Supporting Information for:

Diaza-anthracene antibiotics from a freshwater-derived actinomycete with selective antibacterial activity toward *M. tuberculosis.*

Michael W. Mullowney,[†] Chang Hwa Hwang,[†,‡], Andrew G. Newsome,[†] Xiaomei Wei,[†] Urszula Tanouye,[†] Baojie Wan,[‡] Skylar Carlson,[†] Nanthida Joy Barranis,[†,⊥] Eoghainín Ó hAinmhire,[†,§] Wei-Lun Chen,[†] Kalyanaraman Krishnamoorthy,[∥] John White,[∥] Rachel Blair,[∇] Hyunwoo Lee,[⊥] Joanna Burdette,[†,§] Pradipsinh K. Rathod,[∥] Tanya Parish,[∇] Sanghyun Cho,[‡] Scott G. Franzblau,[†,‡] Brian T. Murphy[†,§,*]

[†]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street (MC 781), Room 539, Chicago, Illinois 60612-7231, United States
[‡]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street (MC 964), Room 412, Chicago, Illinois 60612-7231, United States
[§]Center for Pharmaceutical Biotechnology, College of Pharmacy, University of Illinois at Chicago, Molecular Biology Research Building, 900 South Ashland Avenue (MC 870), Room 3150, Chicago, IL 60607-7173, Illinois 60607-7173, United States
[⊥]Department of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street (MC 865), Room 335, Chicago, IL 60612-7231, United States
[∥]Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, United States
[∇]TB Discovery Research, Infectious Disease Research Institute, 1616 Eastlake Avenue East, Suite 400, Seattle, Washington 98102, United States

[*]E-mail: btmurphy@uic.edu

Table of Contents

Methods

General Experimental Procedures for the Isolation and Characterization of DAQH (1) and DAQJ (2).

UV spectra were measured on a Shimadzu Pharma Spec UV-1700 spectrophotometer. NMR spectra were obtained on a Bruker 600 MHz DRX NMR spectrometer equipped with an inverse 5 mm TXI cryogenic probe with z-axis pfg and XWINNMR version 3.5 operating software, and a 900 (226.2) MHz Bruker AVANCE NMR spectrometer equipped with an inverse 5 mm TCI cryogenic probe with z-axis pfg and TopSpin version 1.3 operating software at the University of Illinois at Chicago Center for Structural Biology. Chemical shifts (δ) are given in ppm and coupling constants (*J*) are reported in Hz. ^{1}H and ^{13}C NMR resonances of **1** and **2** are reported in Table S1. ^{1}H and ^{13}C NMR chemical shifts were referenced to the $CDCl_3$ with vapor TFA solvent signals (δ_H 7.26 ppm and δ_C 77.0 ppm, respectively). High resolution mass spectra (HRMS) were obtained on a Shimadzu IT-TOF LC-MS spectrometer at the University of Illinois at Chicago Research Resources Center. High-performance liquid chromatography (HPLC-UV) data were obtained using a Hewlett-Packard series 1100 system controller and pumps with a Model G1315A diode array detector (DAD) equipped with a reversed-phase C18 column (Phenomenex Luna, 100 × 4.6 mm, 5 μm) at a flow rate of 0.5 mL • min^{-1}. Semi-preparative scale HPLC separations were performed using a Hewlett Packard Series 1050 system with a Phenomenex Luna semi-preparative C18 column (250 × 10 mm, 5 μm) at a flow rate of 2.4 mL • min^{-1}. Preparative scale HPLC separations were performed using a Waters LC4000 System equipped with a Phenomenex Luna preparative C18 column (250 × 21.2 mm, 5 μm) at a flow rate of 16 mL • min^{-1}. Silica gel column chromatography was conducted using Bonna-Angela Technologies Cleanert® silica gel with an average particle size of 40-60 μm and an average pore size of 60 Å.

Fermentation and Extraction.

Strain B026 was grown in 28 × 1 L portions in Fernbach flasks containing high nutrient A1 medium (0.5 L of filtered Lake Michigan water, 0.5 L DI water, 10 g starch, 4 g yeast extract, 2 g peptone, 1 g calcium carbonate, 100 mg potassium bromide, and 40 mg iron sulfate) for 5 days at 21 °C while shaking at 220 rpm. Sterilized Amberlite XAD-16 resin (15 g·L−1) was added to each flask to absorb the extracellular secondary metabolites. The culture medium and resin were shaken for 8 h and filtered using cheesecloth to remove the resin. The resin, cell mass, and cheesecloth were extracted with acetone overnight, concentrated under vacuum, and partitioned between water and ethyl acetate. The organic layer was dried under vacuum to afford 1.1 g of extract.

Isolation and Characterization of DAQH (1) and DAQJ (2).

The organic layer from the liquid-liquid partition was fractionated using silica gel flash column chromatography (100 g of silica) eluting with an isocratic 95% chloroform ($CHCl_3$):5% methanol (MeOH) solvent system to afford 15 fractions. Using bioassay-guided fractionation, it was determined that fraction 2 contained the bioactive constituents, thus it was separated using RP-C18 preparative HPLC (16 mL • min^{-1}, gradient of 50% aqueous acetonitrile (ACN) to 100% ACN for 20 min, followed by an isocratic flow of 100% ACN for 10 min) to afford five fractions. Compounds **1** and **2** were observed in fractions 3 and 4, respectively.

To further isolate **1**, fraction 3 was separated using RP-C18 semi-preparative HPLC (2.4 mL • min^{-1}, gradient of 50% aqueous ACN to 100% ACN for 25 min, followed by an isocratic flow of 100% ACN for 15 min) to afford 4 fractions. Fraction 4 was determined to contain **1** and was further purified using RP-C18 semi-preparative HPLC (2.4 mL • min^{-1}, gradient of 50% aqueous ACN to 100% ACN for 25 min, followed by an isocratic flow of 100% ACN for 15 min) to afford diazaquinomycin H (**1**, t_R 18.6 min, 0.3 mg, 0.00026% yield).

To further isolate **2**, fraction 4 was separated further using RP-C18 semi-preparative HPLC (2.4 mL • min^{-1}, gradient of 50% aqueous ACN to 100% ACN for 25 min, followed by an isocratic flow of 100% ACN for 15 min) to afford 5 fractions. Fraction 3 was separated further using RP-C18 semi-preparative HPLC (2.4 mL • min^{-1}, gradient of 50% aqueous ACN to 100% ACN for 25 min, followed by an isocratic flow of 100% ACN for 15 min) to afford 5 fractions. Fraction 4 was determined to contain **2** and was further purified using RP-C18 semi-preparative HPLC (2.4 mL • min^{-1}, gradient of 50% aqueous ACN to 100% ACN for 25 min, followed by an isocratic flow of 100% ACN for 15 min) to afford diazaquinomycin J (**2**, t_R 22.0 min, 0.3 mg, 0.00026% yield).

***M. tuberculosis* Fermentation for Determination of Minimum Inhibitory Concentration (MIC).**

M. tuberculosis H_{37}Rv ATCC 27294 was purchased from American Type Culture Collection (ATCC) and cultured to late log phase in the 7H12 media, Middlebrook 7H9 broth supplemented with 0.2% (vol/vol) glycerol, 0.05% Tween80, and 10% (vol/vol) oleic acid-albumin-dextrose-catalase (OADC). The culture was harvested and resuspended in phosphate-buffered saline. Suspensions were then filtered through 8 μm filter membranes and frozen at 80 °C. Prior to use of bacterial stocks for the anti-TB assay, CFUs were determined by plating on 7H11 agar media. The MIC is defined here as the lowest concentration resulting in ≥90% growth inhibition of the bacteria relative to untreated controls. MIC against replicating *M. tuberculosis* was measured by the Microplate Alamar Blue Assay (MABA).[1-2]

Low Oxygen Recovery Assay (LORA).

The luciferase reporter gene luxABCDE-recombinant *M. tuberculosis* was prepared as previously reported.[3] The bacteria were adapted to low oxygen conditions during culture in a BioStatQ fermenter. The low oxygen-adapted culture was exposed to test samples in a 96-well microplate for 10 days at 37 °C in a hypoxic environment created with an Anoxomat (WS-8080, MART Microbiology). The cultures were then transferred to a normoxic environment at 37 °C for 28 hours. Viability was assessed by the measurement of luciferase-mediated luminescence. The LORA MIC was defined as the lowest concentration effecting a reduction of luminescence of ≥90% relative to untreated cultures.

Determination of Cytotoxicity.

Vero (ATCC CRL-1586) cells were cultivated in 10% fetal bovine serum (FBS) in Eagle minimum essential medium.[4-5] The culture was incubated at 37 °C under 5% CO_2 in air and then diluted with phosphate-buffered saline to 106 cells/mL. Two-fold serial dilutions of testing samples with a final volume of 200 µL cell culture suspension were prepared in a transparent 96-well plate (Falcon Microtest 96). After 72 h incubation at 37 °C, the medium was removed and monolayers were washed twice with 100 µL of warm Hanks balanced salt solution (HBSS). 100 µL of medium and 20 µL of MTS-PMS (Promega) were added to each well. Plates were then incubated for 3 hours and cytotoxicity was determined by the measurement of absorbance at 490 nm.

Human melanoma MDA-MB-435 cancer cells, human breast MDA-MB-231 cancer cells, human ovarian OVCAR3 cancer cells and human colon HT-29 cancer cells were purchased from the American Type Culture Collection (Manassas, VA). The cell line was propagated at 37 °C in 5% CO_2 in RPMI 1640 medium, supplemented with fetal bovine serum (10%), penicillin (100 units/ml), and streptomycin (100 µg/ml). Cells in log phase growth were harvested by trypsinization followed by two washings to remove all traces of enzyme. A total of 5,000 cells were seeded per well of a 96-well clear, flat-bottom plate (Microtest 96®, Falcon) and incubated overnight (37 °C in 5% CO_2). Samples dissolved in DMSO were then diluted and added to the appropriate wells (concentrations: 20 µg/mL, 4 µg/mL, 0.8 µg/mL, 0.16 µg/mL, 0.032 µg/mL; total volume: 100 µL; DMSO: 0.5%). The cells were incubated in the presence of test substance for 72 h at 37 °C and evaluated for viability with a commercial absorbance assay (CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) that measured viable cells. LC_{50} values are expressed in µg/mL relative to the solvent (DMSO) control.

