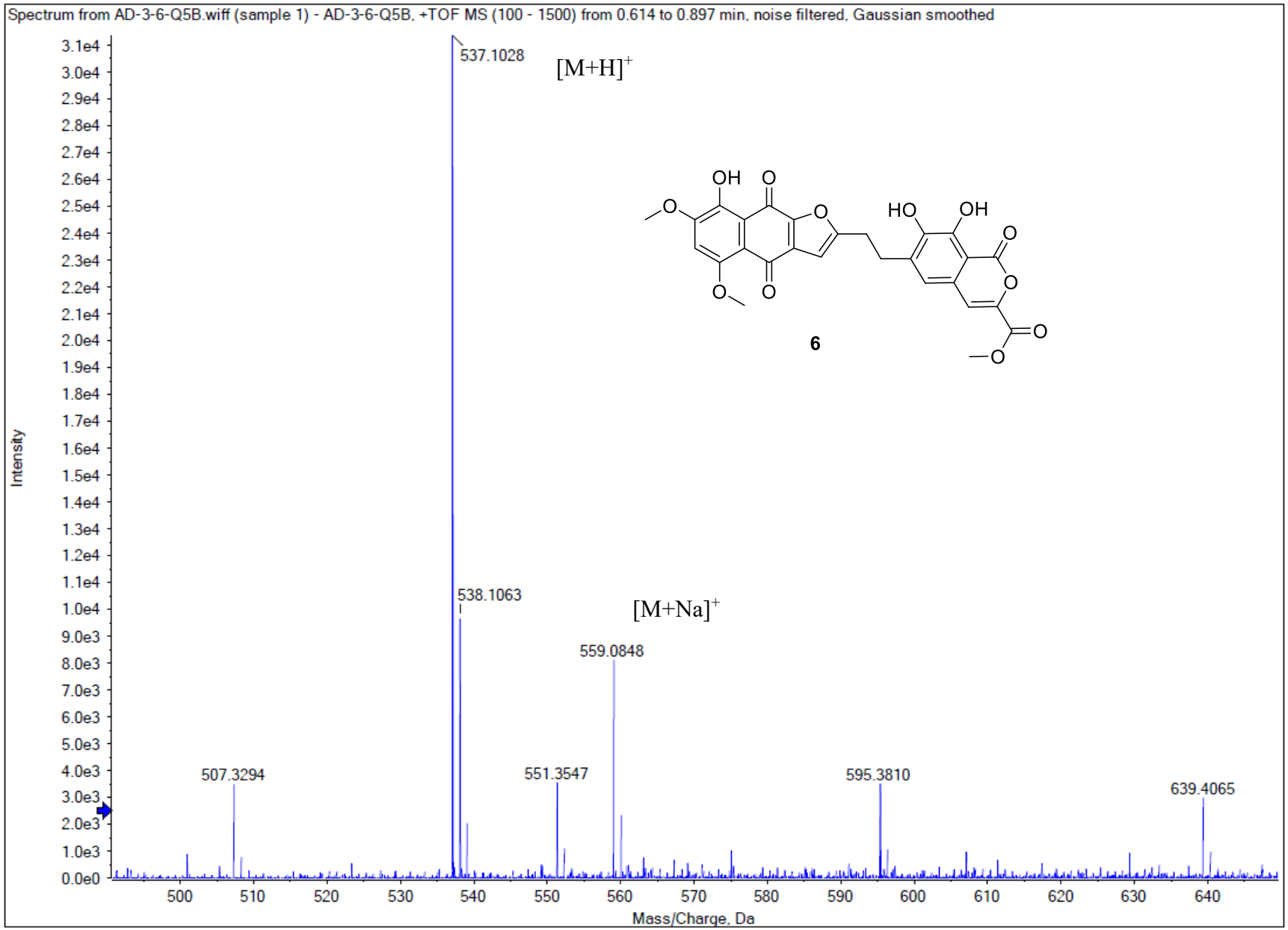

Figure S25. (+)-HRESIMS of α-rubromycin (**6**)

