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Supporting Information

# Lugdunomycin, an Angucycline-Derived Molecule with Unprecedented Chemical Architecture

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## EXPERIMENTAL SECTION

### Microorganisms and culturing conditions

*Streptomyces* sp. QL37 was isolated from soil in the Qinling mountains (P. R. China) as described previously.[1] The strain was deposited to the collection of the Centraal Bureau voor Schimmelcultures (CBS) in Utrecht, The Netherlands, under deposit number 138593. *Streptomyces* sp. QL37 was cultivated on minimal media agar plates (MM) [2] with 0.5% glycerol and 1% mannitol (w/v) as the carbon sources, and in solid R5 agar medium supplemented with 0.8% peptone and 1% mannitol (w/v). Square agar plates (12 cm × 12 cm) were inoculated with 1 x $10^7$ spores from a fresh spore suspension.

### Chromatography and spectroscopy methods

FT-IR was measured on Perkin-Elmer FT-IR Spectrometer Paragon 1000. UV measurements were performed using a Shimadzu UV mini-1240. Optical rotations were measured on a JASCO P-1010 polarimeter. NMR spectra were recorded on a Bruker DMX 600 MHz, either in $CD_3OD$ calibrated to 3.30 ppm or $CDCl_3$ calibrated to 7.27 ppm. Semi-preparative HPLC (pHPLC) was performed with a Shimadzu HPLC system and a 5 mL Rheodyne manual injection loop, equipped with a reversed-phase $C_{18}$ column (Phenomenex Luna $C_{18}$(2) 100 Å 5 micron 250 × 10 mm). All the pHPLC experiments used 2 mL/min flow rate and fraction collection based on detected peak. Silica gel (pore size 60 Å, 230−400 mesh) for open column chromatography was purchased from Sigma-Aldrich (St. Louis, MO, USA). Pre-coated silica gel 60 $F_{254}$ TLC plates (Merck, Darmstadt, Germany) were used for TLC bioautography analysis. PLC silica gel 60 $F_{254}$, 1 mm (Merck, Darmstadt, Germany) was used for preparative TLC separation. Analytical TLC was performed with silica gel 60 (Merck, Darmstadt, Germany) plates and visualized with UV lamp (254 nm and 365 nm) and anisaldehyde/sulfuric acid reagent. All solvents and chemicals were of analytical and HPLC grade.

### Extraction and Isolation of angucyclines from *Streptomyces* sp. QL37

The first round of systematic separation was done on MM culture medium. After seven days of growth, 225 MM agar plates (12 cm × 12 cm) inoculated with *Streptomyces sp*. QL37 were combined and cut into small blocks, which were then homogenated with a pestle. The resultant agar suspension was extracted with ethyl acetate (EtOAc) by soaking overnight at room temperature. The supernatant was filtered and subsequently evaporated under reduced pressure at 38 °C to obtain 2.3 g crude extract. This extract was adsorbed by silica gel and chromatographed on a silica gel (pore size 60 Å, 70−230 mesh, St. Louis, MO, USA) column chromatography employing a gradient elution from *n*-hexane via chloroform to methanol. The Fractions containing lugdunomycin (**1**) were combined thin-layer chromatography (TLC) (Merck, Darmstadt, Germany) using chloroform and methanol (10:1) as solvent system, whereby lugdunomycin was detected as a dark spot at UV 254 nm, with a distinctive blue color when stained with anisaldehyde/sulfuric acid reagent after heating. Lugdunomycin was defatted by partition between methanol and *n*-hexane, and the methanol phase was further purified using a Sephadex LH-20 column (GE Healthcare Life Sciences, Eindhoven, The Netherlands), eluting with methanol. The fraction containing lugdunomycin (**1**) was purified by preparative TLC (Merck, Darmstadt, Germany), migrated with chloroform and methanol (9:1) and detected under UV light at 254 nm. Finally, 0.5 mg pure lugdunomycin (**1**) was obtained. Compounds **2** (3.4 mg), **6**/**7** (27 mg), **8** (3.4 mg), and **11** (1 mg) were isolated from the same cultures.

To obtain angucyclines and derivatives, *Streptomyces* species QL37 was grown on R5 agar plates supplemented with 1% mannitol and 0.8% peptone, using a total volume of 20 L. Compound extraction was the same as described above. Crude extract (20.5 g) adsorbed by silica gel was first chromatographed on a silica gel column chromatography employing gradient elution from *n*-hexane, chloroform, to methanol, to obtain 17 fractions. These were subsequently subjected to HPLC-DAD and UHPLC-ToF-MS analysis, the combination of which was further done by UV spectrum and chemical formulas. 12 final fractions (Fr1—Fr12) were obtained. Fr2 was chromatographed on silica gel eluting with a gradient of chloroform in *n*-hexane, and Sephadex LH-20 eluting with methanol, to afford pure compound **3** (0.5 mg). Fr3 was separated by silica gel eluting with a gradient of chloroform in *n*-hexane, to give 8 subfractions sfr3.1—sfr3.8. Sfr3.3 was purified by Sephadex LH-20, to afford the pure orange compound **13** (20 mg). Fr5 was chromatographed on silica gel eluting with a

gradient of methanol in chloroform, to give 5 subfractions sfr5.1—sfr5.5. Sfr5.2 was separated by Sephadex LH-20 to afford pure compound **15** (10 mg); Sfr5.4 was separated by semi-preparative reversed-phase HPLC (Phenomenex Luna C18 (2) 100 Å 5 micron 250 × 10 mm) on an Agilent 1200 series HPLC (Agilent technologies Inc, Santa Clara, CA, USA), eluting with a gradient of ACN in $H_2O$ from 15% to 80% in 40 min. HPLC peaks were manually collected, resulting in the isolation of compound **8** (2.8 mg), **9** (impure, 1.0 mg), **17** (impure, 0.50 mg), **24** (semi-pure, 0.82 mg), **23** (semi-pure, 1.1 mg), and **25** (semi-pure, 0.90 mg). Fr6 was directly separated by semi-preparative HPLC, eluting with a gradient of ACN in $H_2O$ from 15% to 80% in 40 min, which resulted in the separation of compound **12** (semi-pure, 0.80 mg), **5** (semi-pure, 0.40 mg), **20** (semi-pure, 0.30 mg), a mixture of **22** and **23** (semi-pure, 0.60 mg), and **21** (0.50 mg). Fr7 was directly separated by semi-preparative HPLC, eluting with a gradient of ACN in $H_2O$ from 20% to 85% in 30 min, which resulted in the isolation of **14** (semi-pure, 0.50 mg), and **16** (semi-pure, 0.60 mg). Fr9 was separated by silica gel eluting with a gradient of methanol in chloroform, to give 6 subfractions sfr9.1—sfr9.6. Sfr9.3 was purified by preparative TLC, migrated with chloroform/methanol (5:1) as solvent system, giving pure compounds **18** (5 mg) and **19** (5.5 mg).

Lugdunomycin (**1**): colorless, needle crystal, UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 349 (3.32), 287 (4.06), 250 (4.42) nm; $[\alpha]_D^{20}$ 0 (*c* 0.02, MeOH); IR $\nu_{max}$ 669, 831, 1130, 1203, 1271, 1471, 1558, 1683, 1716, 2324, 2349, 2378, 2850, 2920 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; HRESIMS (positive mode) *m/z* 456.1448 $[M + H - H_2O]^+$ (calcd for $C_{27}H_{22}NO_6$ 456.1453), 474.1553 $[M + H]^+$ (calcd for $C_{27}H_{24}NO_7$ 474.1547).

**X-ray Crystal Structure Determination of lugdunomycin (1).**

Colorless crystals of lugdunomycin (**1**) were obtained from a solvent system of $CHCl_3$/MeOH (10:1). Suitable single crystals for X–ray structural analysis of lugdunomycin were mounted at room temperature in Paratone N inert oil. Single crystal X-ray diffraction data were collected in the home laboratory on a Bruker three circle diffractometer equipped with a Bruker TXS-Mo rotating anode, INCOATEC mirror optics and an APEX II detector. The data were integrated with SAINT and an empirical absorption correction with SADABS [3] was applied. The structure was solved by direct methods.[4] The structure model was refined against all data by full-matrix least-squares methods on $F^2$ with the program shelxl2014.[5] All non-hydrogen-atoms were refined with anisotropic displacement parameters. The hydrogen atoms were refined isotropically on calculated positions using a riding model with Uiso values constrained to 1.2/1.5 $U_{eq}$ of their parent atoms.

**MS/MS-based molecular networking**

Mass spectral networks were assembled as described in reference.[6] Tandem MS spectra were clustered using MS-Clustering [7] that builds consensus spectra for repeatedly observed ions (this was performed using the natural product analysis infrastructure at http://gnps.ucsd.edu). The $MS^2$ spectra were scored based on their similarity; a cosine score of 1 indicates identical spectra, while a cosine score of 0 indicates no similarity. The cosine score threshold to make a match was set to 0.7 and the minimum matched peak was 5. The algorithm assumed a parent peak mass tolerance of 0.1 Da and an $MS^2$ peak tolerance of 0.01 Da. The networks were visualized with Cytoscape software, whereby consensus spectra are represented as nodes connected by edges to aligning nodes. The thickness of the edge indicates the level of similarity between the nodes. The FM3 layout was used to organize and align nodes within the network. The data is available as MSV000079139 and MSV000079279. at http://gnps.ucsd.edu.

**Genome sequencing, assembly, and annotation**

DNA was extracted from *Streptomyces* sp. QL37 as described previously.[8] Genome sequencing and annotation was done essentially as described previously.[9] Illumina/Solexa sequencing on Genome Analyzer IIx and sequencing on PacBio RS were outsourced to BaseClear BV (Leiden, The Netherlands). 100-nt paired-end reads were obtained and the quality of the short reads verified using FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/). Depending on quality, reads were trimmed to various lengths at both ends. Processed raw reads were subsequently used as input for the Velvet assembly algorithm. Genomes were annotated using the RAST server with default options.

Contigs were also annotated using GeneMark.hmm [10] for ORF prediction, BLASTP for putative function prediction and HMMER for protein-domain prediction, manually inspected for some and visualized using Artemis. The genome has been deposited at GenBank under accession PTJS00000000. The biosynthetic gene cluster was identified using the genome mining tool antiSMASH.[11,12] Table S7 shows the gene annotation of the identified angucycline biosynthetic gene cluster (*lug*), which is depicted in Figure S8.

**Creation of a mutant of the *lug* minimal PKS**

Deletion of the minimal PKS genes *lugA-C* was done based on a method published previously.[13] To obtain a construct for gene disruption ~1.5 kb regions up- and downstream of *lugA-C* were amplified from the chromosome of *Streptomyces* sp. QL37 using primer pairs 1+2 and 3+4 (Table S8) and cloned as EcoRI-XbaI and XbaI-BamHI fragments into a derivative of the unstable multi-copy plasmid pWHM3 [14] that harbours *oriT* to allow its conjugative transfer. The apramycin resistance cassette *aac(3)IV* flanked by *loxP* sites was then inserted in-between using an engineered XbaI site. The correct knock-out construct was transformed to the methylase-deficient strain *E. coli* ET12567/pUZ8002,[15] and subsequently further introduced into *Streptomyces* sp. QL37 by conjugation, following the protocol as described.[8] The correct mutant was selected by resistance to apramycin (50 µg/mL) and sensitivity to thiostrepton (10 µg/mL).[16] Polymerase chain reactions (PCR) were performed on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The ZR Plasmid Miniprep-Classic kit (Zymo Research, Irvine, CA, USA) was used for plasmid extraction.

**UHPLC-ToF-MS analysis**

UHPLC-ToF-MS analyses were performed on an UHPLC system (Agilent 1290, Agilent technologies, USA) coupled to an ESI-llQ-TOF spectrometer (micrOTOF-QII, Bruker Daltonics, Germany) in the positive ion mode.[22] The chromatographic separation was done using a Kinetex $C_{18}$ UHPLC 2.6 µm particle size column 150 × 2.0 mm (Phenomenex, USA) at a flow rate of 0.3 mL/min and a column temperature of 30 ºC. Samples (3 µL) were eluted using a gradient of solvent A (water) and B (acetonitrile), both with 0.1% formic acid (v/v). The initial percentage of B was 5%, which was linearly increased to 90% in 19.5 min, followed by a 2 min isocratic period and, then re-equilibrated with original conditions in 2 min. Nitrogen was used as drying and nebulizing gas. The gas flow was set at 10.0 L/min at 250 ºC and the nebulizer pressure was 2.0 bar. The MS data were acquired over *m/z* range of 100–1000. The capillary voltage was 3.5 kV. For internal calibration, a 10 mM solution of sodium formate (Fluka, Steinheim, Germany) was infused. Formic acid, water and acetonitrile were LCMS grade (Optima, Fisher Scientific, NJ, USA).

**HPLC-DAD analysis**

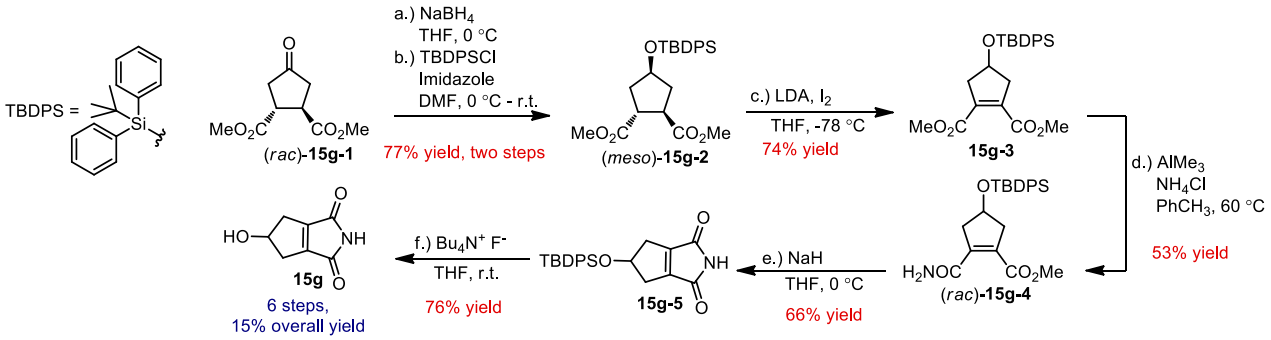
HPLC analysis was performed with an Agilent 1200 series HPLC apparatus (Agilent technologies Inc, Santa Clara, CA, USA), using a 150 × 4.6 mm Luna 5 micron $C_{18}$ (2) 100 Å column equipped with a guard column containing $C_{18}$ 4 × 3 mm cartridges (Phenomenex Inc, Torrance, CA, USA). The mobile phase consisted of water (A) and acetonitrile (B, HPLC grade) in a linear gradient program from 10% B to 100% B in 50 minutes at a flow rate of 1.0 ml/min. Chromatograms were recorded at 210 nm, 254 nm, and 280 nm. The injection volume was 10 µl.

**Organic synthesis of isomaleimycin**

Chemical synthesis of isomaleimycin **15g** (see Scheme 1) was performed using the following reaction steps:

a.) Reduction of ketone **15g-1** to the corresponding alcohol with sodium borohydride.
b.) TBDPS-protection to give **15g-2**.
c.) Desaturation; by enolate formation by means of deprotonation with LDA, followed by *in situ* iodination and subsequent elimination to give cyclopentene di-ester **15g-3**.
d.) Selective mono-amide (**15g-4**) formation by treatment of di-ester **15g-3** with $AlMe_3$ and $NH_4Cl$.
e.) Imide (**15g-5**) formation by deprotonation with sodium hydride.

f.) Deprotection with tetrabutylammonium fluoride to form iso-maleimycin.




**Scheme 1. Chemical synthesis of isomaleimycin 15g.**

**Table S1**. $^{1}H$ and $^{13}C$ NMR data for lugdunomycin (**1**).[a]

| NO. | $\delta_C$ | $\delta_H$ (*J* in Hz) | HMBC [b] | COSY | NOESY |
|---|---|---|---|---|---|
| 1 | 158.8 | | | | |
| 2 | 102.4 | 6.57 (brs) | C-8a, C-4, C-25 | H-4, H-25 | H-25 |
| 3 | 136.2 | | | | |
| 4 | 115.7 | 6.95 (brs) | C-8a, C-2, C-25, C-5 | H-2, H-25 | H-25 |
| 4a | 127.4 | | | | |
| 5 | 127.1 | 7.39 (d, *J* = 8.4 Hz, 1H) | C-4, C-8a, C-7, C-6, C-1*, C-8* | H-6 | |
| 6 | 123.9 | 6.78 (d, *J* = 8.4 Hz, 1H) | C-4a, C-8, C-7, C-9* | H-5 | |
| 7 | 148.2 | | | | |
| 8 | 122.3 | | | | |
| 8a | 129.1 | | | | |
| 9 | 94.8 | | | | |
| 10 | 139.2 | | | | |
| 11 | 119.7 | 6.92 (brd, *J* = 7.8 Hz, 1H) | C-9, C-13, C-15 | H-12, H-13 | |
| 12 | 130.9 | 7.22 (t, *J* = 7.8 Hz, 1H) | C-10, C-14, C-13, C-11 | H-11, H-13 | |
| 13 | 111.1 | 6.96 (brd, *J* = 7.8 Hz, 1H) | C-11, C-15, C-14 | H-12, H-11 | H-26 |
| 14 | 158.4 | | | | |
| 15 | 123.1 | | | | |
| 16 | 62.6 | 5.62 (s) | C-15, C-14, C-10, C-17, C-18, C-21 | | H-18b, H-20a |
| 17 | 59.8 | | | | |
| 18 | 47.9 | 2.64 (m, H-18a); | C-24, C-19, C-20, C-16 | H-18b | H-18b, H-20b |
| | | 1.62 (dd, *J* = 13.8, 3.6 Hz, H-18b) | C-24, C-17, C-16, C-19 | H-19, H-18a | H-20a, H-16, H-19, H-18a |
| 19 | 70.5 | 4.09 (t, *J* =3.6 Hz, 1H) | C-17, C-18, C-20, C-21 | H-18b, H-20b | H-18b, H-20b, H-20a |
| 20 | 47.3 | 2.63 (m, H-20a ) | C-17, C-19, C-18, C-22 | H-20b | H-19, H-18b |
| | | 2.40 (dd, *J* = 14.4, 3.6 Hz, H-20b) | C-22, C-9, C-21 | H-19, H-20a | H-19, H-18a, H-20a |
| 21 | 62.3 | | | | |
| 22 | 182.5 | | | | |
| 23 | -NH | | | | |
| 24 | 182.4 | | | | |
| 25 | 22.1 | 2.46 (brs, 3H) | C-2, C-3, C-4 | H-2, H-4 | H-2, H-4 |
| 26 | 56.1 | 3.90 (s, 3H) | C-14 | | H-13 |

[a] **1** recorded in $CD_3OD$. Proton coupling constants (*J*) in Hz are given in parentheses. $^{1}H$ NMR and $^{13}C$ APT NMR spectra were recorded at 600 MHz. All chemical shift assignments were done on the basis of 1D and 2D NMR techniques. [b] All observed HMBC, COSY, and NOESY correlations are summarized, and long range coupling ($^{4}J_{CH}$) in HMBC was marked with asterisk (*****).

**Table S2.** Crystal Data for lugdunomycin (**1**).

| | | | |
|---|---|---|---|
| **Identification code** | Lugdunomycin | **F(000)** | 1984 |
| **Empirical formula** | $C_{27}H_{23}NO_7$ | **Crystal size** | 0.7 x 0.5 x 0.5 $mm^3$ |
| **Formula weight** | 473.46 | **Theta range for data collection** | 1.241 to 25.340° |
| **Temperature** | 100(2) K | **Index ranges** | -27<=h<=27, -27<=k<=27, -9<=l<=9 |
| **Wavelength** | 0.71073 Å | **Reflections collected** | 31098 |
| **Crystal system** | Tetragonal | **Independent reflections** | 4053 [$R_{int}$ = 0.1666] |
| **Space group** | P 42/n | **Completeness to theta = 25.242°** | 100.0 % |
| **Unit cell dimensions** | a = 23.204(2) Å, α= 90°. | **Refinement method** | Full-matrix least-squares on $F^2$ |
| | b = 23.204(2) Å, β= 90°. | **Data/restraints/parameters** | 4053 / 330 / 321 |
| | c = 8.2073(9) Å, γ = 90°. | **Goodness-of-fit on $F^2$** | 1.036 |
| **Volume** | 4419.1(10) $Å^3$ | **Final R indices [I>2σ(*I*)]** | $R_1$ = 0.0615, wR2 = 0.1134 |
| **Z** | 8 | **R indices (all data)** | $R_1$ = 0.1245, wR2 = 0.1379 |
| **Density (calculated)** | 1.423 $Mg/m^3$ | **Extinction coefficient** | n/a |
| **Absorption coefficient** | 0.104 $mm^{-1}$ | **Largest diff. peak and hole** | 0.287 and -0.236 e.$Å^{-3}$ |

**Table S3.** Culture media with different compositions for lugdunomycin production.

| No. | Media Combination | No. | Media Combination |
|---|---|---|---|
| 1 | NMMP + 0.2% L-asparagine + 50 mM TES buffer + 2% glycerol | 40 | MM + 0.5% yeast extract + 50mM GluNAc |
| 2 | NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% glucose | 41 | MM + 0.8% peptone + 1% mannitol |
| 3 | NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% mannitol | 42 | MM + 0.8% peptone + 1% glucose |
| 4 | NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% xylose | 43 | MM + 0.8% peptone + 1% glycerol |
| 5 | NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% GluNAc | 44 | MM + 0.8% peptone + 50mM GluNAc |
| 6 | NMMP + 0.2% $(NH_4)_2SO_4$ + 50 mM TES buffer + 1% glycerol | 45 | MM + 1% mannitol |
| 7 | NMMP + 0.2% $(NH_4)_2SO_4$ + 50 mM TES buffer + 1% glucose | 46 | MM + 1% glucose |
| 8 | NMMP + 0.2% $(NH_4)_2SO_4$ + 50 mM TES buffer + 1% mannitol | 47 | MM + 1% glycerol |
| 9 | NMMP + 0.2% $(NH_4)_2SO_4$ + 50 mM TES buffer + 1% xylose | 48 | MM + 50mM GluNAc |
| 10 | NMMP + 0.2% $(NH_4)_2SO_4$ + 50 mM TES buffer + 1% GluNAc | 49 | MM + 25mM NaBu + 1% mannitol |
| 11 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% glycerol | 50 | MM + 25mM NaBu + 1% glucose |
| 12 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% glucose | 51 | MM + 25mM NaBu + 1% glycerol |
| 13 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% mannitol | 52 | MM + 25mM NaBu +50mM GluNAc |
| 14 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% xylose | 53 | R5+ proline + NaOH + CaCl2 + 1% glucose |
| 15 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% GluNAc | 54 | R5+ proline + NaOH + CaCl2 + 1% glycerol |
| 16 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% fructose | 55 | R5+ proline + NaOH + CaCl2 + 1% mannitol |
| 17 | NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% maltose | 56 | R5+ proline + NaOH + CaCl2 + 1% 50mM GluNAc |
| 18 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% glycerol | 57 | R5+ proline + NaOH + CaCl2 + without sugar |
| 19 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% glucose | 58 | R5 + 1% glucose |
| 20 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% mannitol | 59 | R5+ 1% glycerol |
| 21 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% xylose | 60 | R5+ 1% mannitol |
| 22 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% GluNAc | 61 | R5 + 50mM GluNAc |
| 23 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% fructose | 62 | R5 + without sugar |
| 24 | NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% maltose | 63 | R5 + 200mM NaCl+ 1% glucose |
| 25 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% glycerol | 64 | R5 + 200mM NaCl+ 1% glycerol |
| 26 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% glucose | 65 | R5 + 200mM NaCl+ 1% mannitol |
| 27 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% mannitol | 66 | R5 + 200mM NaCl + 50mM GluNAc |
| 28 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% xylose | 67 | R5 + 200mM NaCl + without sugar |
| 29 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% GluNAc | 68 | R5 + 0.8% peptone+ 1% glucose |
| 30 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% fructose | 69 | R5 + 0.8% peptone + 1% glycerol |
| 31 | NMMP + 0.2% L-proline + 50 mM TES buffer + 1% maltose | 70 | **R5 + 0.8% peptone + 1% mannitol** |
| 32 | NMMP + 0.2% $(NH_4)_2SO_4$ + phosphate buffer + 1% glycerol + 0.5% mannitol | 71 | R5 + 0.8% peptone + 50mM GluNAc |
| 33 | NMMP + 0.2% $(NH_4)_2SO_4$ + phosphate buffer + 1% glycerol + 0.5% mannitol + 1% SFM | 72 | R5 + 0.8% peptone + without sugar |
| 34 | NMMP + 0.2% $(NH_4)_2SO_4$ + phosphate buffer + 1% glycerol + 0.5% mannitol + 0.5% yeast extract | 73 | R5 + 0.5% Yeast extract+ 1% glucose |
| 35 | NMMP + 0.2% $(NH_4)_2SO_4$ + phosphate buffer + 1% glycerol + 0.5% mannitol + 1% peptone | 74 | R5 + 0.5% Yeast extract + 1% glycerol |
| 36 | NMMP + 0.2% $(NH_4)_2SO_4$ + phosphate buffer + 1% glycerol + 0.5% mannitol + pH 10 | 75 | R5 + 0.5% Yeast extract + 1% mannitol |
| 37 | MM + 0.5% yeast extract + 1% mannitol | 76 | R5 + 0.5% Yeast extract + 50mM GluNAc |
| 38 | MM + 0.5% yeast extract + 1% glucose | 77 | R5 + 0.5% Yeast extract + without sugar |
| 39 | MM + 0.5% yeast extract + 1% glycerol | | |

**Table S4.** $^{13}$C NMR data for new compounds **5**, **9**, **14**－**17**, **21**－**25**.[a]

| position | 5 | 9 | 14 | 15 | 16 | 17 | 21 | 22 | 23 | 24 | 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 155.0 | 197.1 | 134.2 | 157.7 | 157.8 | ? | 152.1 | 151.3 | 185.9 | 176.4 | 176.3 |
| 2 | 117.3 | 52.1 | 115.7 | 104.0 | 104.1 | 134.7 | 112.0 | 109.7 | 134.3 | 46.5 | 46.3 |
| 3 | 140.4 | 74.7 | 135.0 | 137.1 | 137.3 | 149.6 | 134.3 | 132.1 | 148.8 | 72.6 | 71.9 |
| 4 | 120.7 | 74.8 | 124.8 | 115.9 | 116.3 | 184.3 | 118.8 | 117.5 | 183.8 | 41.0 | 48.2 |
| 4a | 136.6 | 154.5 | 123.8 | 126.7 | 126.7 | 128.1 | 130.6 | ? | 121.7 | 139.2 | 146.8 |
| 5 | 132.9 | 119.8 | 138.8 | 127.8 | 128.4 | 130.3 | 130.4 | 128.0 | 129.0 | 132.4 | 137.5 |
| 6 | 130.5 | 165.2 | 132.1 | 121.4 | 121.2 | 122.3 | 118.3 | 117.7 | 121.4 | ? | 127.0 |
| 6a | 79.5 | 118.8 | 182.5 | 150.0 | 150.3 | 164.6 | 151.9 | 153.4 | 167.8 | 113.4 | 132.3 |
| 7 | 68.6 | 188.9 | 168.3 | 170.5 | 168.1 | ? | 59.3 | | | 183.0 | 189.4 |
| 7a | 131.6 | 120.5 | 123.2 | 117.8 | 117.8 | 126.2 | 141.7 | | | 121.5 | 116.6 |
| 8 | 159.1 | 161.5 | 158.0 | 158.2 | 157.9 | 157.6 | 159.9 | | | 162.1 | 163.1 |
| 9 | 116.0 | 118.8 | 113.7 | 113.5 | 113.7 | 116.2 | 114.6 | | | 119.8 | 124.7 |
| 10 | 129.9 | 137.3 | 131.9 | 136.0 | 135.6 | 130.4 | 129.7 | | | 136.7 | 137.4 |
| 11 | 120.0 | 119.8 | 123.0 | 115.4 | 115.4 | 122.5 | 123.9 | | | 120.2 | 119.7 |
| 11a | 133.8 | 138.6 | 131.5 | 148.8 | 147.1 | 135.9 | 123.4 | | | 136.4 | 134.4 |
| 12 | 201.2 | 185.8 | 146.6 | 102.1 | 104.9 | ? | 201.5 | | | 188.0 | 183.5 |
| 12a | 79.5 | 138.2 | 112.8 | 116.6 | 114.6 | 122.8 | 119.9 | 103.4 | 113.0 | 112.9 | 133.7 |
| 12b | 118.6 | 127.0 | 124.8 | 129.3 | 129.6 | 132.6 | 121.8 | 124.3 | 134.5 | 157.3 | 129.8 |
| 3-$CH_3$ | 20.8 | 26.2 | 21.2 | 22.4 | 22.4 | 15.6 | 20.7 | 20.3 | 15.0 | 26.7 | 26.6 |
| 8-$OCH_3$ | 56.1 | 56.6 | 56.0 | 55.9 | 56.0 | 56.0 | 56.0 | | | 56.7 | |
| 7-$OCH_3$ | | | 51.8 | | | | | | | | |
| *N*-$CH_3$ | | | | | 24.3 | | | | | | |

[a] All the compounds were recorded in $CD_3OD$; $^{13}$C NMR chemical shift assignments were done on the basis of APT and/or 2D NMR techniques; "?", $^{13}$C NMR data assignment was failed due to lack of HMBC correlation and/or low amount of isolated compound.

**Table S5.** $^1$H NMR data for new compounds **5**, **9**, **21**, **22**, **23**.[a]

| position | 5 | 9 | 21 | 22 | 23 |
|---|---|---|---|---|---|
| 2 | 6.40, brs | 2.97, d (5.6); 2.91, dd (15.6) | 6.54, brs | 6.60, brs | 6.71, d (1.8) |
| 4 | 6.48, brs | 4.67, s | 7.08, brs | 7.00, brs | |
| 5 | 6.61, d (9.6) | 7.47, s | 7.66, d (9.0) | 7.52, d (8.4) | 7.86, d (8.4) |
| 6 | 5.90, d (9.6) | | 7.11, d (9.0) | 6.98, dd (8.4, 2.4) | 6.94, dd (8.4, 2.4) |
| 7 | 5.00, s | | 5.47, s | | |
| 9 | 7.21, brd (7.8) | 7.53, dd (8.4, 1.2) | 7.16, d (7.8) | | |
| 10 | 7.42, t (7.8) | 7.83, t (8.4) | 7.25, t (7.8) | | |
| 11 | 7.54, d (7.8) | 7.65, dd (8.4, 1.2) | 6.98, d (7.8) | | |
| 12a | | | | 7.38, d (2.4) | 7.20, d (2.4) |
| 3-$CH_3$ | 2.18, s | 1.43, s | 2.33, s | 2.35, brs | 2.11, d (1.2) |
| 8-$OCH_3$ | 3.88, s | 4.04, s | 3.88, s | | |

[a] All the compounds were recorded in $CD_3OD$; Proton coupling constants (*J*) in Hz are given in parentheses. All chemical shift assignments were done on the basis of 1D and 2D NMR techniques.

**Table S6.** $^1$H NMR data for new compounds **14**—**17**, **24**, and **25**[a].

| position | 14 | 15 | 16 | 17 | 24 | 25 |
|---|---|---|---|---|---|---|
| 2 | 7.53, brs | 6.55, brs | 6.61, brs | 6.65, q (1.2) | 2.44, s | 2.39, s |
| 4 | 7.49, brs | 7.08, brs | 7.13, brs | | 3.03, d (13.2); 3.00, d (13.2) | 3.10, d (13.2); 3.04, d (13.2) |
| 5 | 7.80, d (9.6) | 7.60, d (8.4) | 7.65, d (8.4) | 8.03, d (9.0) | 7.34, s | 7.82, dd (7.8, 1.2) |
| 6 | 6.60, d (9.6) | 7.05, d (8.4) | 7.05, d (8.4) | 7.09, d (9.0) | | 8.27, dd (7.8) |
| 9 | 7.31, d (8.4) | 7.14, d (8.4) | 7.16, d (8.4) | 7.28, d (7.8) | 7.57, d (7.8) | 7.34, d (8.4) |
| 10 | 7.62, t (7.8) | 7.52, t (7.8) | 7.51, t (7.8) | 7.34, t (7.8) | 7.84, t (7.8) | 7.75, t (7.8) |
| 11 | 7.37, d (7.2) | 6.65, d (7.2) | 6.67, d (7.2) | 7.02, d (7.8) | 8.00, d (7.8) | 7.81, d (8.4) |
| 12b | | | | | | 8.20, d (1.2) |
| 3-$CH_3$ | 2.58, brs | 2.46, s | 2.48, s | 2.12, d (1.2) | 1.28, s | 1.26, s |
| 8-$OCH_3$ | 3.93, s | 3.99, s | 4.00, s | 3.99, s | 4.04, s | |
| 7-$OCH_3$ | 3.53, s | | | | | |
| *N*-$CH_3$ | | | 2.69, s | | | |

[a] All the compounds were recorded in $CD_3OD$; Proton coupling constants (*J*) in Hz are given in parentheses. All chemical shift assignments were done on the basis of 1D and 2D NMR techniques.

**Table S7.** Gene organization of the angucycline biosynthetic gene cluster (*lug*) in *Streptomyces* sp. QL37. See also Figure S7 for an illustration of the gene cluster.

| ORF | Protein | Gene annotation | aa[1] | Putative function | Nearest homologue | Homology[2] | Accession |
|---|---|---|---|---|---|---|---|
| 1 | ORF1 | Prokka_02560 | 379 | XRE-family regulator | *Streptomyces griseoaurantiacus* M045 | 80% | EGG46652.1 |
| 2 | ORF2 | Prokka_02561 | 284 | ADP-ribose pyrophosphatase | *Streptomyces aurantiacus* JA 4570 | 84% | EPH39897.1 |
| 3 | ORF3 | Prokka_02562 | 144 | hypothetical protein | *Streptomyces aureofaciens* | 76% | WP_052839114.1 |
| 4 | LugM | Prokka_02563 | 199 | NADPH-dependent FMN reductase | *Streptomyces sp.* 303MFCol5.2 | 85% | WP_020127613.1 |
| 5 | LugRI | Prokka_02564 | 280 | XRE family transcriptional regulator | *Streptomyces sviceus* | 81% | WP_007383482.1 |
| 6 | LugRII | Prokka_02565 | 223 | LuxR family transcriptional regulator | *Streptomyces sp.* W007 | 52% | WP_007453015.1 |
| 7 | LugRIII | Prokka_02566 | 239 | TetR family transcriptional regulator | *Streptomyces sp.* W007 | 67% | WP_007453018.1 |
| 8 | LugTI | Prokka_02567 | 493 | putative export protein | *Streptomyces sp.* W007 | 76% | WP_007453020.1 |
| 9 | LugX | Prokka_02568 | 144 | hypothetical protein | --- | --- | --- |
| 10 | LugN | Prokka_02569 | 346 | *O*-methyltransferase | *Streptomyces sp.* W007 | 71% | WP_007453021.1 |
| 11 | LugOI | Prokka_02570 | 490 | Monooxygenase (*urdE*) | *Streptomyces sp.* W007 | 81% | WP_007453024.1 |
| 12 | LugF | Prokka_02571 | 109 | polyketide cyclase (*urdF*) | *Streptomyces sp.* SCC 2136 | 80% | CAH10118.1 |
| 13 | LugA | Prokka_02572 | 427 | polyketide α-ketoacyl synthase II (*urdA*) | *Streptomyces sp.* W007 | 87% | WP_007453026.1 |
| 14 | LugB | Prokka_02573 | 407 | polyketide β-ketoacyl synthase (*urdB*) | *Streptomyces sp.* W007 | 80% | WP_007453027.1 |
| 15 | LugC | Prokka_02574 | 91 | Acyl carrier protein (*urdC*) | *Streptomyces sp.* W007 | 71% | WP_007453028.1 |
| 16 | LugD | Prokka_02575 | 262 | ketoacyl reductase (*urdD*) | *Streptomyces sp.* W007 | 83% | WP_007453030.1 |
| 17 | LugE | Prokka_02576 | 316 | aromatase | *Streptomyces sp.* W007 | 79% | WP_007453031.1 |
| 18 | LugOII | Prokka_02577 | 656 | monooxygenase (*urdM*) | *Streptomyces sp.* W007 | 76% | WP_007453032.1 |
| 19 | LugG | Prokka_02578 | 262 | NAD(P)H dependent dehydrogenase | *Streptomyces sp.* W007 | 76% | WP_007453033.1 |
| 20 | LugH | Prokka_02579 | 527 | methylmalonyl-CoA carboxyltransferase | *Streptomyces sp.* W007 | 87% | WP_007453034.1 |
| 21 | LugI | Prokka_02580 | 79 | putative acetyl-CoA carboxylase | *Streptomyces rapamycinicus* | 61% | WP_020868207.1 |
| 22 | LugTII | Prokka_02581 | 417 | putative MFS-type transporter EfpA | *Streptomyces sp. 303MFCol5.2* | 62% | WP_020130977.1 |
| 23 | LugJ | Prokka_02582 | 195 | NAD(P)H-dependent FMN reductase | *Streptomyces fradiae* | 66% | KDS84998.1 |
| 24 | LugOIII | Prokka_02583 | 214 | putative monooxygenase | *Streptomyces sp.* W007 | 62% | WP_007450404.1 |
| 25 | LugK | Prokka_02584 | 245 | phosphopantetheinyl transferase | *Streptomyces venezuelae* | 51% | WP_015037371.1 |
| 26 | LugRIV | Prokka_02585 | 267 | transcriptional regulatory protein BaeR | *Streptomyces sp.* W007 | 66% | WP_007450396.1 |
| 27 | LugTIII | Prokka_02586 | 458 | MFS transporter | *Streptomyces ochraceiscleroticus* | 76% | WP_051862838.1 |
| 28 | LugL | Prokka_02587 | 307 | thioesterase | *Streptomyces scopuliridis* | 69% | WP_030349255.1 |
| 29 | LugOIV | Prokka_02588 | 275 | oxidoreductase | *Streptomyces sp.* W007 | 70% | WP_050987713.1 |
| 30 | LugRV | Prokka_02589 | 646 | SARP family transcriptional regulator | *Streptomyces sp.* W007 | 57% | WP_007450402.1 |
| 31 | LugOV | Prokka_02590 | 229 | putative dehydrogenase-methyltransferase | *Streptomyces sp.* W007 | 57% | WP_007450403.1 |
| 32 | ORF4 | Prokka_02591 | 425 | phosphoribosyltransferase | *Streptomyces pratensis* | 90% | WP_014156222.1 |

[1] length of the predicted gene product in amino acids;

[2] homology in amino acid identity between predicted gene product of *Streptomyces* sp. QL37 and its nearest homologue.

