Eight-Membered Ring-Containing Jadomycins: Implications for Non-enzymatic Natural Products Biosynthesis

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Experimental Section: Synthetic Methodology and Instrumentation

General Methods

All reagents were purchased from commercial sources and used without further purification unless otherwise stated. Solvents used for all reactions and chromatographic methods were HPLC grade. Flash chromatography was performed using a Biotage SP1™ unit (Biotage®) using pre-packed normal phase silica columns (40 g or 80 g) from SiliCycle®. Glass-backed thin layer chromatography (TLC) plates (SiliCycle®) layered with 250 μm silica were used to monitor reaction progress and assess purity of compounds. Compounds **1**, **1a**-**1f,** and **7** required no visualization reagents or ultraviolet (UV) light as the compounds were highly colored. Compounds **2**-**4** and **6** were visualized under 254 nm and 365 nm UV-light. Compound **5** was visualized using potassium permanganate dip (1.5 g $KMnO_4$, 10 g K_2CO_3, 125 mg NaOH, 200 mL H_2O) followed by heating. All reported R_f values were determined using 250 μm silica TLC plates. Preparative TLC was performed using 20 × 20 cm glass-backed plates (SiliCycle®) layered with 1000 μm or 2000 μm silica. Using the appropriate solvent mixture, the TLC plates were developed, then allowed to dry, then developed and dried again. This process was repeated until good separation was observed (~2-4 cycles). Bands of interest were scraped off the glass backing and eluted using the same solvent used for development. Size-exclusion chromatography was accomplished using Sephadex™ LH-20 (GE Healthcare) resin. SiliaBond® Piperazine (Si-PPZ) was purchased from SiliCycle® as a 0.91 mmol g^{-1} molecular loaded resin. The Si-PPZ was used in the deprotection of **1e** as a heterogeneous Fmoc scavenger.

All compounds were characterized by liquid chromatography tandem-mass spectrometry (LC-MS/MS), high resolution mass spectrometry (HRMS), and 1D- and 2D-nuclear magnetic resonance (NMR) spectroscopy. Low resolution LC-MS/MS spectra were obtained on an

Applied Biosystems hybrid triple quadrupole linear ion trap (2000Qtrap) mass spectrometer using an electrospray ionization (ESI) source. This was coupled with an Agilent 1100 high performance liquid chromatography (HPLC) instrument with a Phenomenex Kinetex 2.6 μm Hilic column (150 mm × 2.10 mm). Samples were prepared in methanol and 5 μL aliquots were injected onto the column. Elution of compounds was accomplished using an isocratic gradient of (7:3) CH_3CN : 2 mM ammonium acetate in water (pH 5.5) with a flow rate of 120 μL min^{-1} for 10 min. For all jadomycins, the instrument was used in positive mode (ESI+). Enhanced product ionization (EPI) was performed with a capillary voltage of +4500 kV, declustering potential +80 V, and curtain gas 10 arbitrary units. EPI scans were conducted over a range of 300-900 *m/z* scanning for $[M+H]^+$ and the appropriate jadomycin fragmentation. Scans were conducted using two steps, 300.0 amu to 320 amu (0.005 s) and 300.0 amu to 900.0 amu (0.150 s). Spectra were analyzed using Analyst software version 1.4.1 (Applied Biosystems). HRMS traces of all jadomycins were recorded on a Bruker Daltonics MicroTOF Focus Mass Spectrometer using an ESI+ source, with the exception of **1e** which required ESI-. All ultra-violet-visible (UV-vis) spectroscopy was carried out on a SpectraMax Plus Microplate Reader (Molecular Devices), and analyzed using SoftMax® Pro Version 4.8 Software. Samples were dissolved in methanol placed in a quartz cuvette (1 cm path length) and scanned over a range of 280-700 nm using 1 nm intervals. Two separate dilutions were used in each case (concentrations are listed with the appropriate characterization data) to calculate a series of extinction coefficients (ε) from several maximal absorbance wavelengths (λ_{max}).