Human ovarian OVCAR4 and Kuramochi cancer cells were maintained in RPMI 1640 (11875-093, Life-technologies) supplemented with 10% FBS (16000-044, Life-technologies) and 1% penicillin/streptomycin. Non-cancerous murine ovarian surface

epithelium (MOSE) and murine oviductal epithelium (MOE) cells were maintained as previously reported.[6] Concentration-response experiments were performed as previously described for 72 hours.[7]

MIC Determination against Drug-Resistant *M. tuberculosis* Isolates.

M. tuberculosis strains individually resistant to rifampin (RMP, ATCC 35838), isoniazid (INH, ATCC 35822), streptomycin (SM, ATCC 35820), cycloserine (CS, ATCC 35826) and kanamycin (KM, ATCC 35827) were obtained from the American Type Culture Collection (ATCC). Cultures were prepared and MICs against drug-resistant *M. tuberculosis* isolates were determined by the MABA as described above for *M. tuberculosis* H_{37}Rv.

MIC Determination against Non-Tuberculous Mycobacteria (NTM).

Mycobacterium smegmatis (mc^2155), *Mycobacterium abscessus* (ATCC19977), *Mycobacterium chelonae* (ATCC35752), *Mycobacterium avium* (ATCC15769), *Mycobacterium marinum* (ATCC927), *Mycobacterium kansasii* (ATCC12478), and *Mycobacterium bovis* BCG (ATCC35734) were obtained from the American Type Culture Collection (ATCC). Cultures were prepared and MICs against these mycobacteria were determined by the MABA as described above for *M. tuberculosis* H_{37}Rv. *M. abscessus* was incubated with 7H12 medium at 37 °C for 3 days and for an additional 4 hours after adding 12 μL of 20% Tween80 and 20 μL of Alamar blue dye. *M. bovis* was incubated with 7H12 medium at 37 °C for 7 days, and an additional 1 day of incubation after adding 12 μL of 20% Tween80 and 20 μL of Alamar blue dye. *M. chelonae* was incubated with 7H9 medium at 30 °C for 3 days, plus an additional 6 days of incubation after adding 12 μL of 20% Tween80 and 20 μL of Alamar blue dye. *M. marinum* was incubated with 7H9 medium at 30 °C for 5 days, and an additional 1 day of incubation after adding 12 μL of 20% Tween80 and 20 μL of Alamar blue dye. *M. avium* and *M. kansasii* were incubated with 7H9 media at 37 °C for 6 days, plus an additional day of incubation after adding 12 μL of 20% Tween80 and 20 μL of Alamar blue dye. Viability was assessed by measuring fluorescence at 530 nm excitation/590 nm emission with a Victor^3 multilabel reader (PerkinElmer).

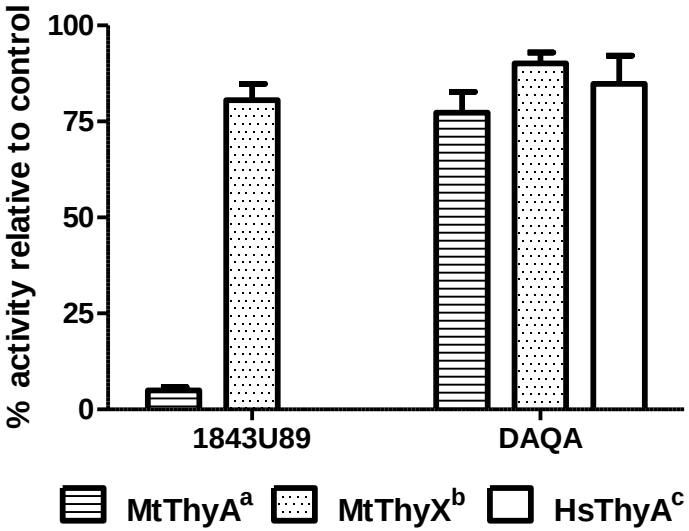
MIC Determination against Gram-Positive and Gram-Negative Bacteria.

The following Gram-positive and Gram-negative reference organisms were obtained from the ATCC: Methicillin-sensitive *Staphylococcus aureus* (ATCC29213), methicillin-resistant *Staphylococcus aureus* (ATCC33591), *Enterococcus faecalis*

(ATCC29212), *Enterococcus faecium* (ATCC19434), vancomycin-resistant *Enterococcus faecium* (ATCC51559), *Streptococcus pneumoniae* (ATCC49619), *Streptococcus pyogenes* Rosenbach (ATCCBAA-1633), *Bacillus cereus* (ATCC14579), *Bacillus cereus* (ATCC10987), *Bacillus thuringiensis* serovar konkukian str. 97-27, *Acinetobacter baumannii* (ATCC19606), *Escherichia coli* (ATCC25922), *Enterobacter cloacae* (ATCC13047), *Klebsiella pneumoniae* (ATCC13883), *Pseudomonas aeruginosa* (ATCC27853), and *Proteus mirabilis* (ATCC29906). *Bacillus subtilis* subsp. subtilis 168 is a laboratory strain and *Bacillus anthracis* Sterne was obtained from Philip C. Hanna at the University of Michigan, Ann Arbor. Positive controls piperacillin, ampicillin, vancomycin and gentamicin were obtained from Sigma-Aldrich, while daptomycin was obtained from Fisher Scientific; each was used to prepare a stock solution of at least 1280 μg/mL on the same day of each experiment. Inocula for MSSA, MRSA, *E. faecalis*, *E. faecium*, VRE, *A. baumannii*, *E. coli*, *E. cloacae*, *K. pneumoniae*, *P. aeruginosa*, and *P. mirabilis* consisted of 5 x 10^5 CFU/mL of each bacterial species with determination of MICs by broth microdilution in duplicate according to Clinical Laboratory Standards Institute (CLSI) guidelines with growth scored by visual observation of turbidity.[8] The MIC against *S. pneumoniae* (ATCC49619) was determined by broth microdilution method using a 4 x 10^5 CFU/mL inoculum as described in the National Committee on Clinical Laboratory Standards[9-10] with viability assessed by spectrophotometric readout at 490 nm using a Victor^3 multilabel reader (PerkinElmer). Inocula for *S. pyogenes* consisted of approximately 5 x 10^6 CFU/mL with determination of MICs by broth microdilution with growth scored by optical density (OD) at 625 nm using a Biotek Synergy 2 Microplate Reader. Each *Bacillus* inoculum consisted of 1 x 10^5 to 2 x 10^5 CFU/mL, with determination of MICs by broth microdilution and growth scored by visual observation of turbidity.

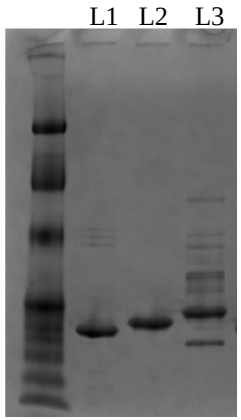
Studies toward Mechanism of Action of DAQA (3)

Figure S1. Activity of MtThyA, MtThyX, and HsThyA Enzymes in the Presence of 9 µM **3** and 9 µM 1843U89 (Positive Control).



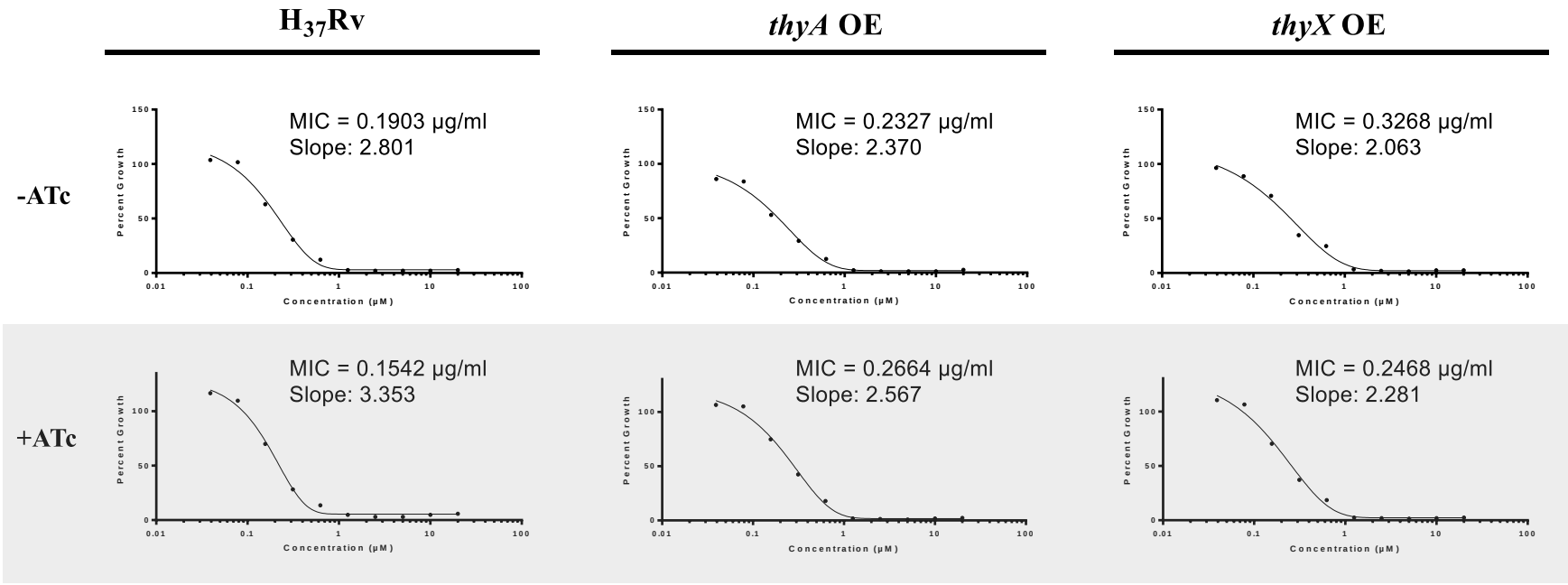
[a] *M. tuberculosis* thymidylate synthase A; [b] *M. tuberculosis* thymidylate synthase X; [c] human thymidylate synthase A

Figure S2. Purity of MtThyA, MtThyX, and HsThyA Enzymes.



Lane 1: MtThyA
Lane 2: MtThyX
Lane 3: HsThyA

Figure S3. Concentration-Response Curves of **3** against *M. tuberculosis* Anhydrotetracycline-Inducible *thyA* and *thyX* Overexpression Mutants.



Structure Elucidation of DAQH (1) and DAQJ (2).