**Table S8.** Primers used for gene knockout of minimal PKS genes *lugA-C*.

| primer NO. | Primer name | sequence (5'→ 3') [#] |
|---|---|---|
| 1 | MinPKS_LF_Fw | ctag**GAATTC**CCGCCACCACCGAGCTCTTC |
| 2 | MinPKS_LF_RV | GAAGTTATCCATCACC**TCTAGA**GATACCGGTGATGACGACCC |
| 3 | MinPKS_RF_Fw | GAAGTTATCGCGCATC**TCTAGA**GCCGAGCAGCTCGACCGTTAC |
| 4 | MinPKS_RF_Rv | ctag**GGATCC**CTGCCCTTGTCGAGAAGCAGTG |

[#]Restriction sites used for cloning are underlined and in bold face. GAATTC, EcoRI; TCTAGA, XbaI; GGATCC, BamHI.

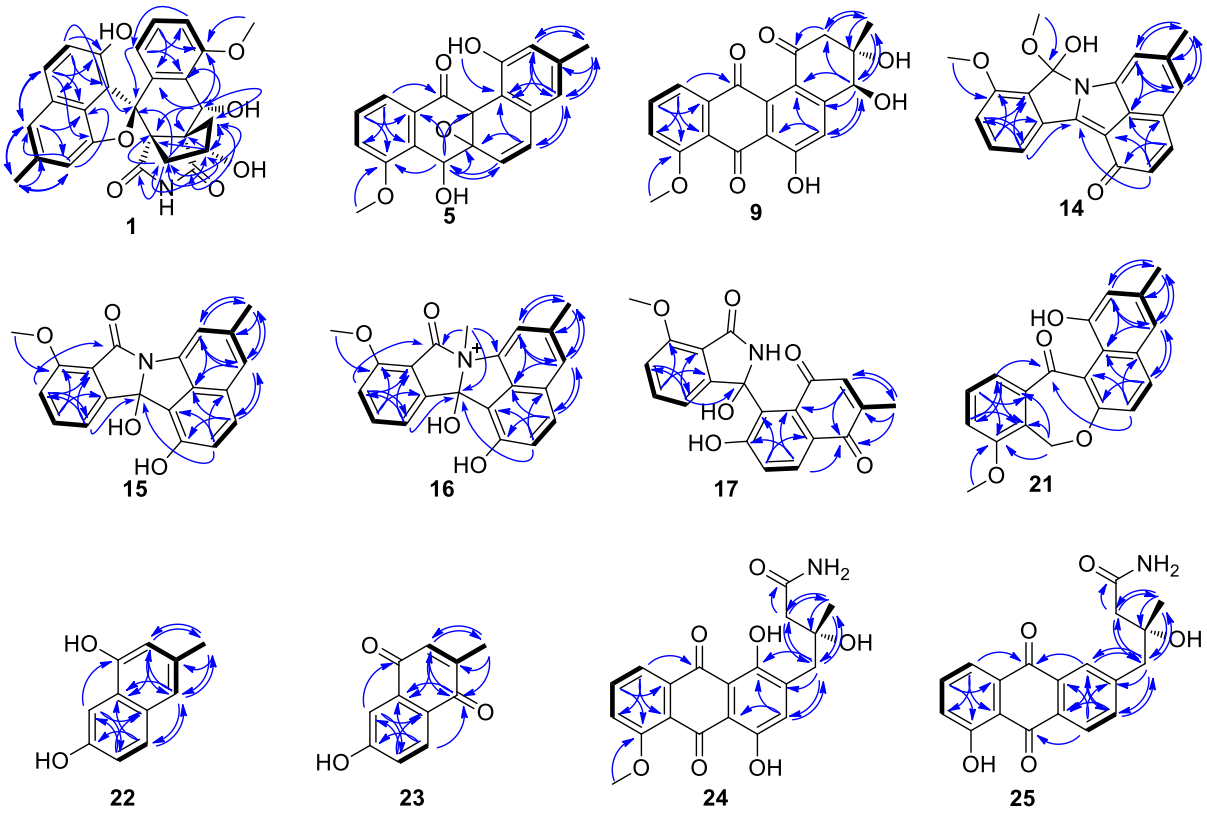
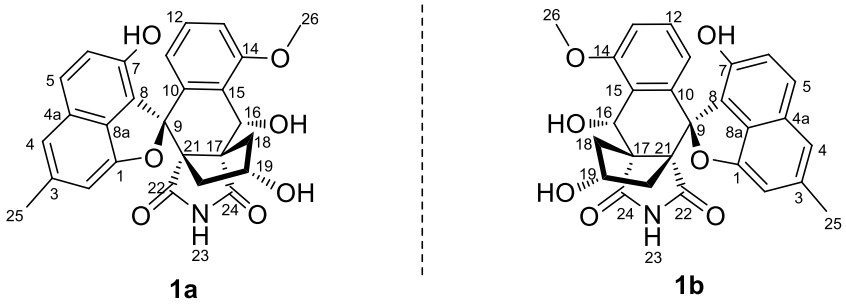



**Figure S1. 2D NMR correlations of lugdunomycin (1), and new angucyclines 5, 9, 14—17, 21—25.** Displayed are HMBC (↷) and COSY (—) correlations for determination of planar structure.

**A**




**B**

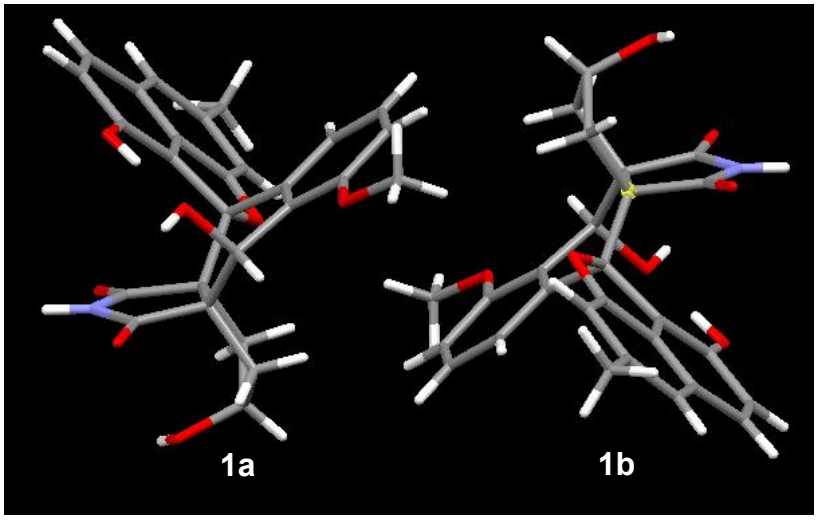



**Figure S2. Enantiomers of lugdunomycin (1). A**) ChemDraw depiction of the two enantiomers **1a** and **1b.** The absolute configurations of five chiral centers in **1a** are 9*R*, 16*S*, 17*R*, 19*S*, and 21*S*, while the stereochemistry is 9*S*, 16*R*, 17*S*, 19*R*, and 21*R* for **1b**. **B**) The X-ray crystallographic structures of two enantiomers **1a** and **1b**. This shown image for mirror symmetry is presented by Mercury (version 3.3) software.

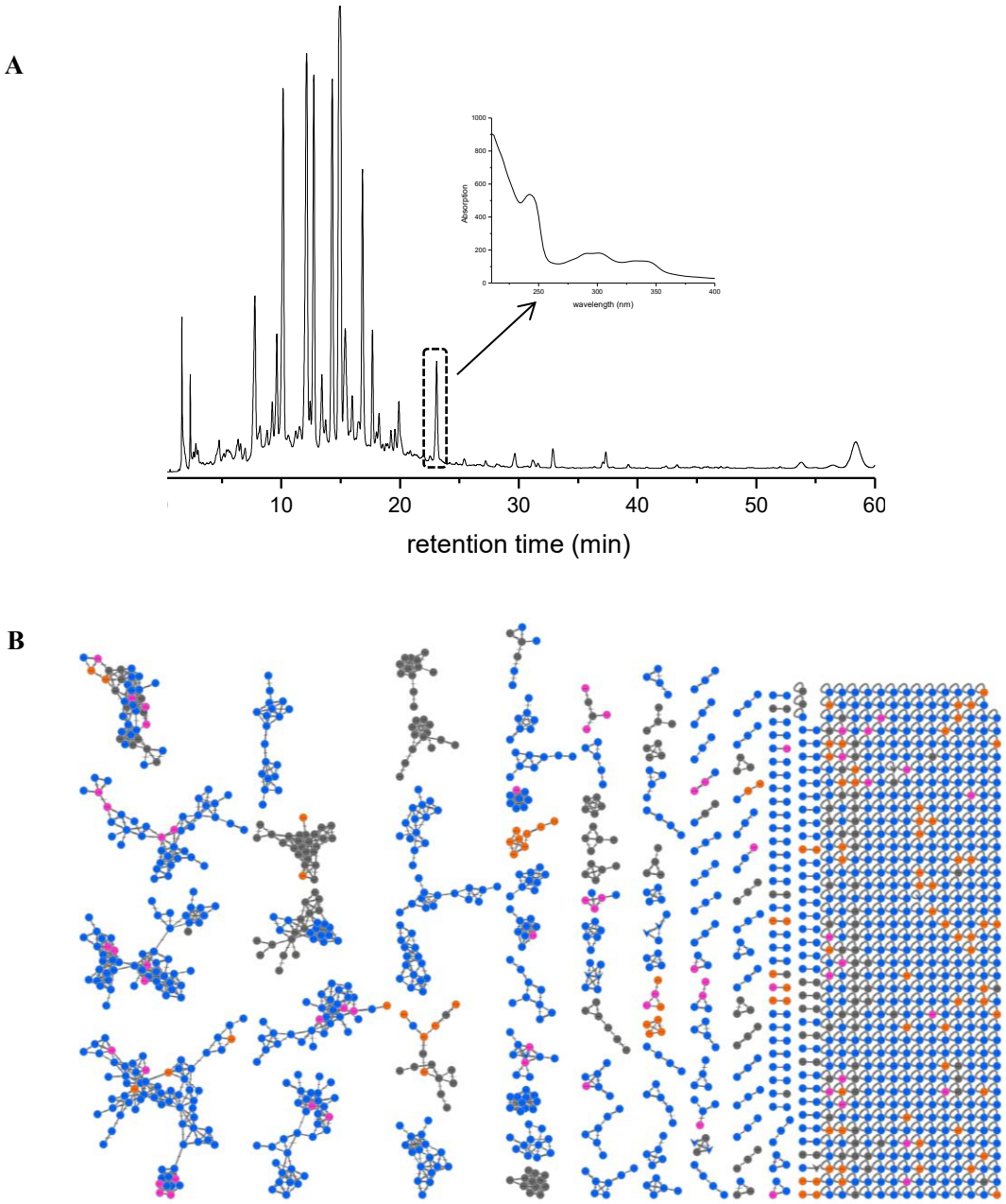



**Figure S3. Metabolic analysis of crude extracts from *Streptomyces* sp. QL37. A**) HPLC-DAD analysis (254 nm) of *Streptomyces sp*. QL37 grown on R5 + 0.8% peptone + 1% mannitol. The compound in dashed box gives a UV spectrum similar to that of lugdunomycin (see S9). **B**) $MS^2$-based molecular networking analysis of crude extracts harvested from QL37 R5 + 0.8% peptone + 1% mannitol and MM + 0.5% mannitol + 1% glycerol. Related compounds cluster together to form structural families, and this observation suggests the presence of as yet unidentified metabolites, especially new angucyclines produced by *Streptomyces* sp. QL37.

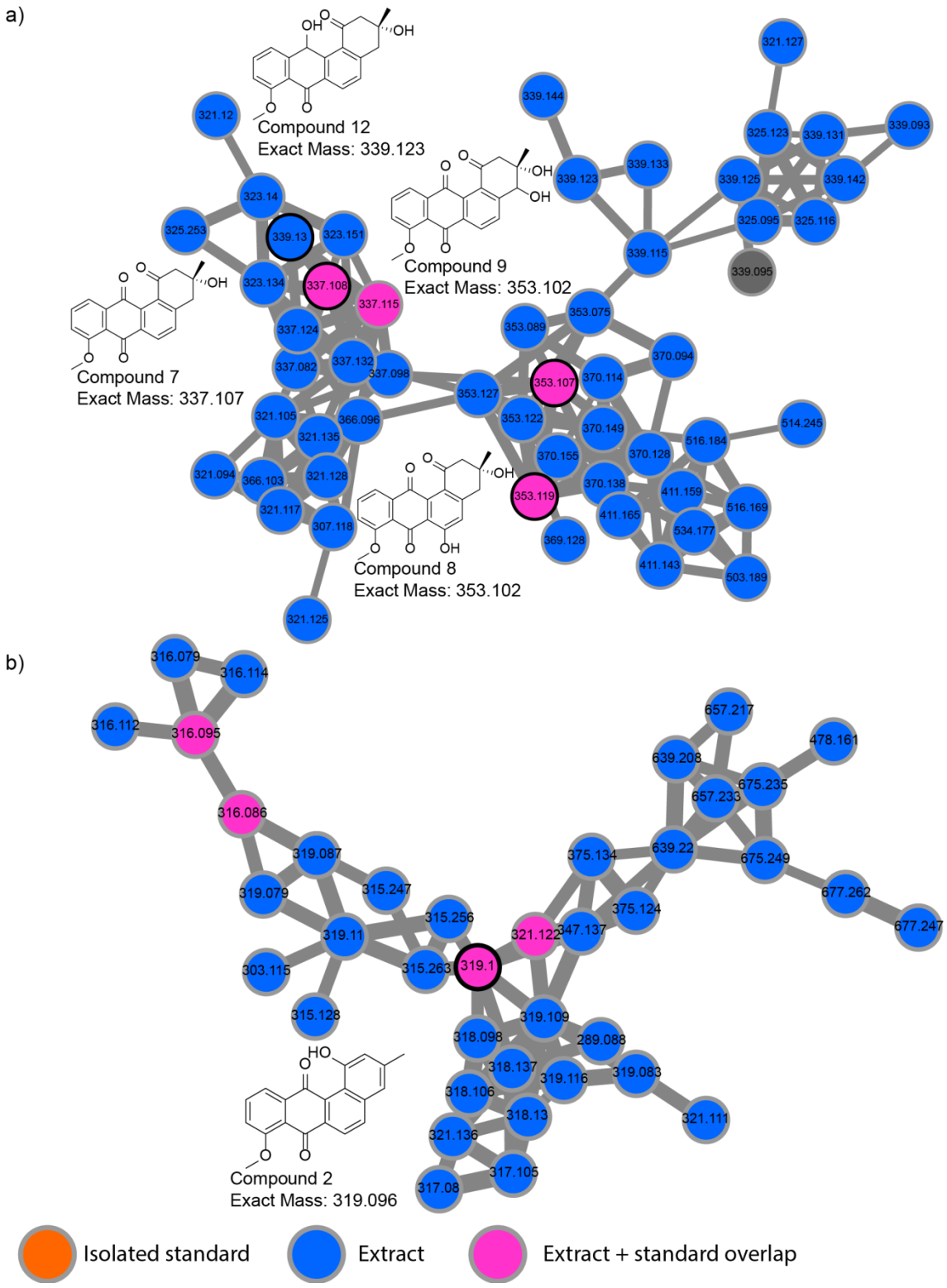



**Figure S4. Subnetwork for unrearranged angucyclines.**

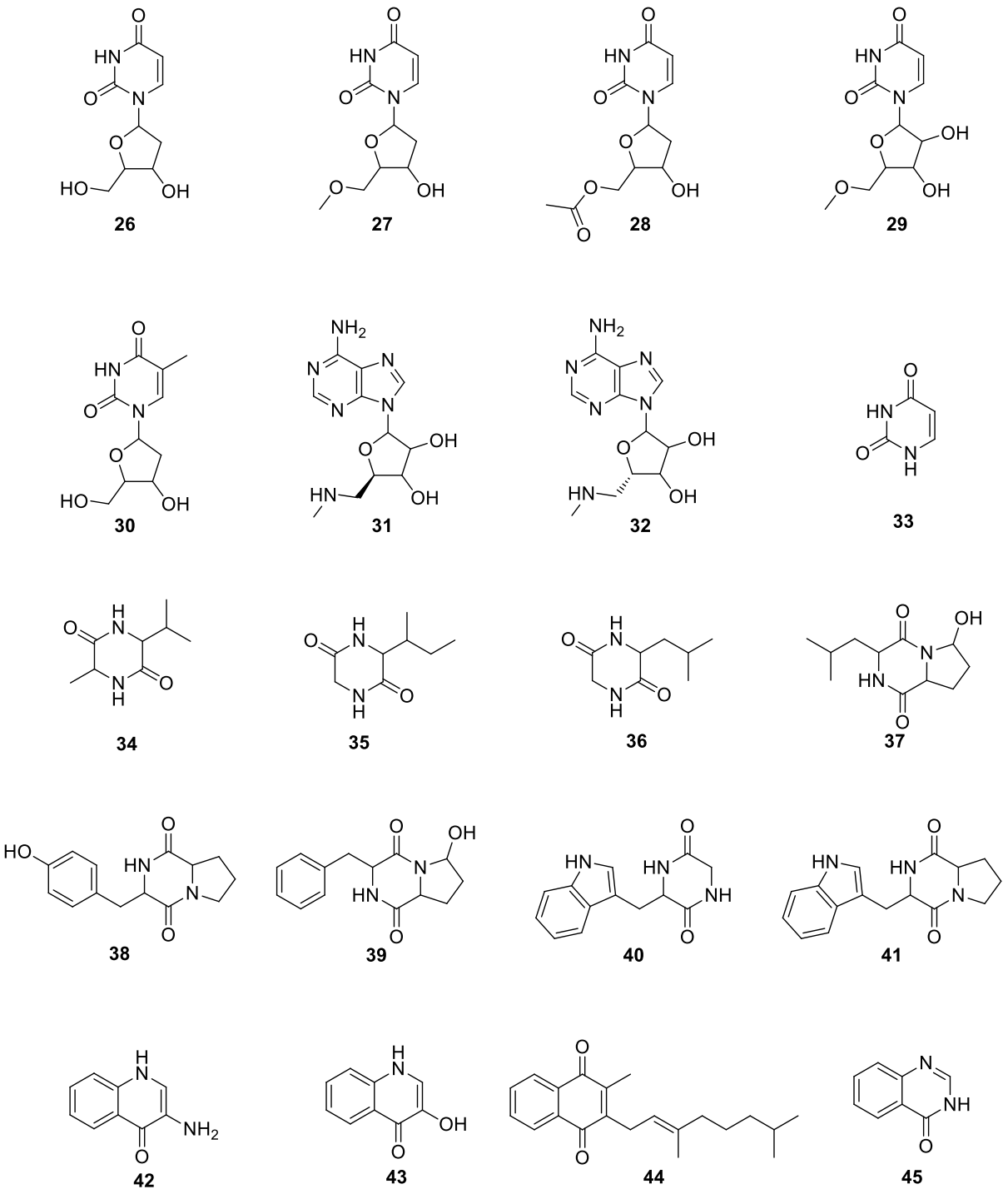
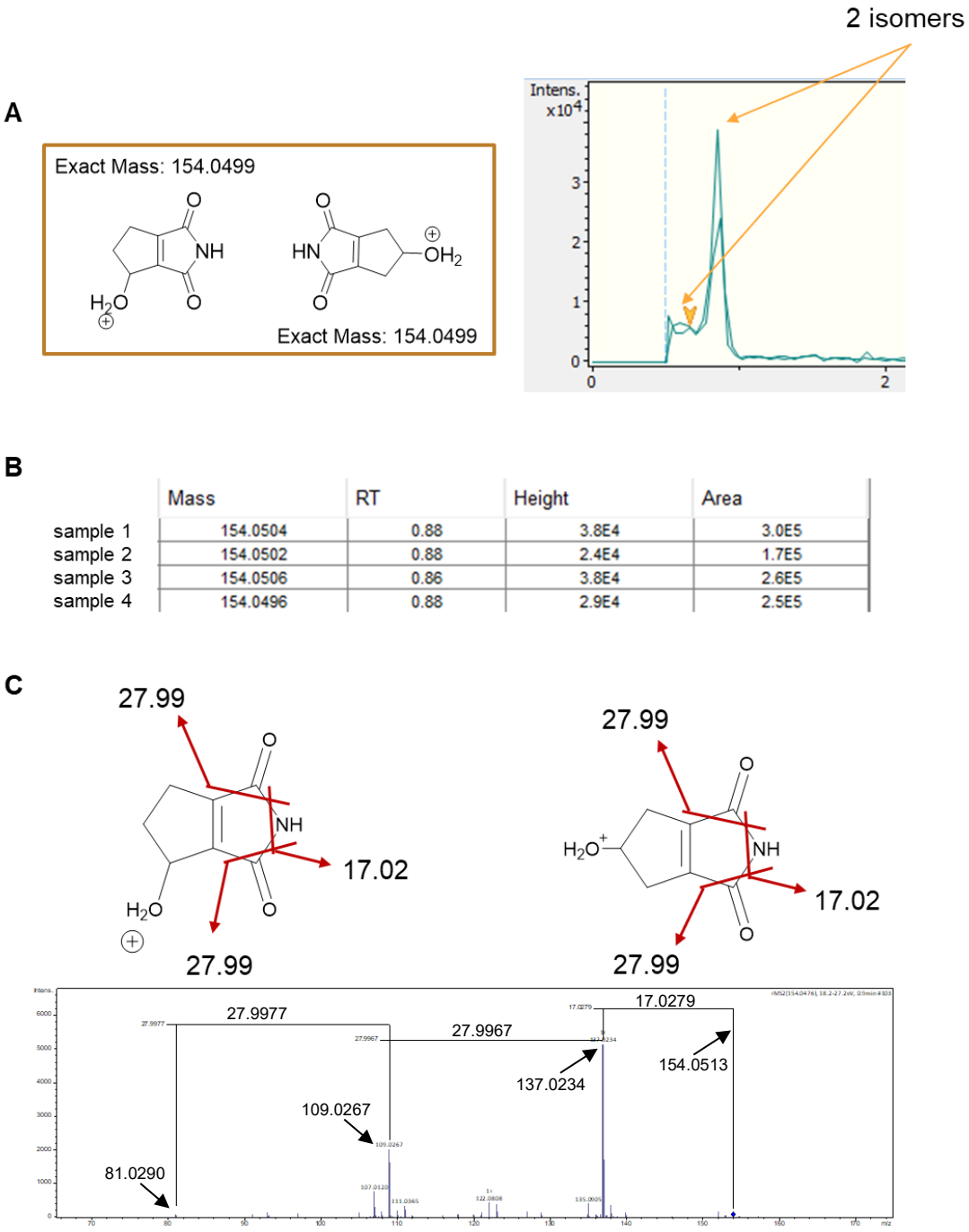



**Figure S5. Structure of compounds 26－45 identified from *Streptomyces* sp. QL37.**




| | Mass | RT | Height | Area |
|---|---|---|---|---|
| sample 1 | 154.0504 | 0.88 | 3.8E4 | 3.0E5 |
| sample 2 | 154.0502 | 0.88 | 2.4E4 | 1.7E5 |
| sample 3 | 154.0506 | 0.86 | 3.8E4 | 2.6E5 |
| sample 4 | 154.0496 | 0.88 | 2.9E4 | 2.5E5 |

**Figure S6. Identification of maleimycin.** Two stereoisomers of maleimycin with a mass of 154.0499 (A) were identified in four independent samples of *Streptomyces* sp. QL37 (B). MS/MS fragmentation is shown in (C).

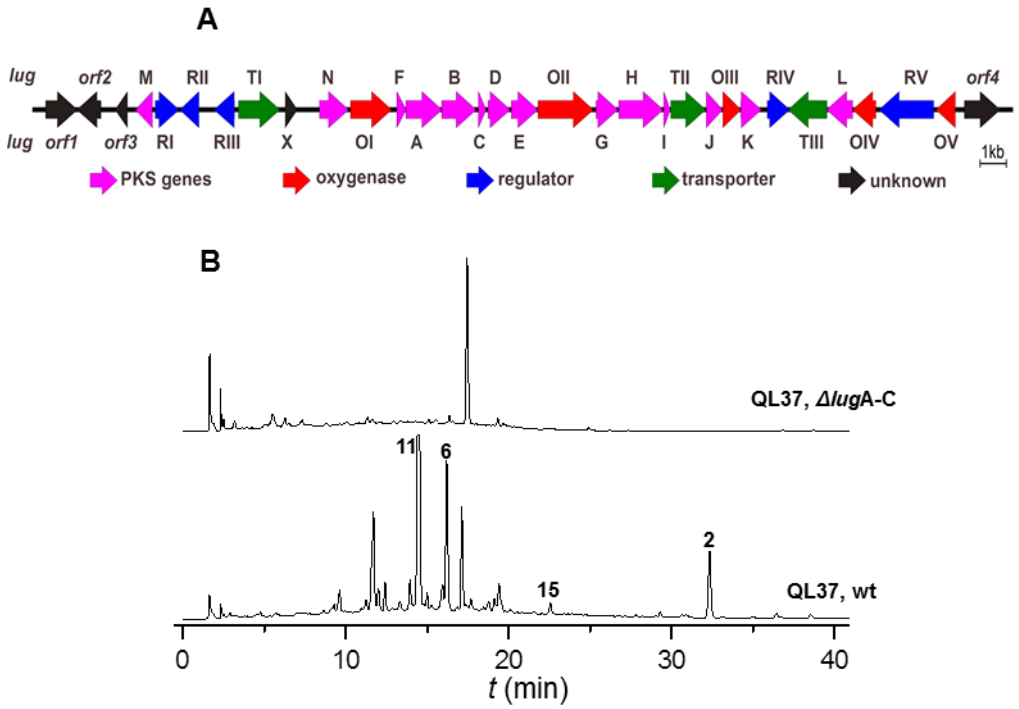



**Figure S7. The *lug* gene cluster is responsible for lugdunomycin and angucyclines production in *Streptomyces* sp. QL37.** (**A**) Organization of the type II PKS gene cluster (*lug*) in *Streptomyces* sp. QL37, and the annotation of the respective genes is displayed in Table S7. **(B)** HPLC-UV analysis (254 nm) showed that inactivation of *lugA-C* abolished production of angucyclines and derivatives in wild type (wt) *Streptomyces* sp. QL37. Strains were grown in R5 + 0.8% peptone + 1% mannitol medium. Numbers correspond to the molecules shown in Figure 1.

**Spectra list of new compounds**

S39. HSQC spectrum (600 MHz) of **16** in $CD_3OD$.
S40. HMBC spectrum (600 MHz) of **16** in $CD_3OD$.
S41. HRESIMS spectrum of **16**.
S42. UV spectrum of **16**.

S43. $^1H$ NMR spectrum (600 MHz) of **17** in $CD_3OD$.
S44. HSQC spectrum (600 MHz) of **17** in $CD_3OD$.
S45. HMBC spectrum (600 MHz) of **17** in $CD_3OD$.
S46. $^1H$-$^1H$ COSY spectrum (600 MHz) of **17** in $CD_3OD$.
S47. HRESIMS spectrum of **17**.
S48. UV spectrum of **17**.

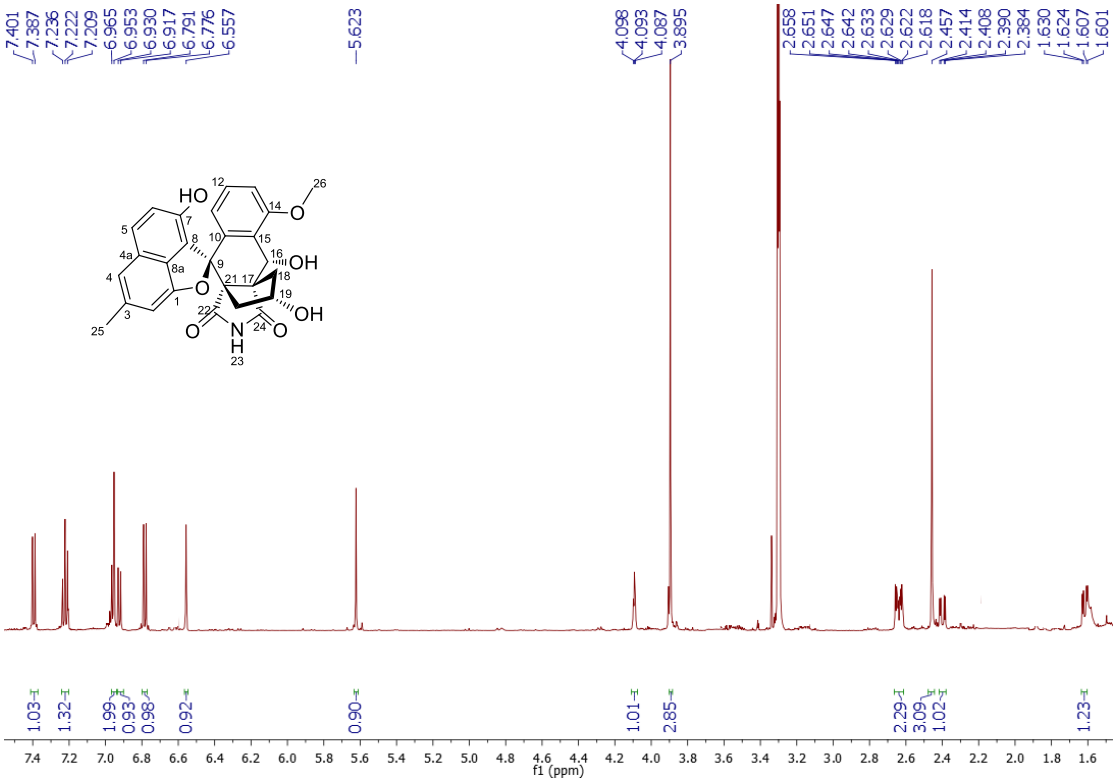
S49. $^1H$ NMR spectrum (600 MHz) of **21** in $CD_3OD$.
S50. HSQC spectrum (600 MHz) of **21** in $CD_3OD$.
S51. HMBC spectrum (600 MHz) of **21** in $CD_3OD$.
S52. $^1H$-$^1H$ COSY spectrum (600 MHz) of **21** in $CD_3OD$.
S53. HRESIMS spectrum of **21**.
S54. UV spectrum of **21**.

S55. $^1H$ NMR spectrum (600 MHz) of **22** and **23** in $CD_3OD$.
S56. HSQC spectrum (600 MHz) of **22** and **23** in $CD_3OD$.
S57. HMBC spectrum (600 MHz) of **22** and **23** in $CD_3OD$.
S58. $^1H$-$^1H$ COSY spectrum (600 MHz) of **22** and **23** in $CD_3OD$.
S59. HRESIMS spectrum of **22**.
S60. UV spectrum of **22**.
S61. HRESIMS spectrum of **23**.
S62. UV spectrum of **23**.

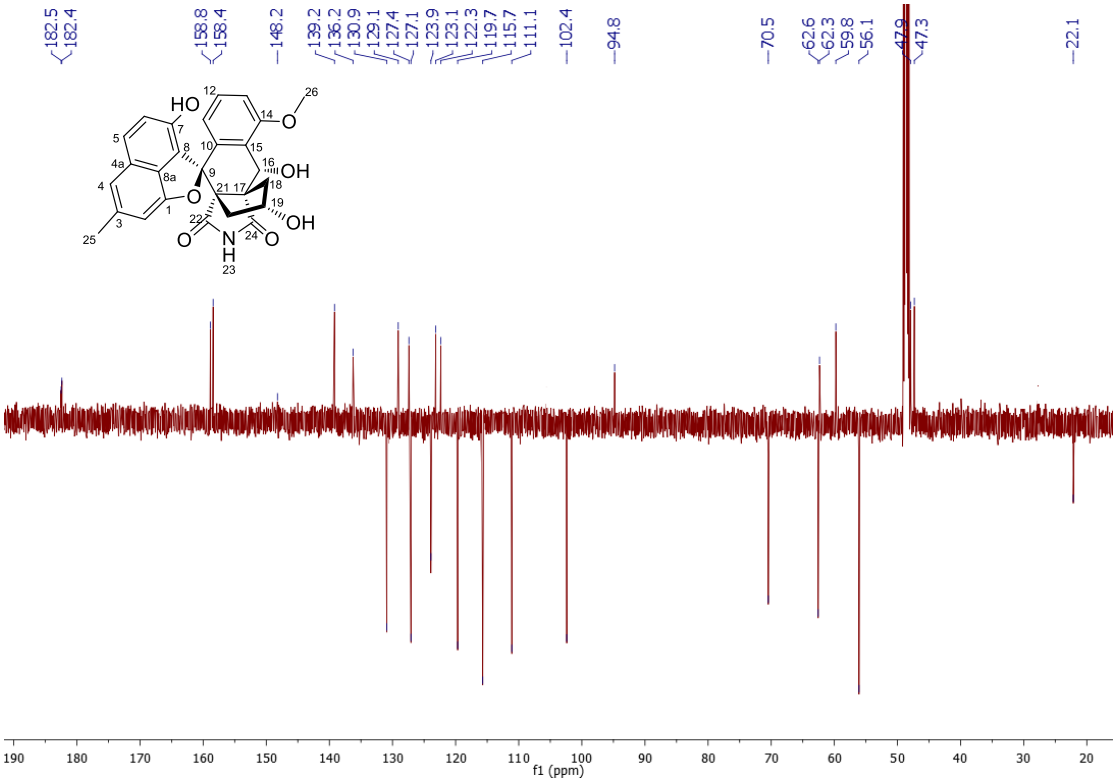
S63. $^1H$ NMR spectrum (600 MHz) of **24** in $CD_3OD$.
S64. APT spectrum (600 MHz) of **24** in $CD_3OD$.
S65. HSQC spectrum (600 MHz) of **24** in $CD_3OD$.
S66. HMBC spectrum (600 MHz) of **24** in $CD_3OD$.
S67. $^1H$-$^1H$ COSY spectrum (600 MHz) of **24** in $CD_3OD$.
S68. HRESIMS spectrum of **24**.
S69. UV spectrum of **24**.

S70. $^1H$ NMR spectrum (600 MHz) of **25** in $CD_3OD$.
S71. HSQC spectrum (600 MHz) of **25** in $CD_3OD$.
S72. HMBC spectrum (600 MHz) of **25** in $CD_3OD$.
S73. $^1H$-$^1H$ COSY spectrum (600 MHz) of **25** in $CD_3OD$.
S74. HRESIMS spectrum of **25**.
S75. UV spectrum of **25**.