NMR analyses of activated acids **2-7** and rotating frame nuclear Overhauser effect spectroscopy (ROESY) of **1a-1f** were performed on a Bruker AV 500 MHz Spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz) equipped with an auto-tune and match (ATMA) broadband observe

(BBFO) SmartProbe located at the Nuclear Magnetic Resonance Research Resource (NMR-3) facility (Dalhousie University). NMR spectra of all jadomycins (**1**, **1a**-**1f**) were recorded using a Bruker AV-III 700 MHz Spectrometer (^{1}H: 700 MHz, ^{13}C: 150 MHz) equipped with an ATMA 5 mm TCI cryoprobe located at the Canadian National Research Council Institute for Marine Biosciences (NRC-IMB) in Halifax, Nova Scotia. All spectra were recorded in MeOD-d_4, $CDCl_3$, or CD_2Cl_2. Appropriate solvents used in each case can be found with the accompanying supplemental NMR-spectra. Chemical shifts (δ) were given in ppm, and calibrated to residual solvent peaks (MeOD: 3.31 ppm; $CDCl_3$: 7.24 ppm; CD_2Cl_2: 5.32 ppm). Structural characterization and signal assignments were accomplished using ^{1}H-NMR chemical shifts and multiplicities, and ^{13}C-NMR chemical shifts. In addition, ^{1}H-^{1}H correlated spectroscopy (COSY), ^{1}H-^{13}C heteronuclear single quantum coherence (HQSC) NMR, ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) NMR, and ^{1}H-^{1}H ROESY experiments were used in the NMR analyses.

*HPLC Method: Compounds **1**, **1a-1c**, **1e-1f**, **8** and **9a***

HPLC analyses of jadomycin analogues **1**, **1a-1c**, **1e-1f, 8 and 9a** were performed on a Hewlett Packard Series 1050 instrument with an Agilent Zorbax 5 μm Rx-C18 column (4.6 × 150 mm). Elution of the compounds was monitored at an absorbance of 254 nm using an isocratic gradient of 9:1 (A:B) over 0.5 min followed by an increasing linear gradient from 9:1 (A:B) to 4:6 (A:B) over 7.5 min, followed by an isocratic gradient of 4:6 (A:B) for an additional 2 min. This was then followed by a decreasing linear gradient from 4:6 (A:B) to 9:1 (A:B) over 1 min, ending with an isocratic gradient of 9:1 (A:B) over 4 min (total time 15 min; flow rate of 1 ml/min).

Buffer A was an aqueous buffer comprised of 12 mM Bu_4NBr, 10 mM KH_2PO_4, and 5% HPLC grade CH_3CN (pH 4.0) and B was HPLC grade CH_3CN.

*HPLC Method: Compound **1d***

HPLC of jadomycin analogue **1d** was performed on a Hewlett Packard Series 1050 instrument with an Agilent Zorbax 5 μm Rx-C18 column (4.6 × 150 mm). Elution of the compound was monitored at an absorbance of 254 nm using an isocratic gradient of 9:1 (A:B) over 0.5 min followed by an increasing linear gradient from 9:1 (A:B) to 2:8 (A:B) over 4.5 min, followed by an isocratic gradient of 2:8 (A:B) for an additional 15 min. This was then followed by a decreasing linear gradient from 2:8 (A:B) to 9:1 (A:B) over 1 min, ending with an isocratic gradient of 9:1 (A:B) over 9 min (total time 30 min; flow rate of 1 ml/min). Buffer A was an aqueous buffer comprised of 12 mM Bu_4NBr, 10 mM KH_2PO_4, and 5% HPLC grade CH_3CN (pH 4.0) and B was HPLC grade CH_3CN.