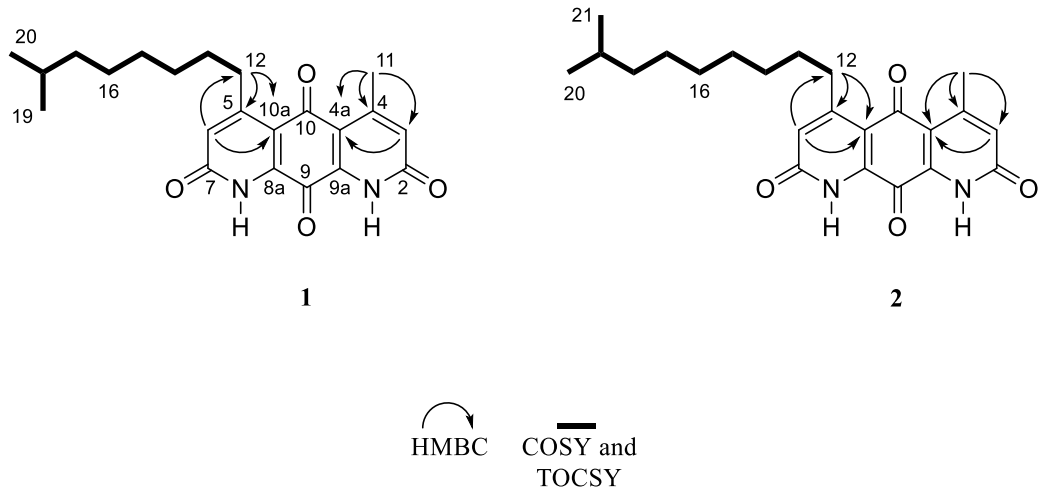
Following a series of chromatographic steps, **1** was obtained as red powder. Combined HRMS and NMR experiments allowed for the assignment of the molecular formula as $C_{22}H_{27}N_2O_4$. The UV absorption profile of **1** displayed characteristics of the diazaquinomycins as previously reported.[7] Combined NMR and high resolution IT-TOF MS experiments of **1** established the molecular formula as $C_{22}H_{27}N_2O_4$, which was indicative of eleven degrees of unsaturation. Analysis revealed that **1** lacked the symmetry of the previously reported DAQA (**3**) or DAQC.[11-13] A resonance at δ_H 7.00 (2H, H-3, H-6) observed in the ^{1}H NMR spectrum, determined by integration to represent two hydrogens, indicated a less substituted anthraquinone compound when compared to known DAQs. A triplet signal at δ_H 3.13 (2H, H-12) indicated one β-substituted methylene group and a singlet at δ_H 2.77 (3H, H-11) revealed one aromate-bound, β-substituted methyl group. A doublet with an integration of six at δ_H 0.87 (6H, H-19, H-20) and a methine multiplet resonance at δ_H 1.53 (1H, H-18) provided evidence of the isononyl group. Multiplet signals at δ_H 1.60 (2H, H-13), δ_H 1.47 (2H, H-14), δ_H 1.35 (2H, H-15), δ_H 1.31 (2H, H-16) and δ_H 1.19 (2H, H-17) were indicative of the methylene groups that constituted the remainder of the aliphatic side chain.

Analysis of ^{13}C NMR data suggested the presence of two quinone carbonyls (δ_C 180.1, C-10; 172.9, C-9), two near overlapping lactam carbonyls (δ_C 163.2 and 163.0; C-2 and C-7), two methine alkene carbons (δ_C 127.6, C-6; 128.6, C-3), six quaternary carbons, two of which were interchangeable (δ_C 160.1, C-5; 155.6, C-4; 136.3 and 136.8, C-8a and C-9a; 118.0, C-4a; 117.5, C-10a), six methylene carbons, three of which were overlapping (δ_C 39.1, C-17; 35.0, C-12; 29.7, C-15; 29.7, C-14; 29.7, C-13; 27.4, C-16), and three methyl carbons (δ_C 23.1, C-11; 22.8, C-19 and C-20) (Table S1). Given that the molecular formula afforded 11 degrees of unsaturation and the molecule contained 4 carbonyls, 6 quaternary alkene carbons, and 2 methine alkene carbons, the remaining degrees were satisfied by the fused ring system. Key HMBC, COSY, and 1D-TOCSY correlations are given in Figure S4. Interpretation of COSY and 1D-TOCSY data defined one distinct aliphatic spin system (H_2-12 to H_3-20). Connectivity of the aliphatic side chain and the methyl group to opposing β-substituted positions of the core ring system was established using HMBC correlations (Figures S4 and S8).

HMBC correlations between H_3-11 and C-3, C-4 and C-4a, as well as correlations between the H_2-12 and C-5, C-6, C-10a gave evidence for the connectivity of the alkyl groups to opposing sides of the anthracene core skeleton of **1**. Two lactam carbonyl resonances, C-2 and C-7, were observed in the ^{13}C DEPTQ spectrum; due to overlap it was not possible to distinguish between them in an HMBC experiment (Table S1). Similarly, two quaternary carbon resonances, C-8a and C-9a, were observed in the ^{13}C DEPTQ spectrum but were indistinguishable by an HMBC experiment.

Following a series of chromatographic steps, **2** was obtained as red powder. Structure elucidation of **2** was executed in the same fashion as **1**, using a similar series of 1D-TOCSY experiments to identify the 14 Da increase in molecular weight as an additional methylene in the aliphatic side chain (Figure S16).

Figure S4. Key 2D NMR Correlations of **1** and **2**.



1 **2**

HMBC COSY and TOCSY

Table S1. ^{1}H and ^{13}C NMR Chemical Shift Data ($CDCl_3$–1% CF_3CO_2D) of **1** and **2**.

Position	**1**		**2**	
	^{13}C [a]	^{1}H mult. (J, Hz) [b]	^{13}C [a]	^{1}H mult. (J, Hz) [b]
1		8.05 s		8.03 s
2	163.2 [d]		161.0	
3	128.6	7.00 s	128.9 [c]	6.93 s
4	155.6		154.6	
4a	118.0		118.3	
5	160.1		160.2 [c]	
6	127.6	7.00 s	128.0 [c]	6.93 s
7	163.0 [d]		160.9 [c]	
8		8.05 s		8.03 s
8a	136.3 [e]		136.2	
9	172.9		173.0	
9a	136.8 [e]		136.8	
10	180.1		180.0	
10a	117.5		117.8	
11	23.1	2.77 s	23.0	2.74 s
12	35.0	3.13 t (7.7)	35.0	3.10 t (7.0)
13	29.7 [f]	1.60 p (7.5)	29.9	1.59 m
14	29.7 [f]	1.47 p (7.4)	29.8	1.45 m
15	29.7 [f]	1.35 m	29.7	1.35 m
16	27.4	1.31 m	29.6	1.31 m
17	39.1	1.19 q (6.9)	27.5	1.28 m
18	28.1	1.53 m	39.1	1.17 m
19	22.8	0.87 d (6.6)	28.1	1.52 m
20	22.8	0.87 d (6.6)	22.8	0.86 d (6.6)
21			22.8	0.86 d (6.6)

[a] 226.2 MHz; [b] 900 MHz; [c] shifts obtained through HMBC and/or HSQC NMR experiments; [d, e, f] shifts indistinguishable by HMBC and HSQC NMR experiments.
s = singlet; t = triplet; q = quartet; p = pentet; m = multiplet.

Figure S5. ^{1}H NMR Spectrum (900 MHz) of **1** in $CDCl_3$–1% CF_3CO_2D.

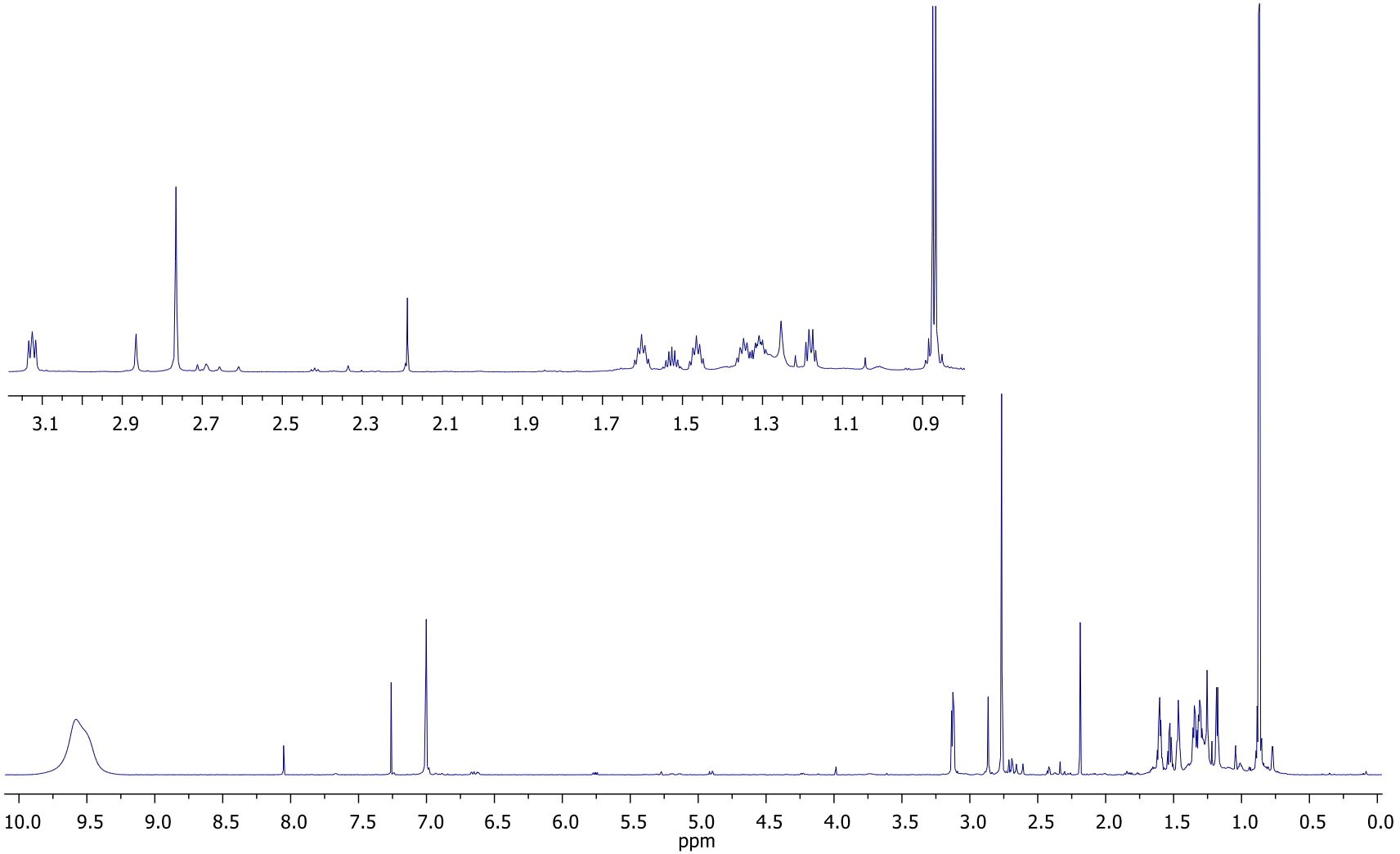


Figure S6. ^{13}C DEPTQ Spectrum (226.2 MHz) of **1** in $CDCl_3$–1% CF_3CO_2D.