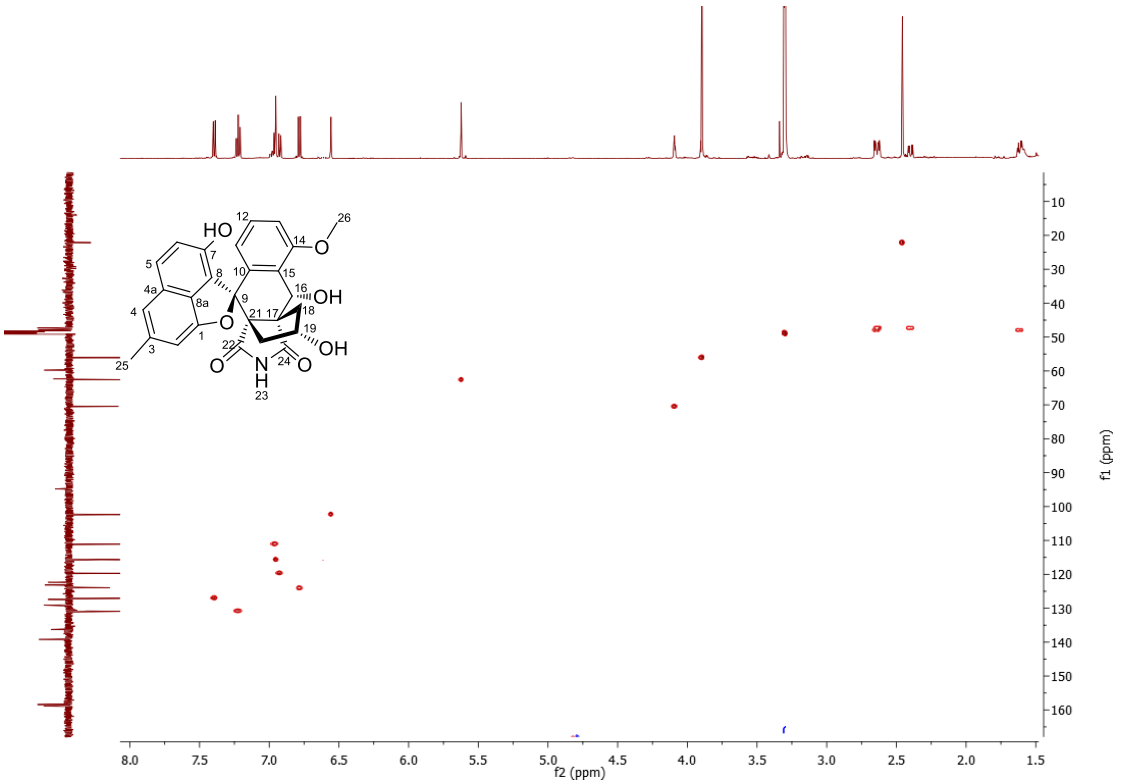
S1. $^{1}$H NMR spectrum (600 MHz) of lugdunomycin (**1**) in $CD_3OD$.



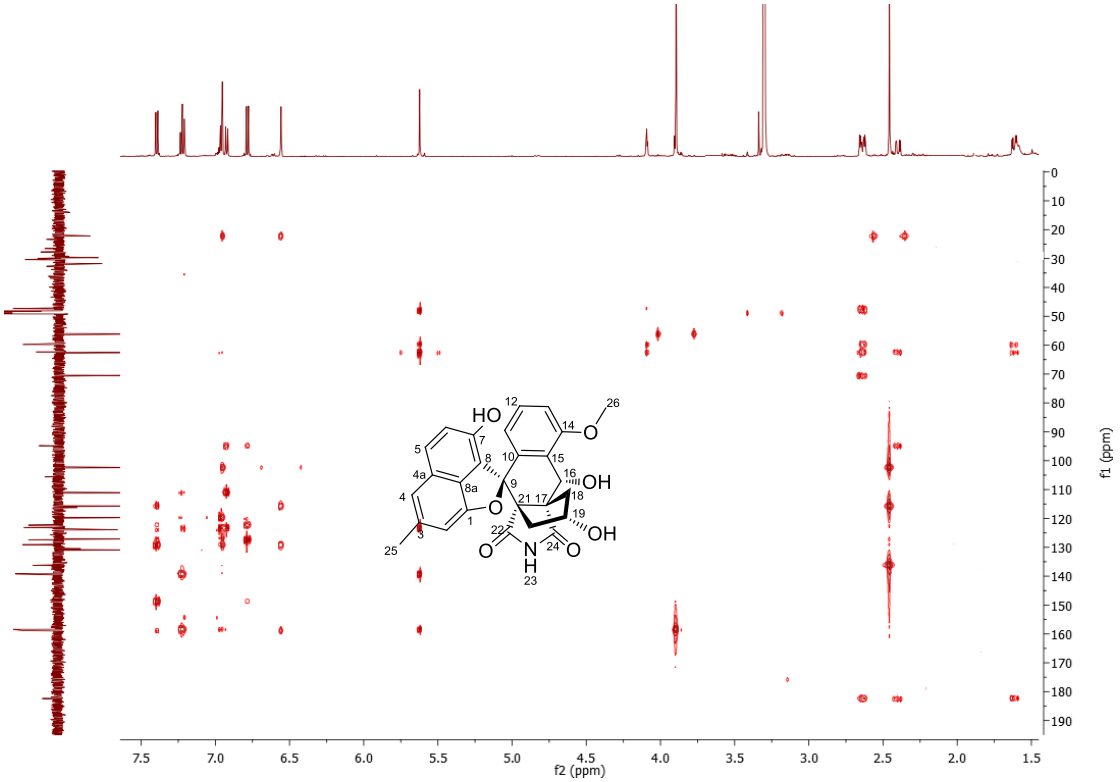
S2. APT NMR spectrum (150 MHz) of lugdunomycin (**1**) in $CD_3OD$.



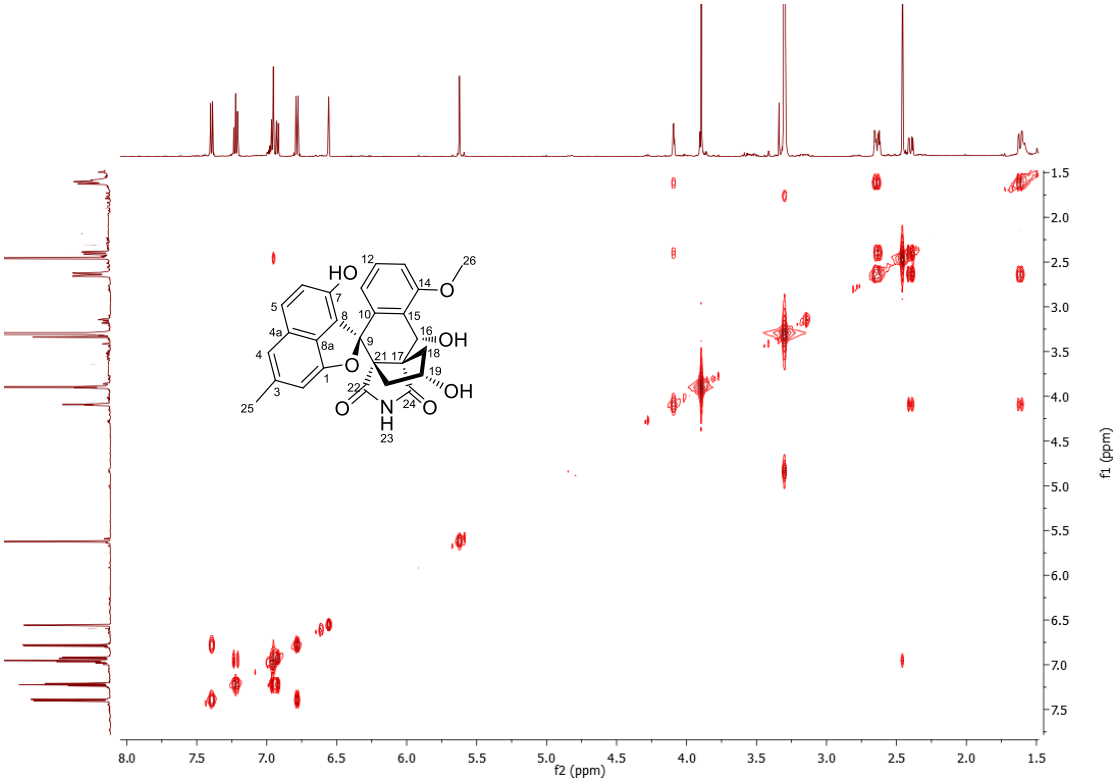
S3. HSQC spectrum (600 MHz) of lugdunomycin (**1**) in $CD_3OD$.



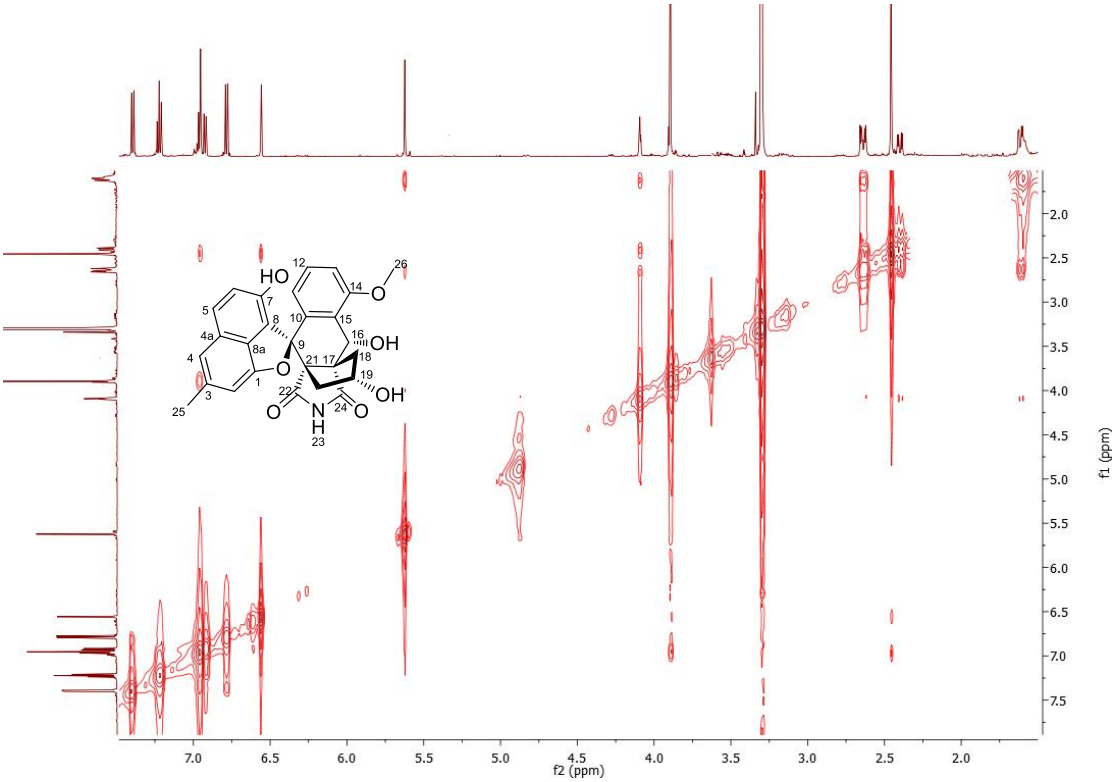
S4. HMBC spectrum (600 MHz) of lugdunomycin (**1**) in $CD_3OD$.



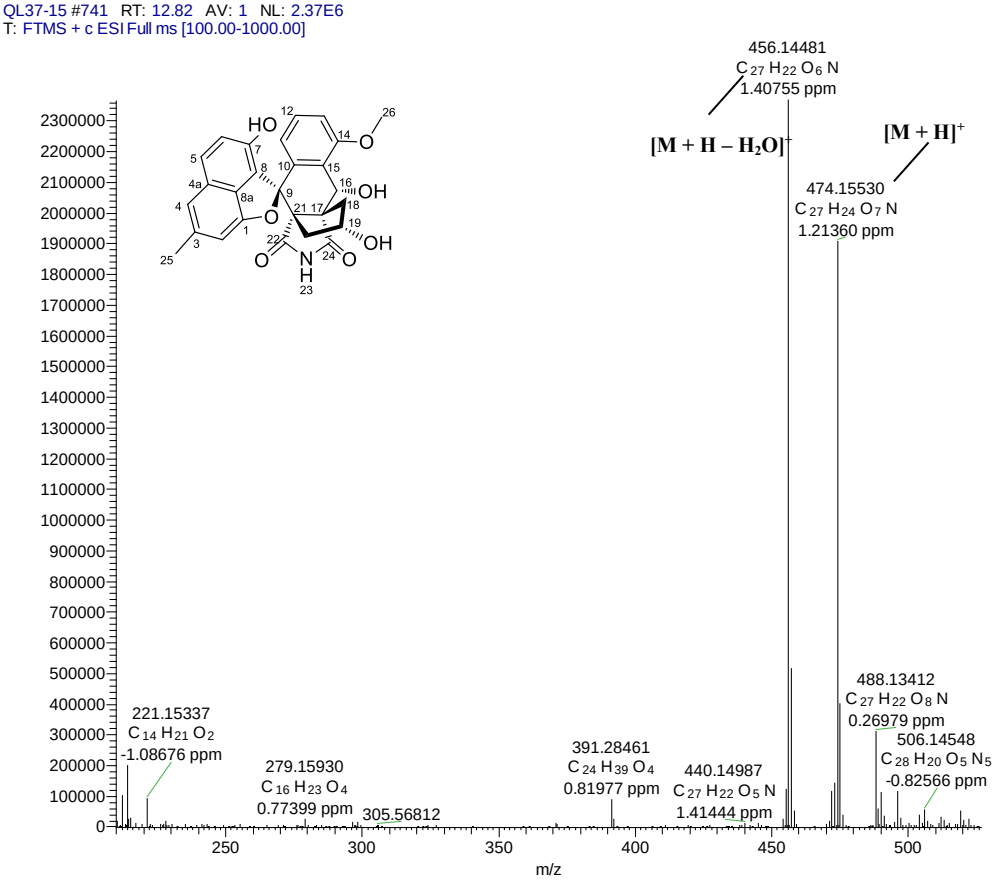
S5. $^{1}$H-$^{1}$H COSY spectrum (600 MHz) of lugdunomycin (**1**) in $CD_3OD$.



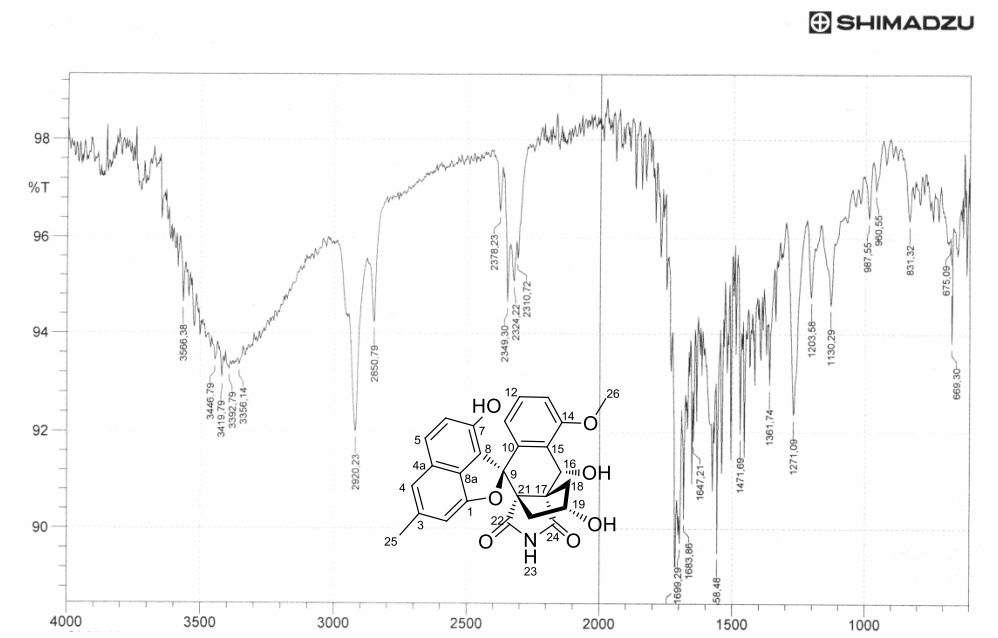
S6. $^{1}$H-$^{1}$H NOSEY spectrum (600 MHz) of lugdunomycin (**1**) in $CD_3OD$.



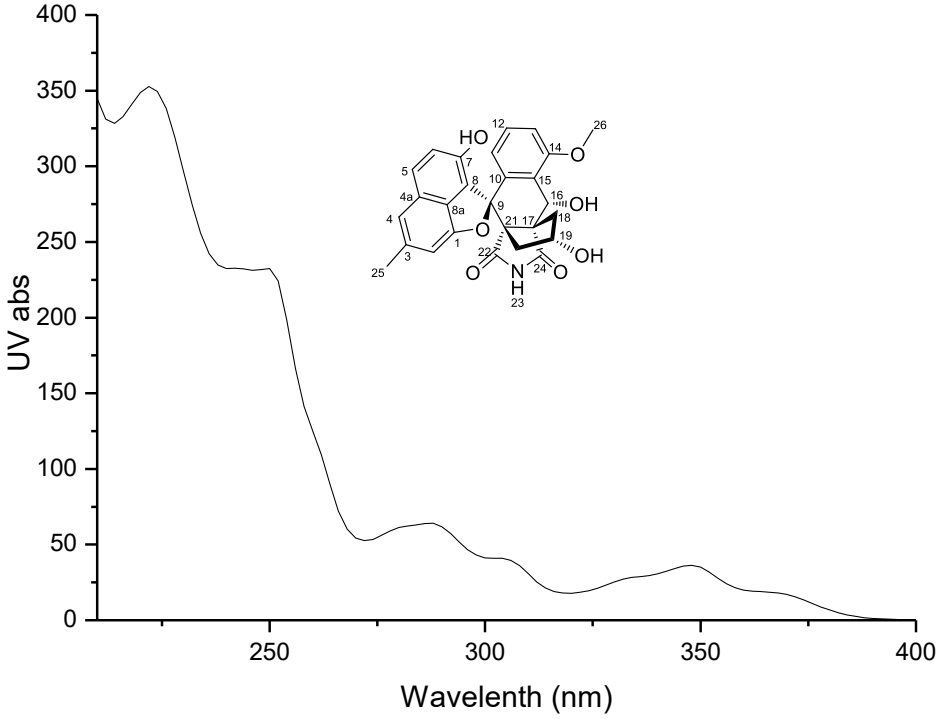
S7. HRESIMS spectrum of lugdunomycin (**1**).



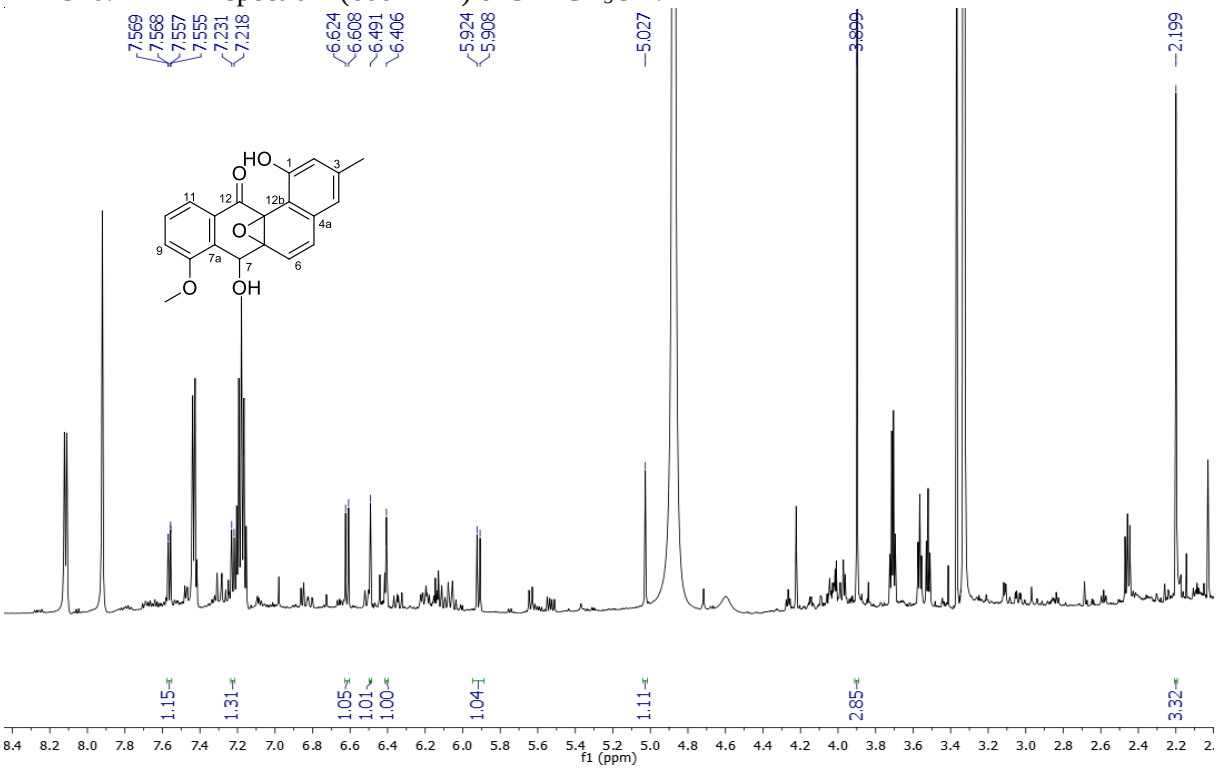
S8. IR spectrum of lugdunomycin (**1**).



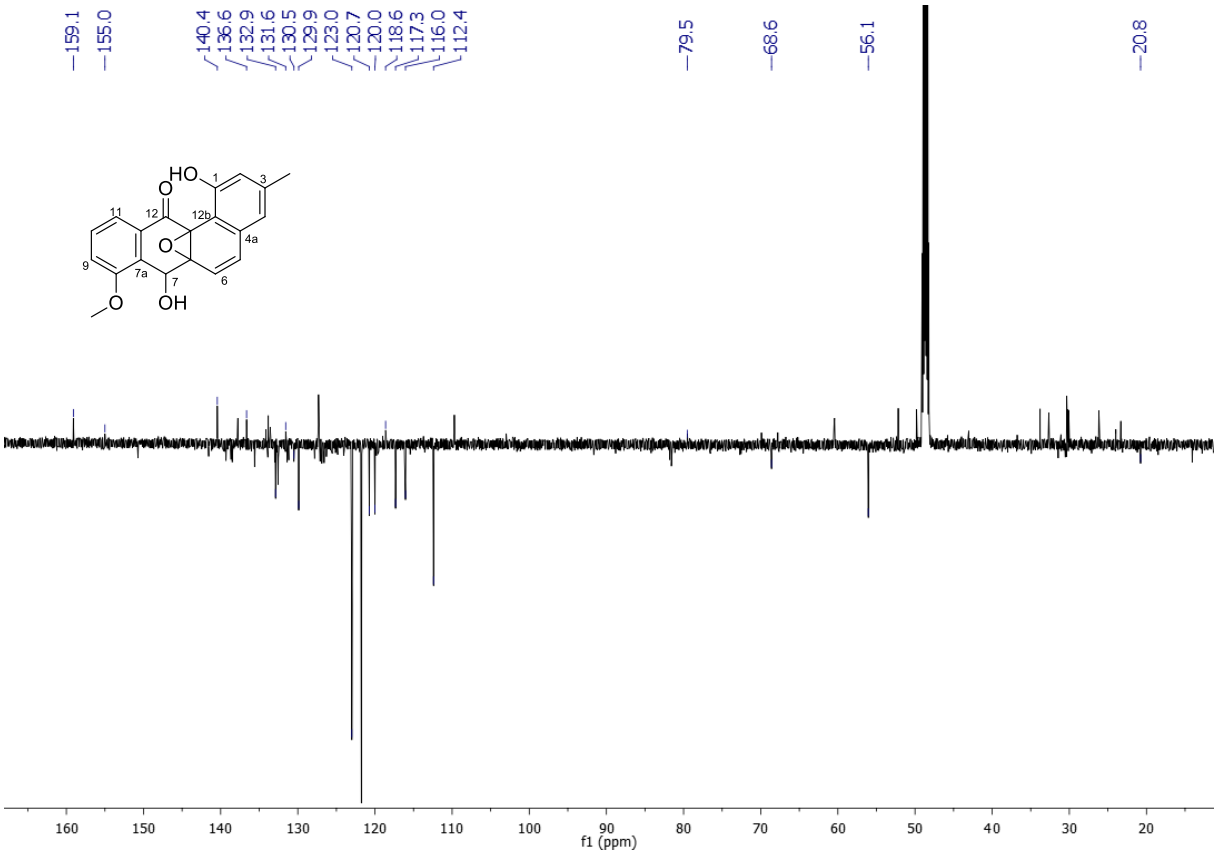
S9. UV spectrum of lugdunomycin (**1**).



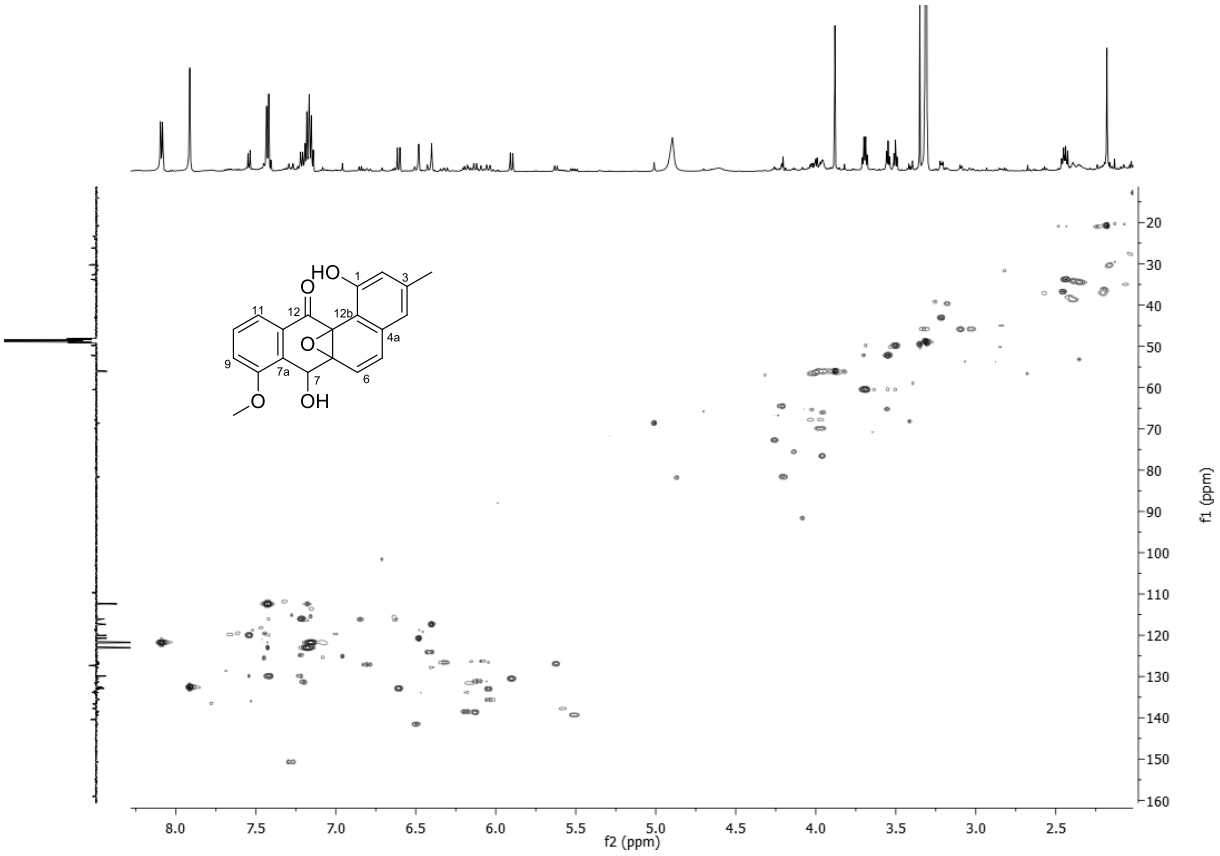
S10.[1]H NMR spectrum (600 MHz) of **5** in $CD_3OD$.



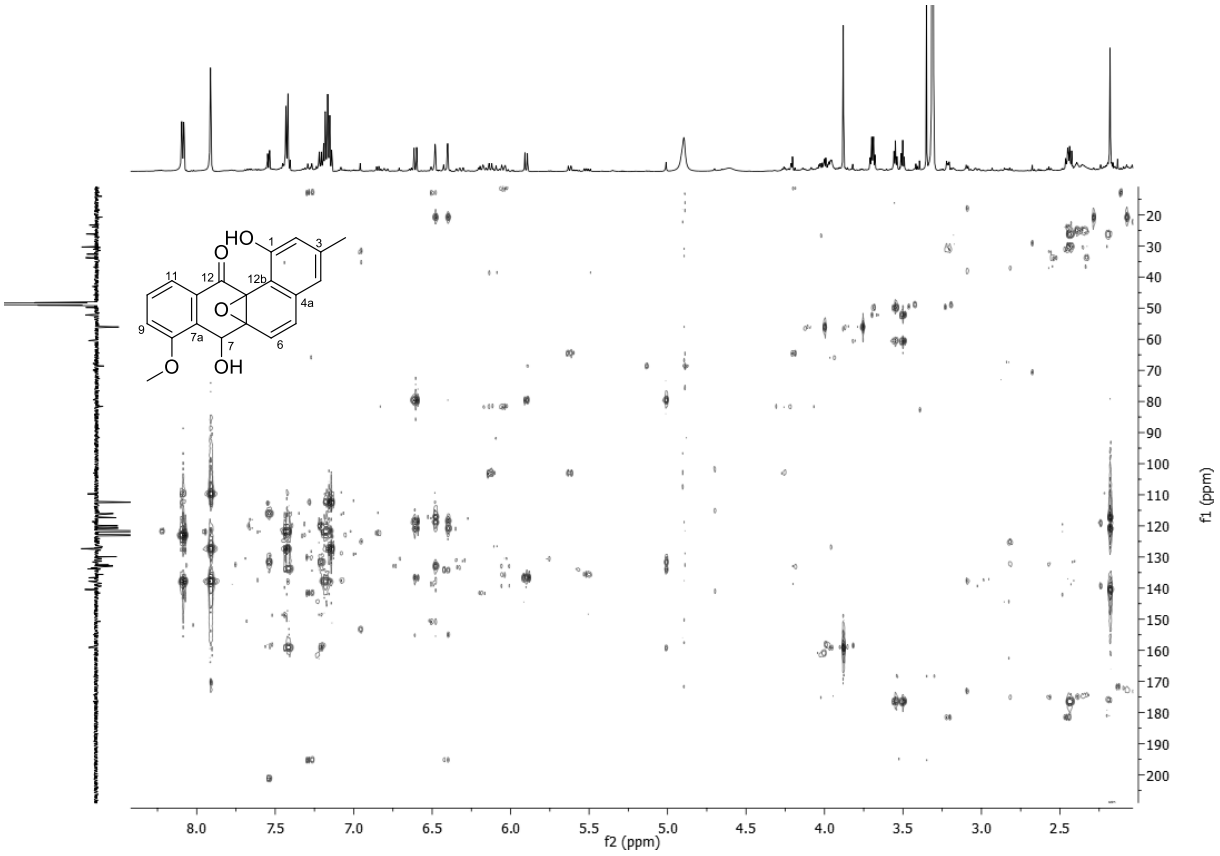
S11. APT spectrum (600 MHz) of **5** in $CD_3OD$.



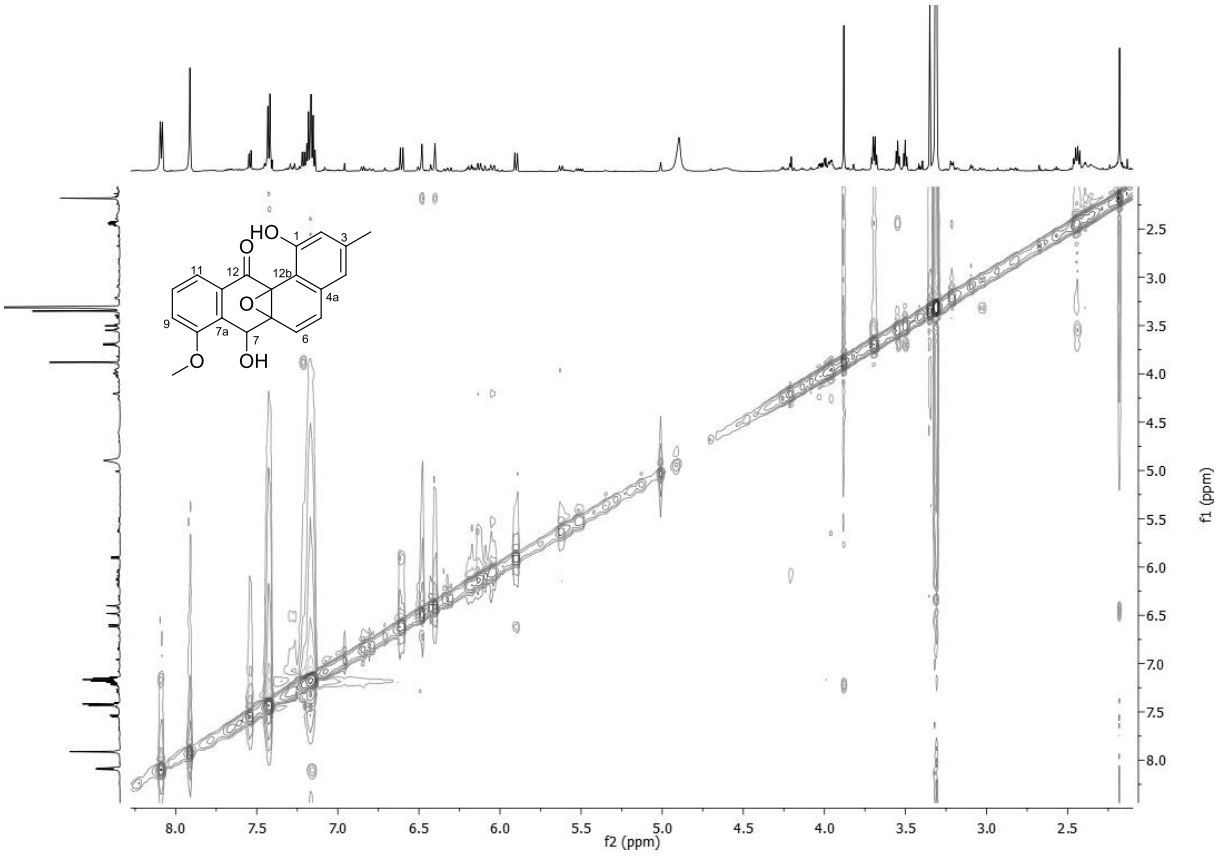
S12. HSQC spectrum (600 MHz) of **5** in $CD_3OD$.



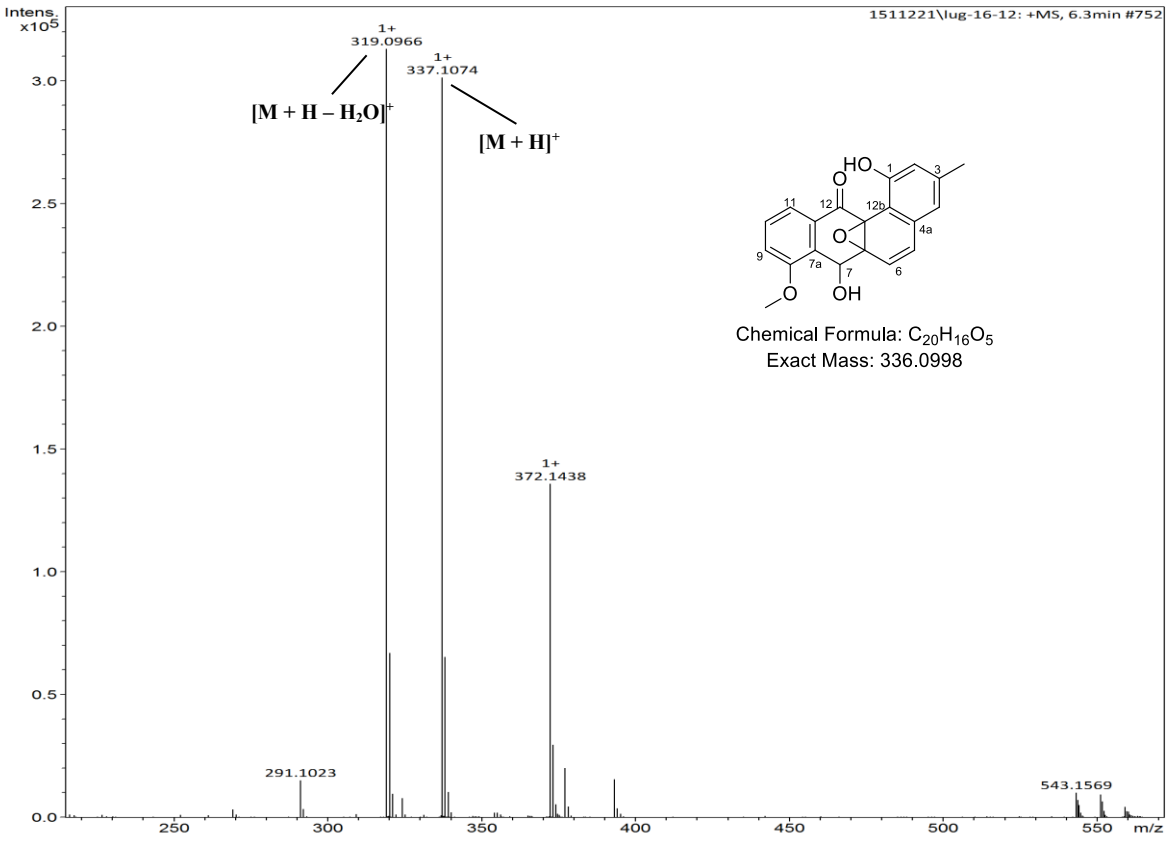
S13. HMBC spectrum (600 MHz) of **5** in $CD_3OD$.