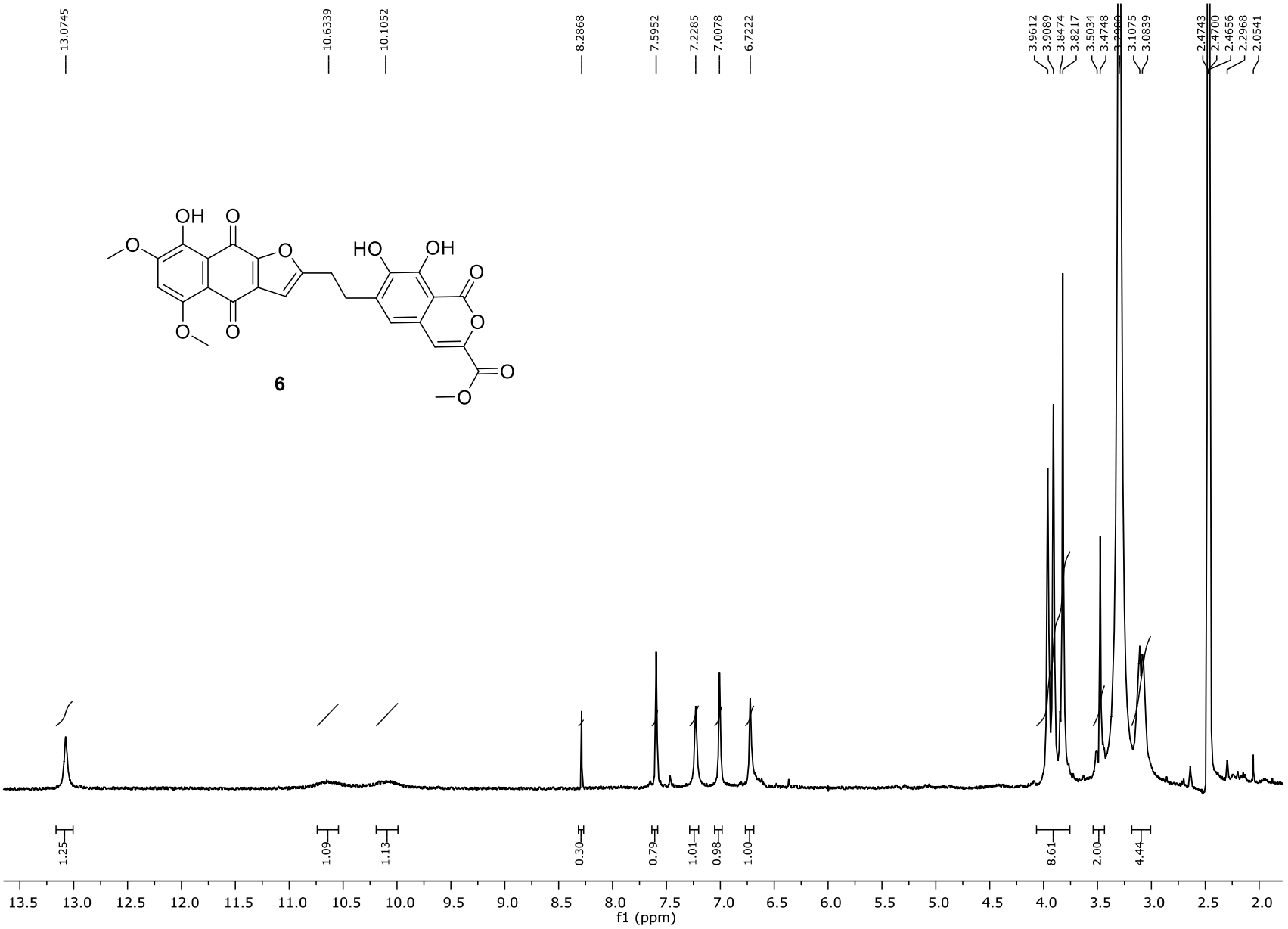

Figure S26. ^{1}H-NMR (DMSO-d_6, 400 MHz) of α-rubromycin (**6**)

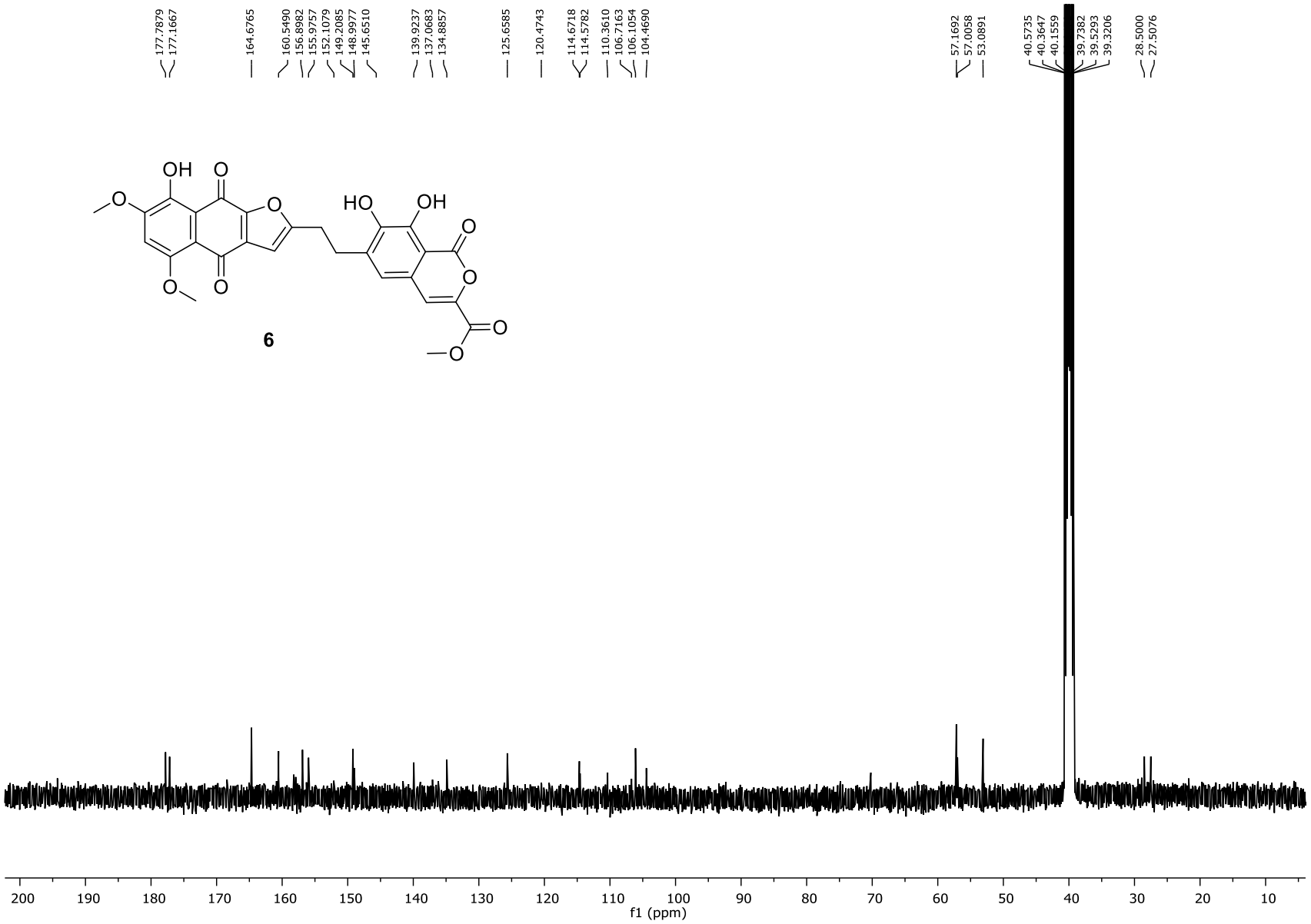

Figure S27. ^{13}C-NMR (DMSO-d_6, 100 MHz) of α-rubromycin (**6**)