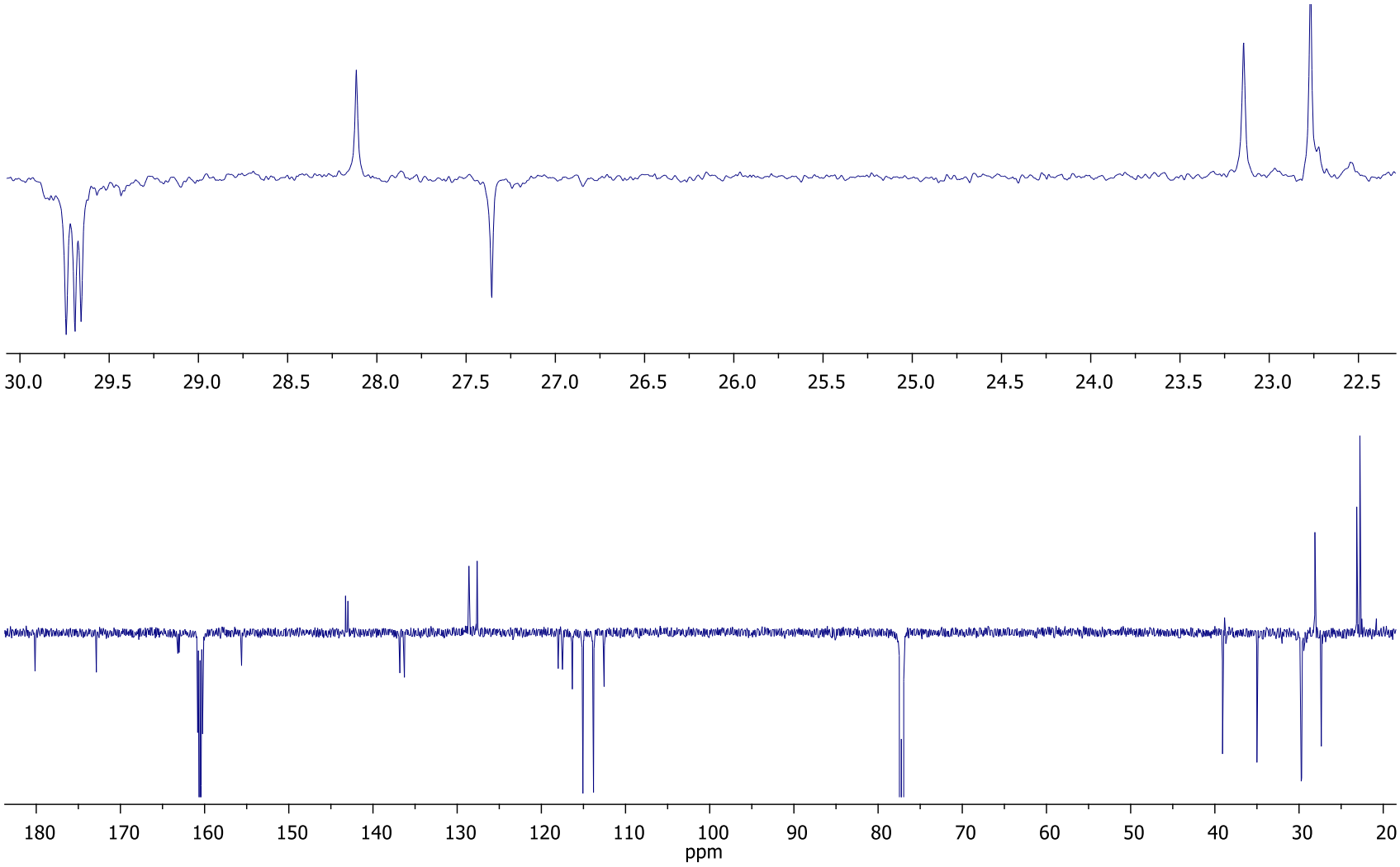


Figure S7. COSY Spectrum (600 MHz) of **1** in $CDCl_3$–1% CF_3CO_2D.

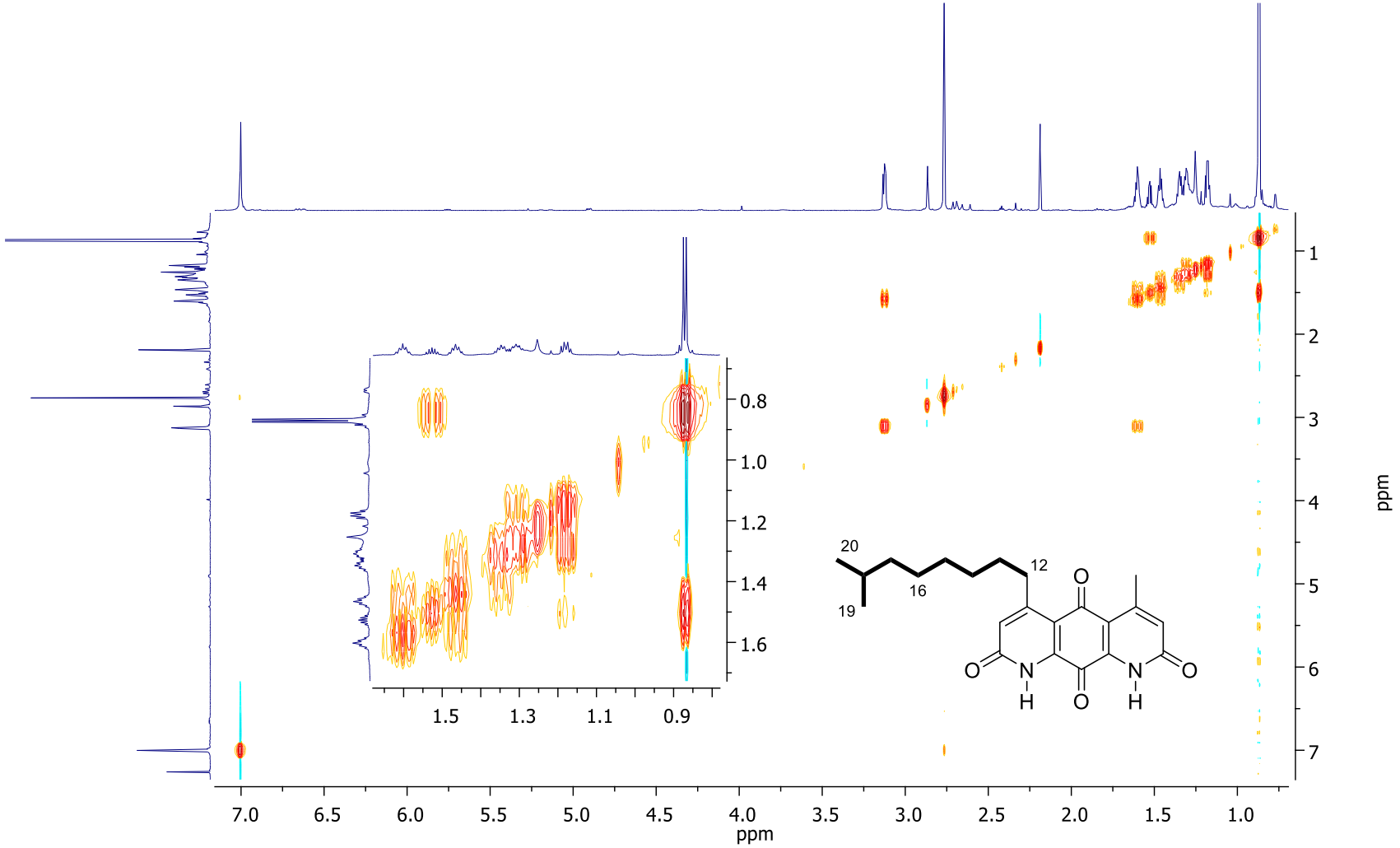


Figure S8. HMBC spectrum (600 MHz) of **1** in $CDCl_3$–1% CF_3CO_2D.