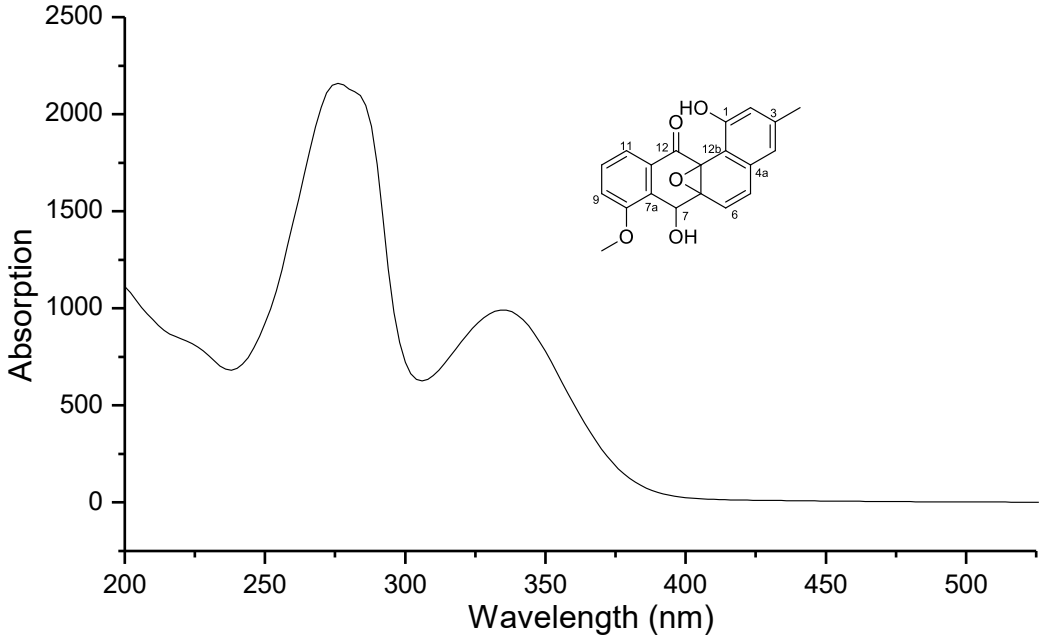
S14. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **5** in $CD_3OD$.



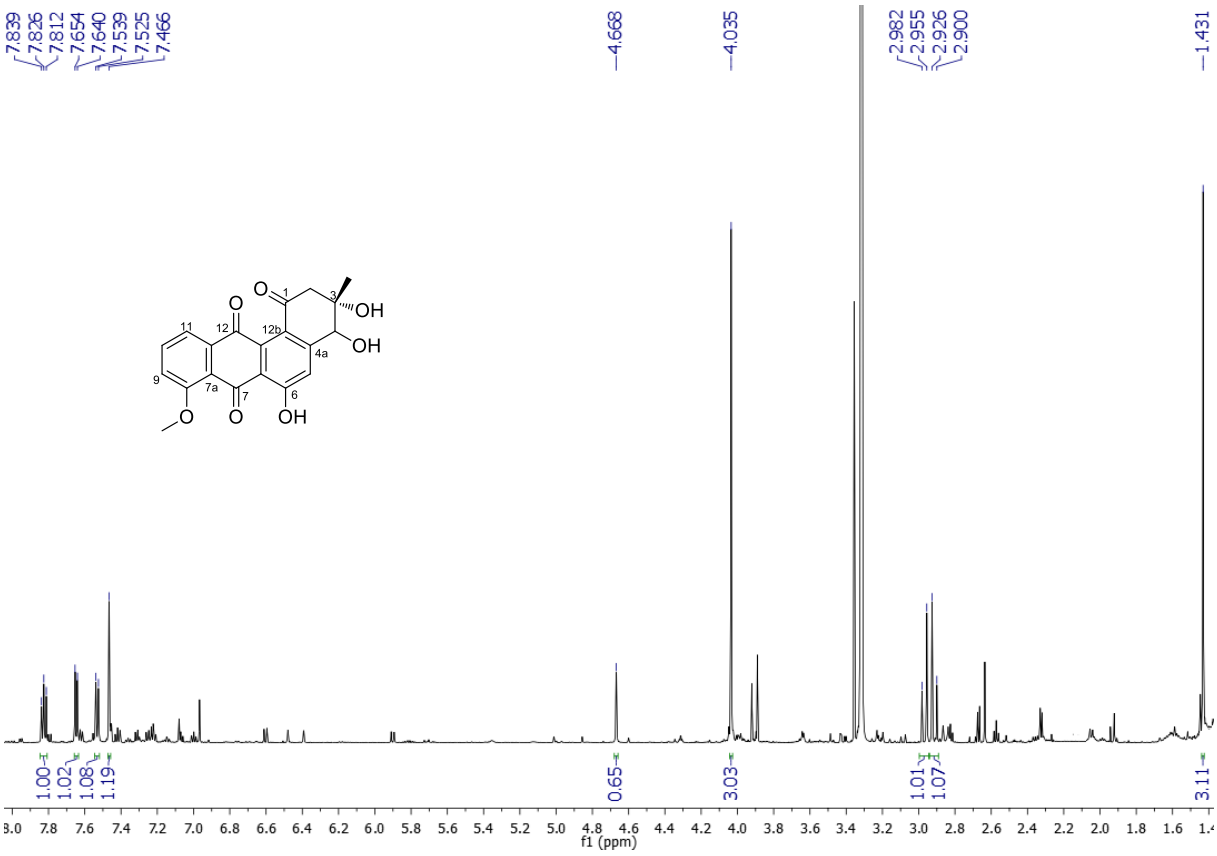
S15. HRESIMS spectrum of **5**



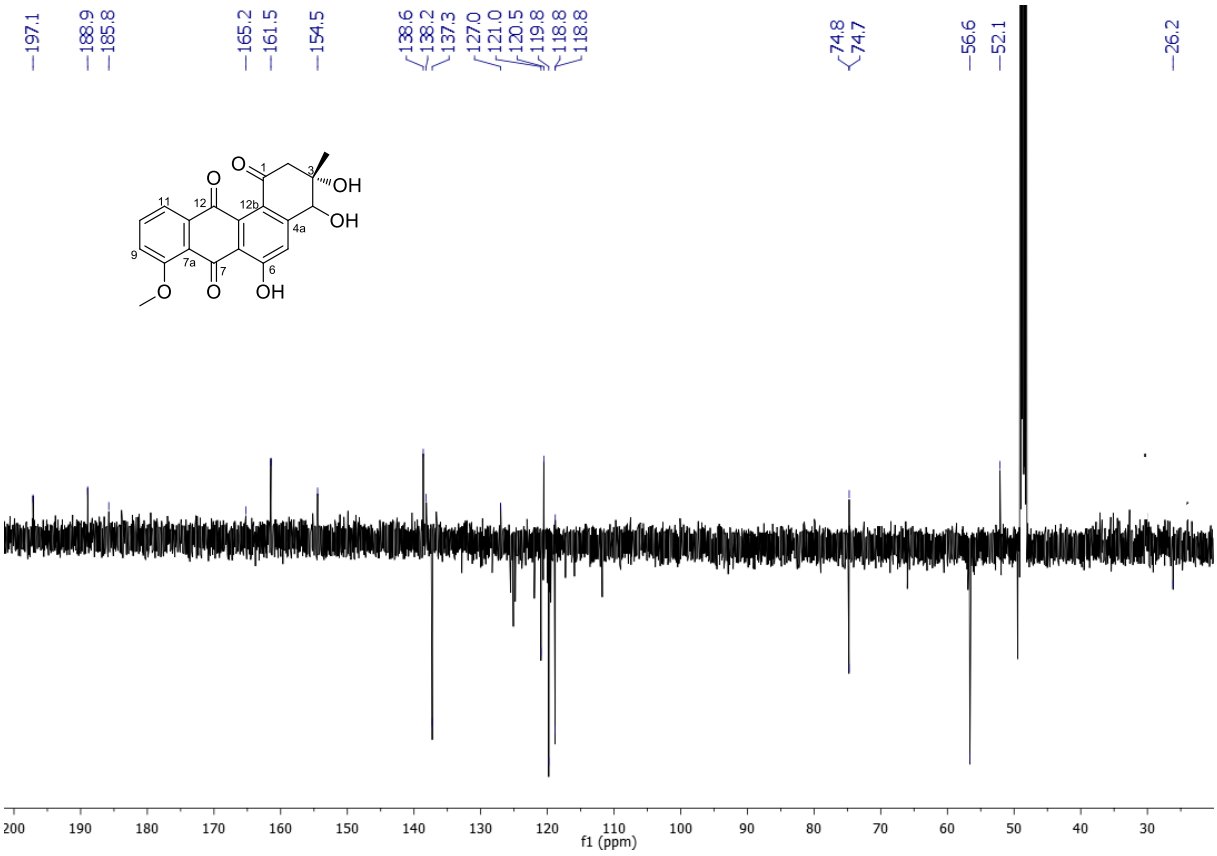
S16. UV spectrum of **5**



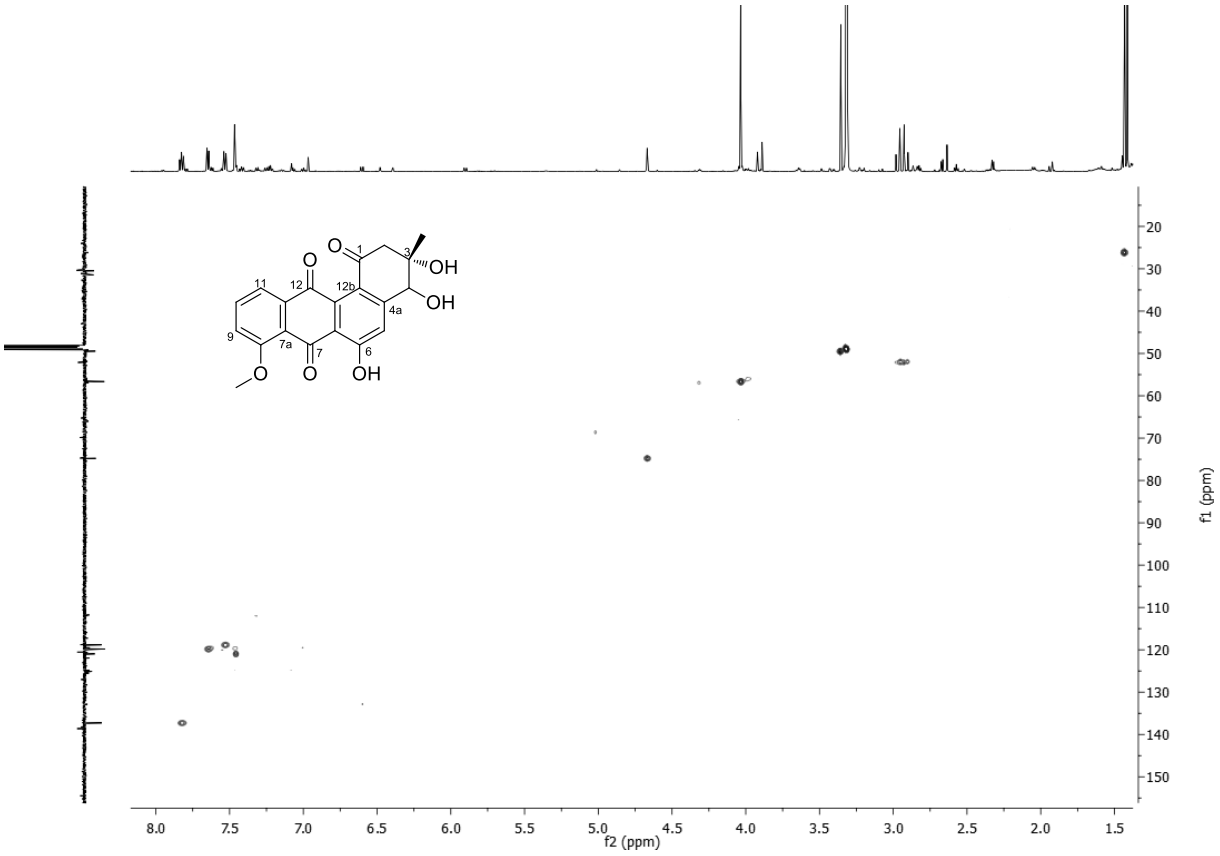
S17. $^{1}$H NMR spectrum (600 MHz) of **9** in $CD_3OD$.



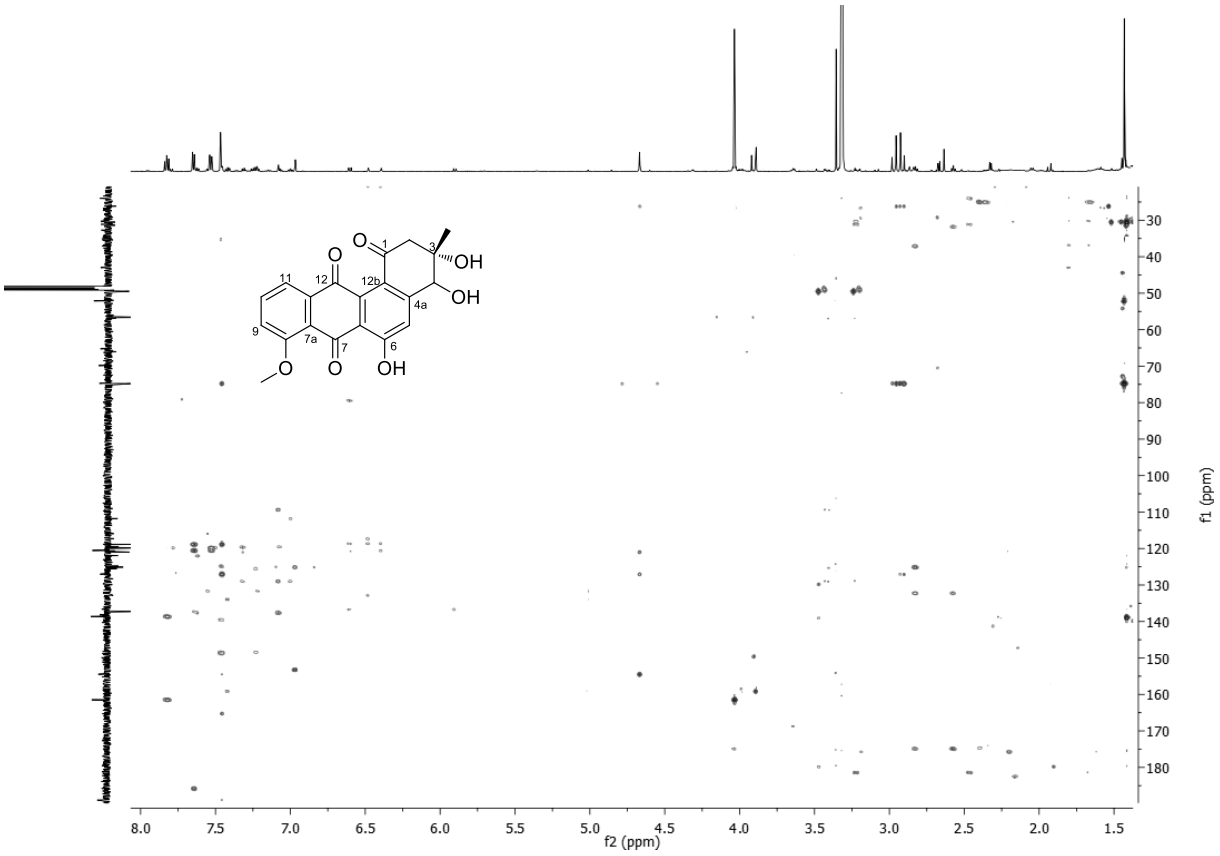
S18. APT spectrum (600 MHz) of **9** in $CD_3OD$.



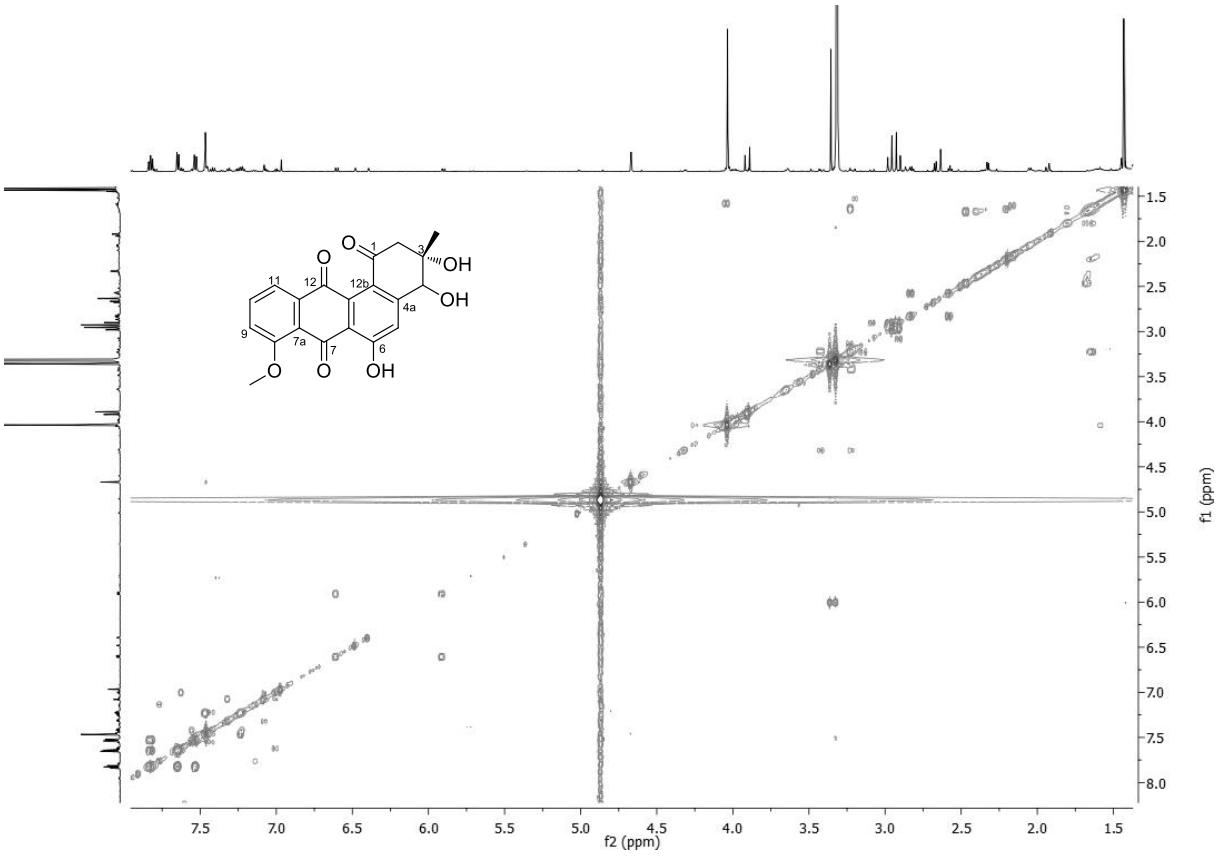
S19. HSQC spectrum (600 MHz) of **9** in $CD_3OD$.



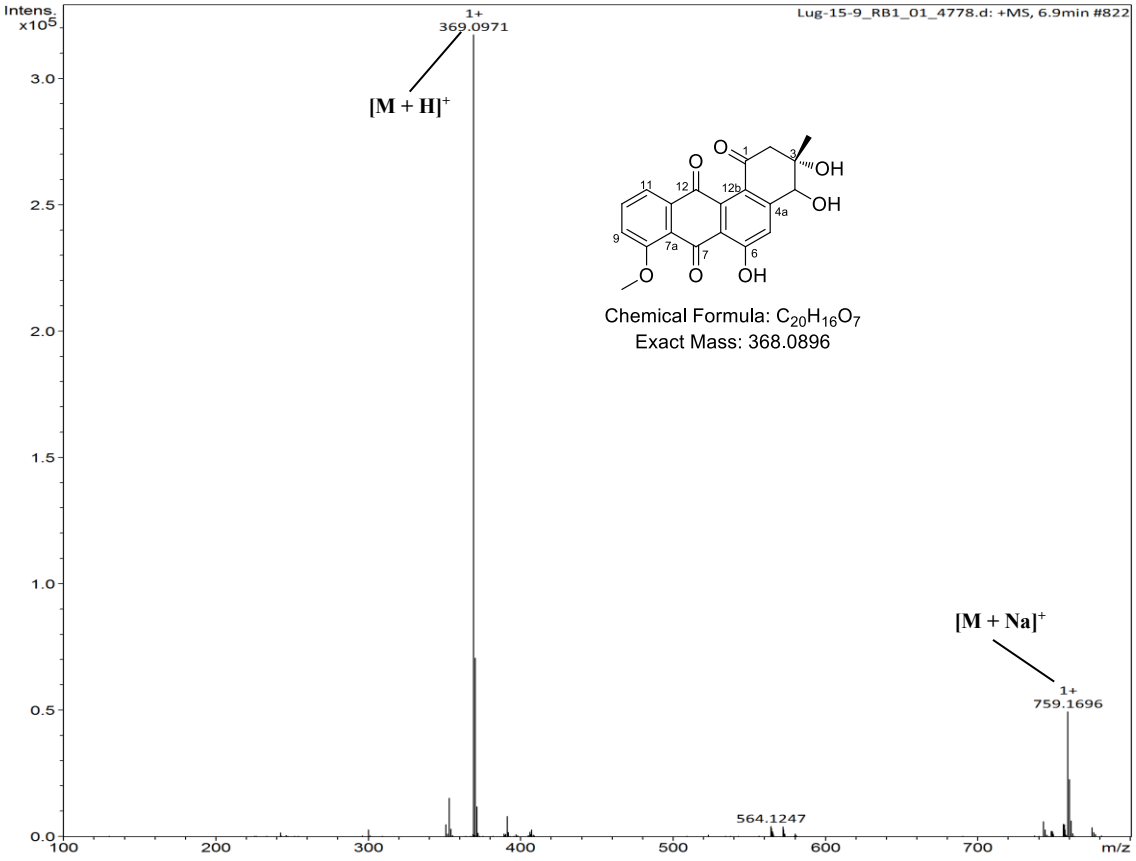
S20. HMBC spectrum (600 MHz) of **9** in $CD_3OD$.



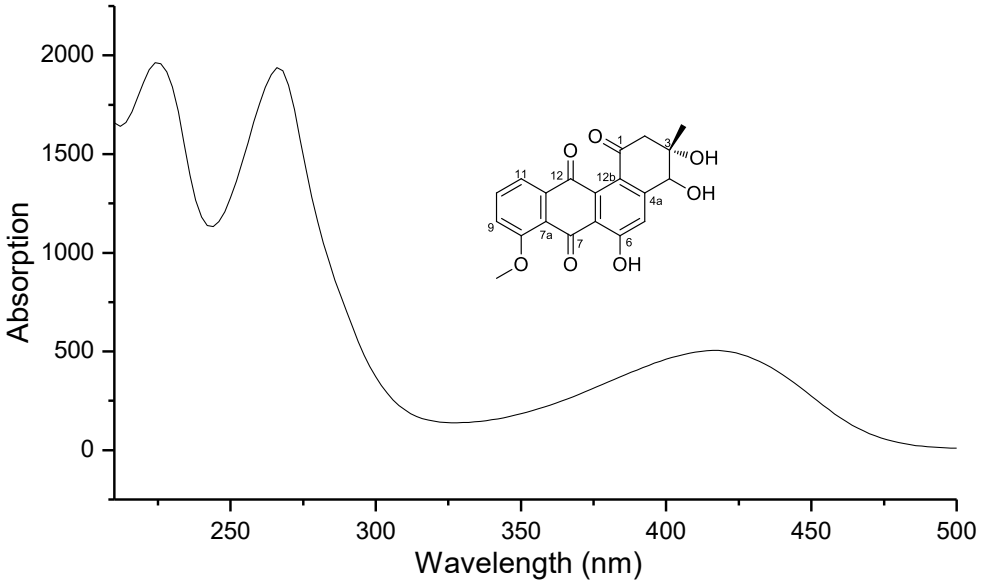
S21. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **9** in $CD_3OD$.



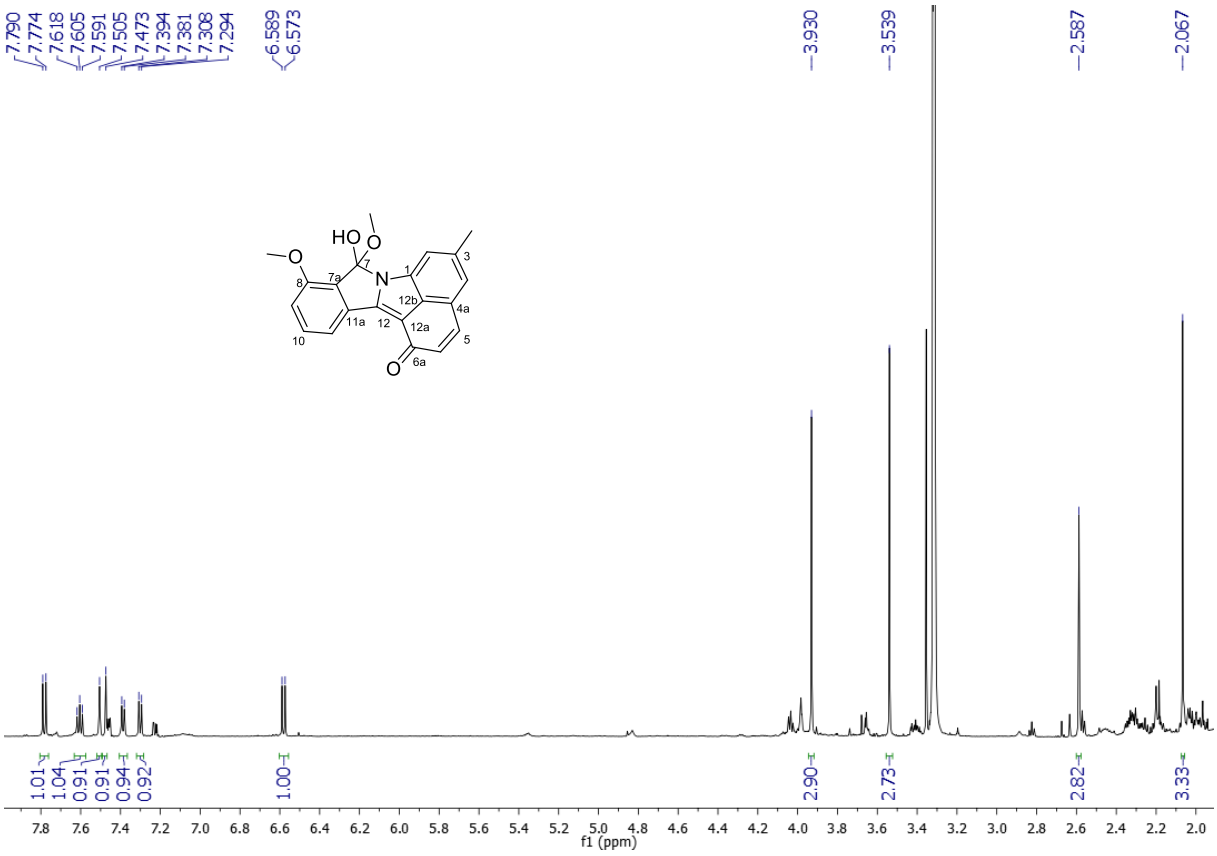
S22. HRESIMS spectrum of **9**.



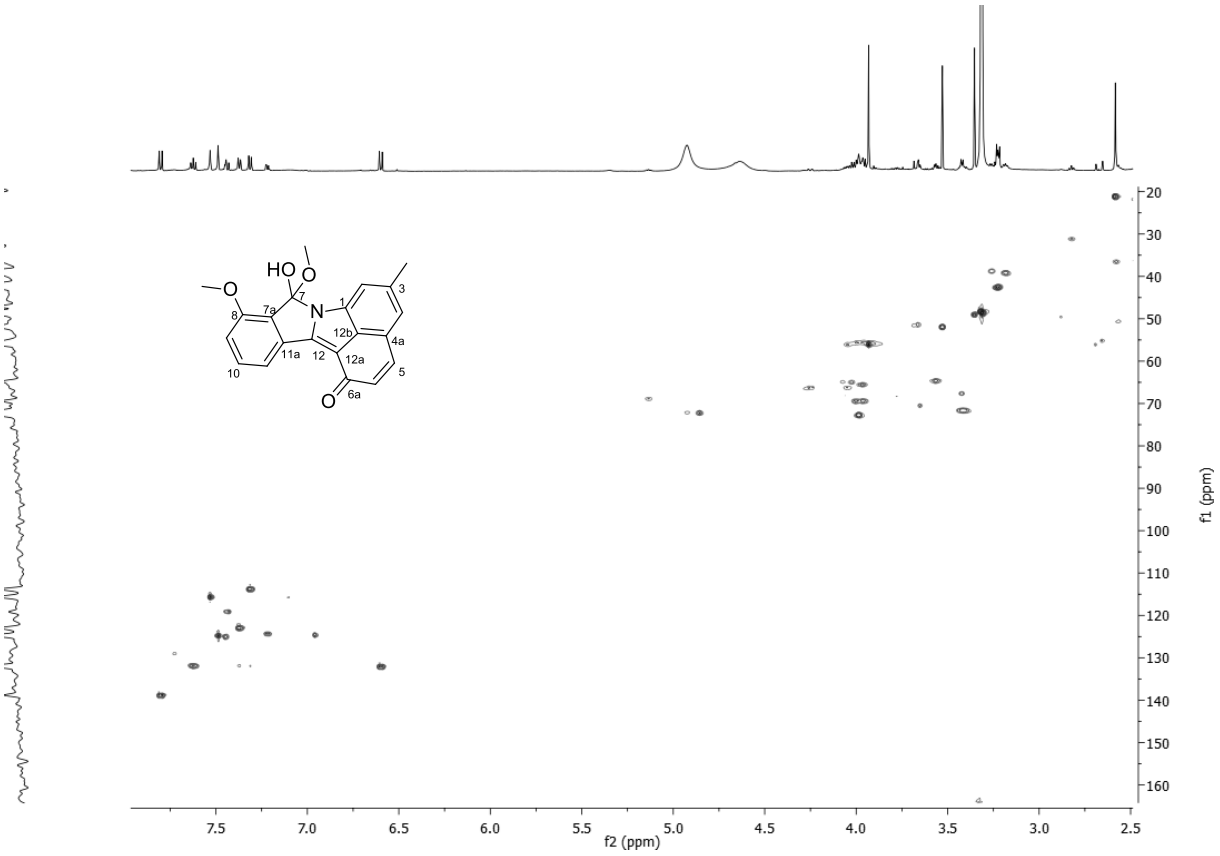
S23. UV spectrum of **9**.



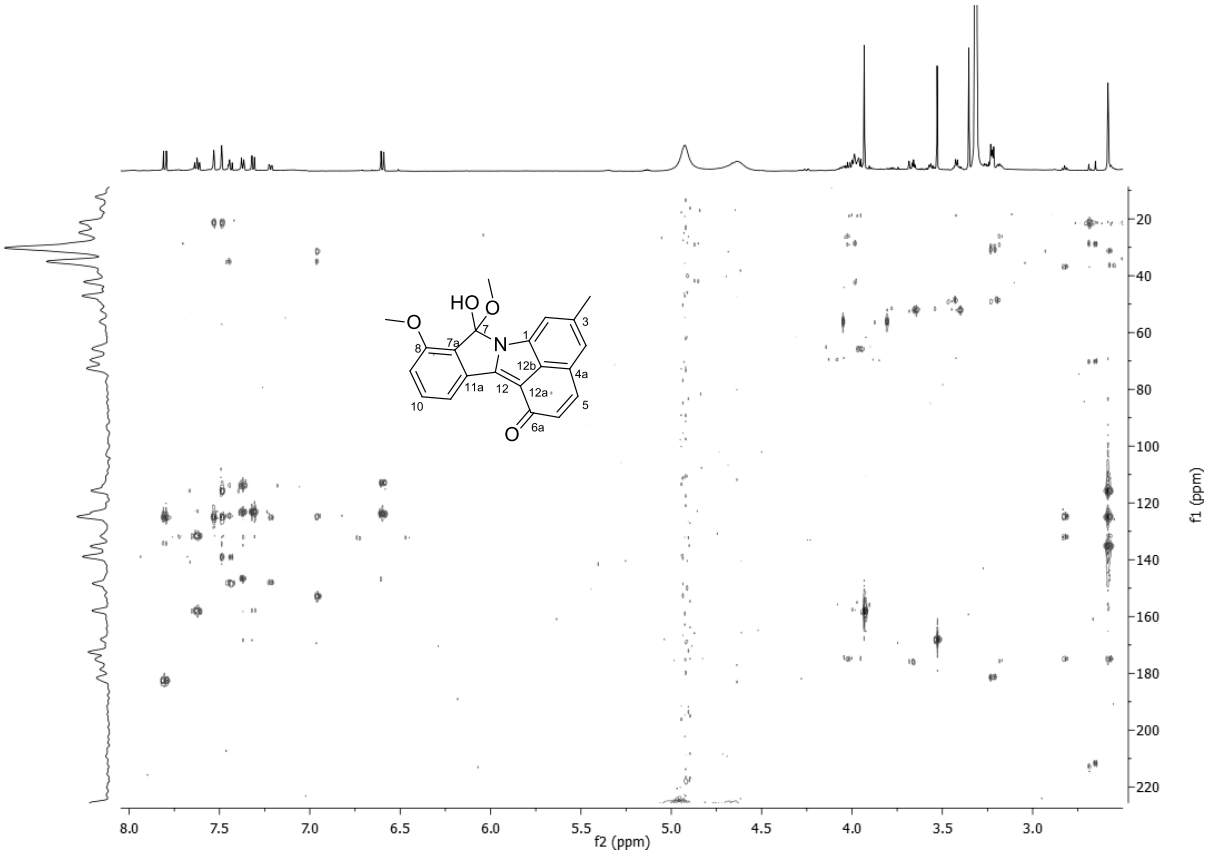
S24. $^{1}$H NMR spectrum (600 MHz) of **14** in $CD_3OD$.



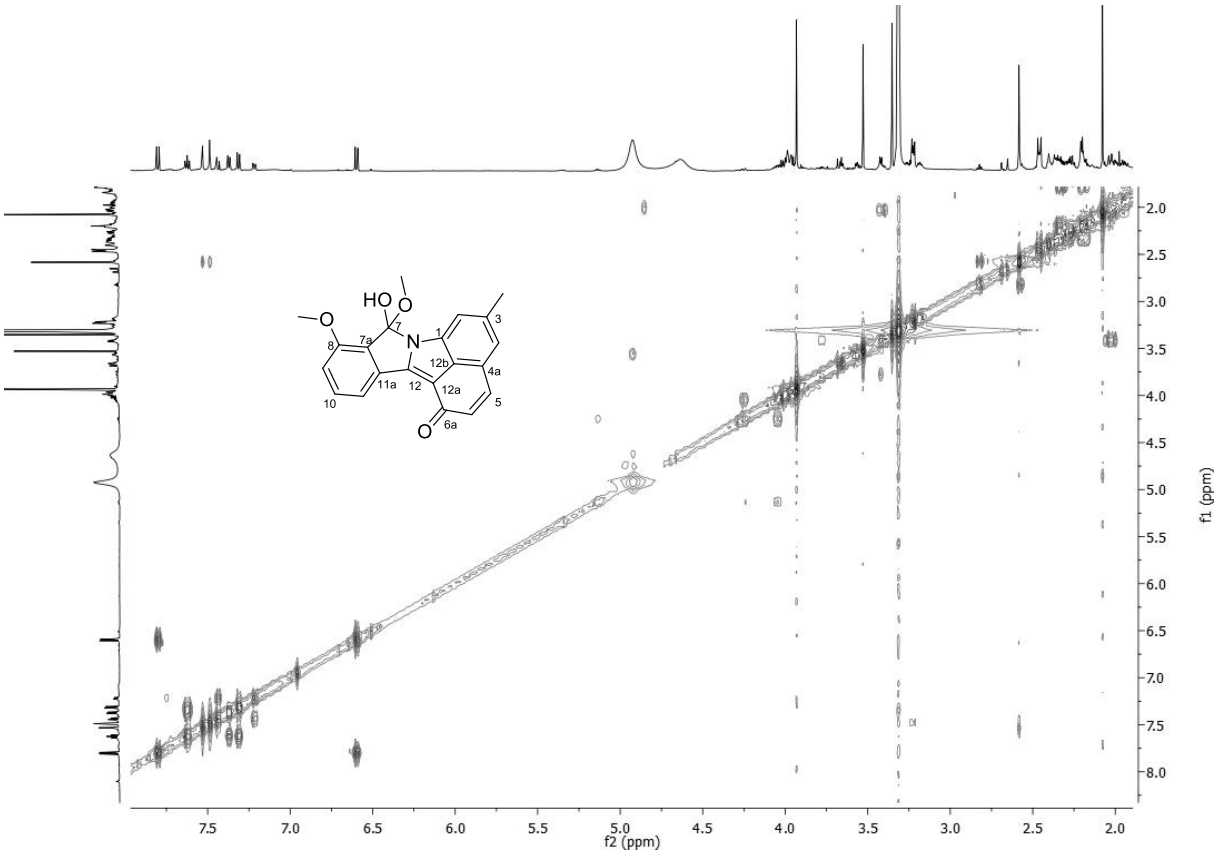
S25. HSQC spectrum (600 MHz) of **14** in $CD_3OD$.