Media and Growth Conditions: Jadomycin Oct (1), Jadomycin AVA (8), and Jadomycin DOct (9) Productions

Media Composition

All media was prepared with distilled water unless otherwise stated. MYM broth [maltose 4 g/L, yeast extract 4 g/L, malt extract 10 g/L]; MYM agar [maltose (4 g/L), yeast extract (4 g/L), malt extract (10 g/L), agar 15 (g/L), pH 7.0]; MSM media [$MgSO_4$ (0.4 g/L), MOPS (3.77 g/L), salt solution (9 mL 1% w/v NaCl, 1% w/v $CaCl_2$), $FeSO_4{\cdot}7H_2O$ (4.5 mL 0.2% w/v), trace mineral solution (4.5 mL), pH 7.5]. Trace mineral solution [$ZnSO_4{\cdot}7H_2O$ (880 mg/L), $CuSO_4{\cdot}5H_2O$ (39 mg/L), $MnSO_4{\cdot}4H_2O$ (6.1 mg/L), H_3BO_3 (5.7 mg/L), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (3.7 mg/L).

250 mL fractions of each broth solution were prepared into 1 L glass Erlenmeyer flasks. One hundred twenty five mL of MYM agar was prepared in 250 mL glass Erlenmeyer flasks. Agar solutions were supplemented with 50 $\mu g mL^{-1}$ apramycin sulfate before being poured into standard petri dishes while molten. All media was adjusted to pH 7 or 7.5 with 5 M NaOH or 5 M HCl as required. All solutions were autoclaved at 120 $^{\circ}$C for 20 minutes prior to use.

*Bacteria Maintenance and Growth: Jadomycin Oct (**1**), Jadomycin AVA (**8**) and Jadomycin DOct (**9**) Production*

Streptomyces venezuelae ISP5230 (ATCC 10712) VS1099 was maintained on MYM agar plates supplemented with apramycin for 1-3 weeks for use in jadomycin production fermentations. For long term storage, spore solutions were stored in a 20% glycerol solution at -70°C. Jadomycin Oct (**1**) fermentations were carried out using modified conditions for jadomycin production previously established in the Jakeman laboratory.[1] A 1 × 1 cm lawn of *S. venezuelae* ISP5230 VS1099 was used to inoculate 250 mL MYM media (4 × 250 mL in 4-1 L flasks per growth). Growths were incubated at 30°C with agitation (250 RPM) for 16-24 hours. The cell suspension was centrifuged at 3750 RPM (4°C) for 30-45 minutes. Supernatant was removed and the cell pellet was washed with 100 mL MSM containing no amino acid. The washing step was repeated twice more to ensure removal of all nutrient rich MYM. The cell pellet was re-suspended in 100 mL MSM without amino acid. Autoclaved MSM media containing L-Ornithine (60 mM, 8 × 250 mL in 8-1 L flasks) or D-Ornithine (60 mM, 3 × 66 mL in 2-250 mL flasks) were supplemented with glucose (33 mM) and phosphate (50 μM) before being inoculated with the pre-growth *S. venezuelae* ISP5230 VS1099 cell suspension to an initial OD_{600} of 0.6. For jadomycin AVA (**8**) growths, MSM without amino acid (3 × 66 mL in 2-250 mL flasks) was supplemented with glucose (33 mM) and phosphate (50 μM) and a 6 M filter sterilized solution of 5-aminovaleric

acid to a final concentration of 60 mM. before being inoculated with the pre-growth *S. venezuelae* ISP5230 VS1099 cell suspension to an initial OD_{600} of 0.6. Growths were immediately ethanol shocked with 100% ethanol (3% v/v) and incubated at 30°C with agitation (250 RPM) for 48 hours. After 24 hours, the pH of the media was readjusted to a pH of 7.5. Bacterial growths were monitored by absorbance at 600 nm (OD_{600}), jadomycin and colored natural product production was monitored by absorbance of clear growth solution at 526 nm (Figure S1).