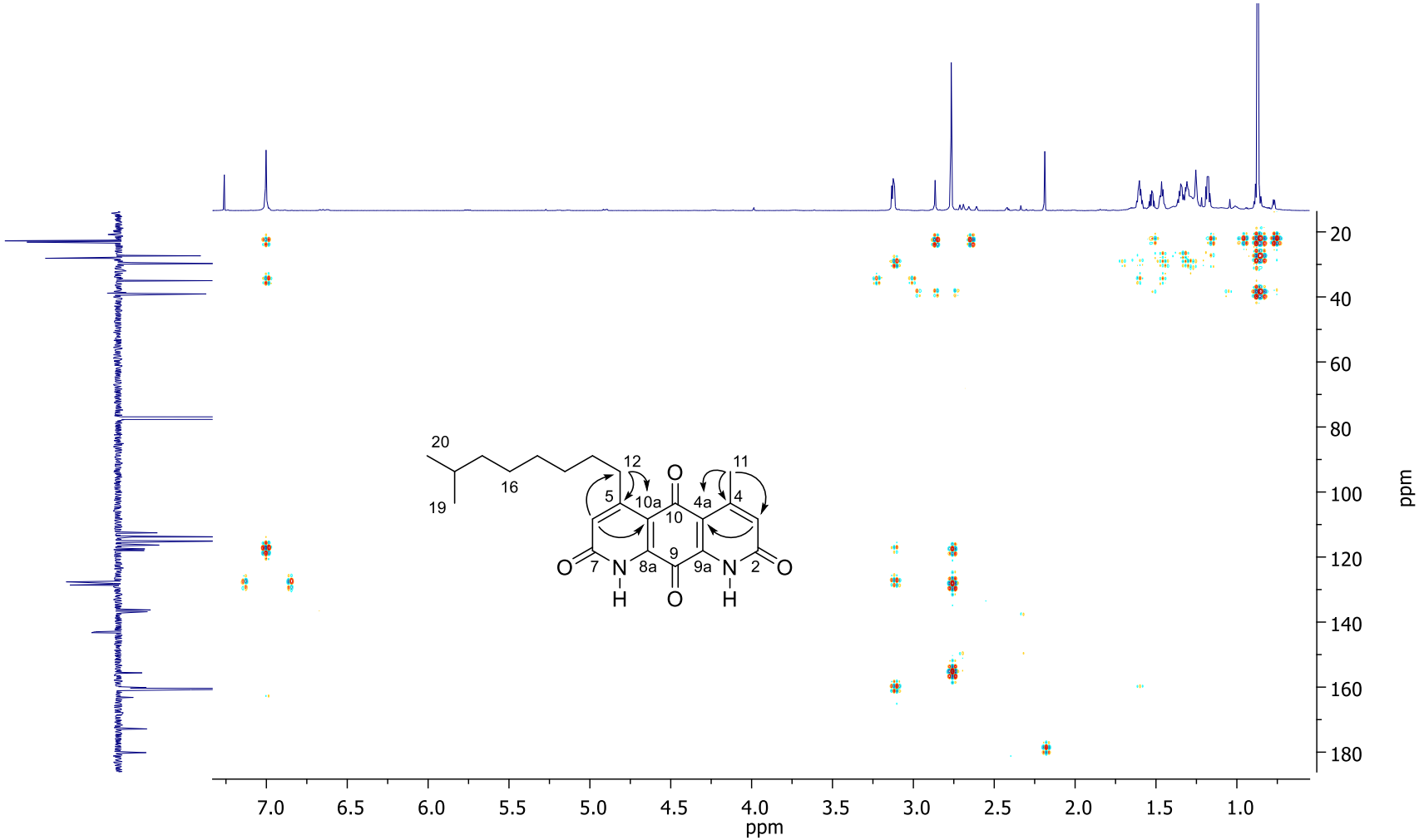
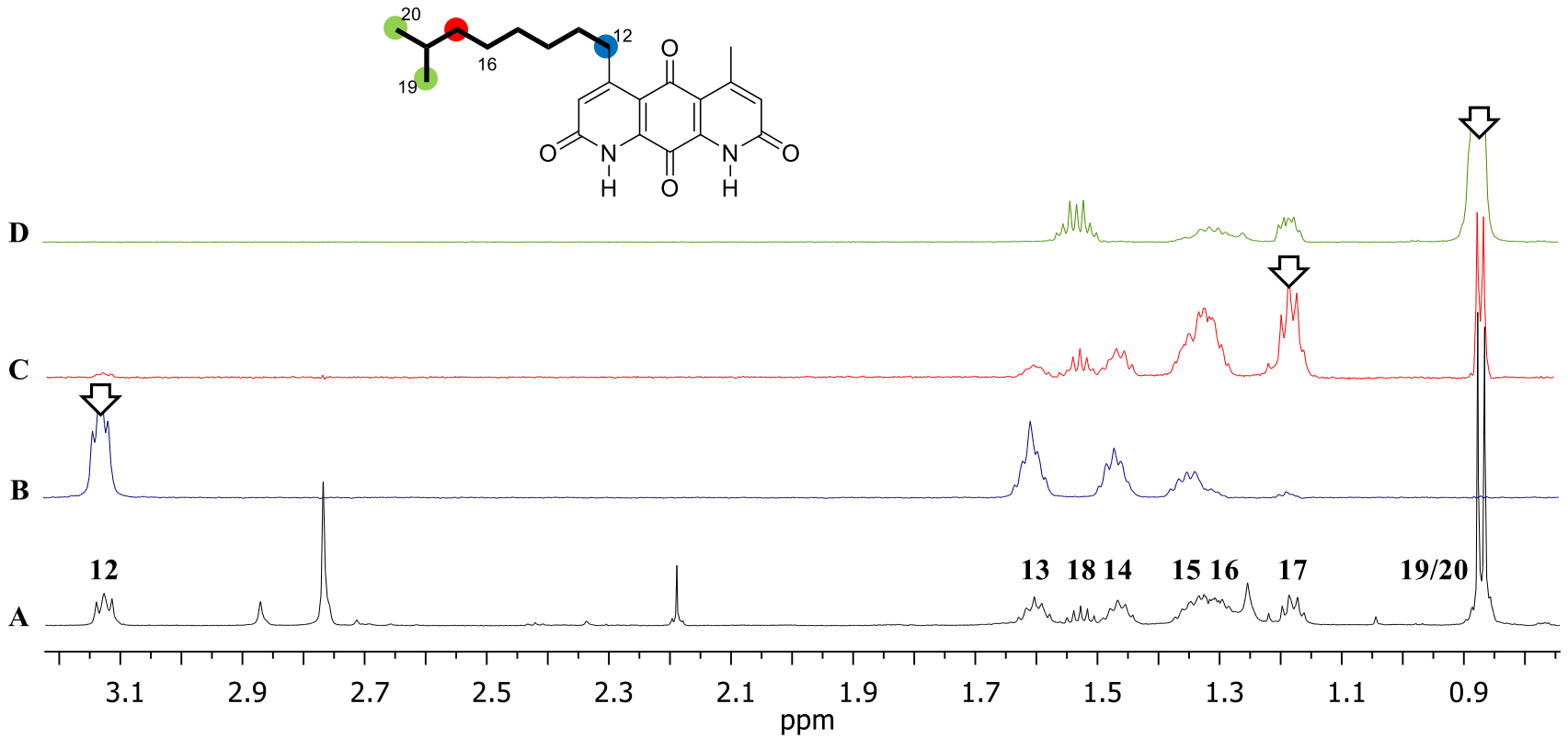
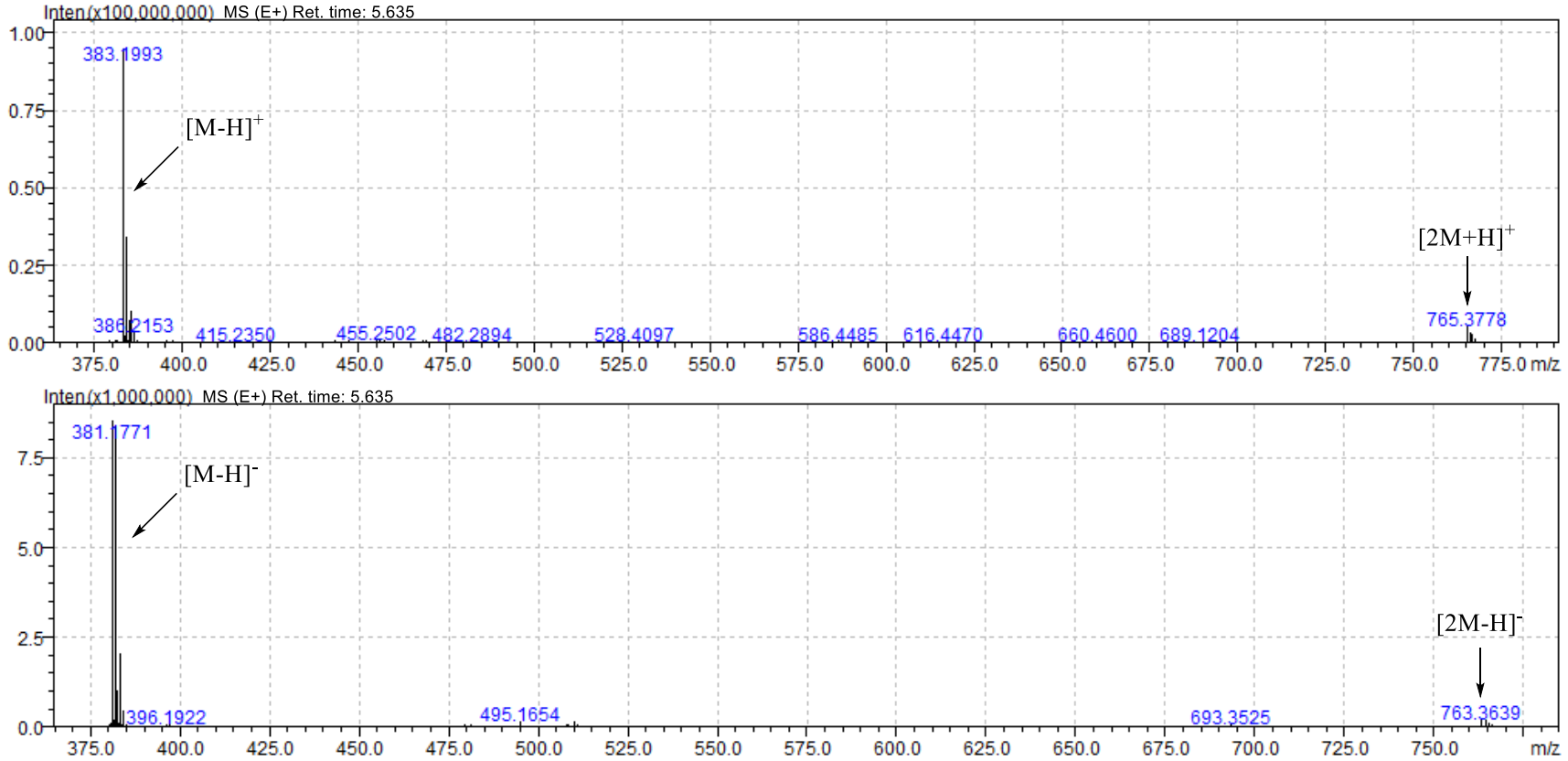


Figure S9. 1D-TOCSY Spectra (600 MHz) of **1** in $CDCl_3$–1% CF_3CO_2D.



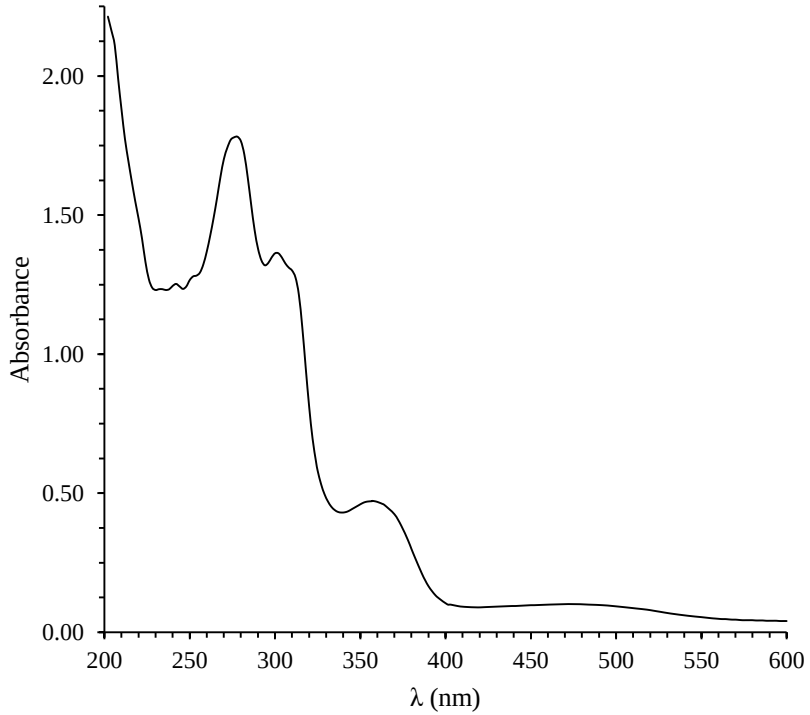
Arrows indicate irradiated resonances. (A) Expansion of ^{1}H NMR spectrum (600 MHz) of **1**; (B) Expansion of 1D TOCSY spectrum of **1** (irradiation of δ_H 3.13); (C) Expansion of 1D TOCSY spectrum of **1** (irradiation of δ_H 1.19); (D) Expansion of 1D TOCSY spectrum of **1** (irradiation of δ_H 0.87).