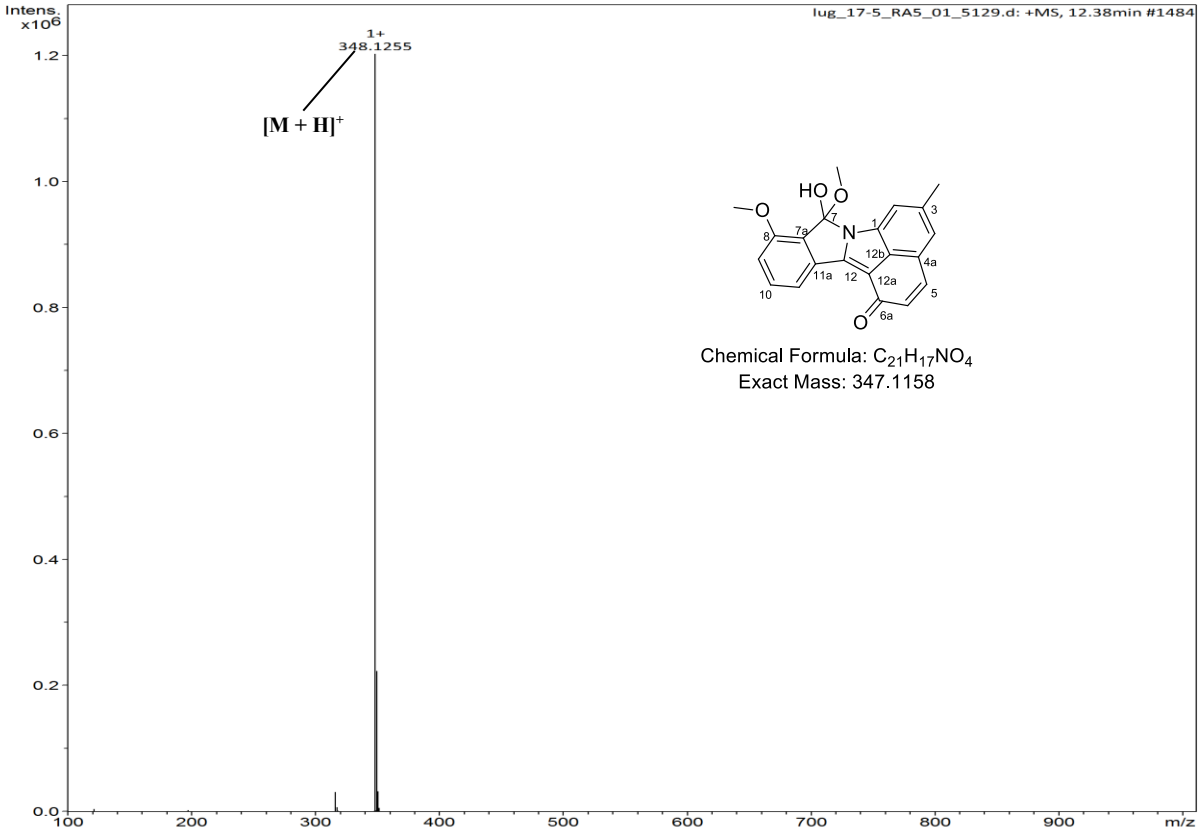
S26. HMBC spectrum (600 MHz) of **14** in $CD_3OD$.



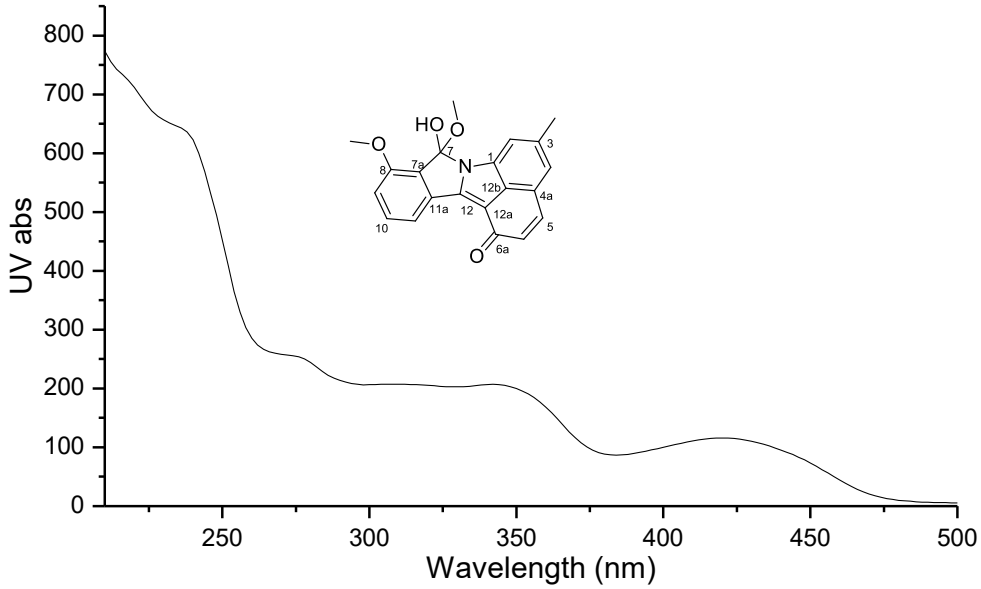
S27. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **14** in $CD_3OD$.



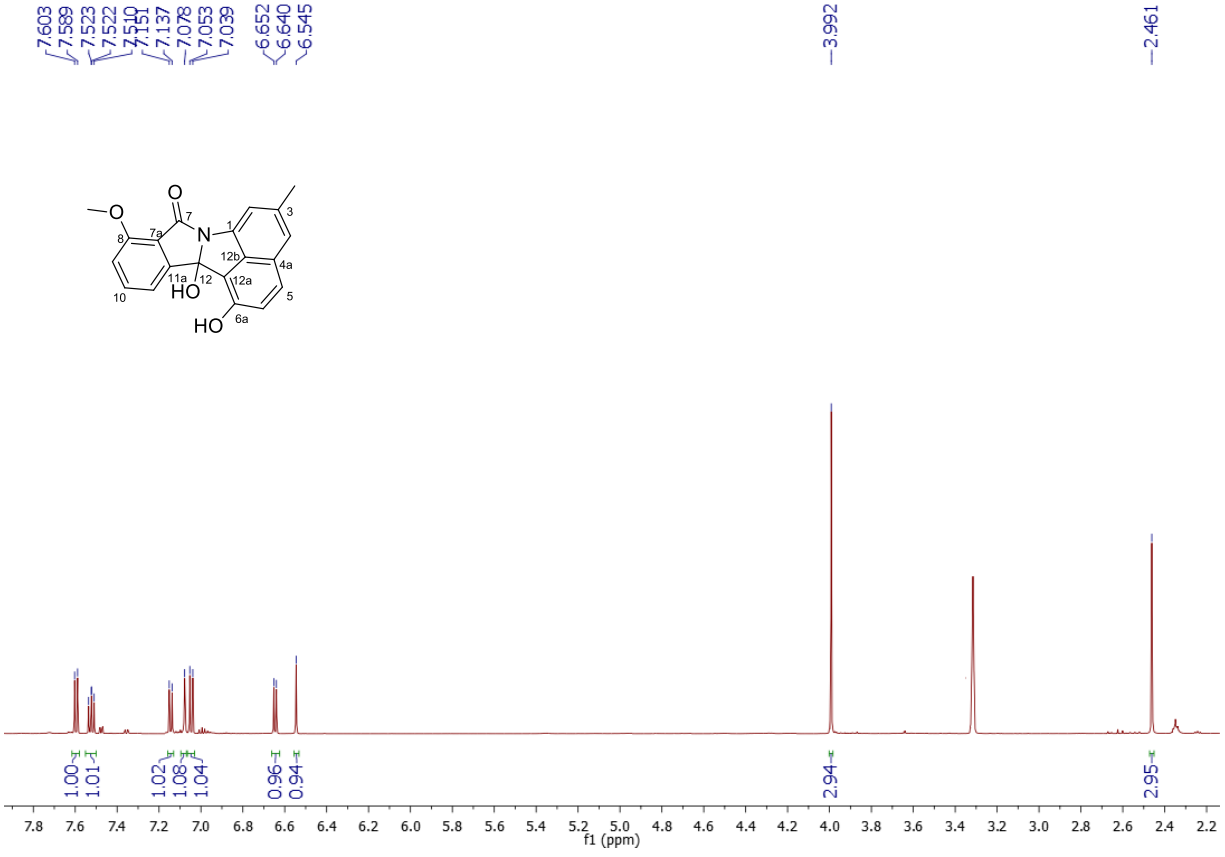
S28. HRESIMS spectrum of **14**.



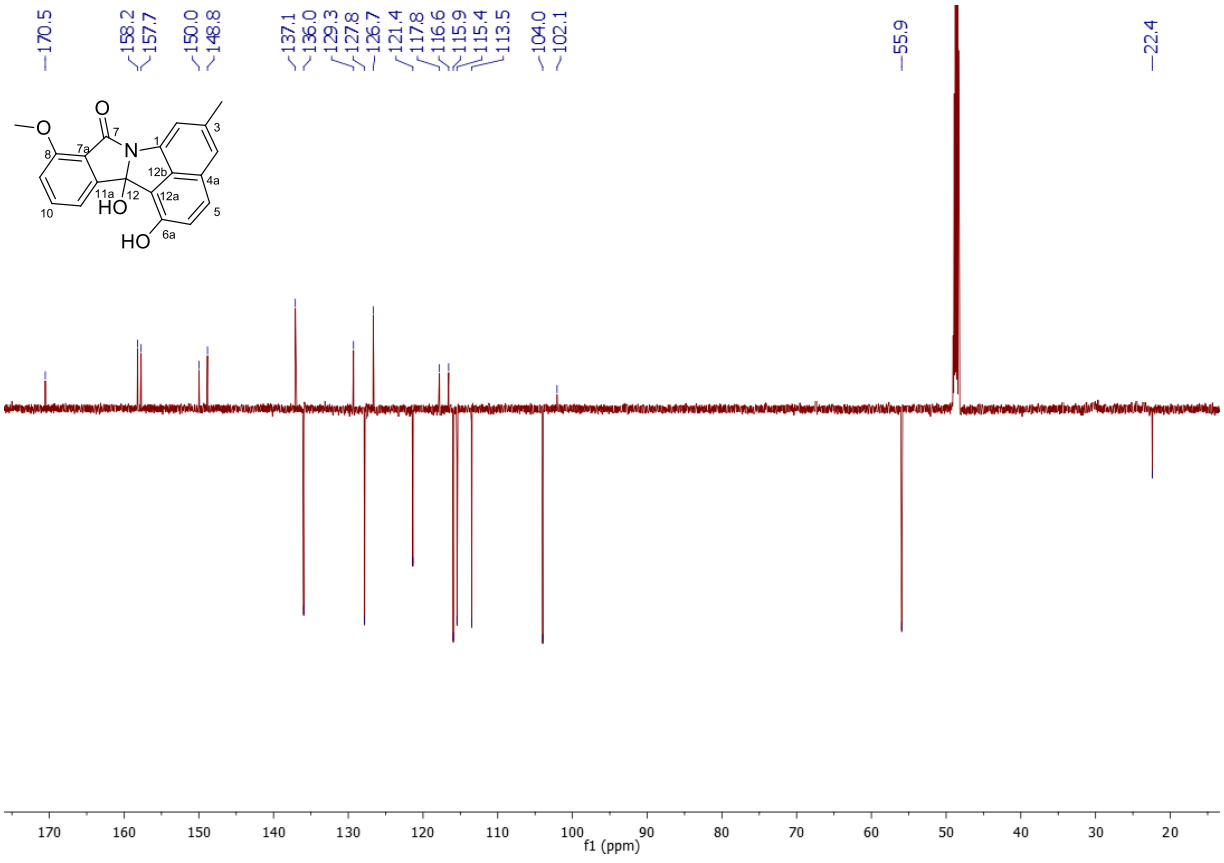
S29. UV spectrum of **14**.



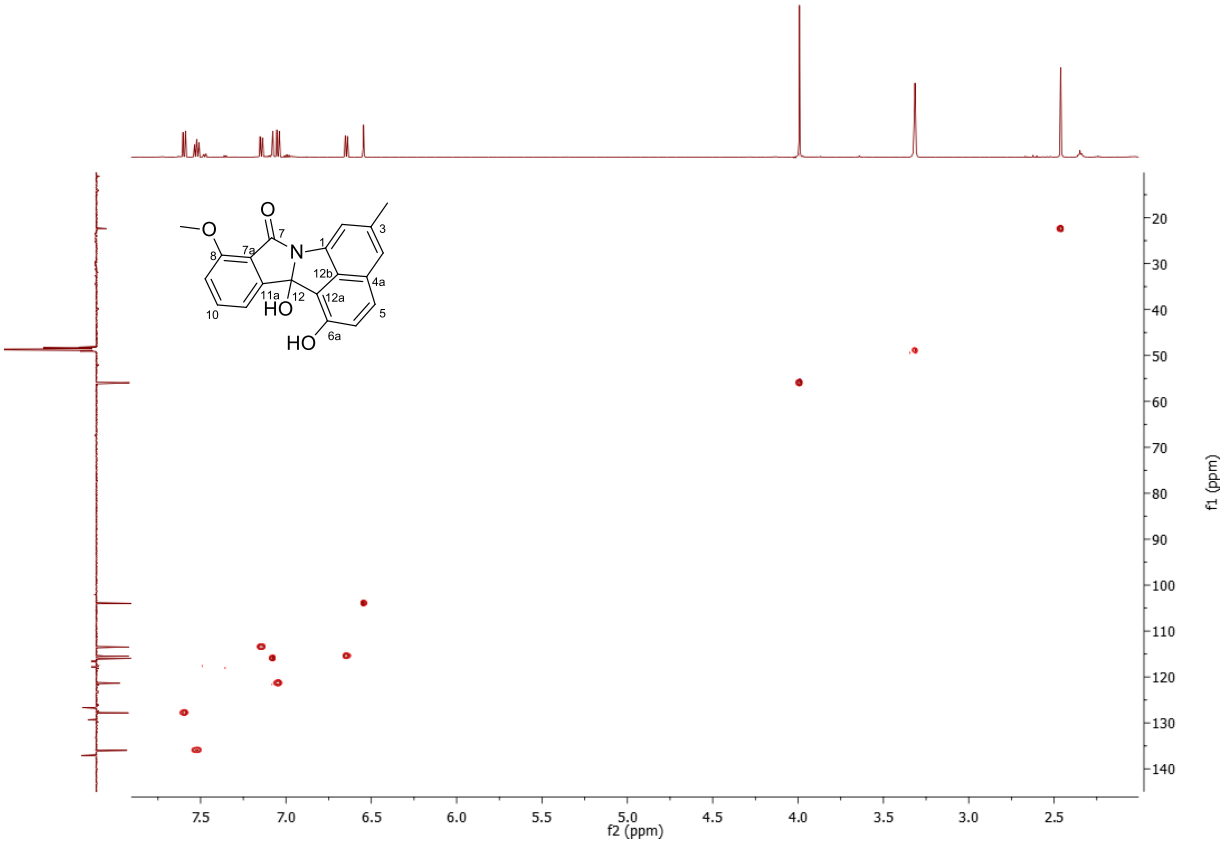
S30. $^{1}H$ NMR spectrum (600 MHz) of **15** in $CD_3OD$.



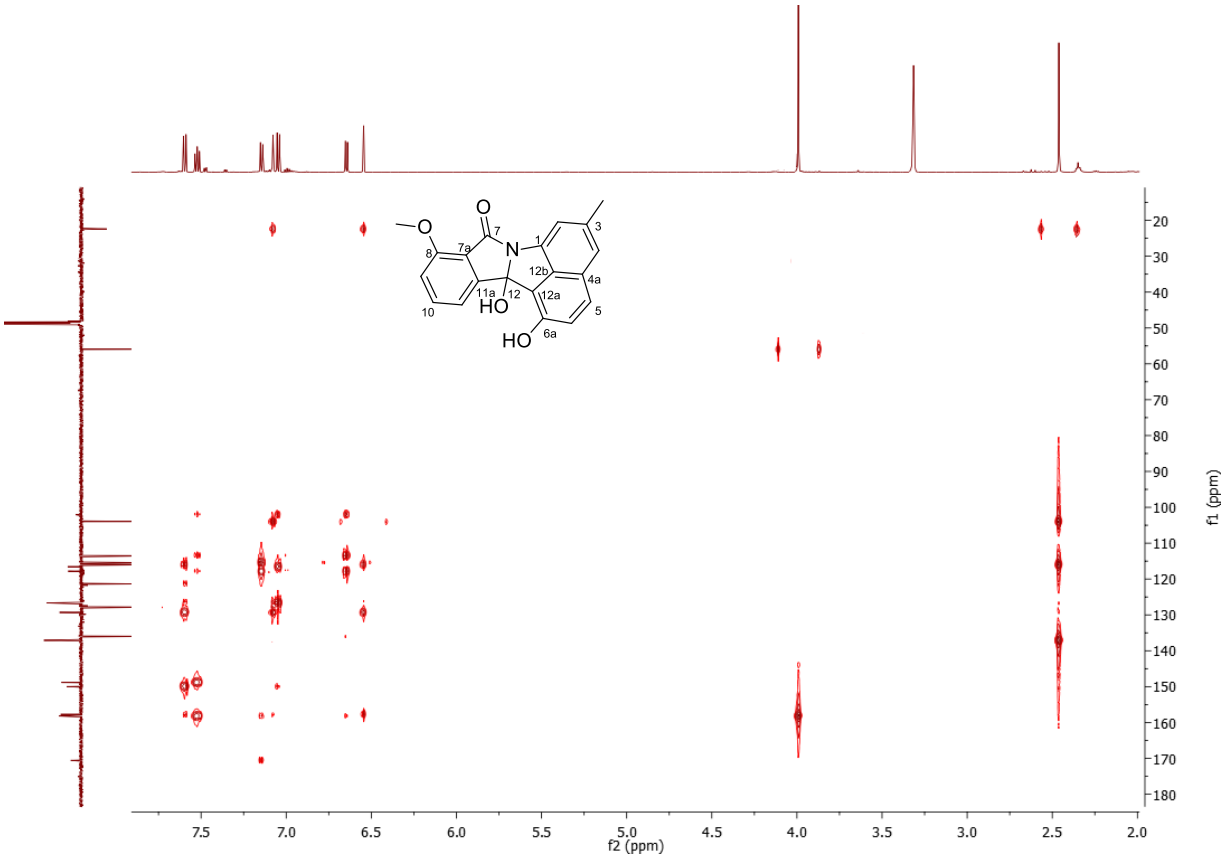
S31. APT spectrum (600 MHz) of **15** in $CD_3OD$.



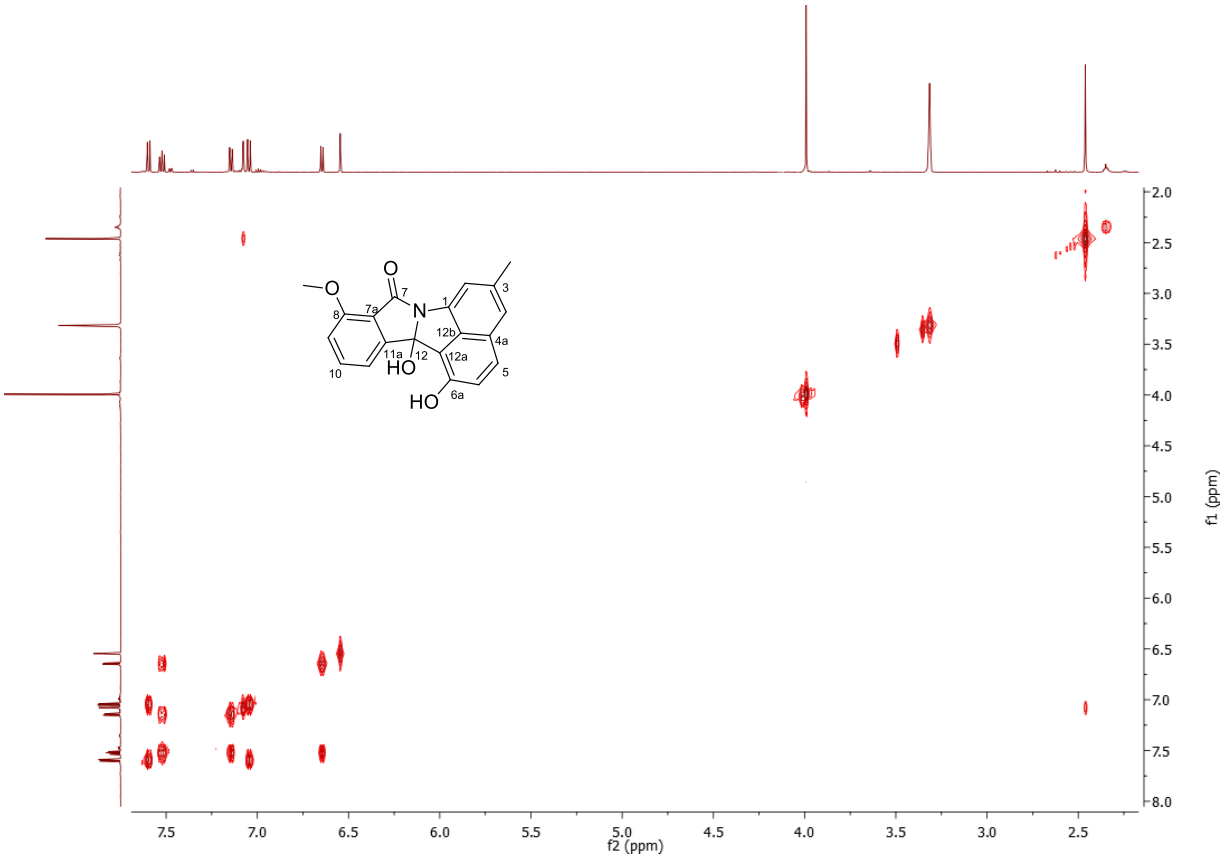
S32. HSQC spectrum (600 MHz) of **15** in $CD_3OD$.



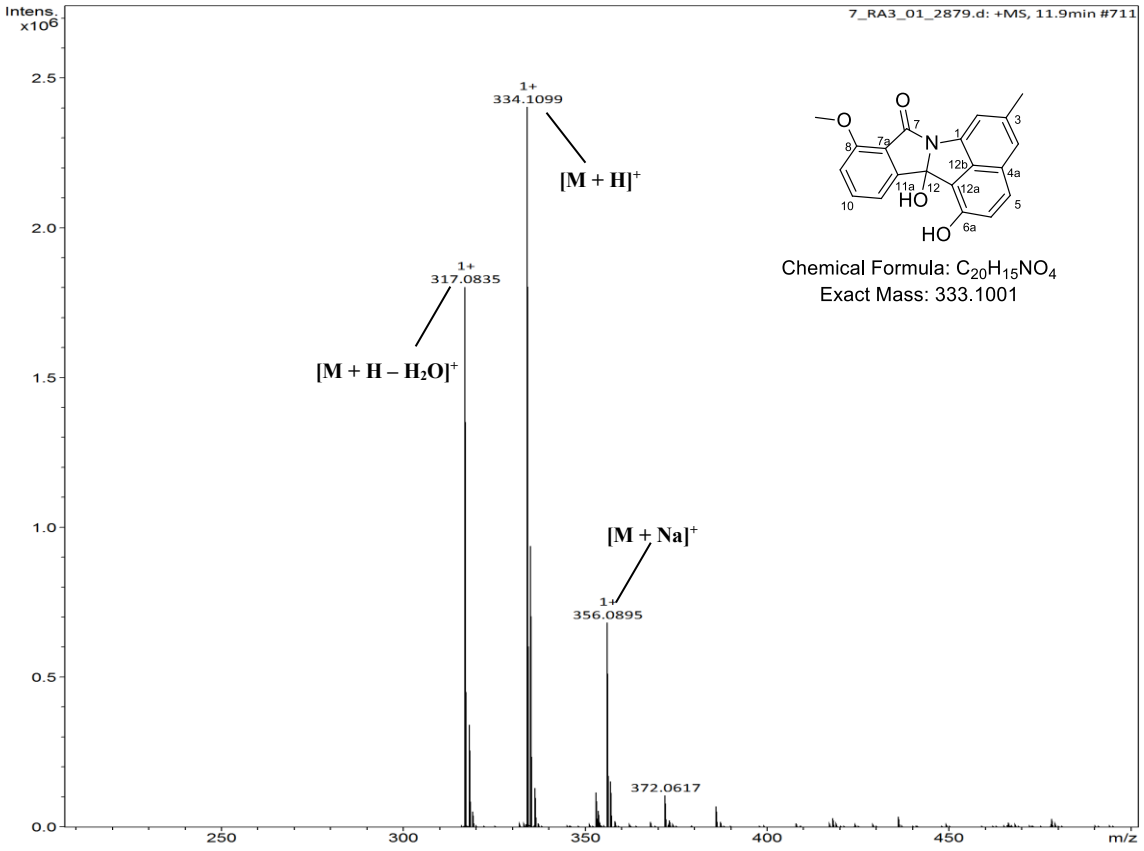
S33. HMBC spectrum (600 MHz) of **15** in $CD_3OD$.



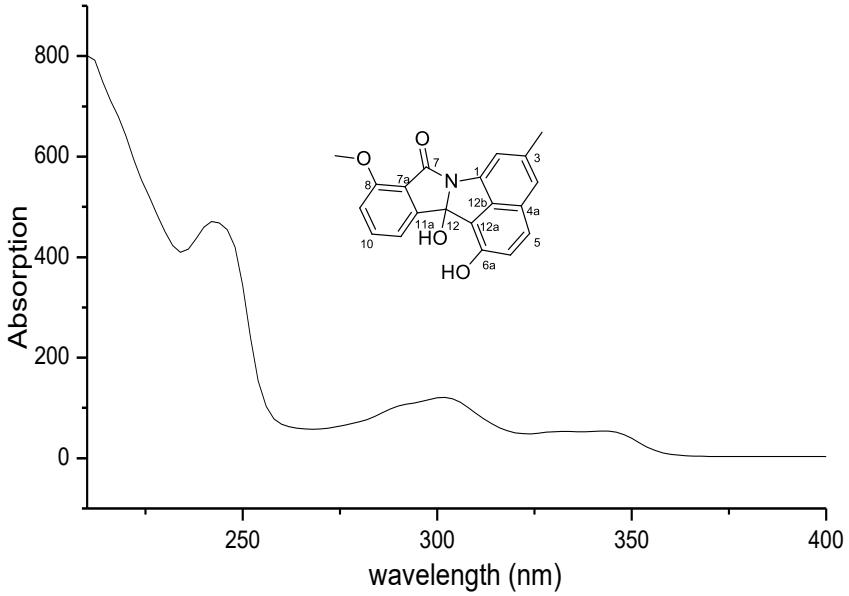
S34. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **15** in $CD_3OD$.



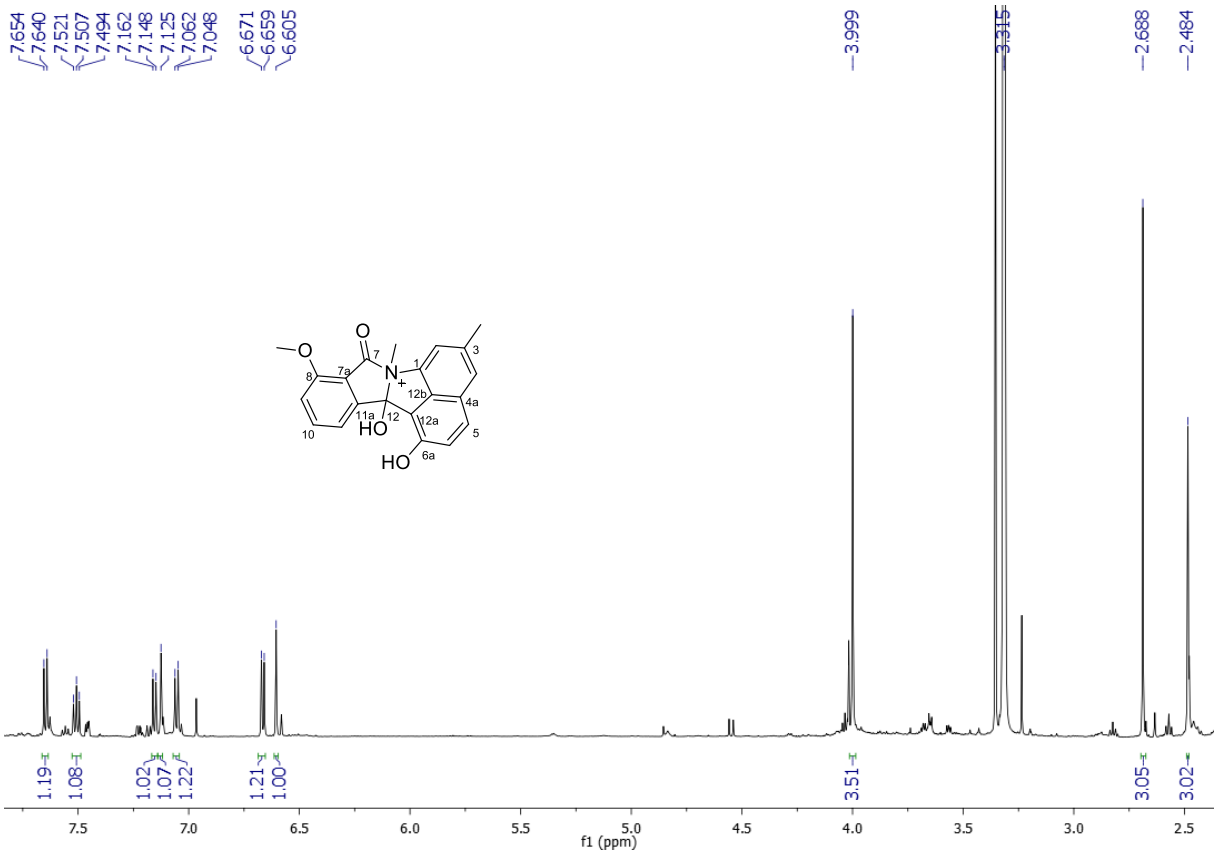
S35. HRESIMS spectrum of **15**.



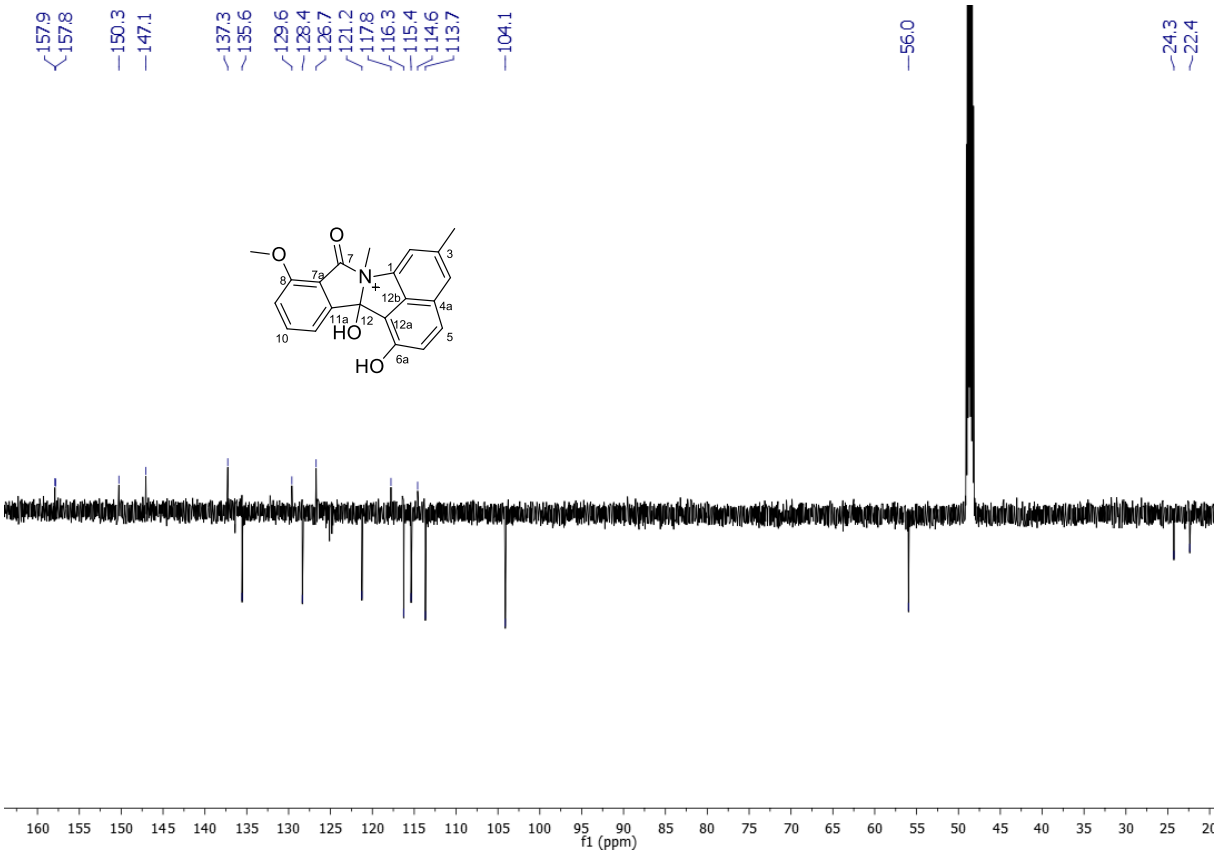
S36. UV spectrum of **15**.



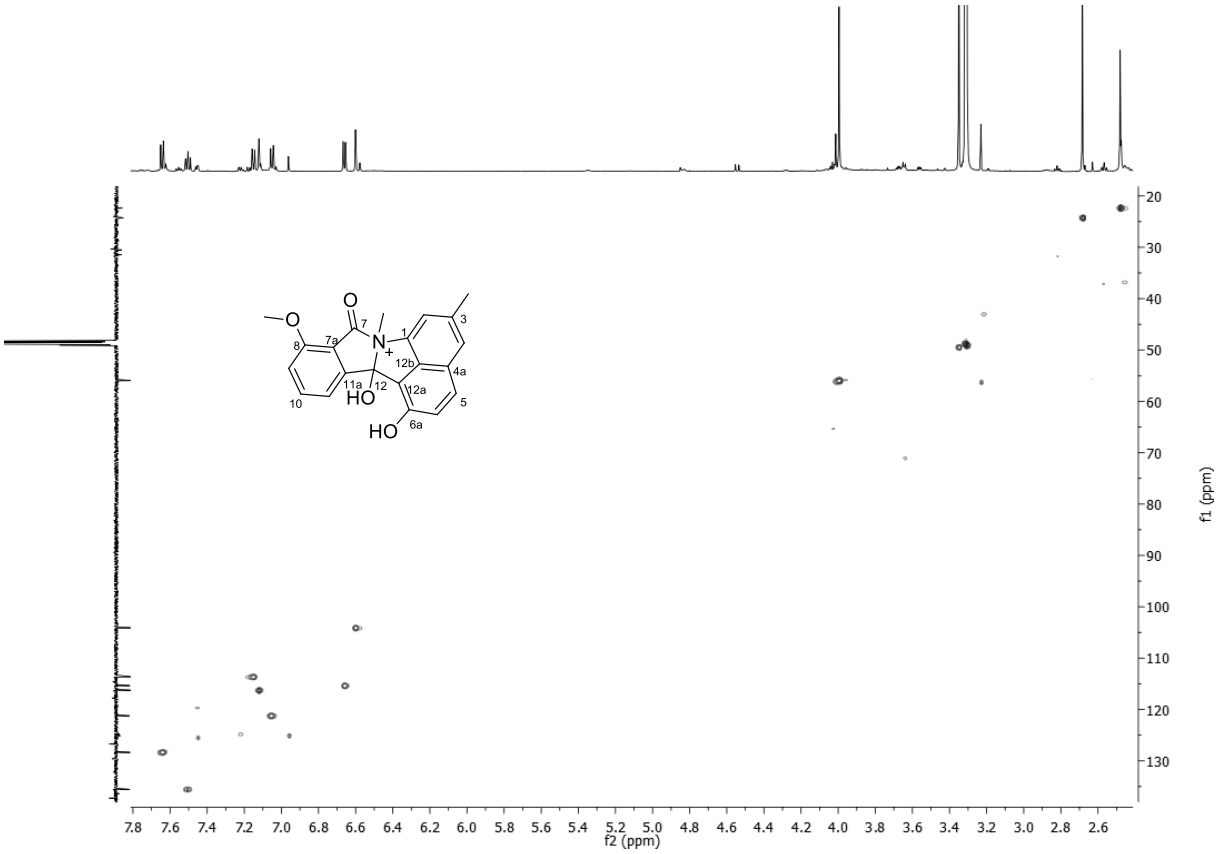
S37. $^{1}$H NMR spectrum (600 MHz) of **16** in $CD_3OD$.