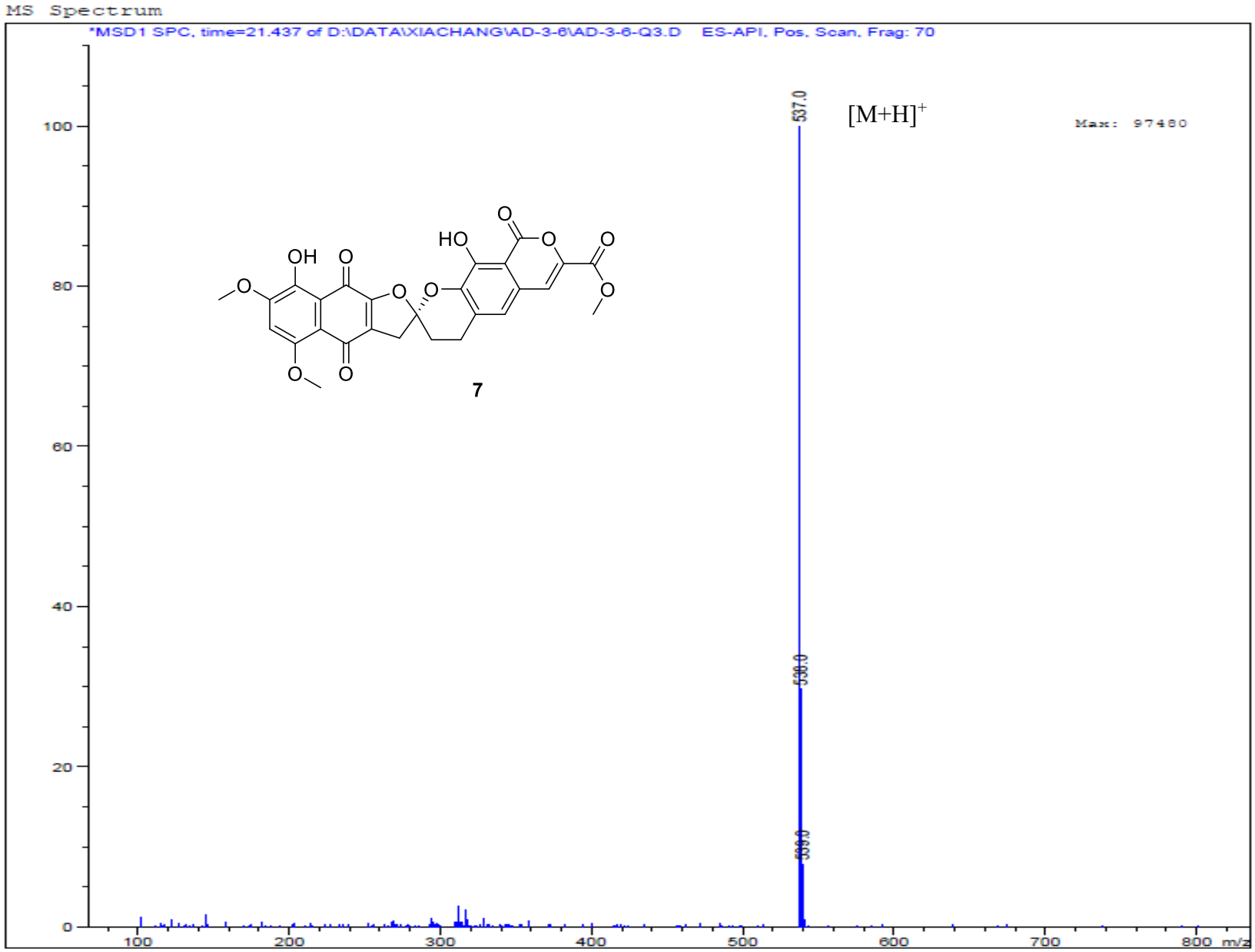

Figure S28. (+)-ESI-MS of β-rubromycin (**7**)