Figure S10. Expanded HR-ESI-ITTOF Mass Spectrum of **1**.



HRESI-ITTOF MS *m/z* 383.1993 $[M + H]^+$ (calcd. for $C_{22}H_{27}N_2O_4$: 383.1971), *m/z* 381.1771 $[M - H]^-$ (calcd. for $C_{22}H_{25}N_2O_4$: 381.1820), *m/z* 765.3778 $[2M + H]^+$ (calcd. for $C_{44}H_{53}N_4O_8$: 765.3863), and *m/z* 763.3639 $[2M - H]^-$ (calcd. for $C_{44}H_{51}N_4O_8$: 763.3712).

Figure S11. UV Spectrum of **1** in Methanol.



UV (MeOH) λ_{max} (log ε) = 278 (3.83), 301 (3.72), 357 (3.26) and a broad absorption with maximum at 472 (2.59) nm.

Figure S12. ^{1}H NMR Spectrum (900 MHz) of **2** in $CDCl_3$–1% CF_3CO_2D.

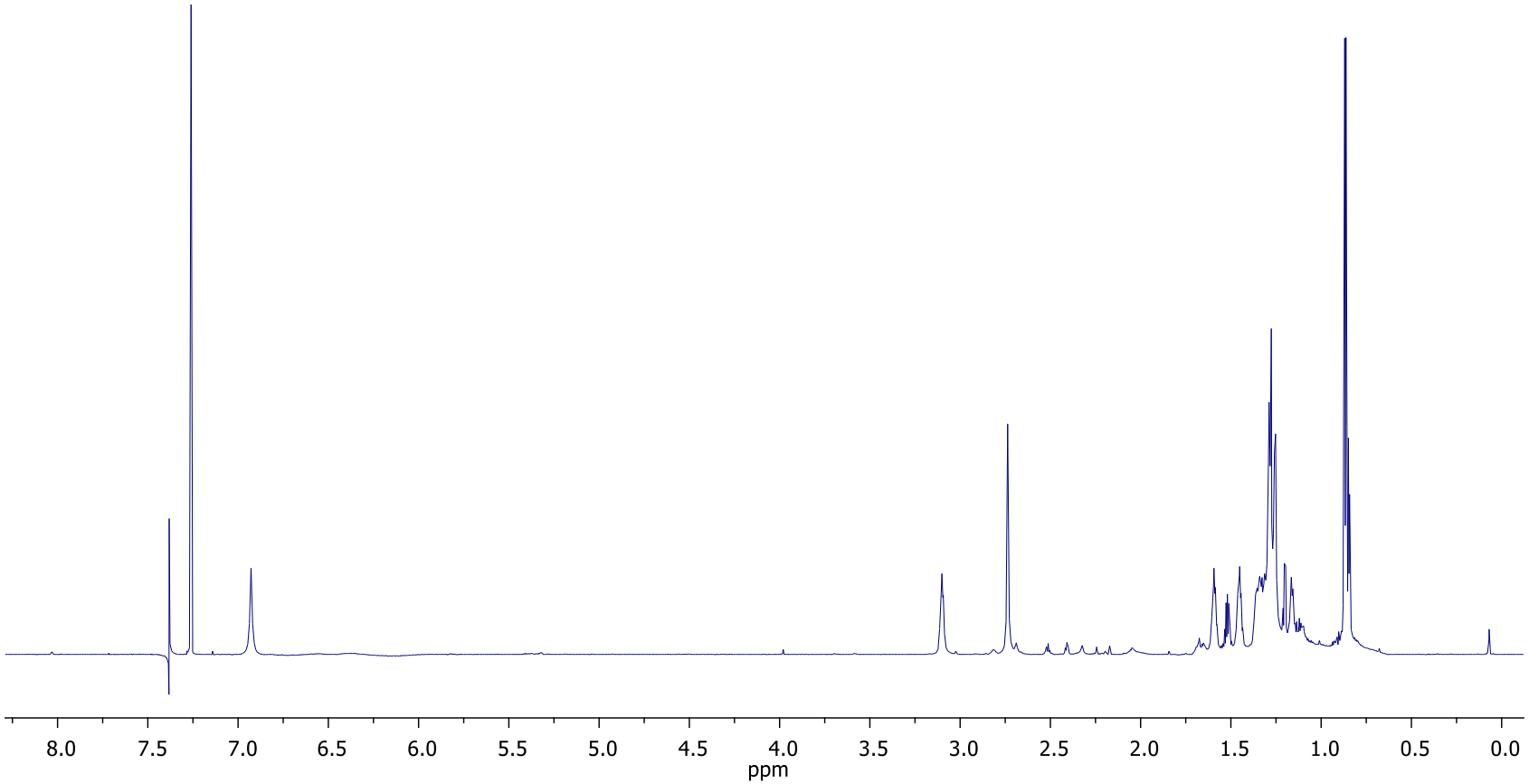


Figure S13. ^{13}C DEPTQ Spectrum (226.2 MHz) of **2** in $CDCl_3$–1% CF_3CO_2D.

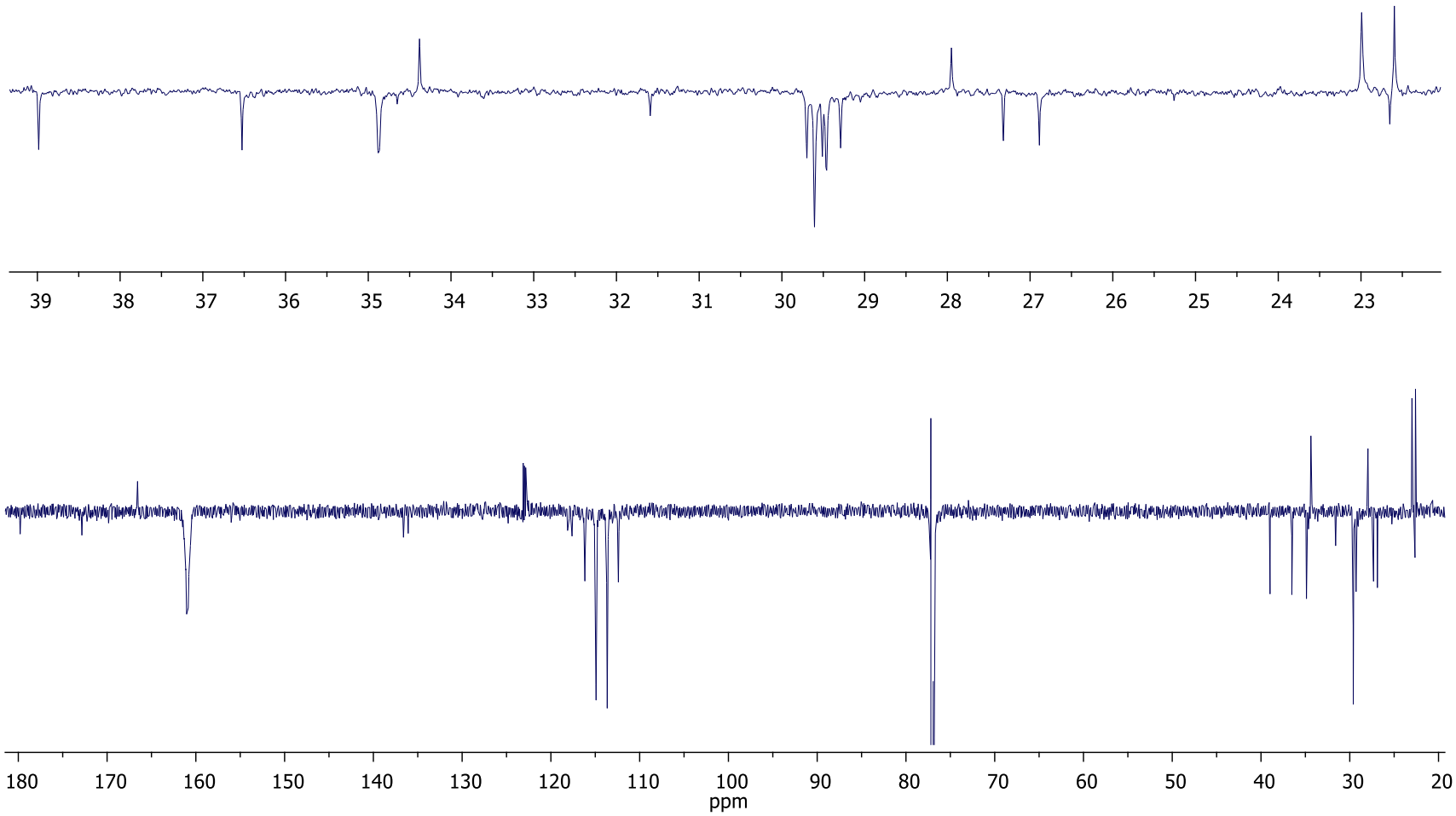


Figure S14. COSY Spectrum (600 MHz) of **2** in $CDCl_3$–1% CF_3CO_2D.