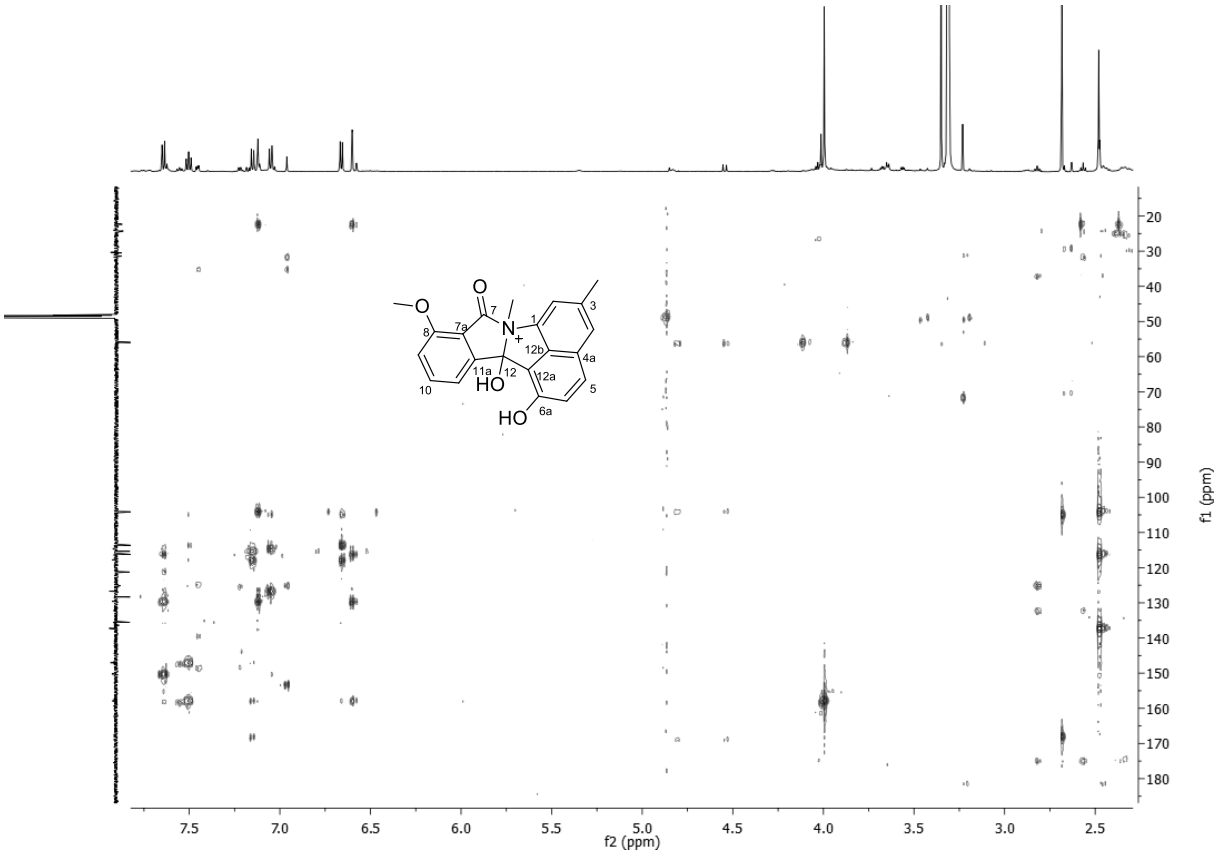
S38. APT spectrum (600 MHz) of **16** in $CD_3OD$.



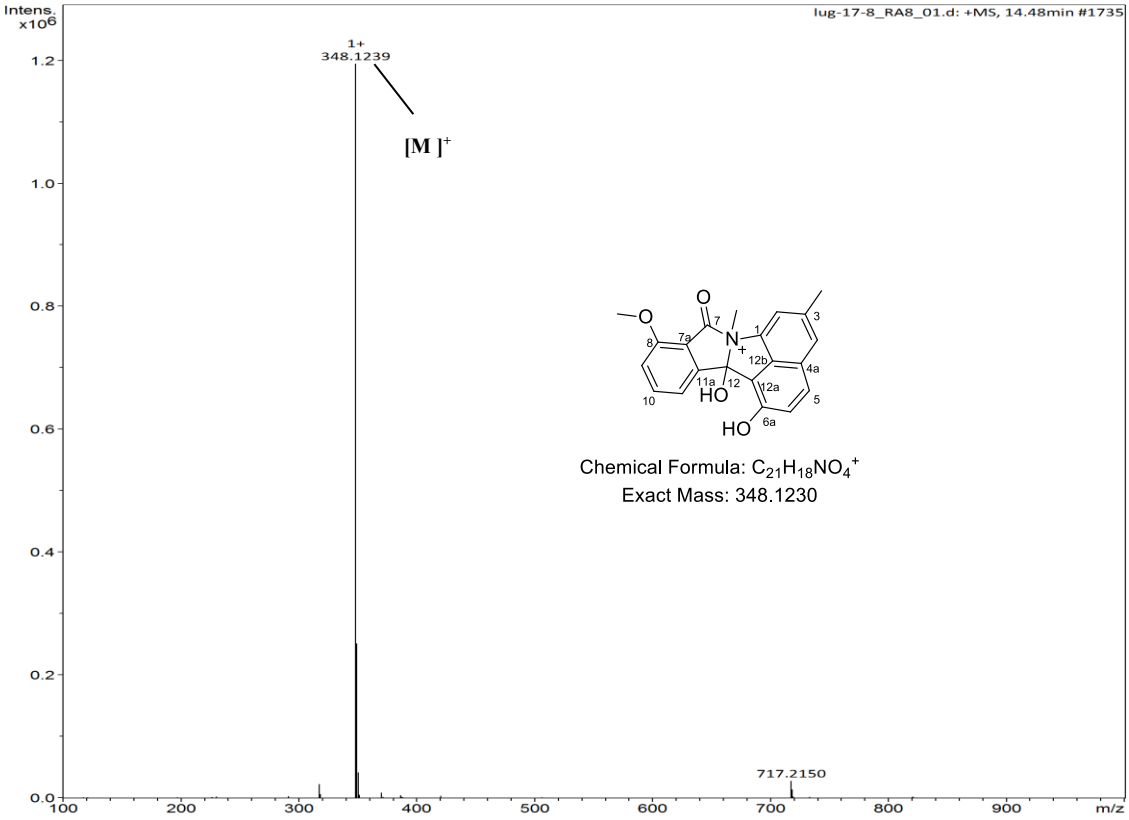
S39. HSQC spectrum (600 MHz) of **16** in $CD_3OD$.



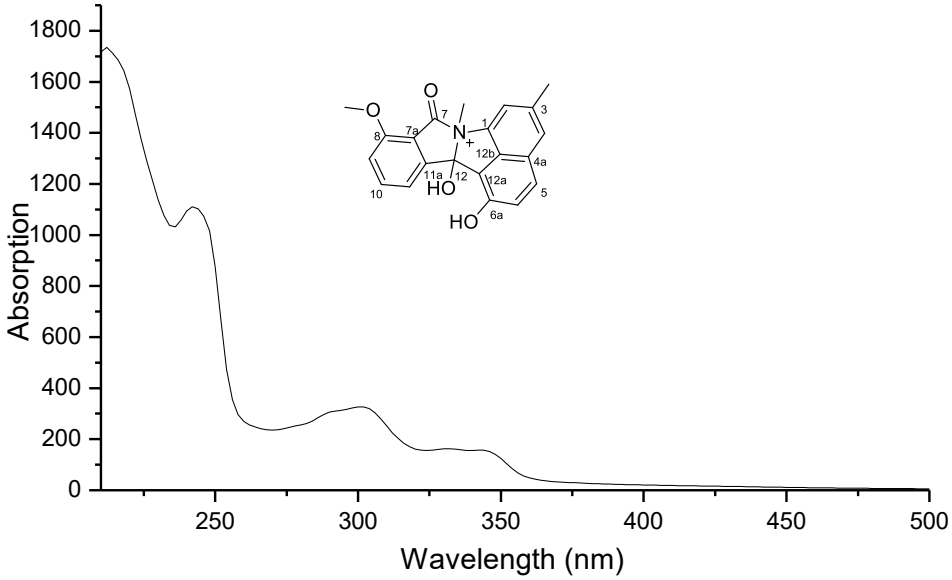
S40. HMBC spectrum (600 MHz) of **16** in $CD_3OD$.



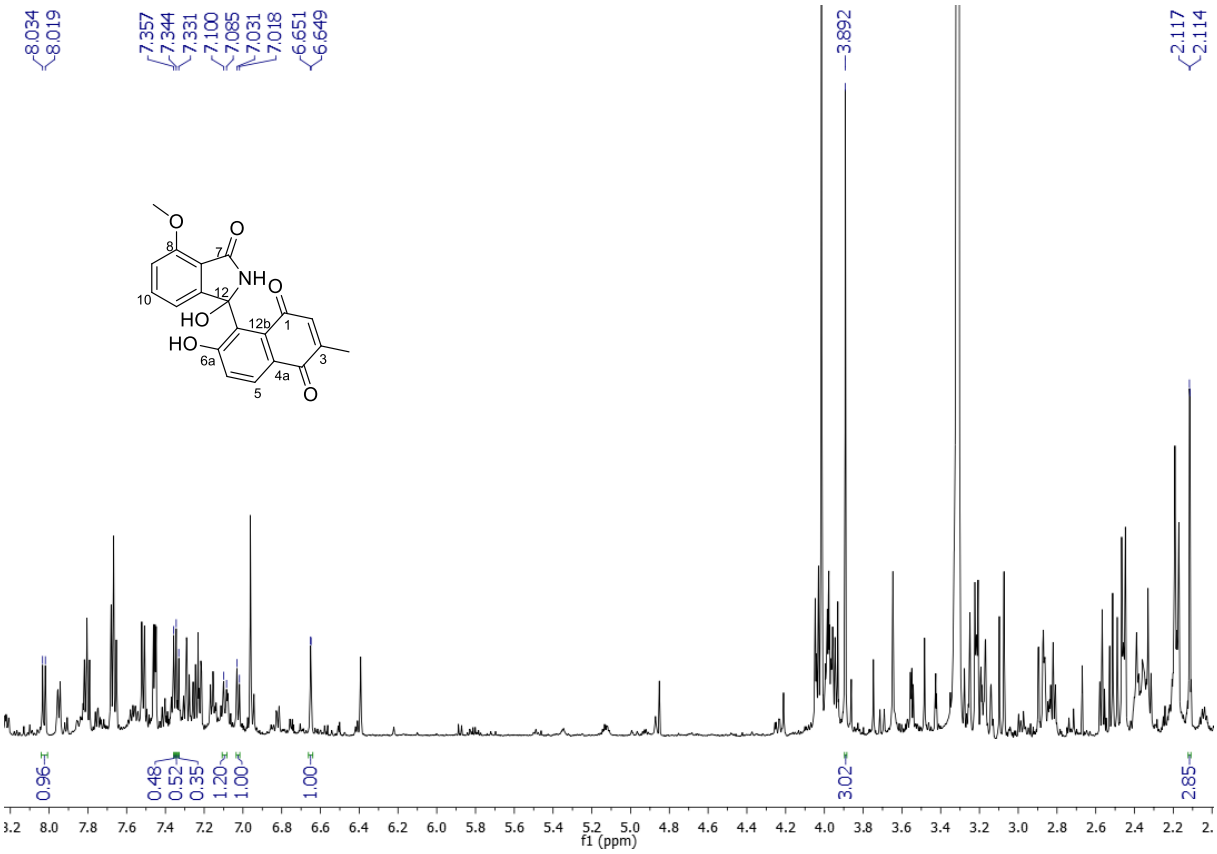
S41. HRESIMS spectrum of **16**.



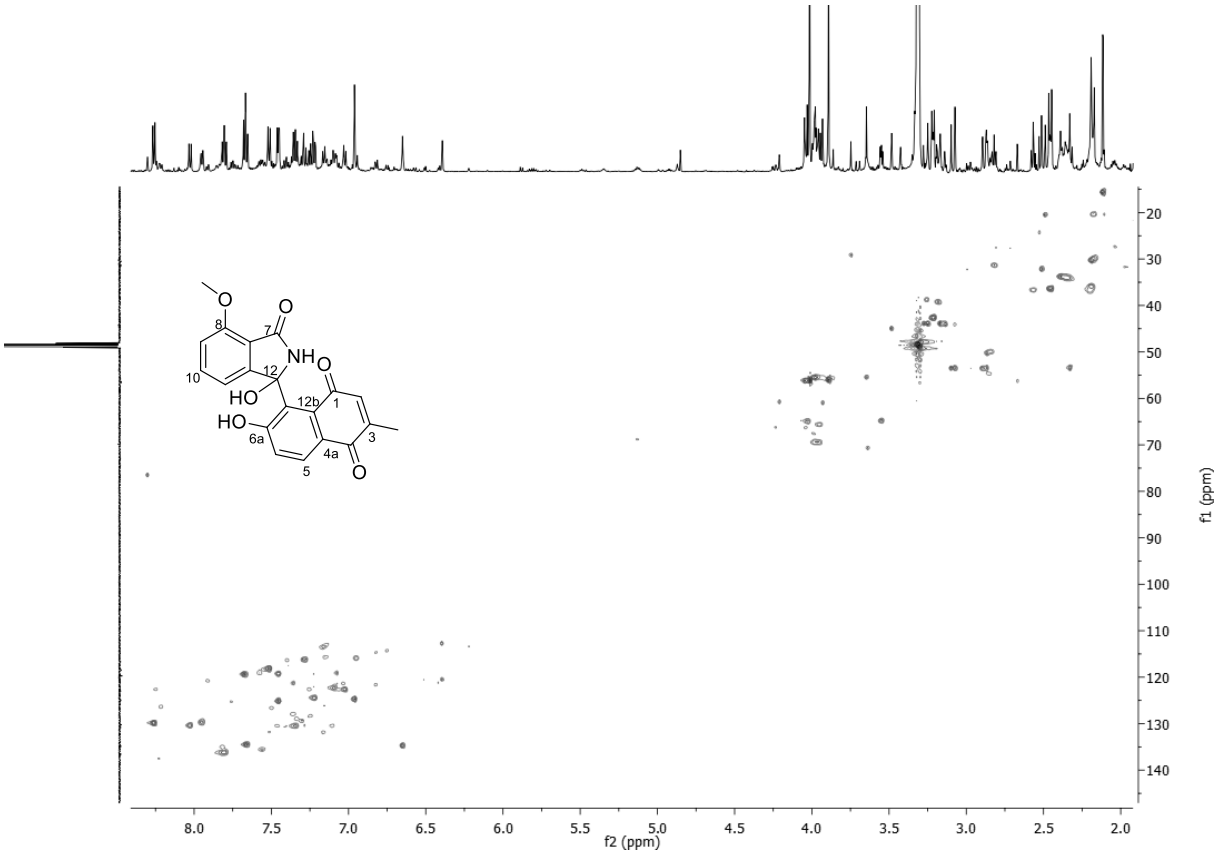
S42. UV spectrum of **16**.



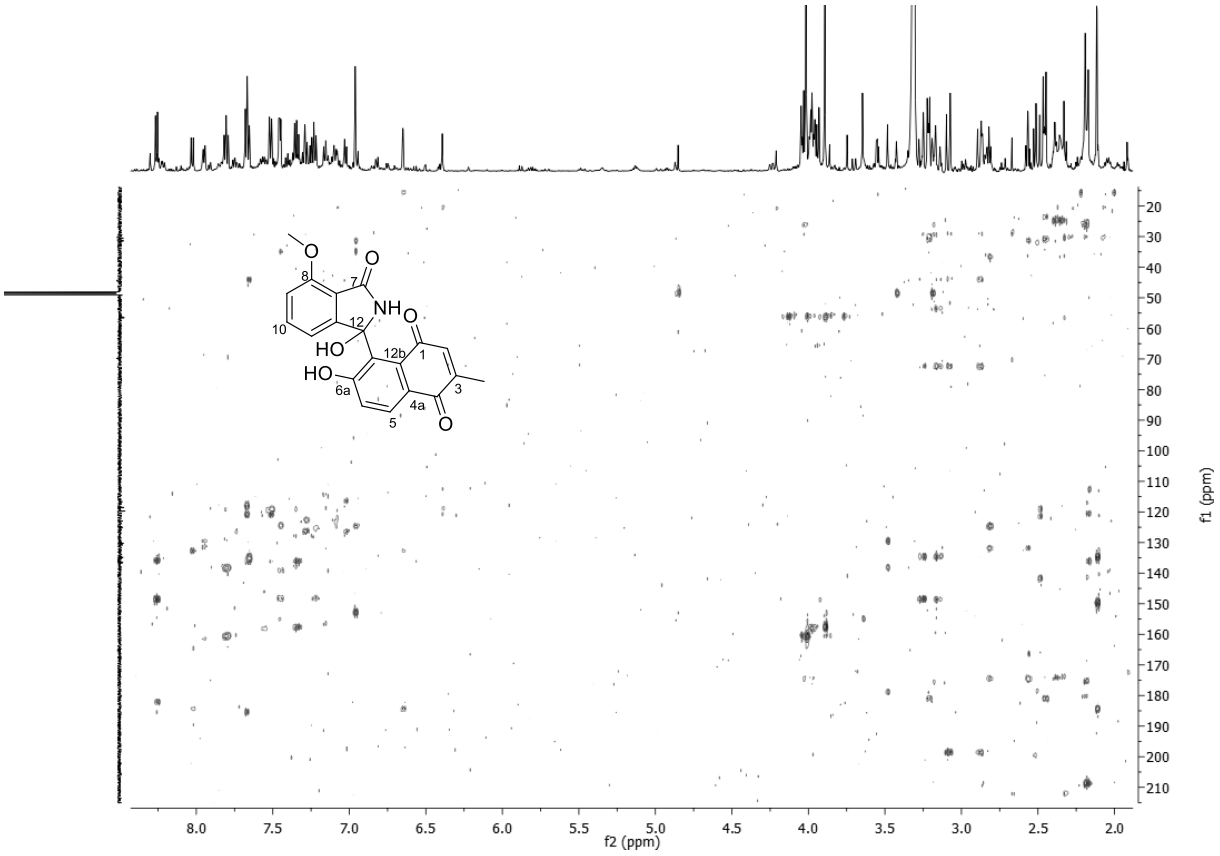
S43. $^{1}$H NMR spectrum (600 MHz) of **17** in $CD_3OD$.



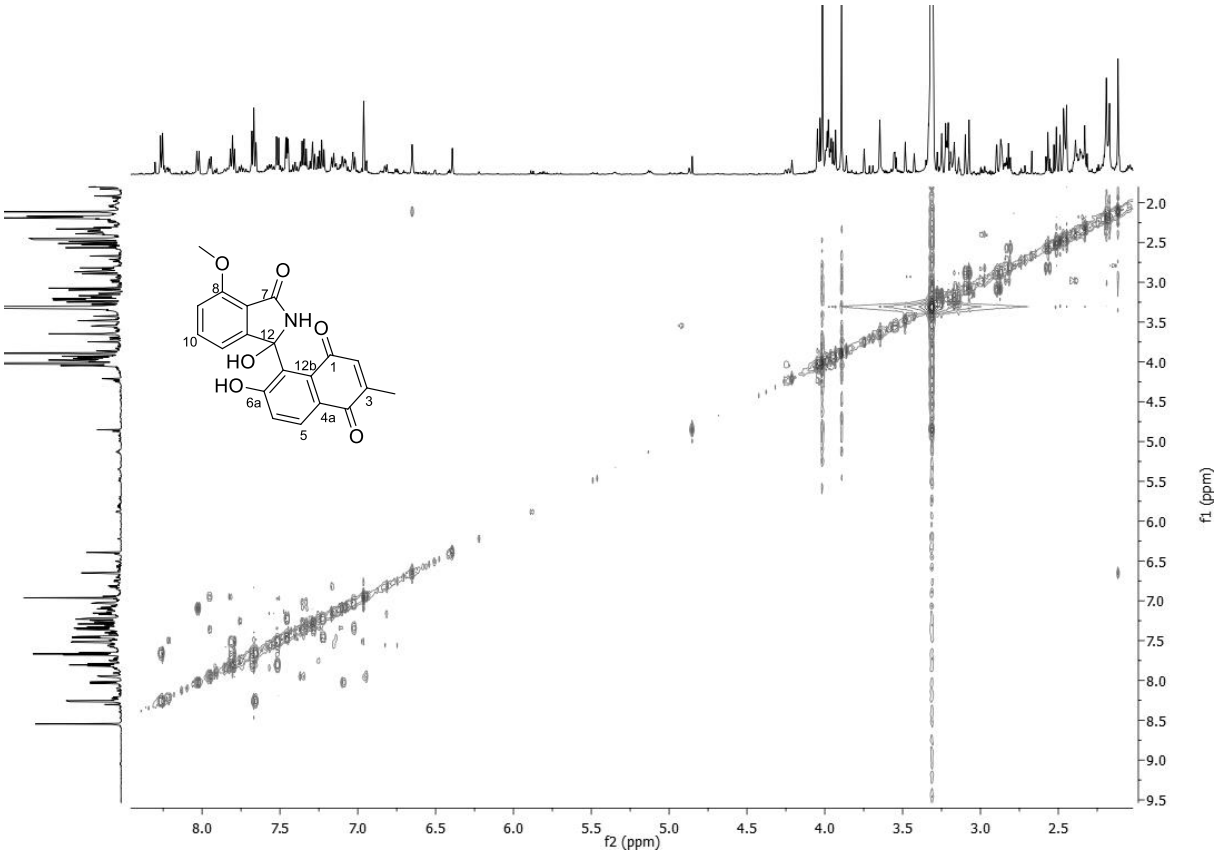
S44. HSQC spectrum (600 MHz) of **17** in $CD_3OD$.



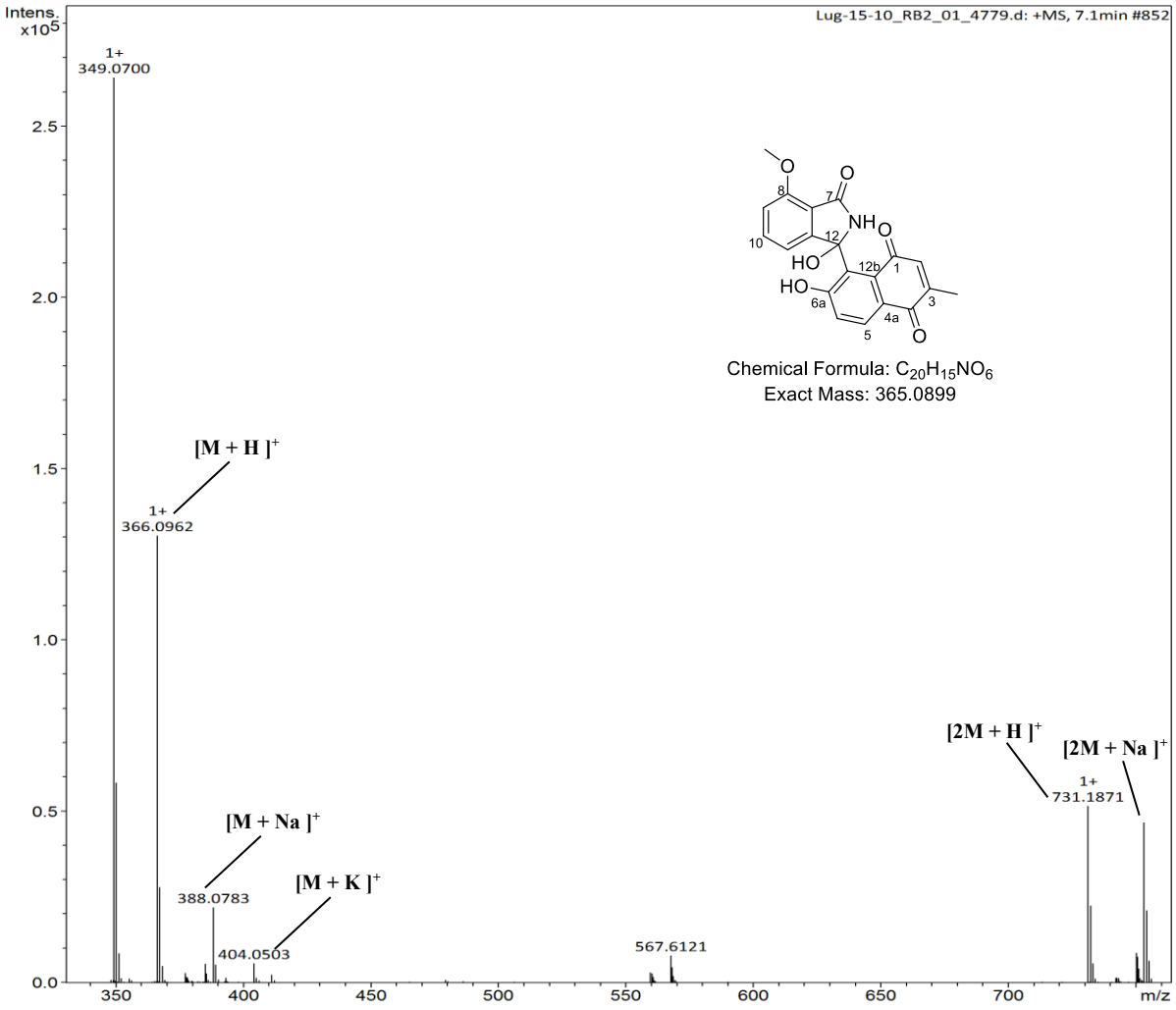
S45. HMBC spectrum (600 MHz) of **17** in $CD_3OD$.



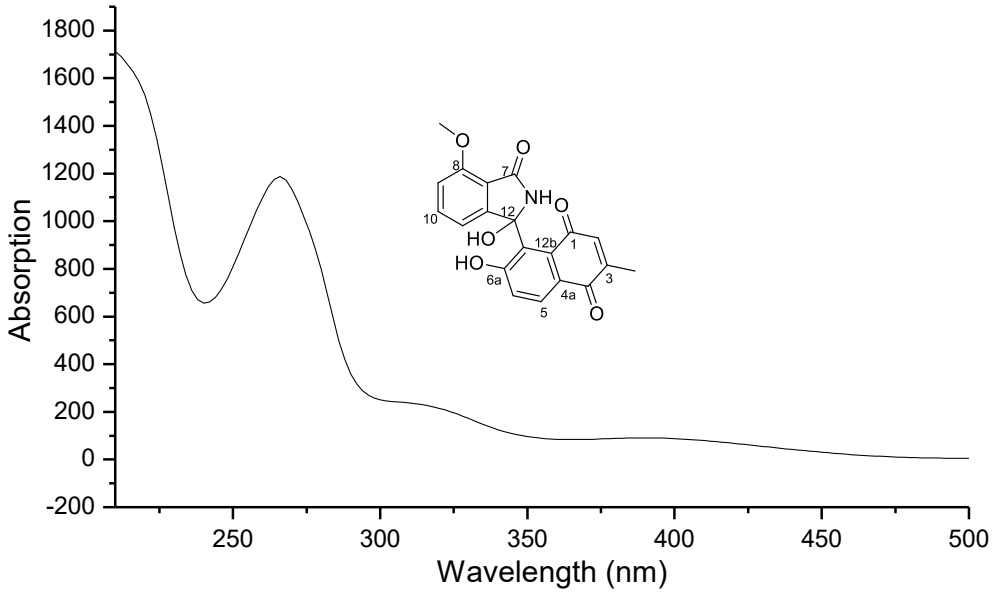
S46. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **17** in $CD_3OD$.



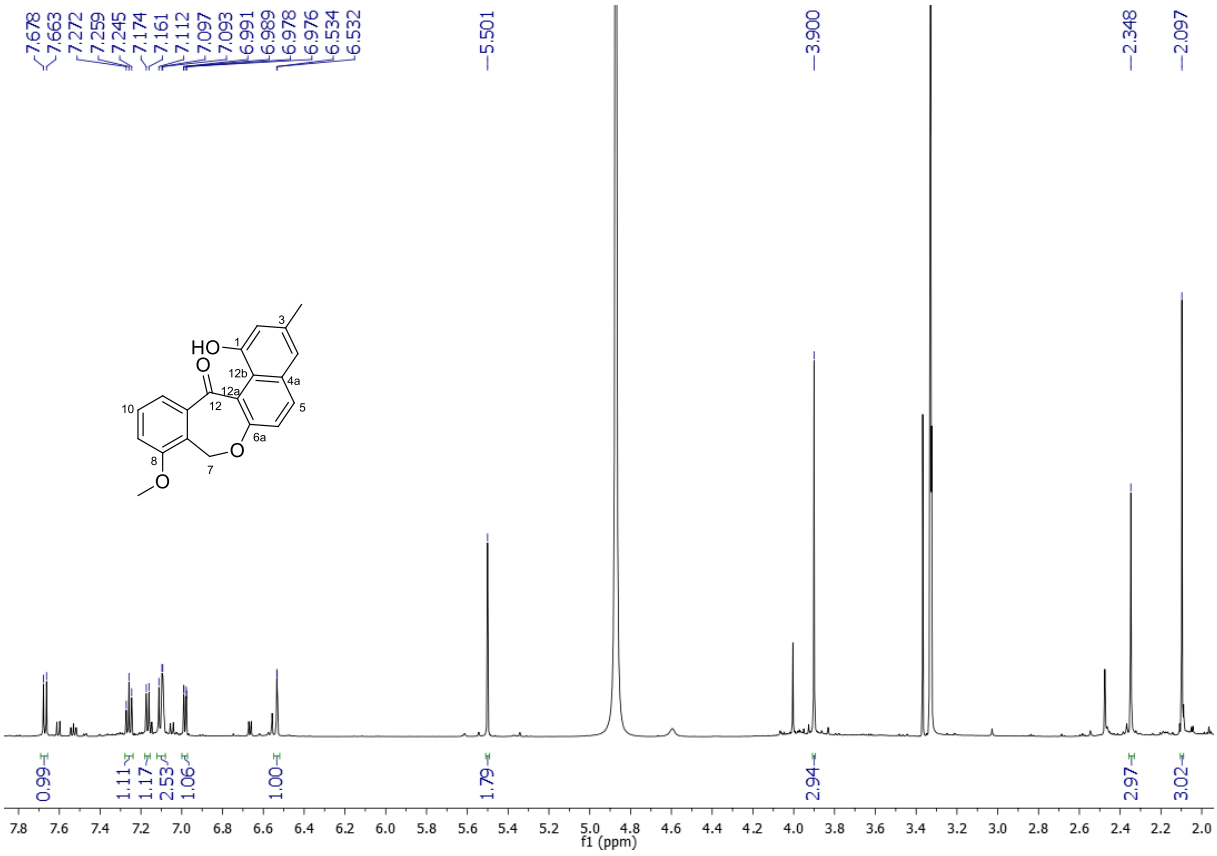
S47. HRESIMS spectrum of **17**.



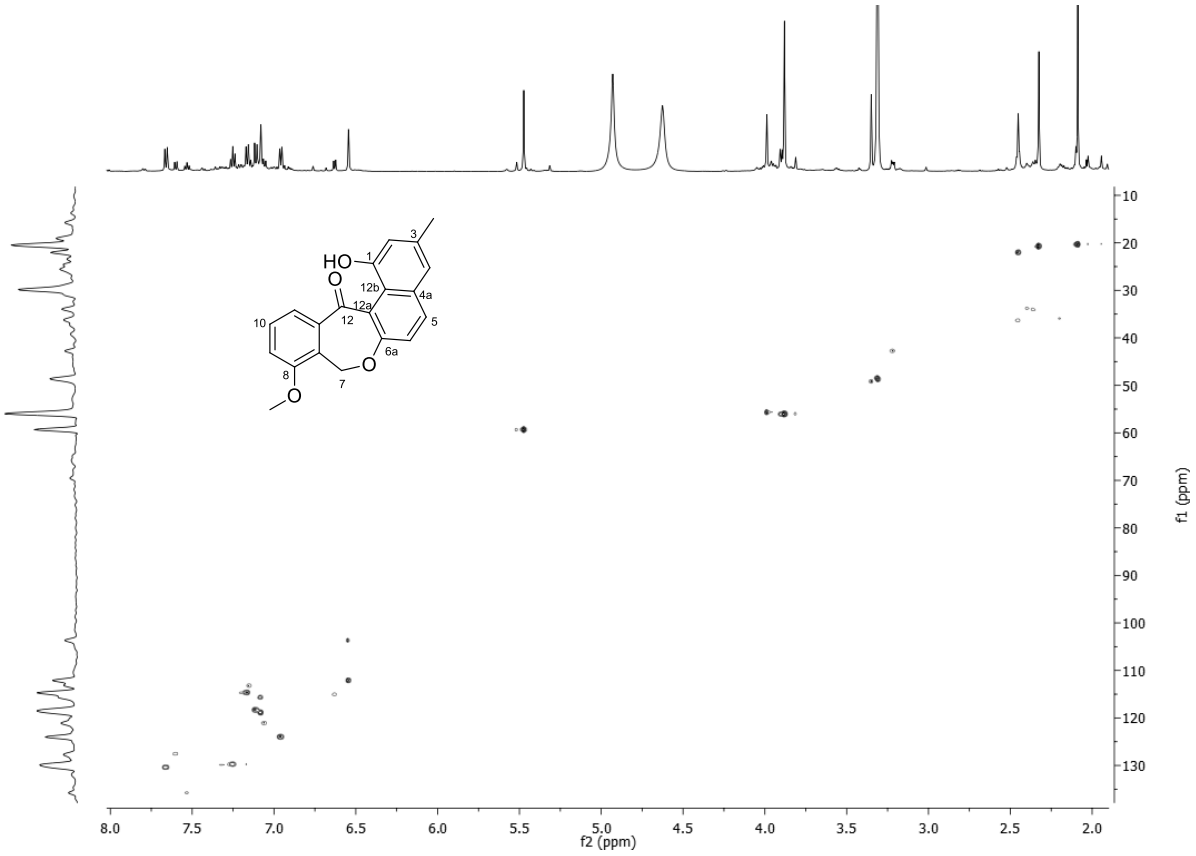
S48. UV spectrum of **17**.



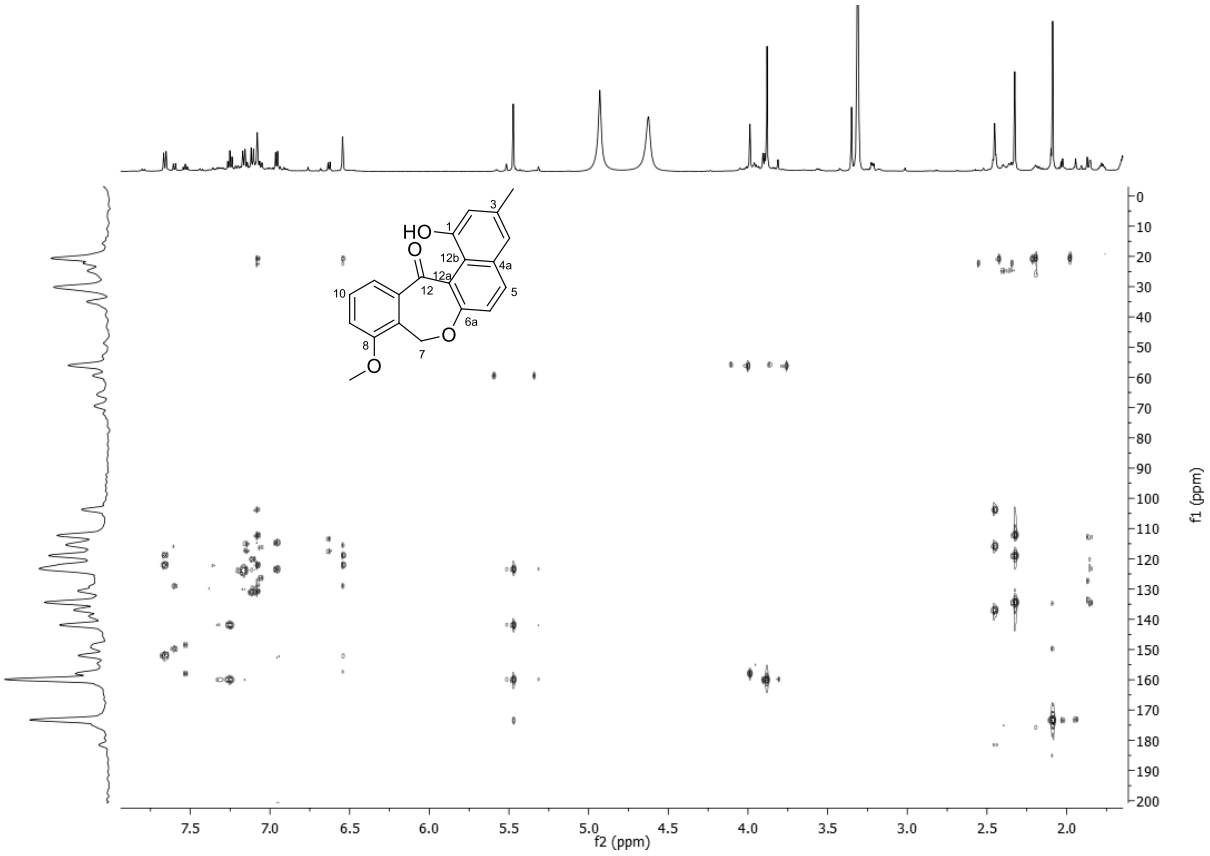
S49. $^{1}$H NMR spectrum (600 MHz) of **21** in $CD_3OD$.