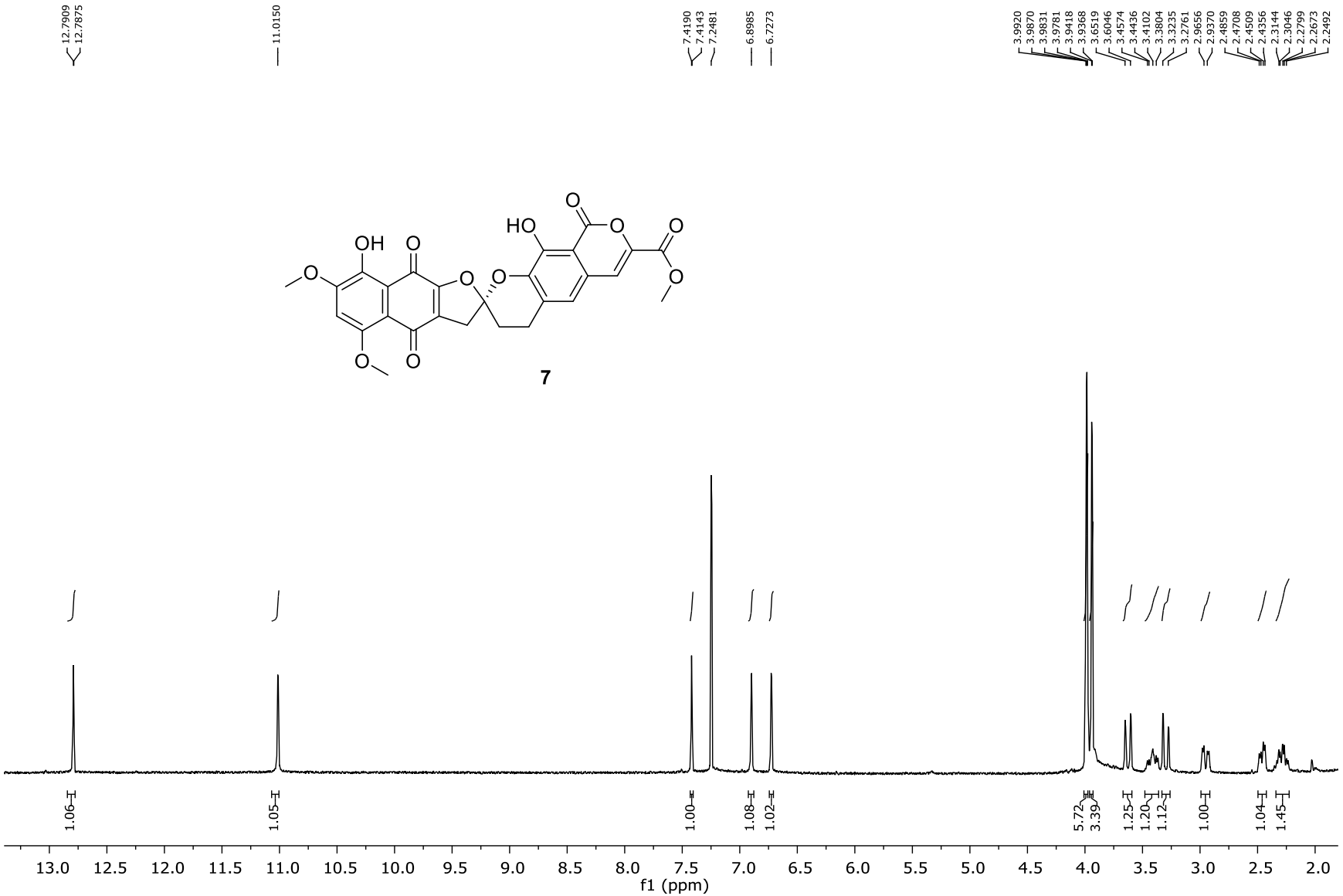

Figure S29. ^{1}H-NMR ($CDCl_3$, 400 MHz) of β-rubromycin (**7**)

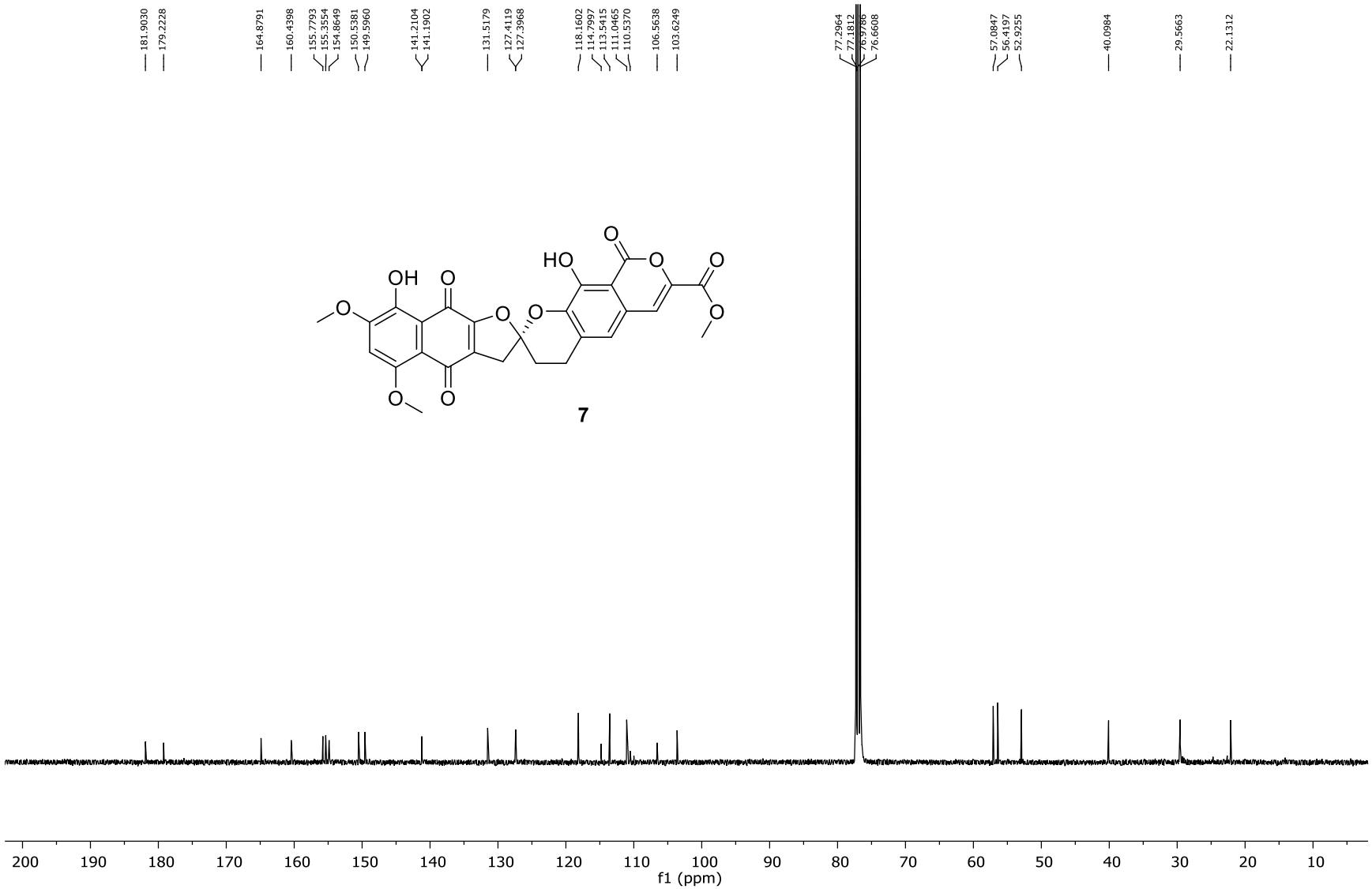

Figure S30. ^{13}C-NMR ($CDCl_3$, 100 MHz) of β-rubromycin (**7**)