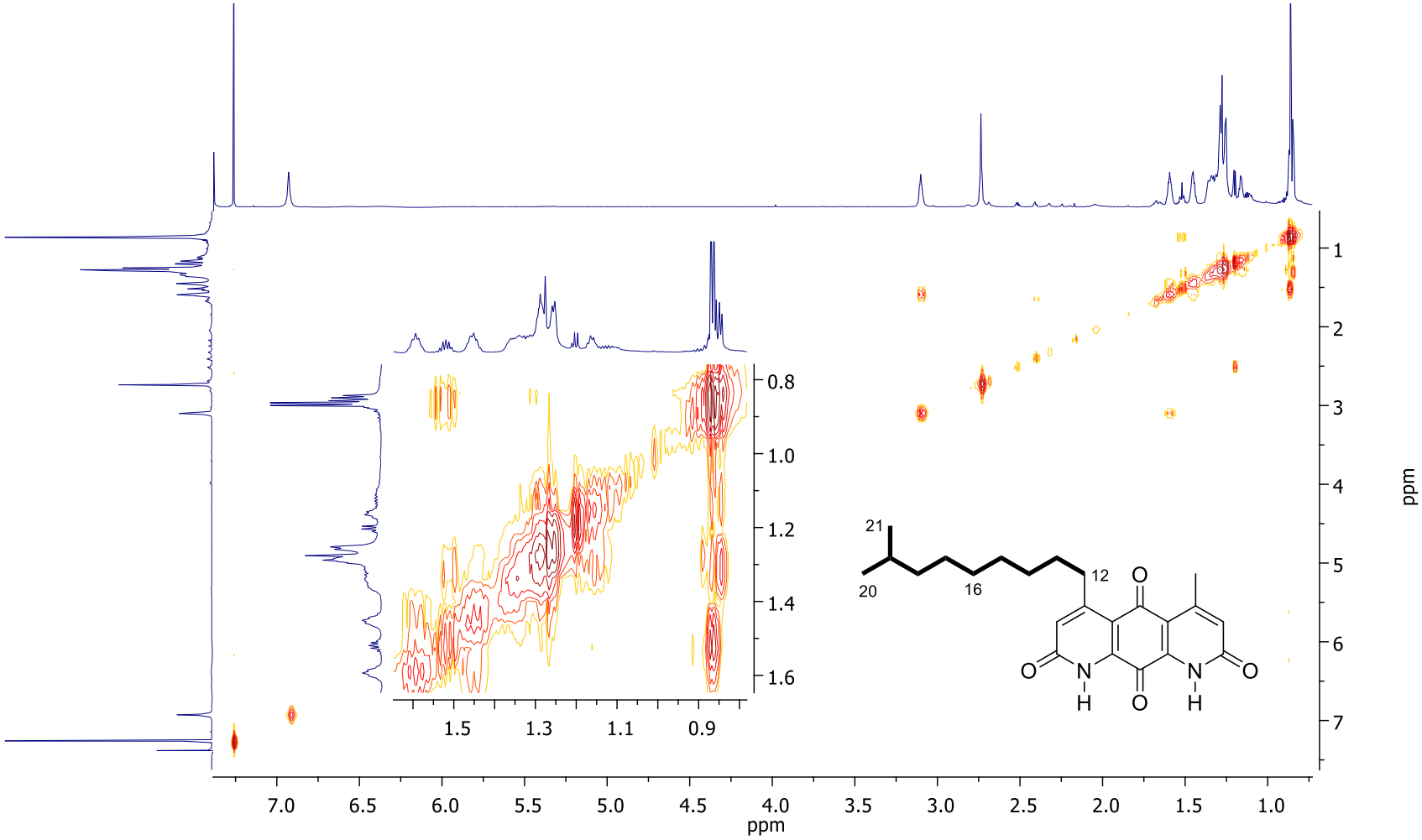


Figure S15. HMBC Spectrum (600 MHz) of **2** $CDCl_3$–1% CF_3CO_2D.

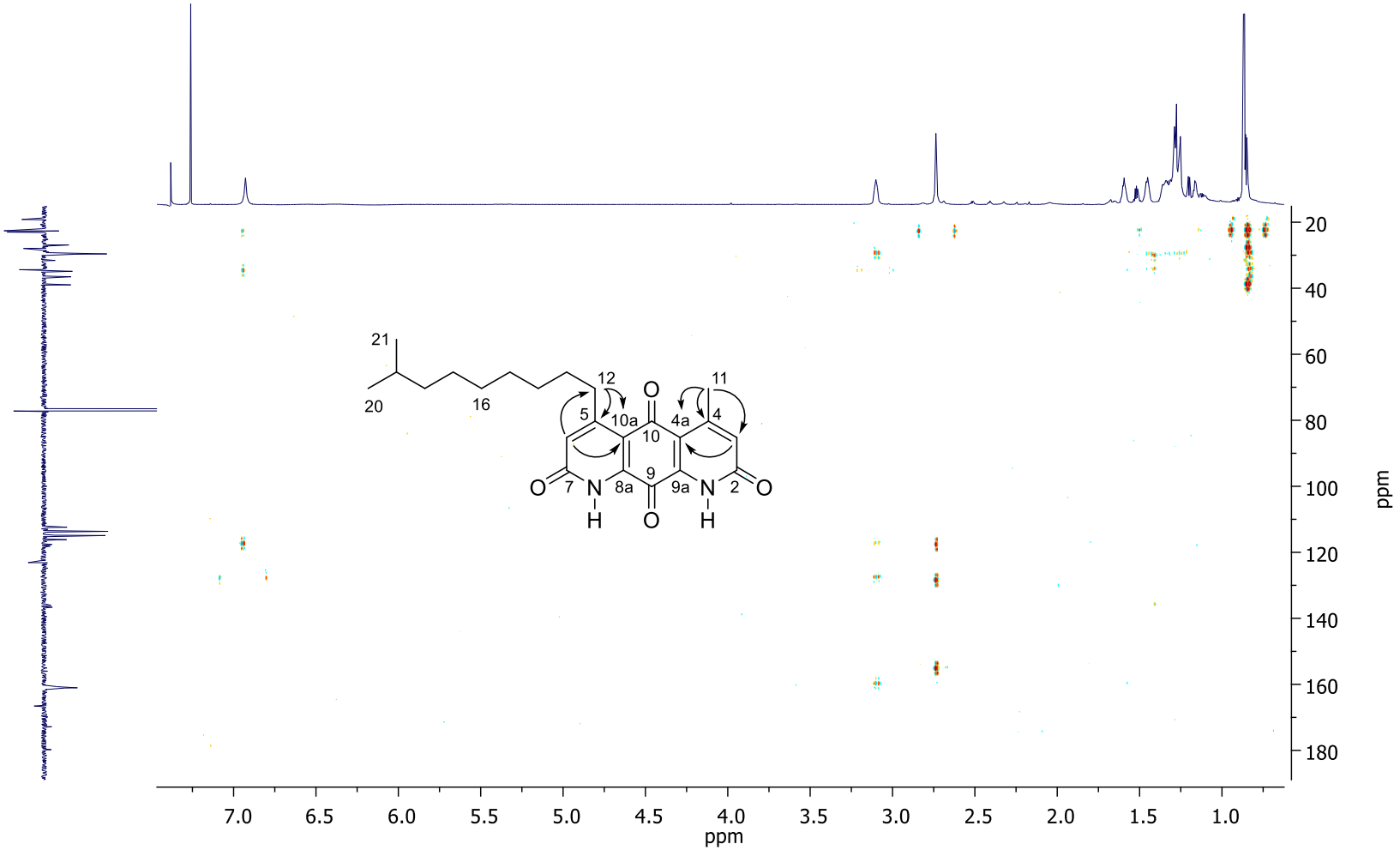
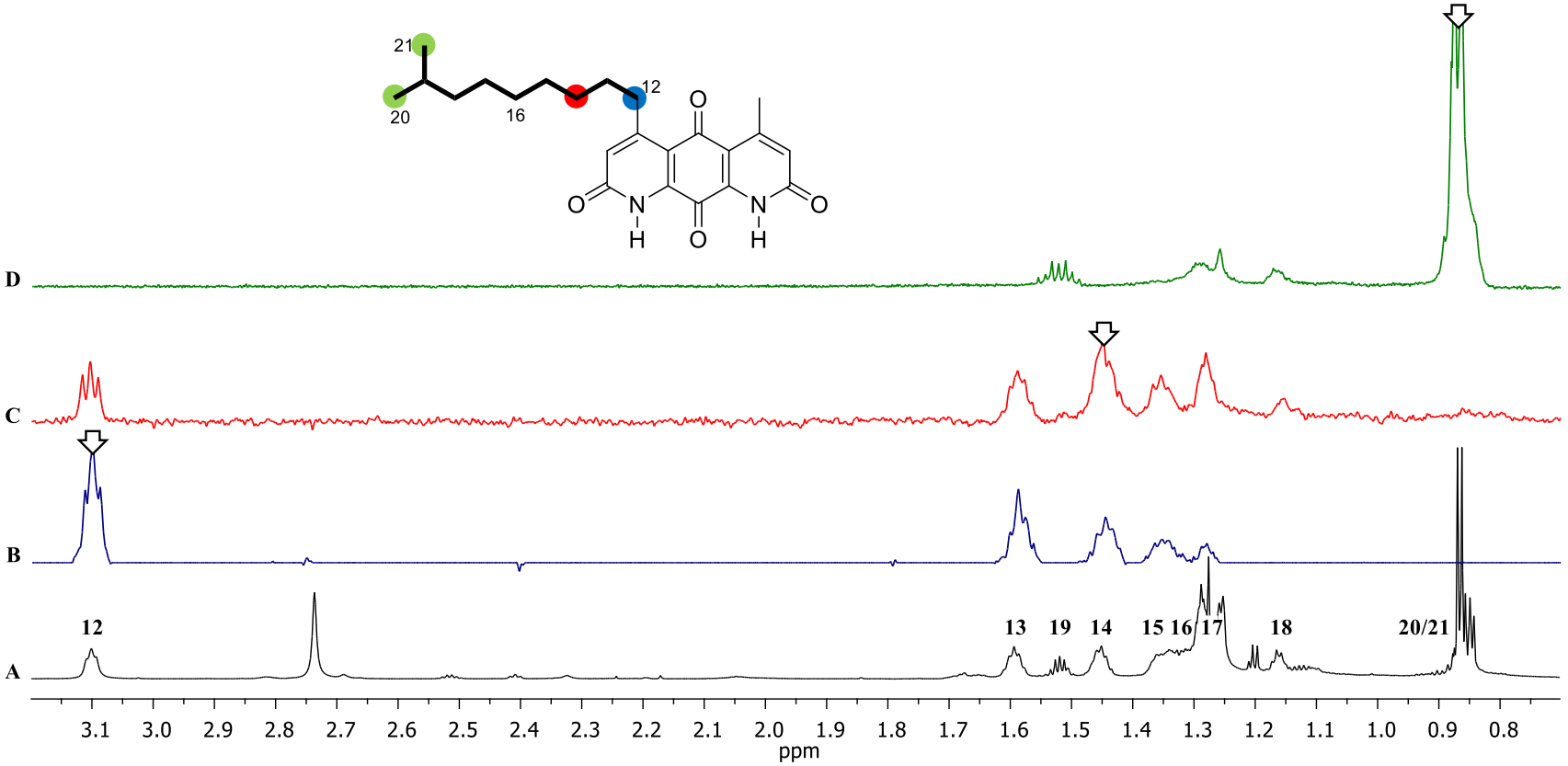
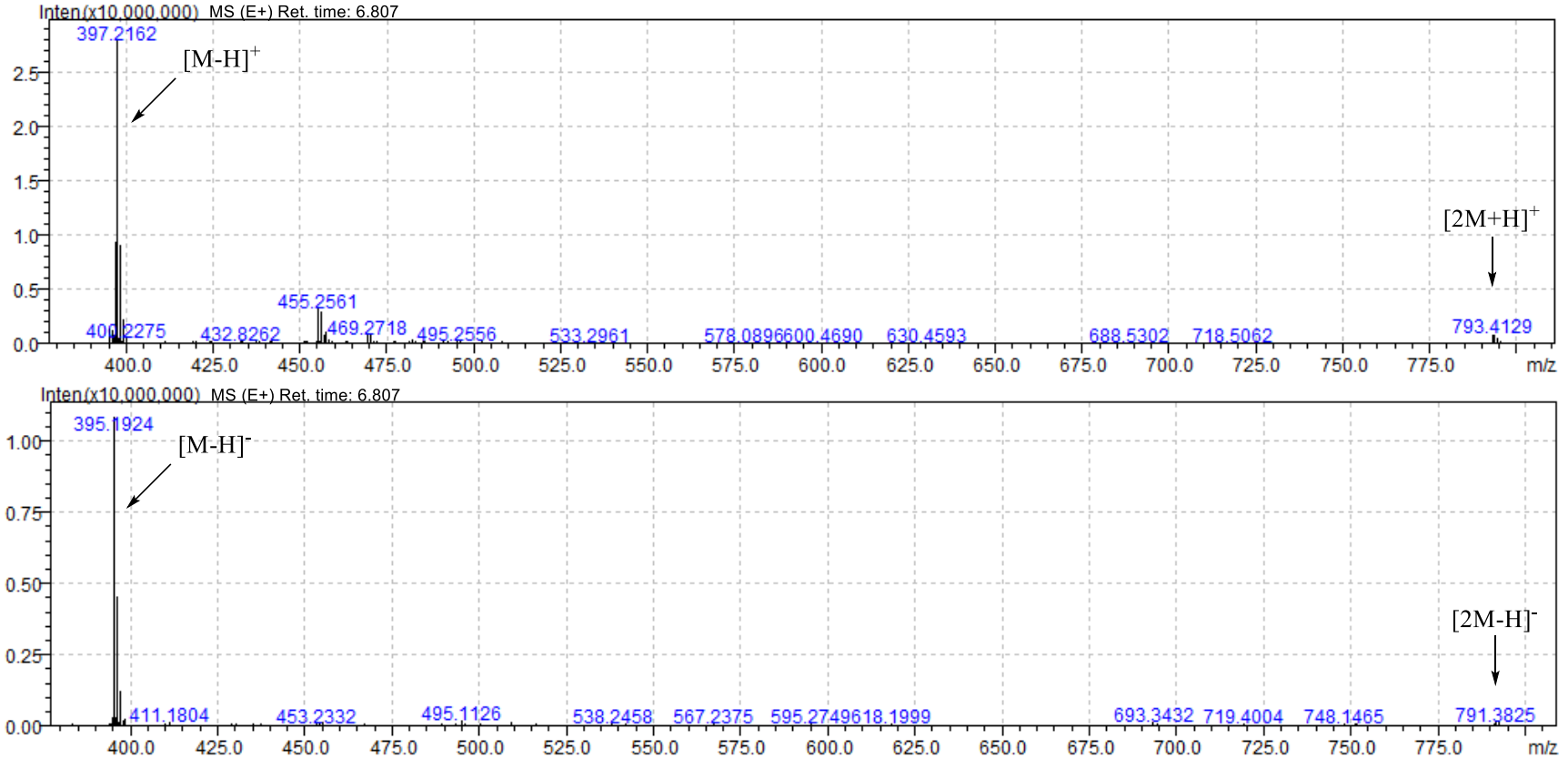