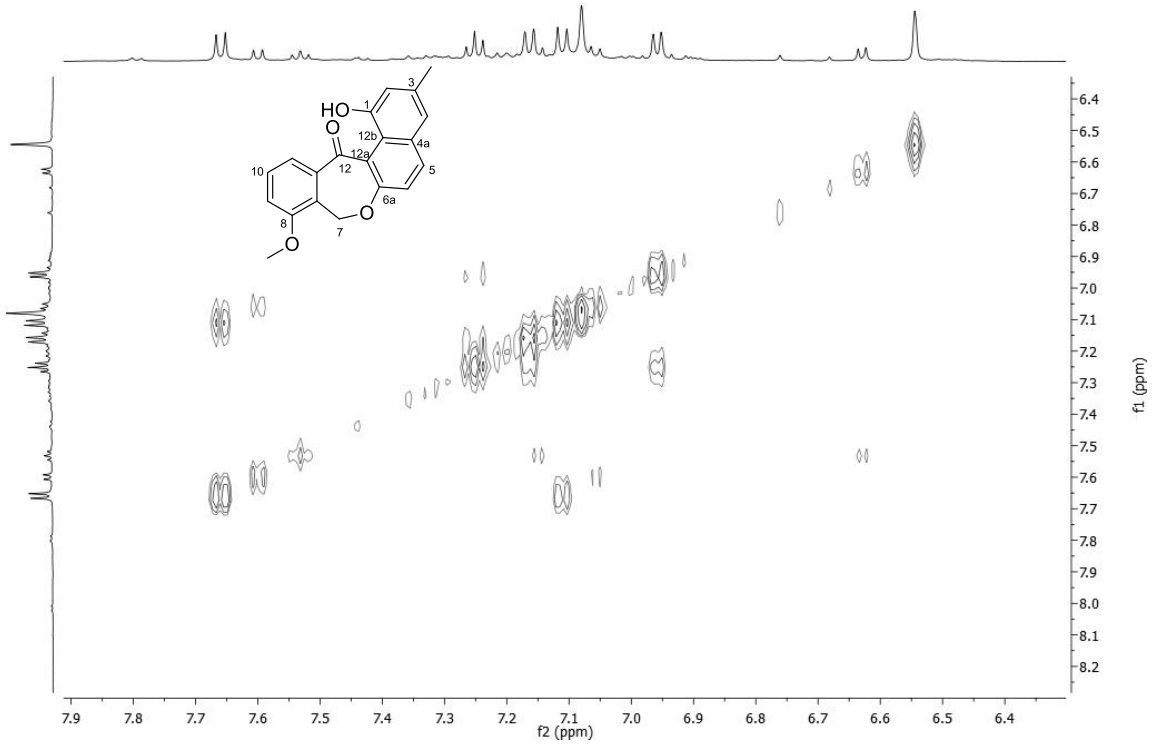
S50. HSQC spectrum (600 MHz) of **21** in $CD_3OD$.



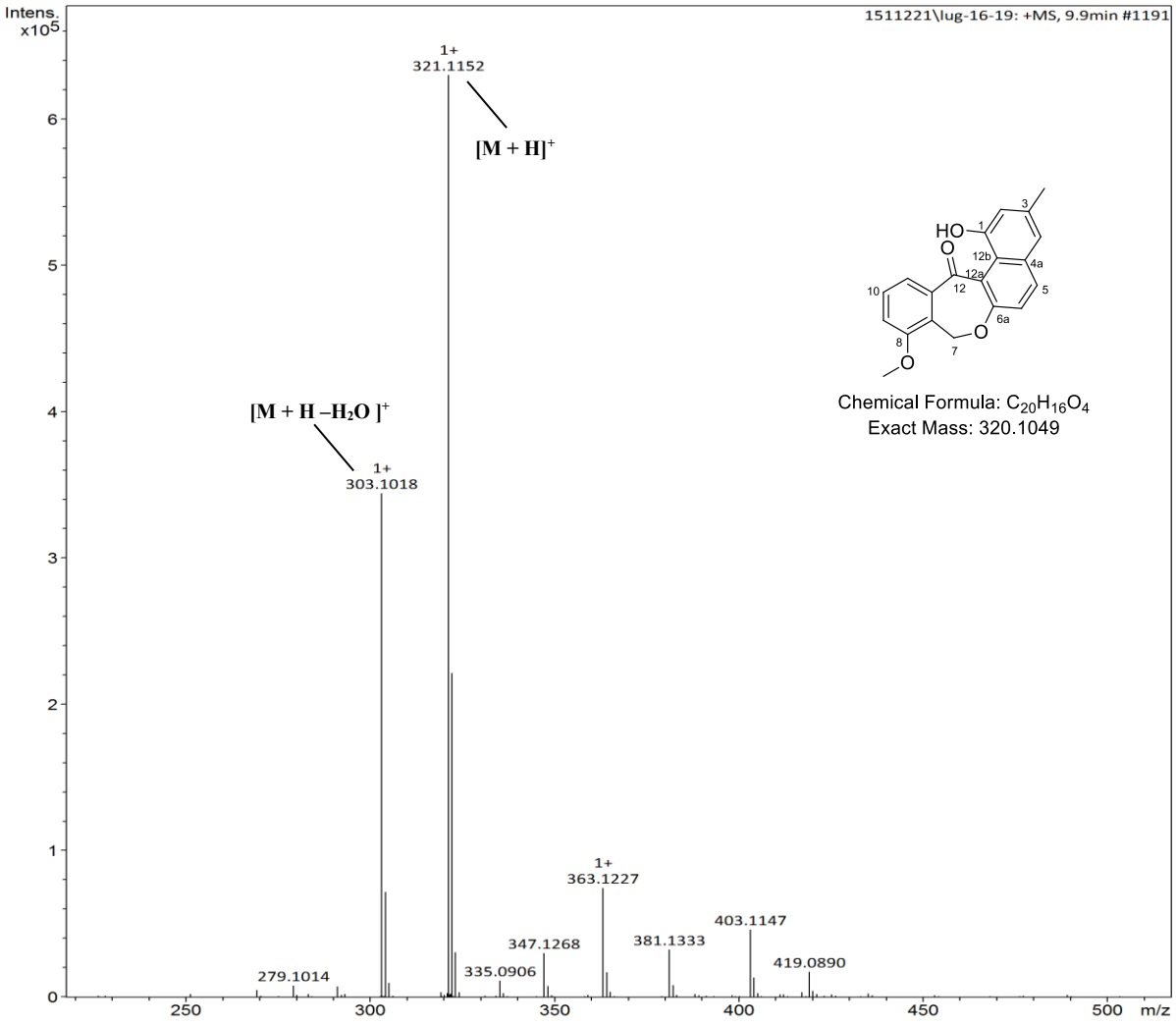
S51. HMBC spectrum (600 MHz) of **21** in $CD_3OD$.



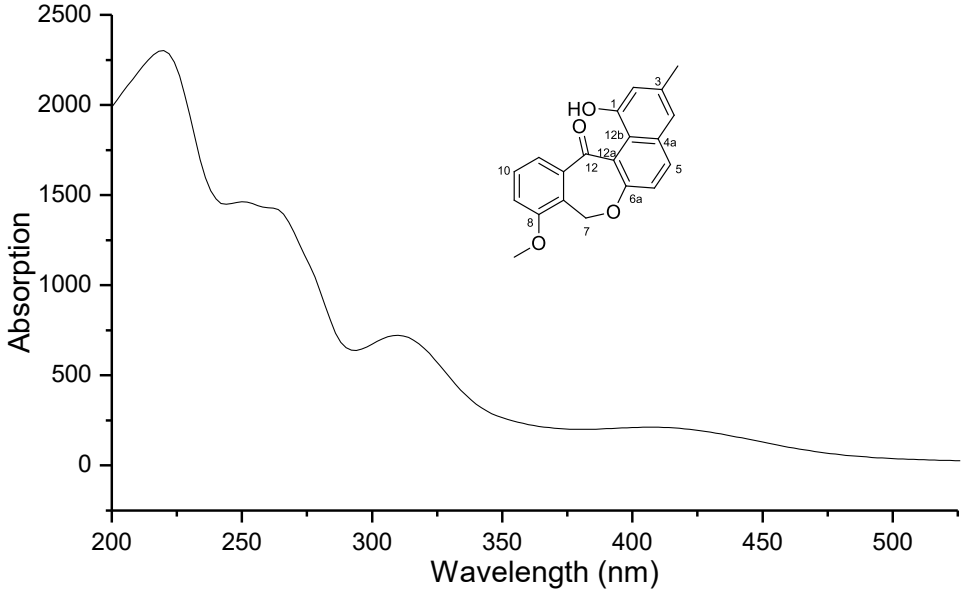
S52. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **21** in $CD_3OD$.



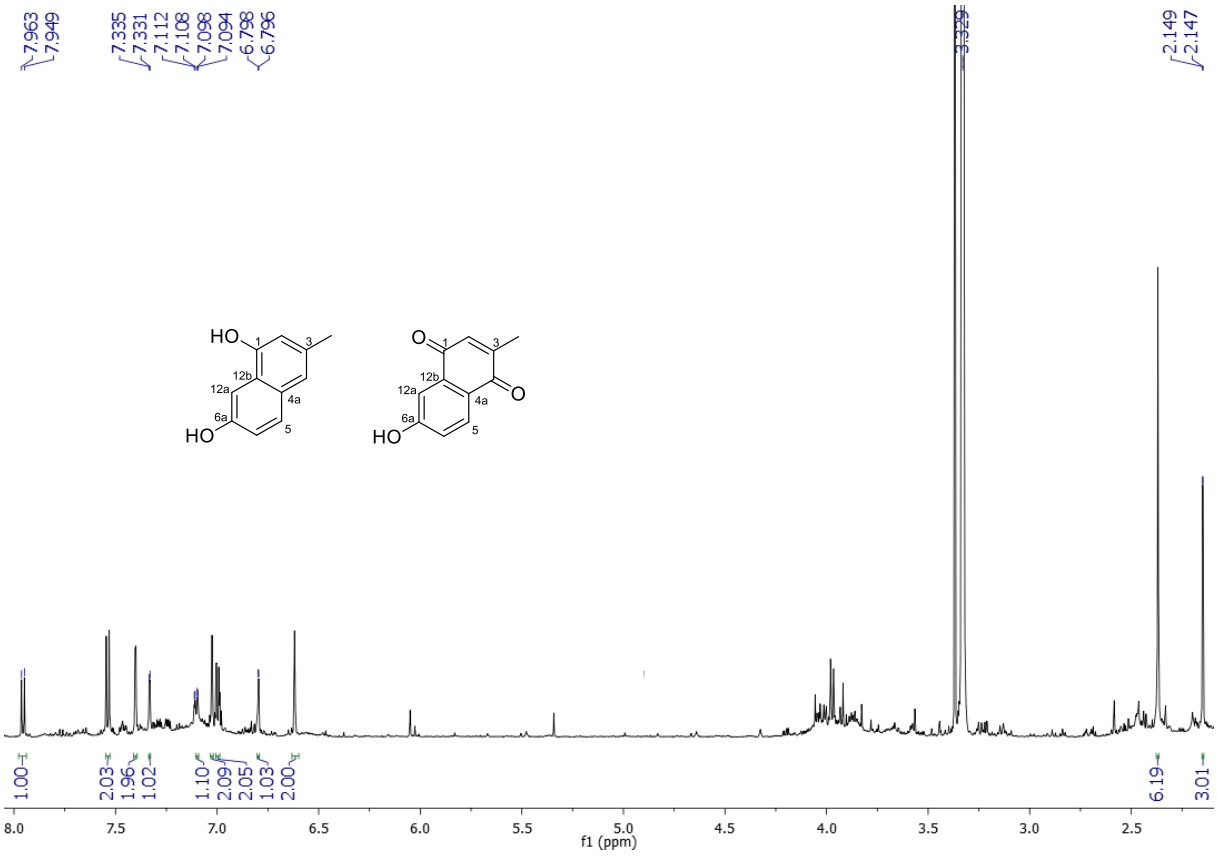
S53. HRESIMS spectrum of **21**.



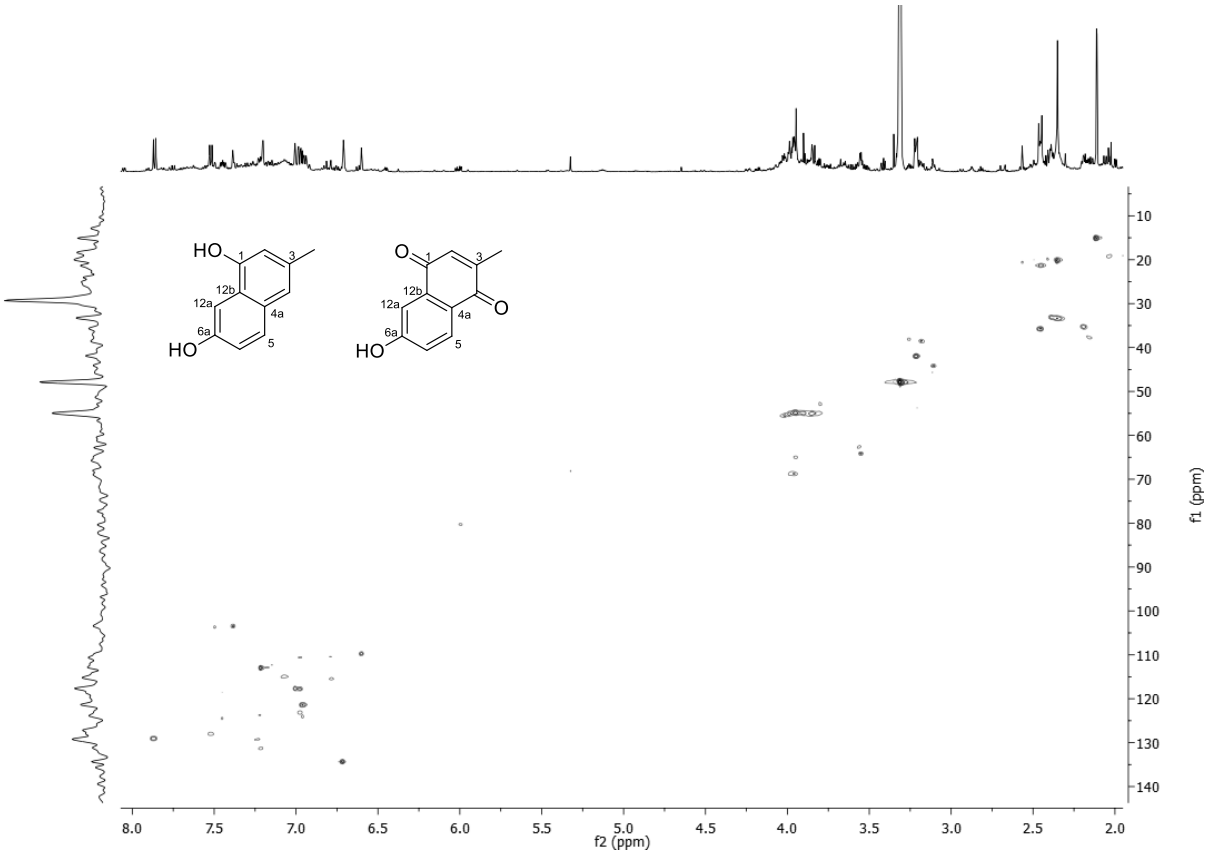
S54. UV spectrum of **21**.



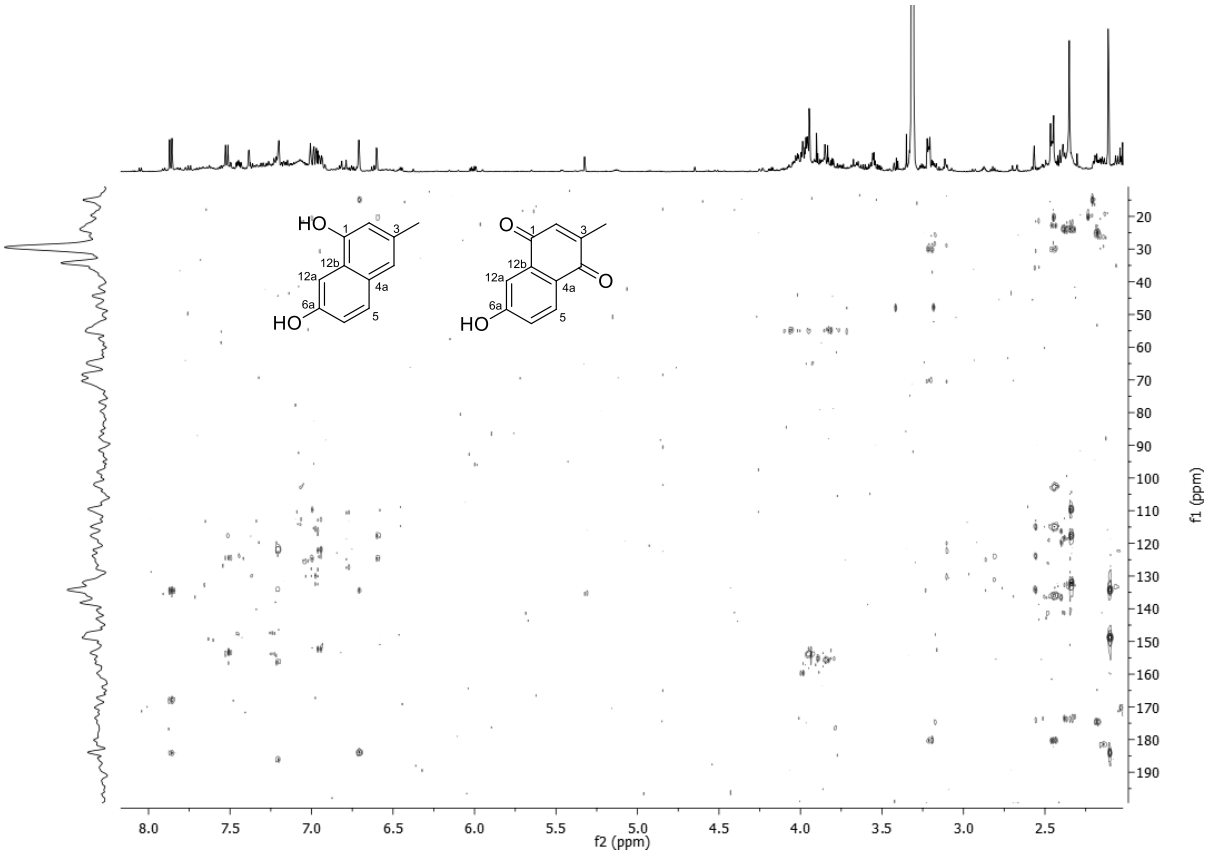
S55. $^{1}$H NMR spectrum (600 MHz) of **22** and **23** in $CD_3OD$.



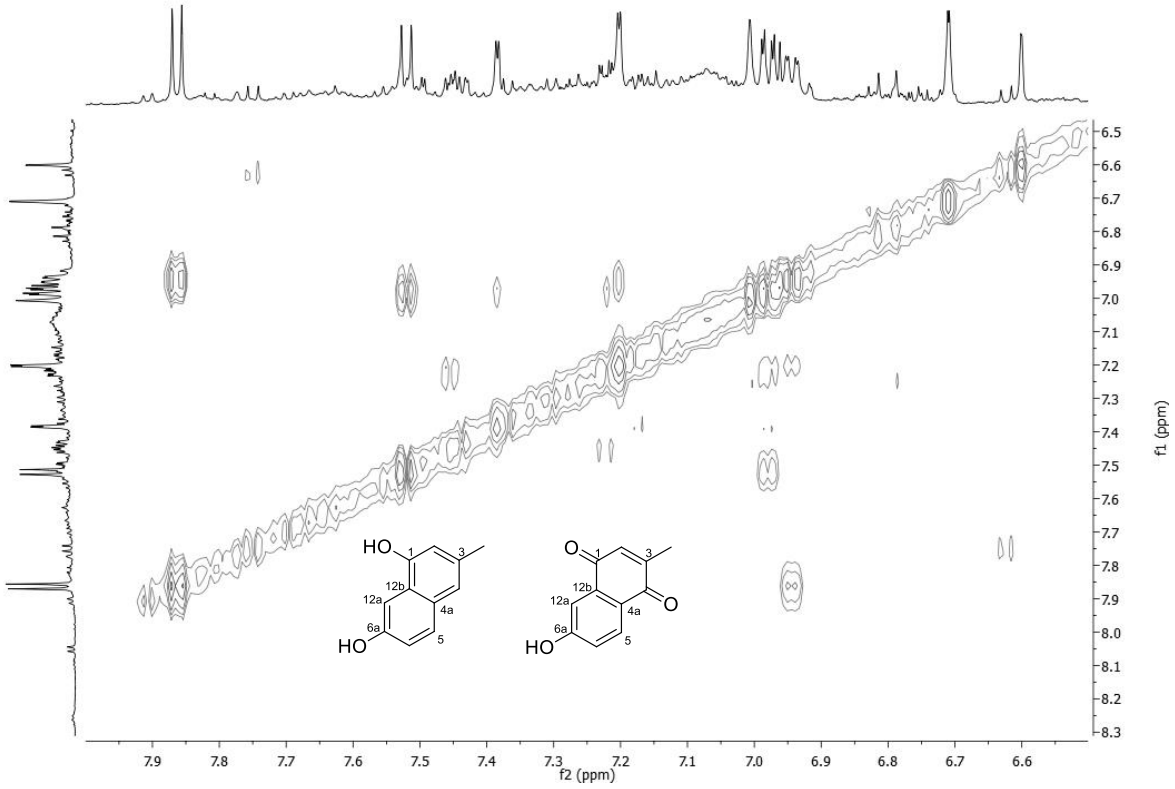
S56. HSQC spectrum (600 MHz) of **22** and **23** in $CD_3OD$.



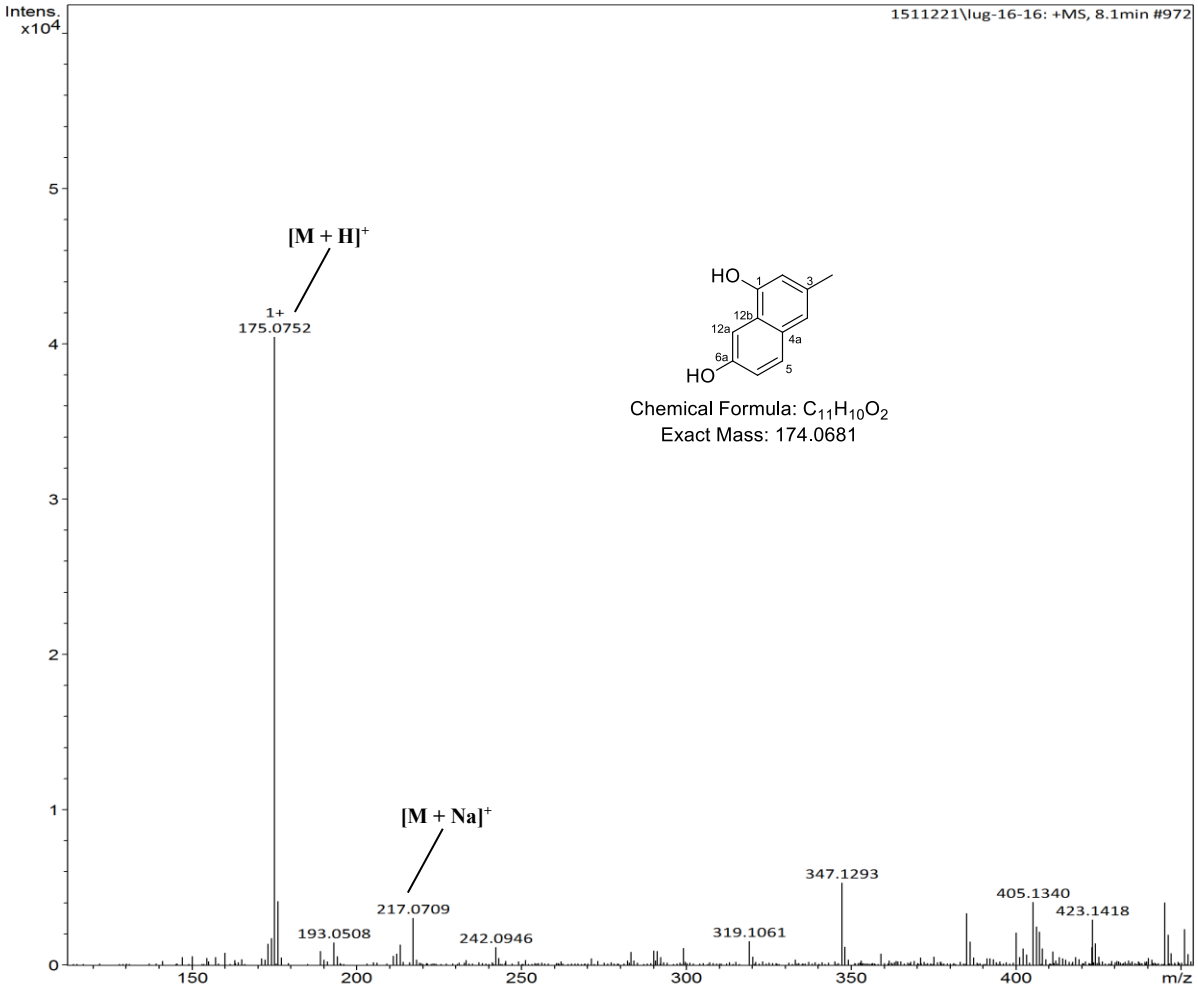
S57. HMBC spectrum (600 MHz) of **22** and **23** in $CD_3OD$.



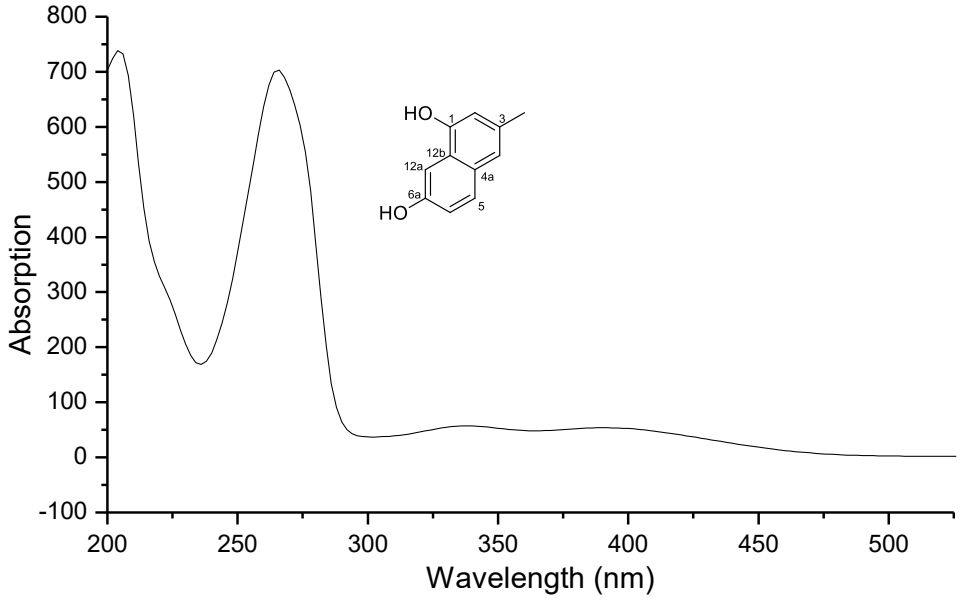
S58. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **22** and **23** in $CD_3OD$.



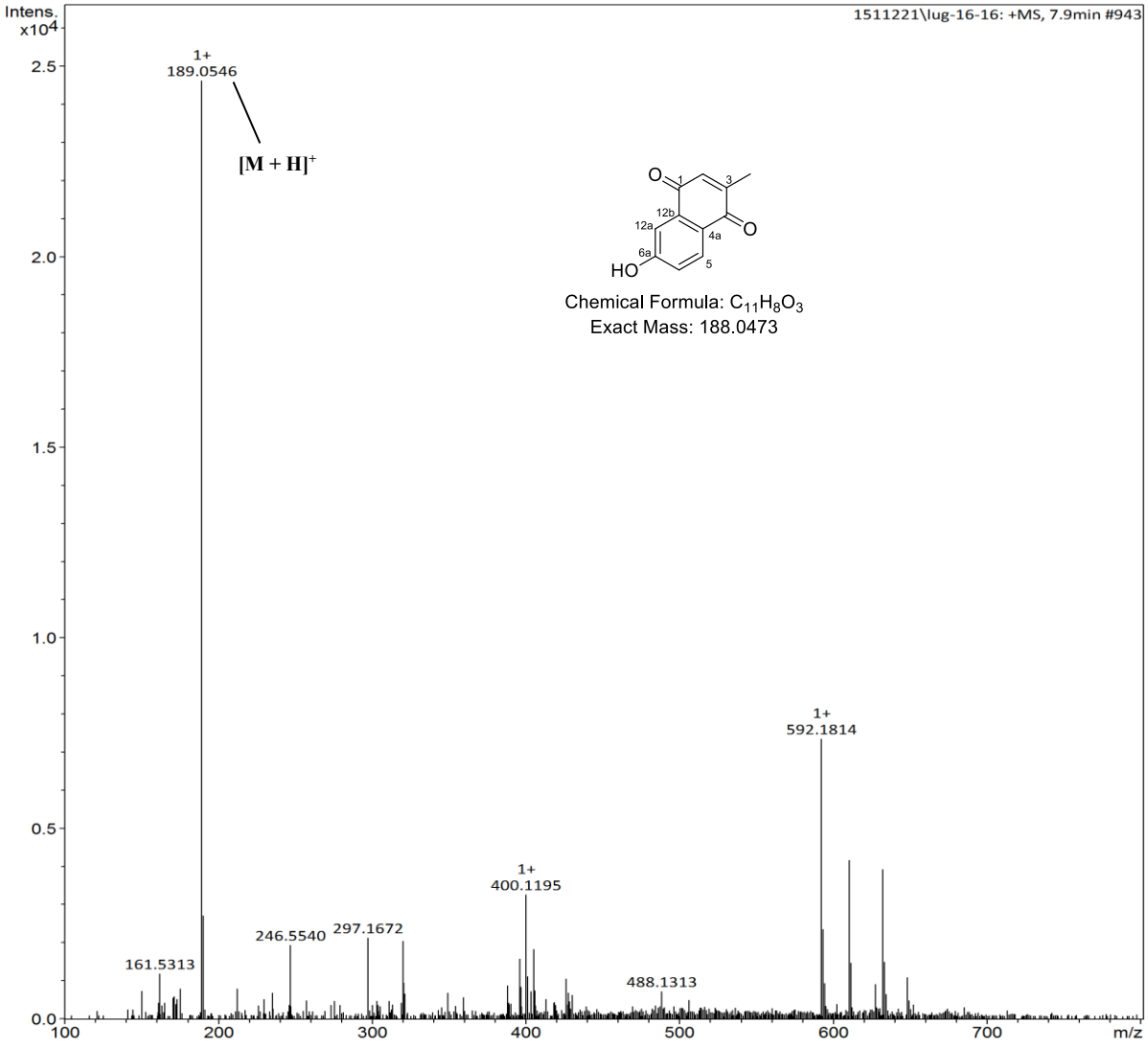
S59. HRESIMS spectrum of **22**.



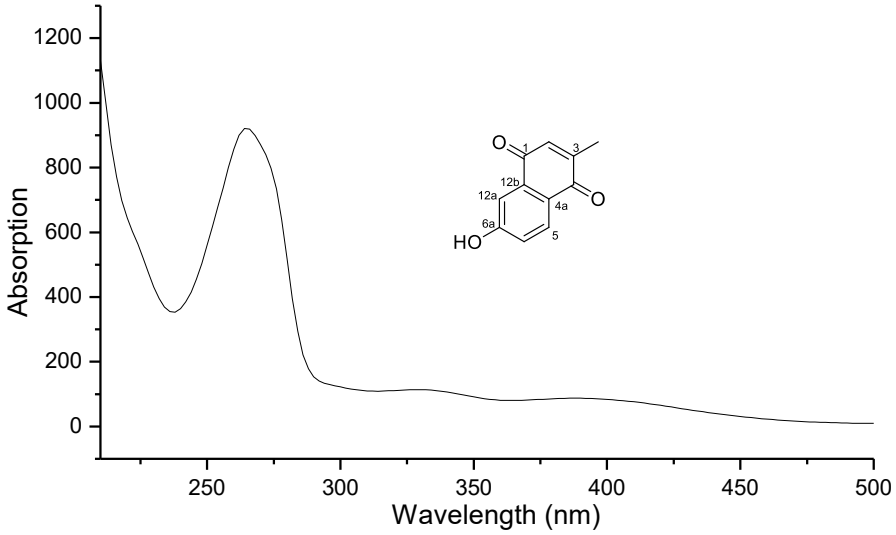
S60. UV spectrum of **22**.



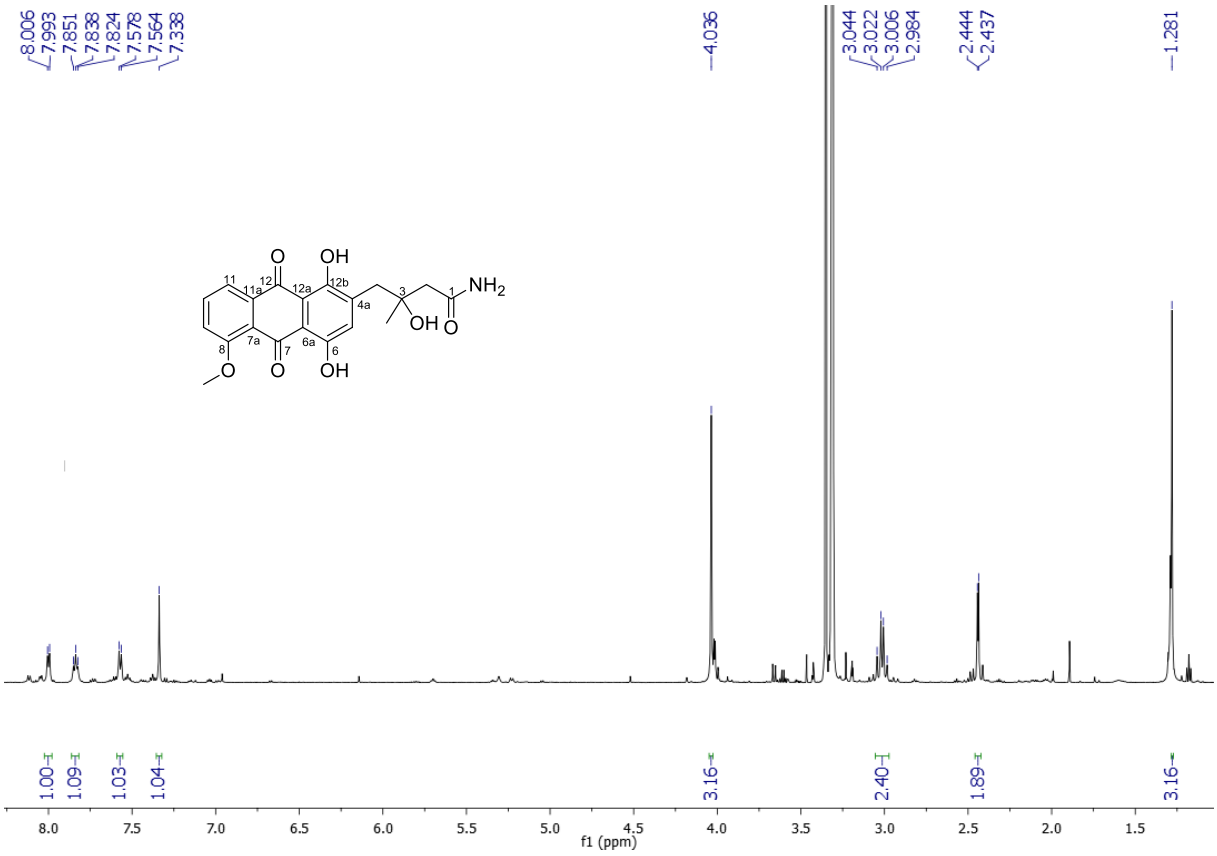
S61. HRESIMS spectrum of **23**.