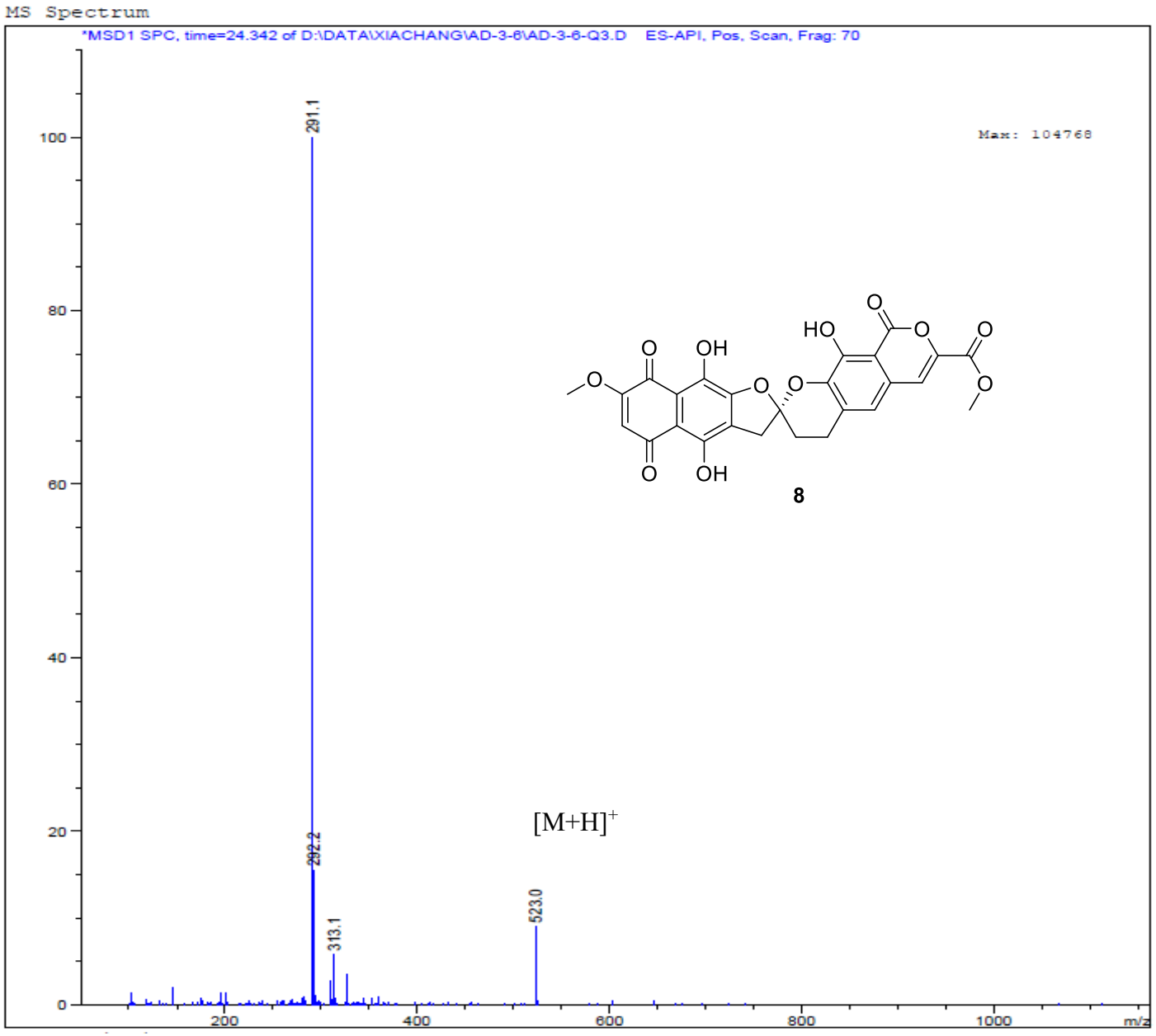

Figure S31. (+)-ESI-MS of γ-rubromycin (**8**)