Figure S16. 1D-TOCSY Spectra (600 MHz) of **2** in $CDCl_3$–1% CF_3CO_2D.



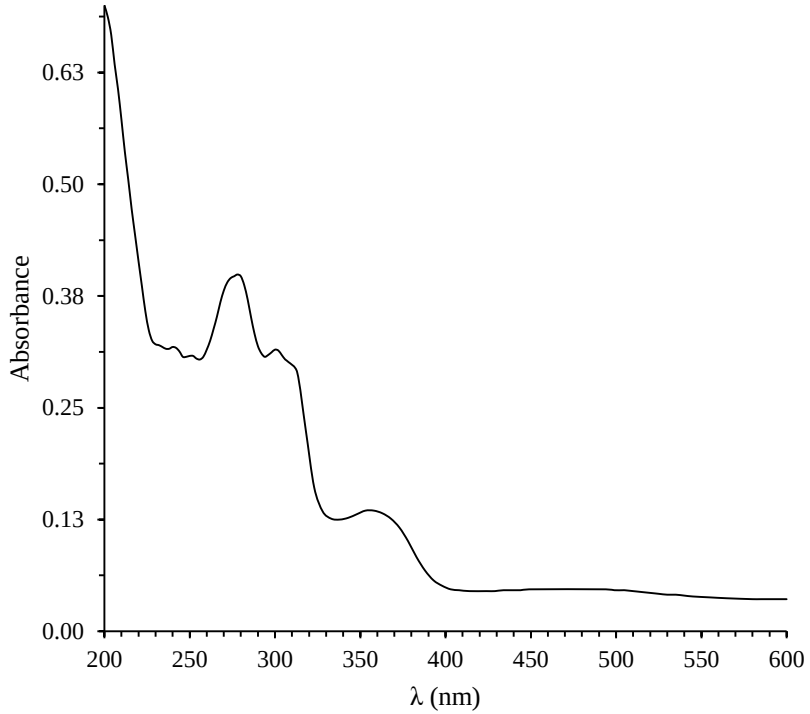
Arrows indicate irradiated resonances. (A) Expansion of ^{1}H NMR spectrum (600 MHz) of **2**; (B) Expansion of 1D TOCSY spectrum of **2** (irradiation of δ_H 3.10); (C) Expansion of 1D TOCSY spectrum of **2** (irradiation of δ_H 1.45); (D) Expansion of 1D TOCSY spectrum of **2** (irradiation of δ_H 0.86).

Figure S17. Expanded HR-ESI-ITTOF Mass Spectrum of **2**.



HRESI-ITTOF MS *m/z* 397.2162 $[M + H]^+$ (calcd. for $C_{23}H_{29}N_2O_4$: 397.2127), *m/z* 395.1924 $[M - H]^-$ (calcd. for $C_{23}H_{27}N_2O_4$: 395.1976), *m/z* 793.4129 $[2M + H]^+$ (calcd. for $C_{46}H_{57}N_4O_8$: 793.4176), and *m/z* 791.3825 $[2M - H]^-$ (calcd. for $C_{46}H_{55}N_4O_8$: 791.4025).

Figure S18. UV Spectrum of **2** in Methanol.



UV (MeOH) λ_{max} (log ε) = 278 (3.37), 300 (3.26), 356 (2.90) and a broad absorption with maximum at 472 (2.44) nm.

REFERENCES

1. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *J. Clin. Microbiol.* **1998,** *36*, 362-366.
2. Collins, L.; Franzblau, S. G. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1997,** *41*, 1004-1009.
3. Cho, S. H.; Warit, S.; Wan, B.; Hwang, C. H.; Pauli, G. F.; Franzblau, S. G. Low-oxygen-recovery assay for high-throughput screening of compounds against nonreplicating *Mycobacterium tuberculosis*. *Antimicrob. Ag. Chemother.* **2007,** *51*, 1380-1385.
4. Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau, S. G. *In vitro* and *in vivo* activities of macrolide derivatives against Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **2005,** *49*, 1447-1454.
5. Cantrell, C. L.; Lu, T.; Fronczek, F. R.; Fischer, N. H.; Adams, L. B.; Franzblau, S. G. Antimycobacterial cycloartanes from *Borrichia frutescens*. *J. Nat. Prod.* **1996,** *59*, 1131-1136.
6. Ó hAinmhire, E.; Quartuccio, S. M.; Cheng, W.; Ahmed, R. A.; King, S. M.; Burdette, J. E. Mutation or loss of p53 differentially modifies TGFβ action in ovarian cancer. *PLoS ONE* **2014,** *9*, e89553.
7. Mullowney, M. W.; Ó hAinmhire, E.; Shaikh, A.; Wei, X.; Tanouye, U.; Santarsiero, B. D.; Burdette, J. E.; Murphy, B. T. Diazaquinomycins E-G, novel diaza-anthracene analogs from a marine-derived *Streptomyces* sp. *Mar. Drugs* **2014,** *12*, 3574-3586.
8. Clinical and Laboratory Standards Institute (CLSI); *Performance Standards for Antimicrobial Susceptibility Testing; Tewnty-Fourth Informational Supplement. CLSI document M100-S24*; CLSI: Wayne, PA. 2014.
9. National Committee for Clinical Laboratory Standards (NCCLS); *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Second Edition: Approved Standard M7-A2*; NCCLS: Villanova, PA. 1990.
10. National Committee for Clinical Laboratory Standards (NCCLS); *Performance Standards for Antimicrobial Susceptibility Testing; Third Informational Supplement M100-S3*; NCCLS: Villanova, PA. 1991.
11. Maskey, R. P.; Grun-Wollny, I.; Laatsch, H. Isolation and structure elucidation of diazaquinomycin C from a terrestrial *Streptomyces* sp. and confirmation of the akashin structure. *Nat. Prod. Res.* **2005,** *19*, 137-142.
12. Omura, S.; Iwai, Y.; Hinotozawa, K.; Tanaka, H.; Takahashi, Y.; Nakagawa, A. OM-704 A, a new antibiotic active against gram-positive bacteria produced by *Streptomyces* sp. *J. Antibiot.* **1982,** *35*, 1425-1429.
13. Omura, S.; Nakagawa, A.; Aoyama, H.; Hinotozawa, K.; Sano, H. The structures of diazaquinomycins A and B, new antibiotic metabolites. *Tetrahedron Lett.* **1983,** *24*, 3643-3646.