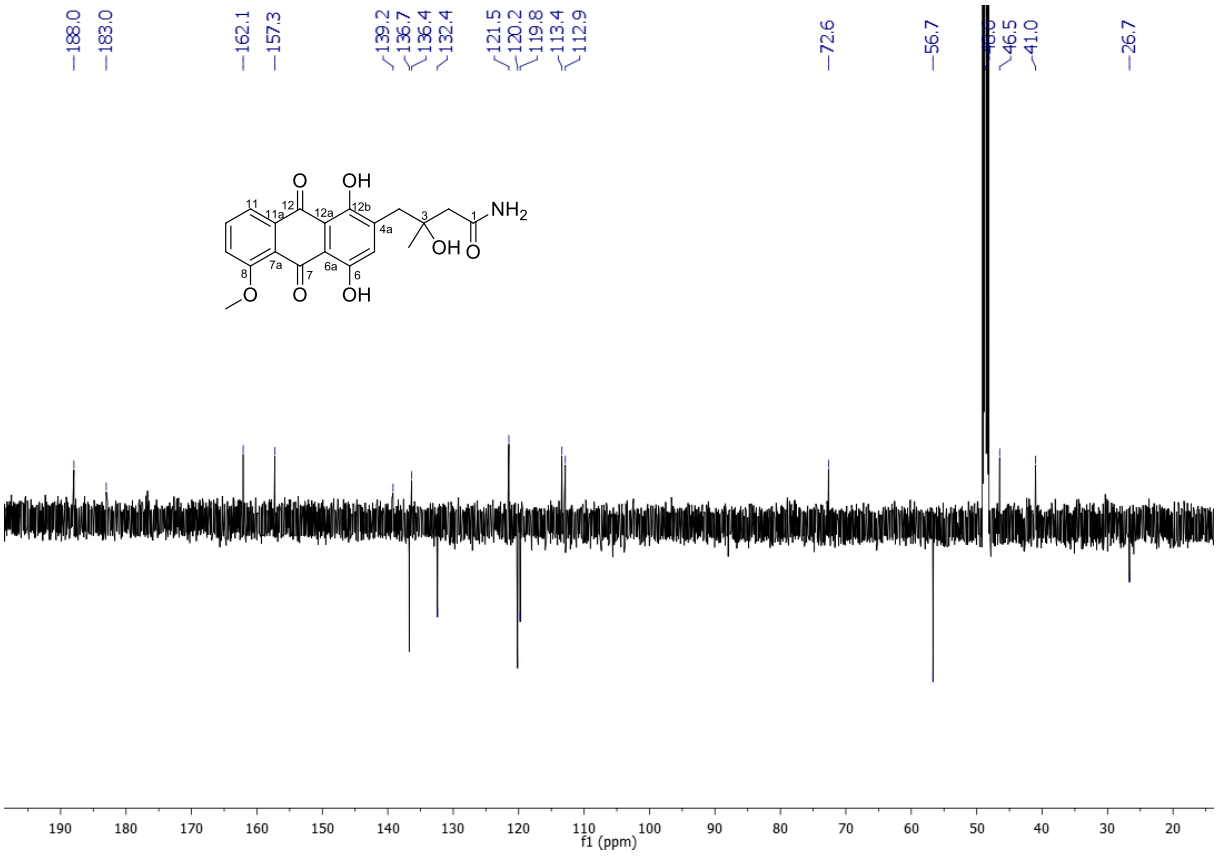
S62. UV spectrum of **23**.



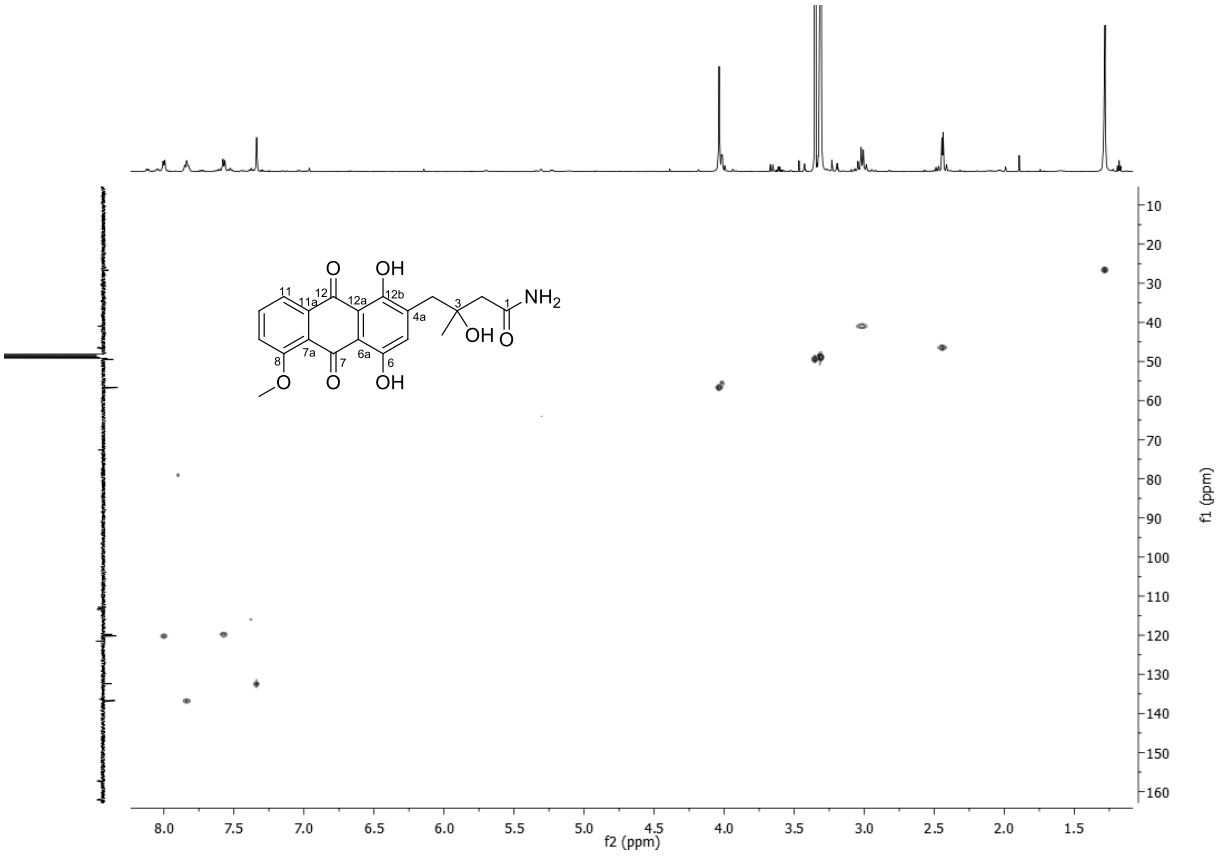
S63. $^{1}$H NMR spectrum (600 MHz) of **24** in $CD_3OD$.



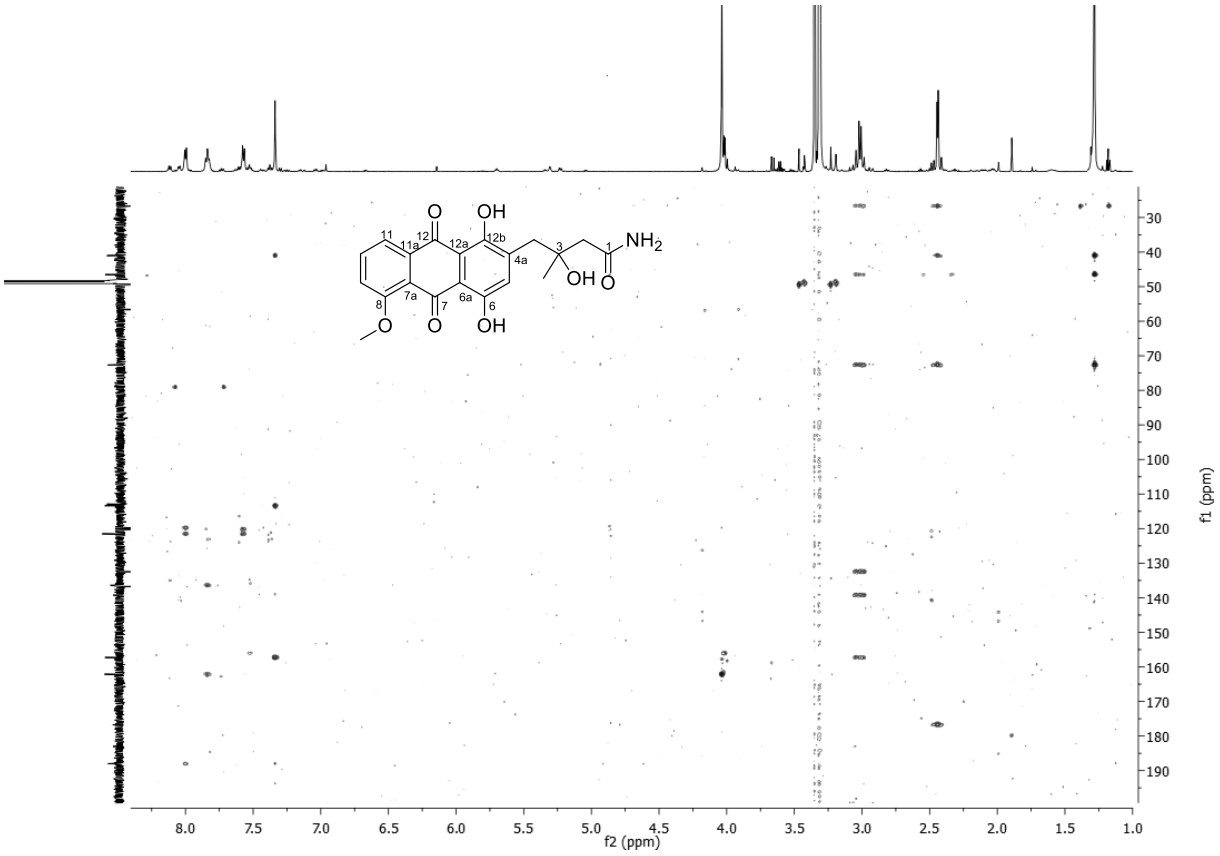
S64. APT spectrum (600 MHz) of **24** in $CD_3OD$.



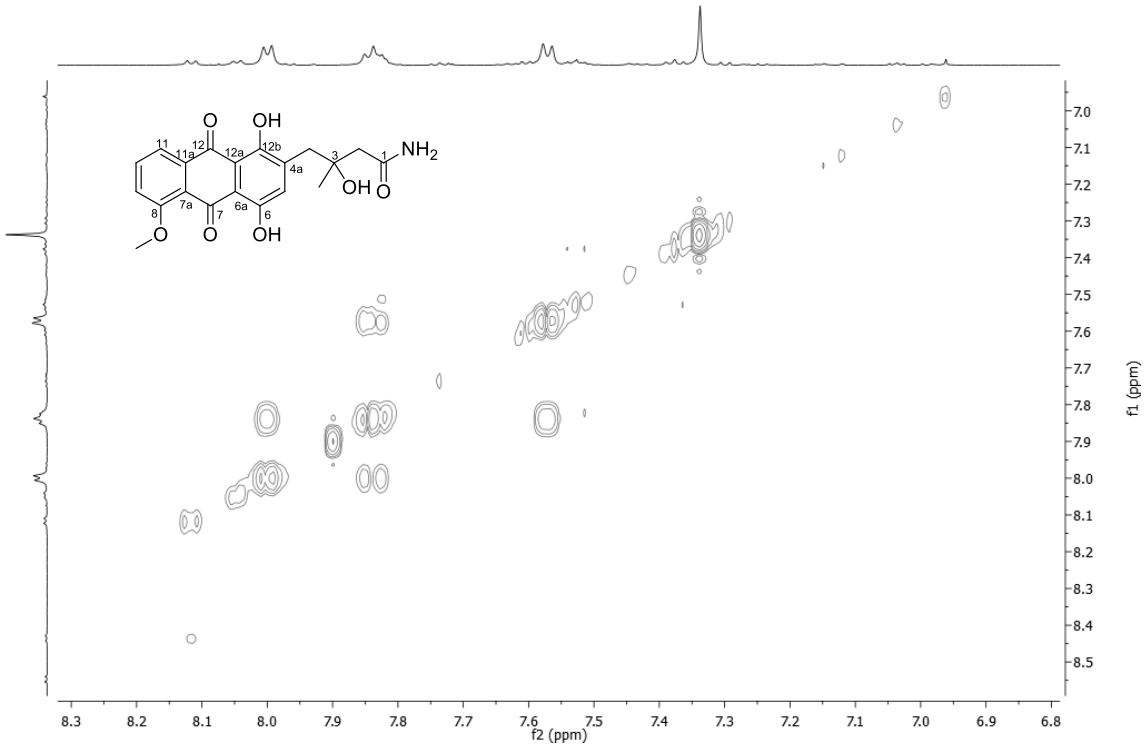
S65. HSQC spectrum (600 MHz) of **24** in $CD_3OD$.



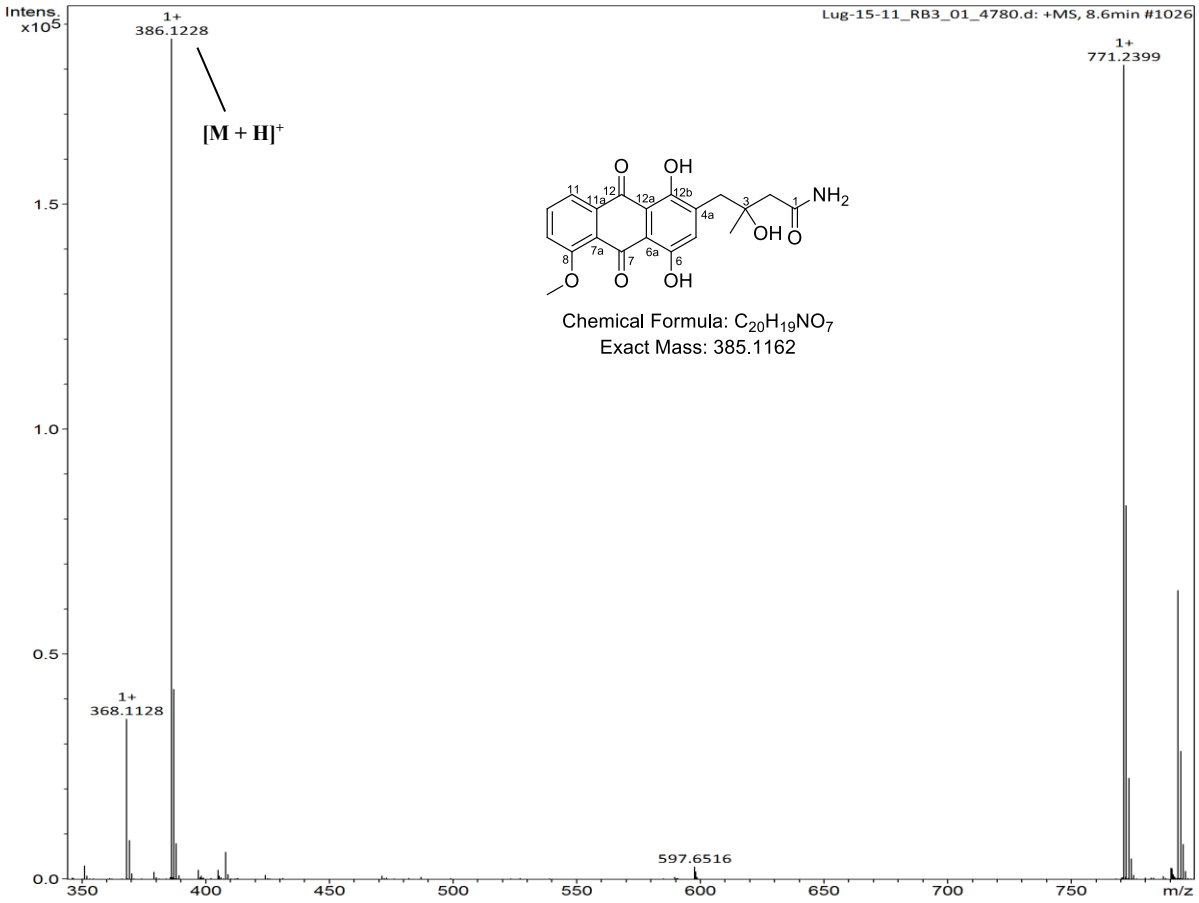
S66. HMBC spectrum (600 MHz) of **24** in $CD_3OD$.



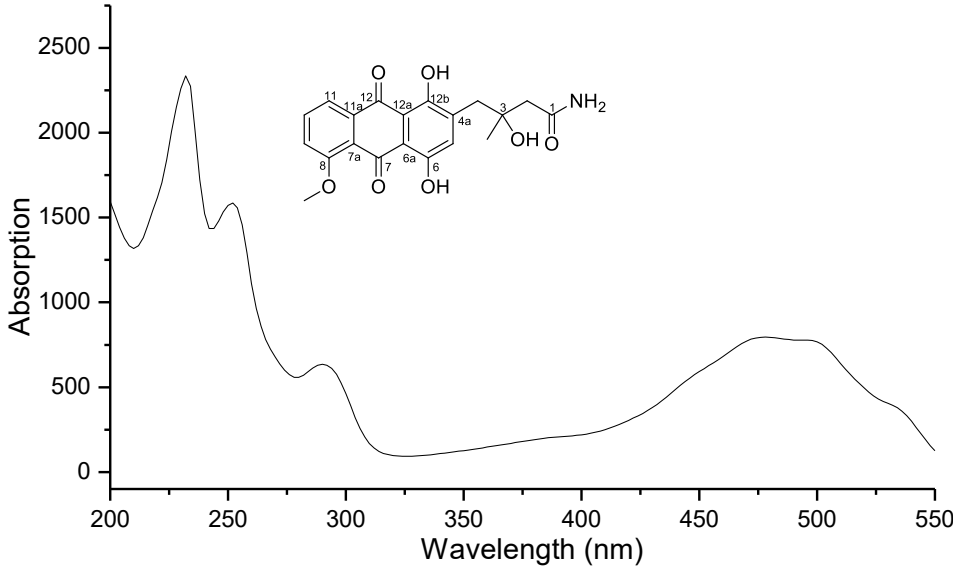
S67. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **24** in $CD_3OD$.



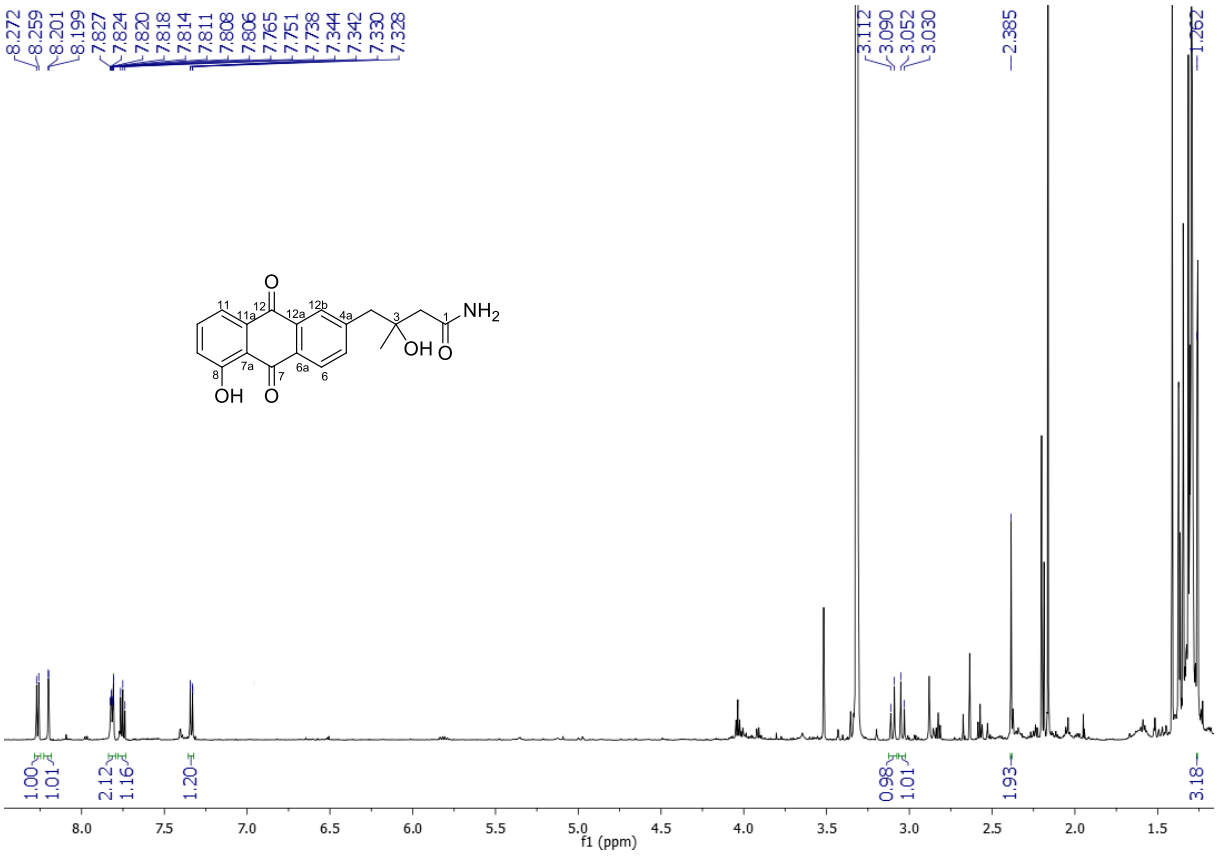
S68. HRESIMS spectrum of **24**.



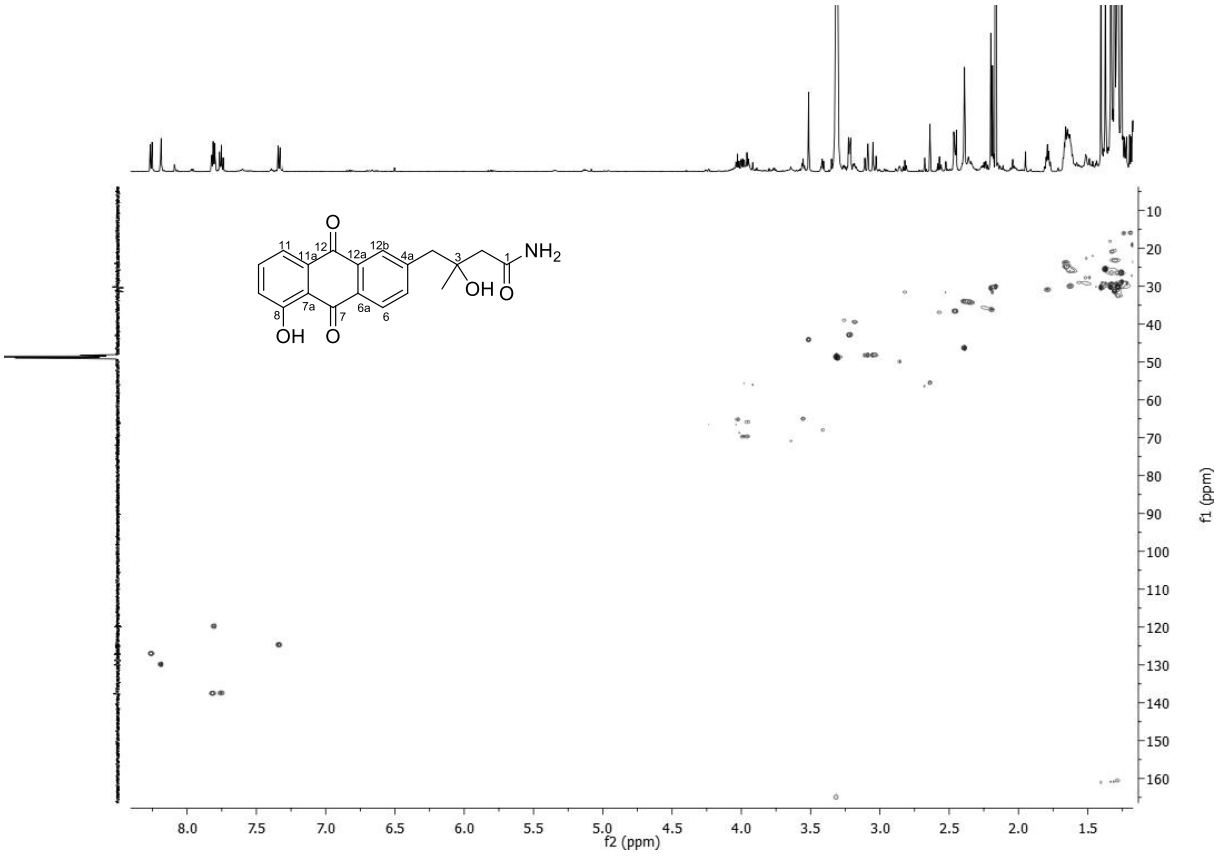
S69. UV spectrum of **24**.



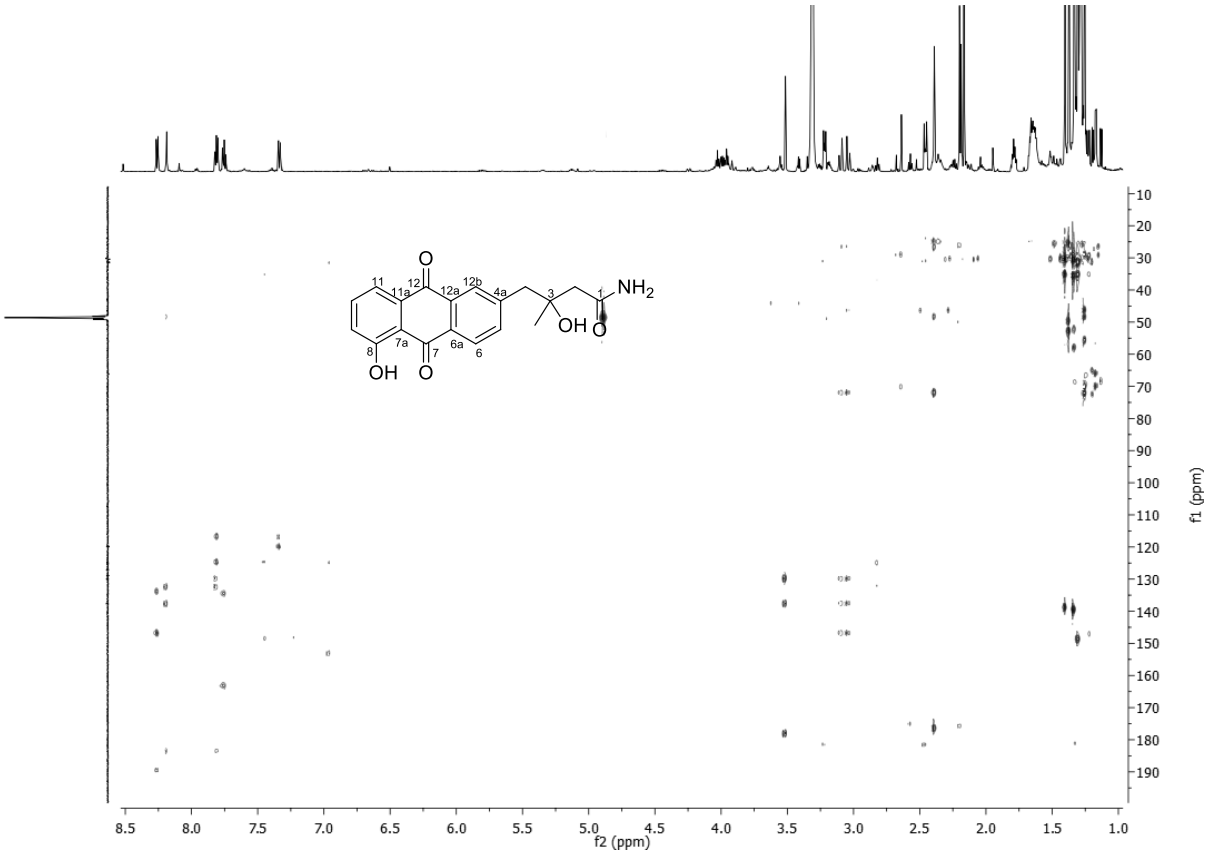
S70. $^{1}$H NMR spectrum (600 MHz) of **25** in $CD_3OD$.



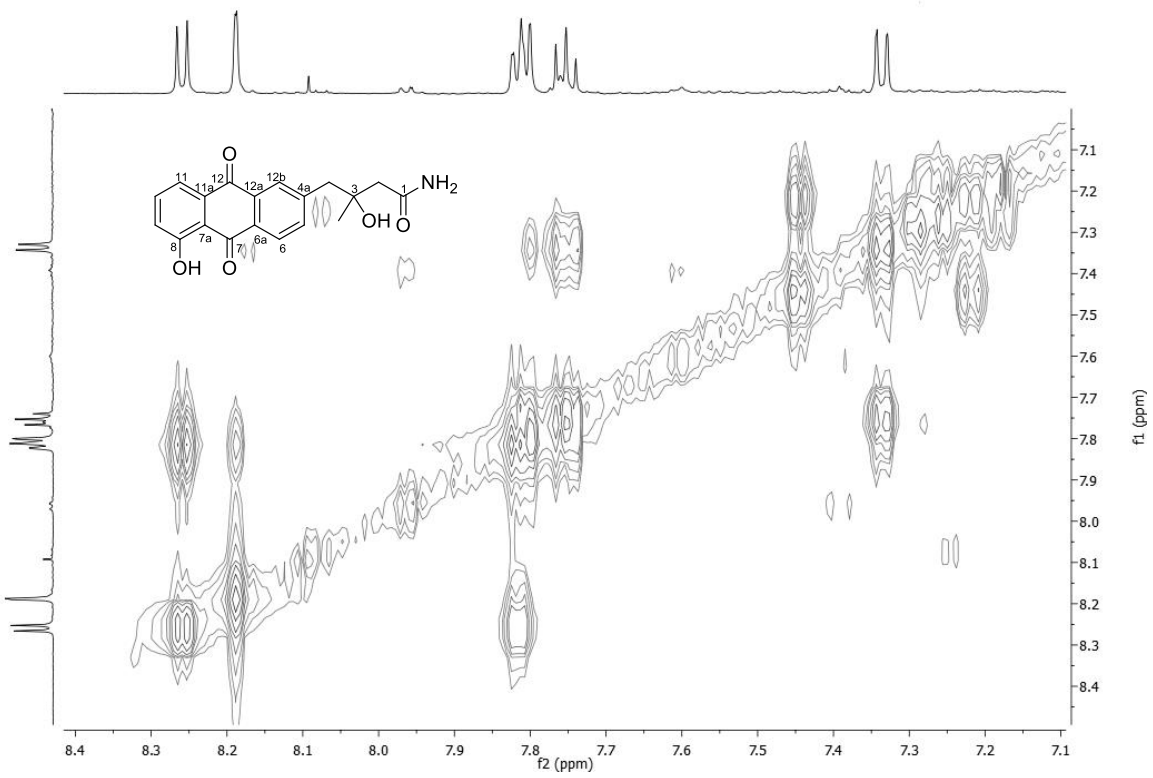
S71. HSQC spectrum (600 MHz) of **25** in $CD_3OD$.



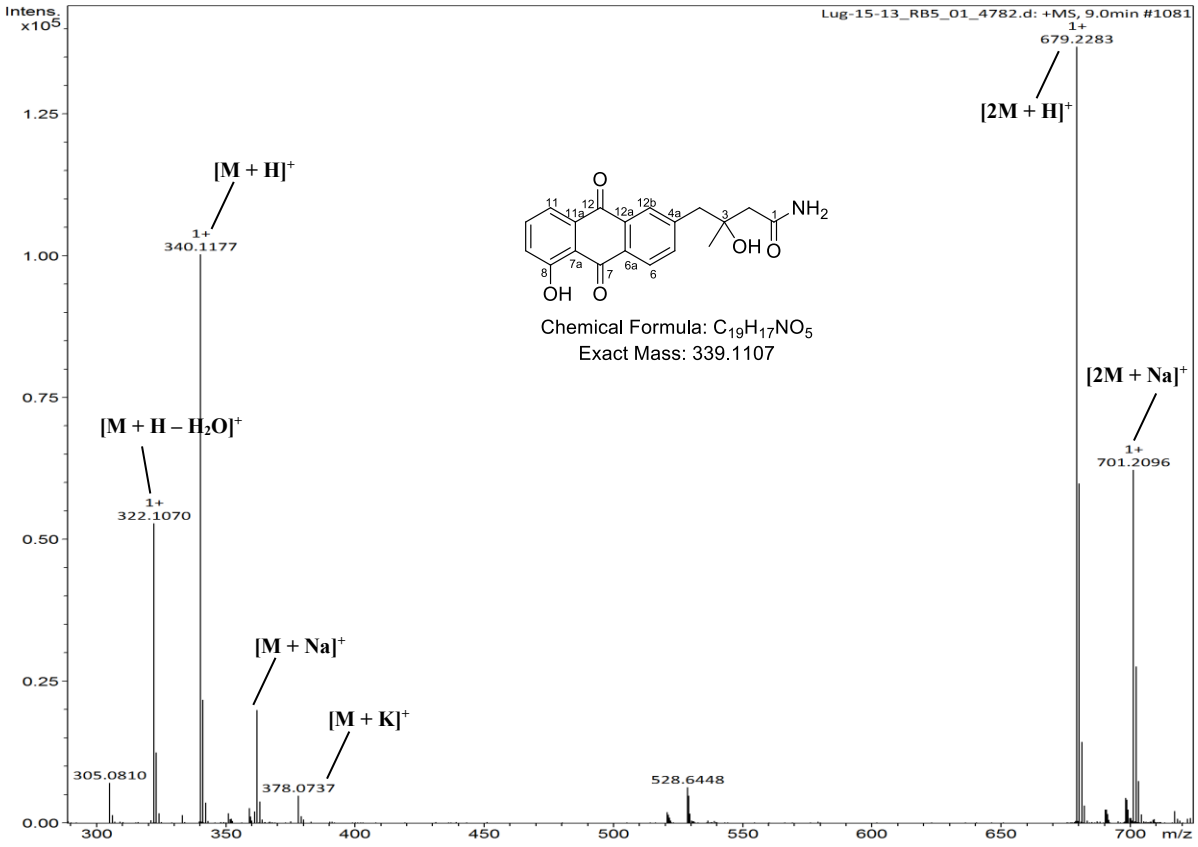
S72. HMBC spectrum (600 MHz) of **25** in $CD_3OD$.



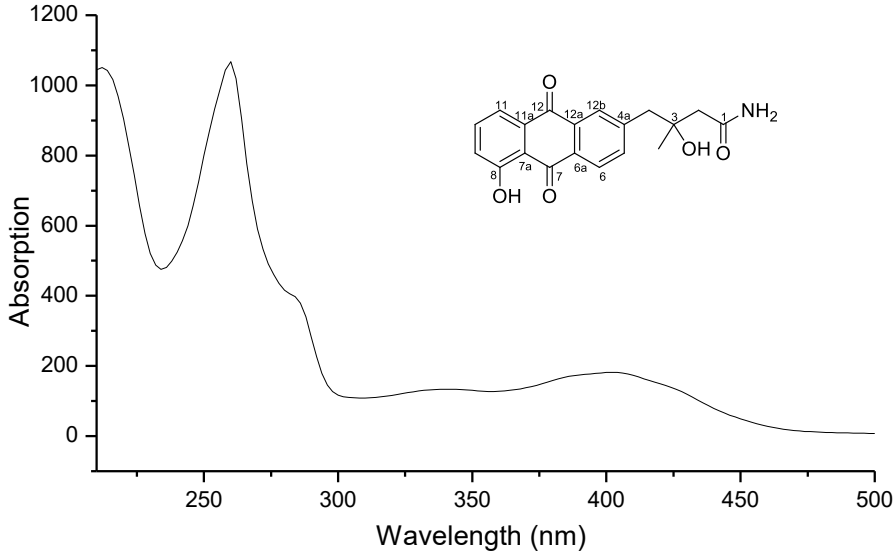
S73. $^{1}H$-$^{1}H$ COSY spectrum (600 MHz) of **25** in $CD_3OD$.



S74. HRESIMS spectrum of **25**.



S75. UV spectrum of **25**.



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