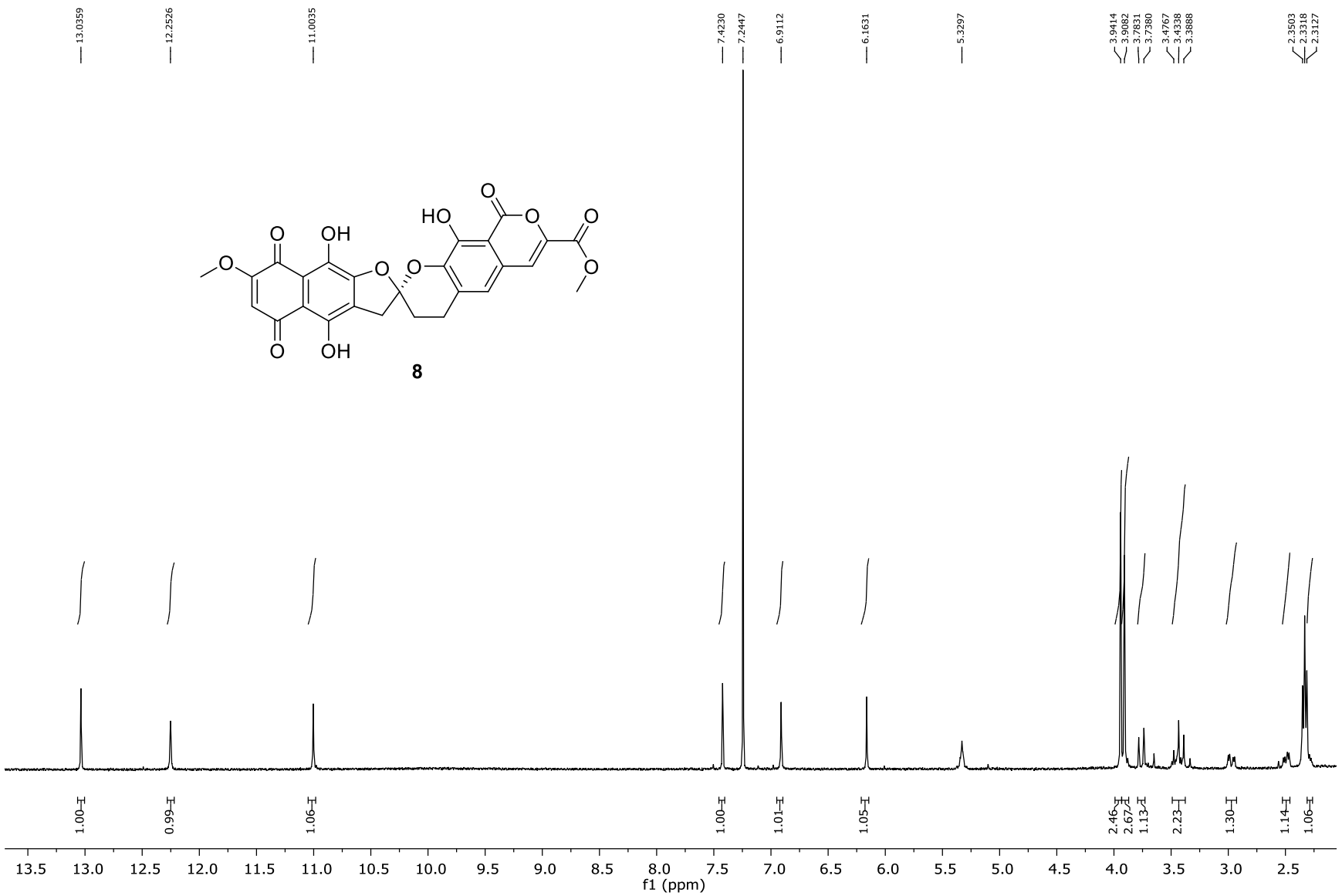

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Figure S32. ^{1}H-NMR (DMSO-d_6, 500 MHz) of γ-rubromycin (**8**)

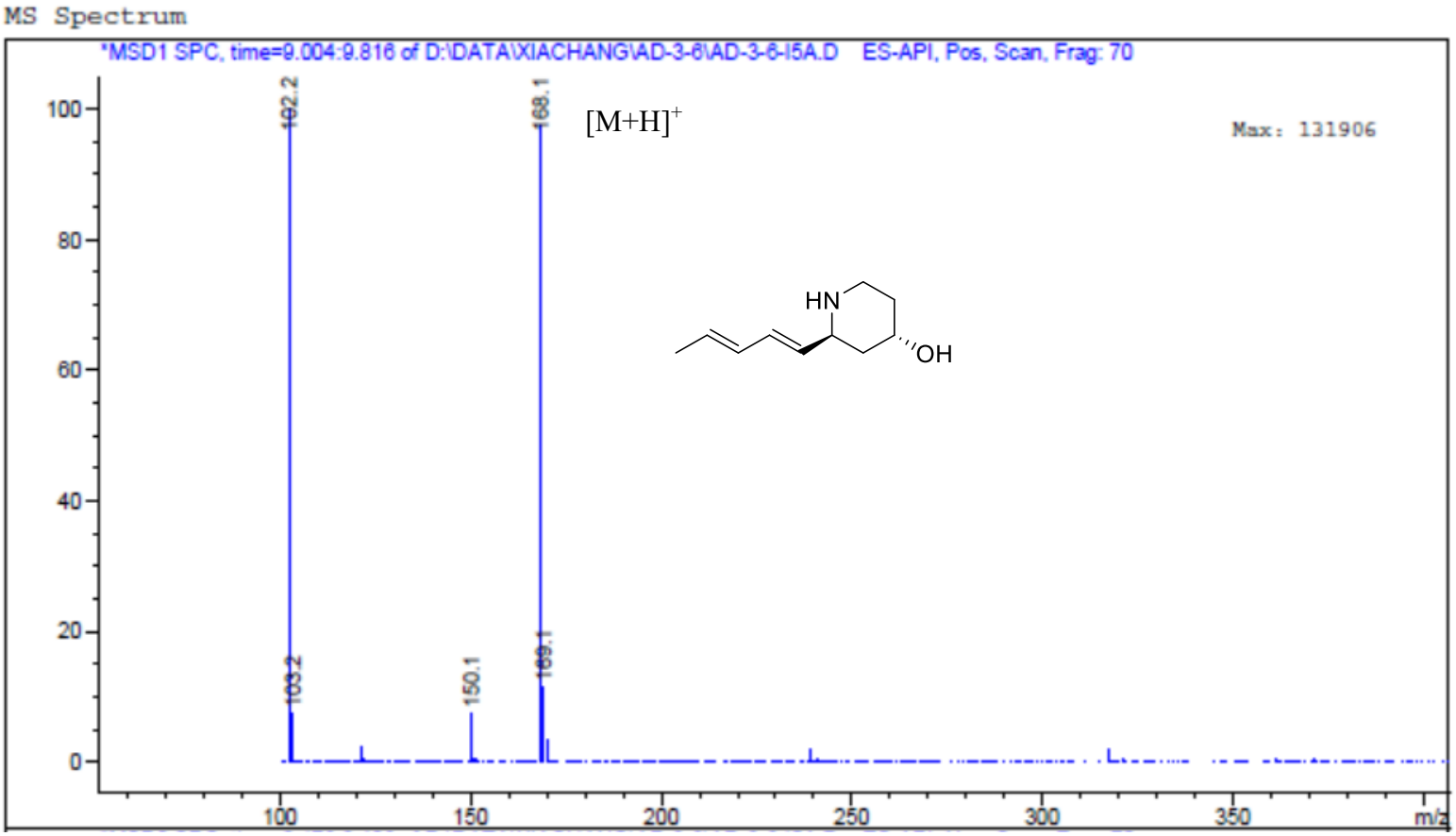

Figure S33. (+)-ESI-MS of [2α(1*E*,3*E*),4β]-2-(1,3-pentadienyl)-4-piperidinol (**9**)