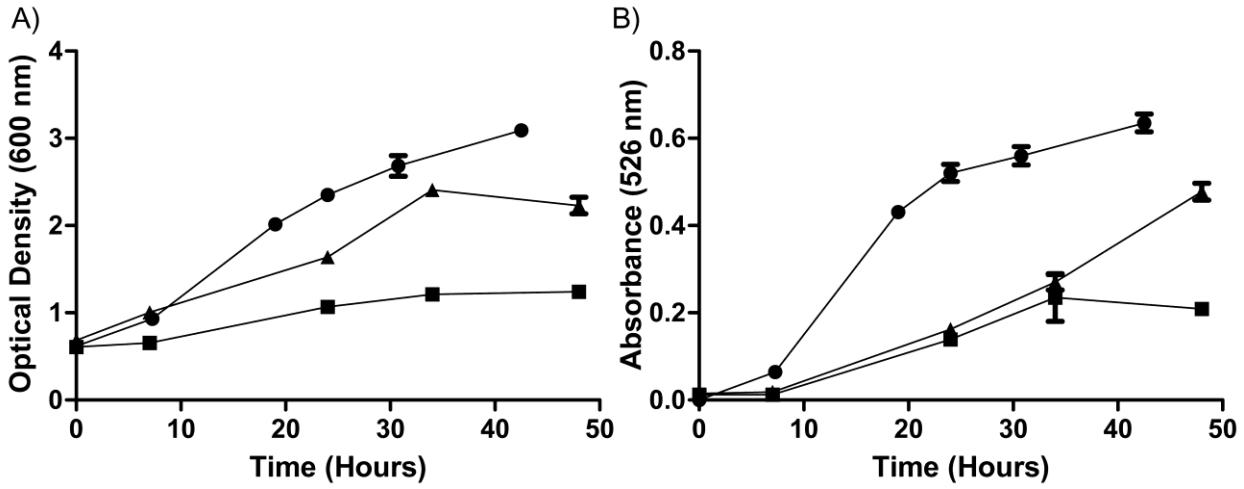


Figure S1. (A) *S. venezuelae* ISP5230 VS1099 growth curves measured at 600 nm (OD_{600}) in the presence of L-ornithine (●), D-ornithine (■), and 5-aminovaleric acid (▲) as the sole nitrogen sources; (B) *S. venezuelae* ISP5230 VS1099 growth curves measured at 526 nm (Abs_{526}) estimating production of colored compounds.

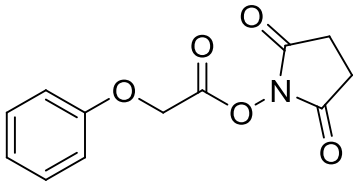
Crude Growth Extraction

Bacterial cells were removed via suction filtration through Whatman No. 5 filter paper, followed by 0.45 μm then 0.22 μm Millipore Durapore® membrane filters. The clear media was passed through a reversed-phase SiliCycle® phenyl column [70 g (jadomycin Oct) or 2 g (jadomycin AVA and jadomycin DOct)], and washed with distilled water until flow through was colorless [~4-8 L (jadomycin Oct) or ~500-1000 mL (jadomycin AVA and jadomycin DOct)] to remove

all water soluble material. Remaining material was eluted with 100% methanol and dried *in vacuo*. No further extractions were performed on **8**, methodology for purification can be found with the accompanying compound information. For **1** and **9**, the crude mixtures were dissolved in minimal H_2O and extracted with equal volumes of EtOAc three times. For **1**, the aqueous layer was fractioned into 4 equal aliquots (corresponding to ~ 500 mL of growth solution each) and dried *in vacuo* yielding an aqueous extract of ~100 mgL^{-1}. For purposes of derivatization of **1**, the aqueous layer was not purified further. For purposes of characterizing **1** the crude extracts were purified further. Methodology for purification can be found with the accompanying compound information. For **9**, the aqueous layer was dried *in vacuo* yielding an aqueous extract of ~20 mgL^{-1}. For purposes of derivatization of **9**, the aqueous layer was not purified further.