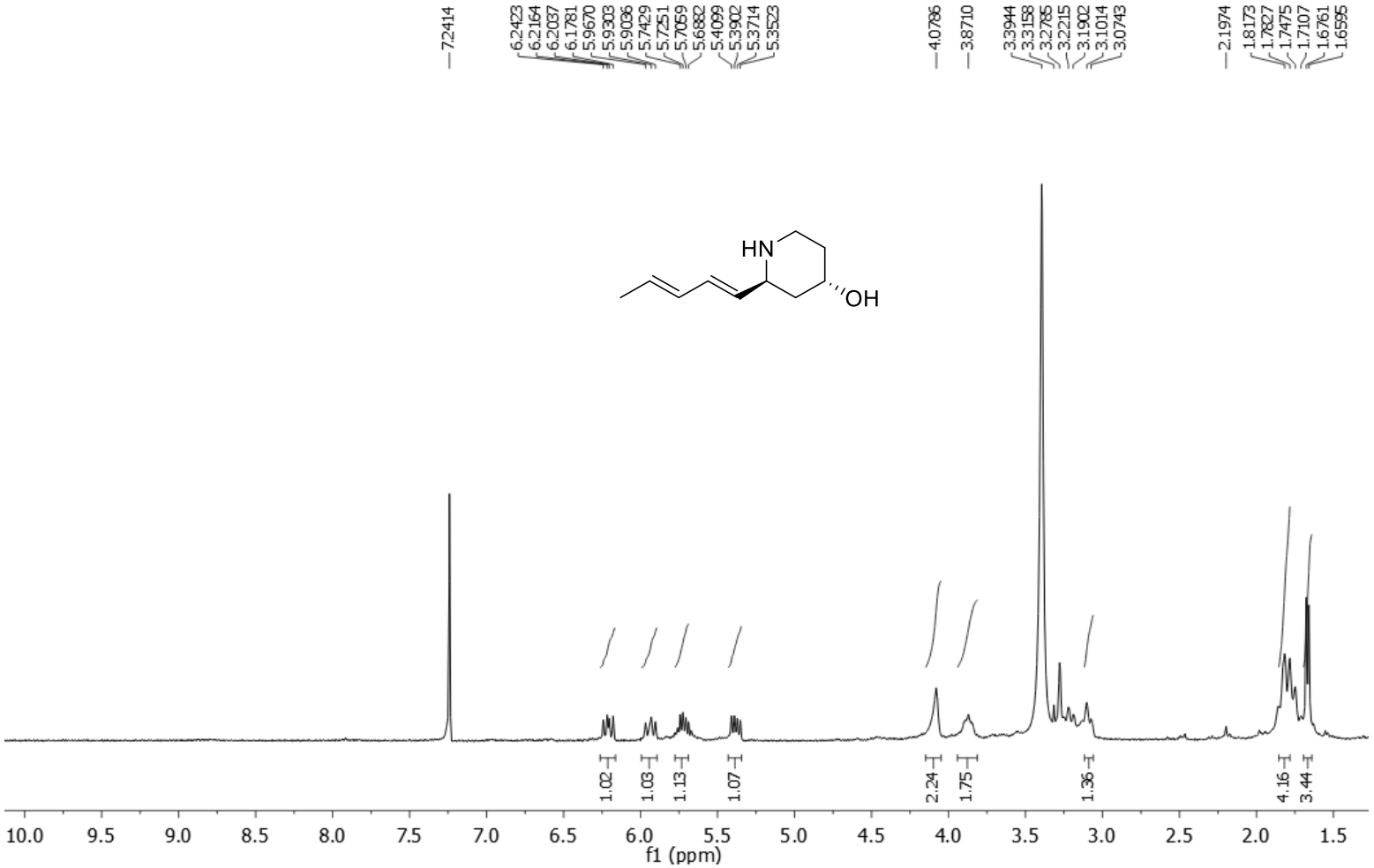

Figure S34. ^{1}H-NMR ($CDCl_3/CD_3OD$, 400 MHz) of [2α(1*E*,3*E*),4β]-2-(1,3-pentadienyl)-4-piperidinol (**9**)

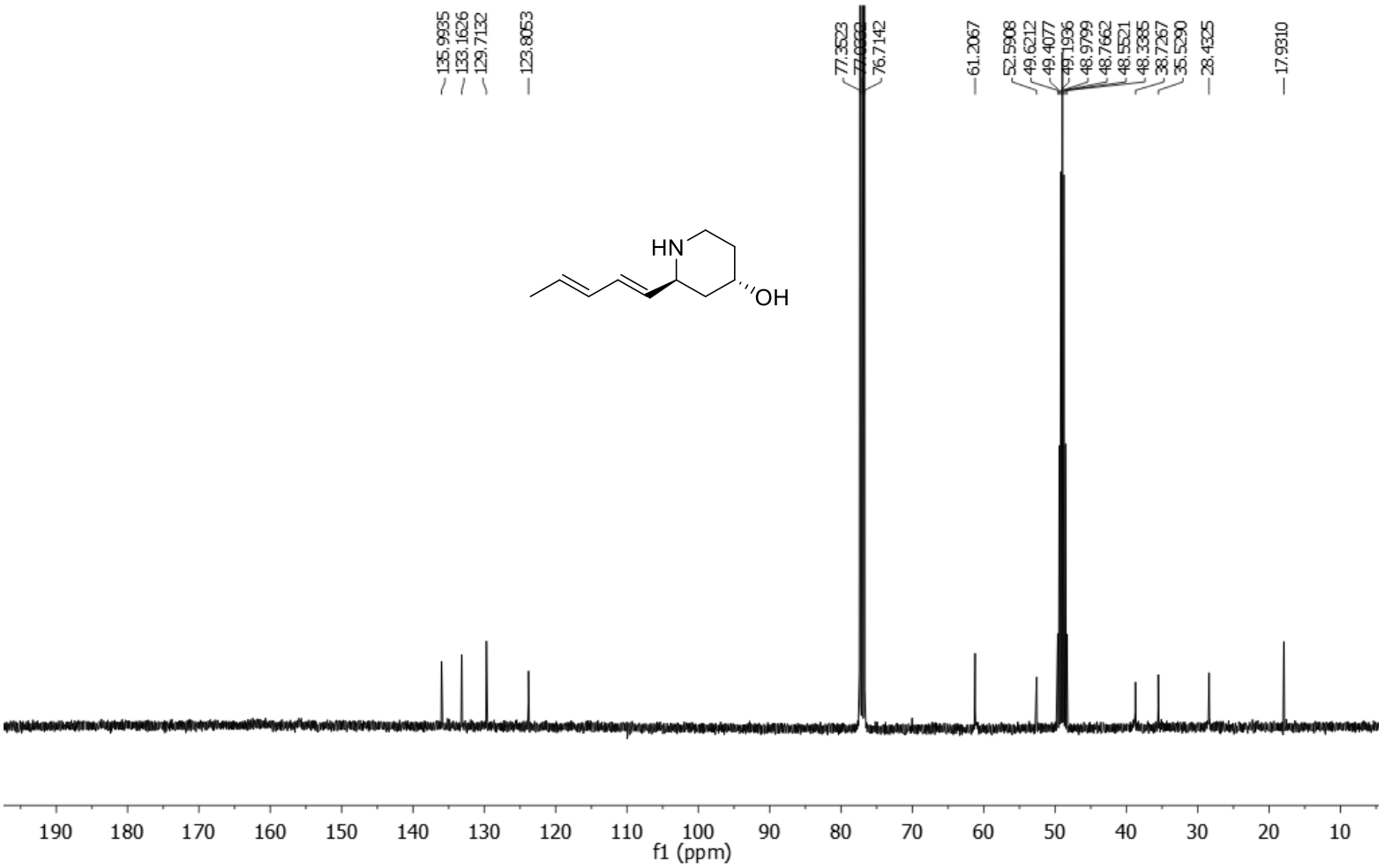

Figure S35. ^{13}C-NMR ($CDCl_3/CD_3OD$, 100 MHz) of [2α(1*E*,3*E*),4β]-2-(1,3-pentadienyl)-4-piperidinol (**9**)

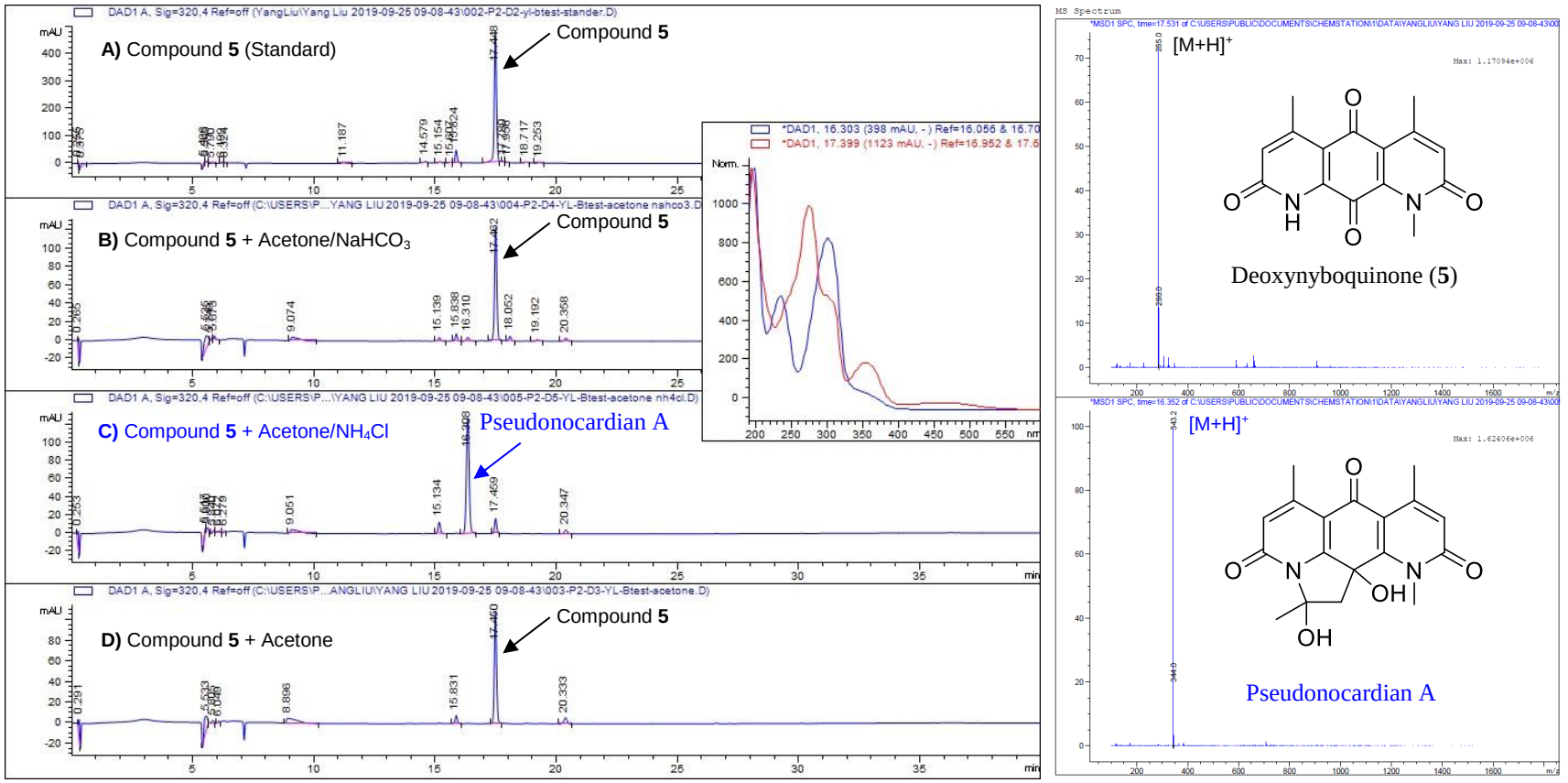

Figure S36. HPLC-UV/MS analyses of standard **5** and putative product pseudonocardian A. **A)** Standard sample of deoxynyboquinone (**5**). **B)** Aqueous saturated $NaHCO_3$ (100 µL) was added to a solution of **5** (0.3 mg) in acetone (100 µL) and mixture stirred at room temperature overnight followed by analysis via HPLC-MS**. C)** Aqueous saturated NH_4Cl (100 µL) was added to a solution of **5** (0.3 mg) in acetone (100 µL) and mixture stirred at room temperature overnight followed by analysis via HPLC-MS. The new reaction product displayed a molecular weight (MW 342) and UV/vis profile consistent with pseudonocardian A. **D)** As a control, a solution of **5** (0.3 mg) in acetone (100 µL) was stirred at room temperature overnight followed by analysis via HPLC-MS. HPLC-conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5%-100% B; 30-35 min, 100% B; 35-36 min, 100%-5% B; 36-40 min, 5 % B; Phenomenex C18 column (250 × 4.6 mm, 5 *µ*m); 320 nm. UV-vis inset of full wavelength scan (190-600 nm). **Compound 5:** (+)-ESI-MS: *m/z* 285 $[M + H]^+$; **Pseudonocardian A:** (+)-ESI-MS: *m/z* 343 $[M + H]^+$