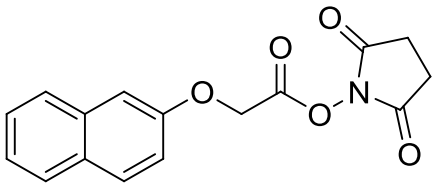
Succinimidyl Ester Preparation

*Phenoxyacetic acid N-hydroxysuccinimide ester (**2**)*



Compound **2** was purchased from Sigma and used without further purification.

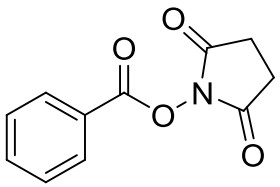
*(2-Naphthoxy)acetic acid N-hydroxysuccinimide ester (**3**)*



Compound **3** was synthesized using modified literature methodology.[2] *N*-hydroxysuccinimide (289 mg, 2.51 mmol) was added to a 50 mL round bottom flask with a stir bar, and placed under an N_2 atmosphere. (2-naphthoxy)acetic acid (524 mg, 2.59 mmol) was dissolved in 10 mL of anhydrous EtOAc under an N_2 atmosphere and added, with gentle stirring, to the flask containing *N*-hydroxysuccinimide and cooled to 0°C. A solution of *N*,*N*-dicyclohexylcarbodiimide (DCC) (506 mg, 2.45 mmol) dissolved in 10 mL anhydrous EtOAc was then added drop-wise to the mixture with gentle stirring. Upon complete addition of DCC, the reaction flask was removed from the ice bath and the reaction was allowed to proceed for 16 hours. The reaction mixture was filtered through #5 Whatman filter paper to remove 1,3-dicyclohexylurea (DCU) precipitate, and the filtrate was dried. The residue was then suspended in 50 mL of anhydrous ether, filtered, and washed with an additional 75 mL of ether (3 × 25 mL). The material was dried, yielding the compound as a white powder (688 mg, 92% yield). The pure material was stored at 4°C under desiccation and used without further purification. ^{1}H NMR ($CDCl_3$, 700 MHz) δ: 7.80-7.78 (m, 3 × CH), 7.47 (t, *J* = 7.49 Hz), 7.38 (t, *J* =7.49 Hz), 7.23 (dd, *J* = 9.0 Hz, 2.5 Hz), 7.19 (d, *J* = 2.5

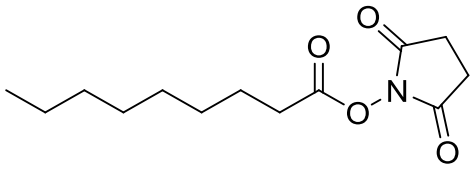
Hz), 5.10 (s), 2.85 (bs); ^{13}C NMR ($CDCl_3$, 176 MHz) δ: 168.8, 164.7, 155.2, 134.3, 130.1, 129.8, 127.8, 127.3, 126.8, 124.6, 118.4, 107.6, 63.4, 25.7 ppm. These data are consistent with literature values.[2]

*Benzoic acid N-hydroxysuccinimide ester (**4**)*



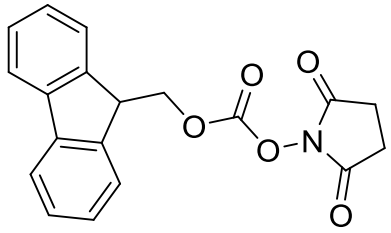
Compound **4** was synthesized using modified literature methodology.[2] *N*-hydroxysuccinimide (1.064 g, 9.2 mmol) was added to a 50 mL round bottom flask with a stir bar, and placed under an N_2 atmosphere. Benzoic acid (1.060 g, 8.7 mmol) was dissolved in 15 mL of anhydrous EtOAc under an N_2 atmosphere and added, with gentle stirring, to the flask containing the *N*-hydroxysuccinimide and cooled to 0°C. A solution of DCC (3.586, 17.0 mmol) dissolved in 15 mL anhydrous EtOAc was added drop-wise to the mixture with gentle stirring. Upon complete addition of DCC, the reaction flask was removed from the ice bath and the reaction was allowed to proceed for 16 hours. The reaction mixture was filtered through #5 Whatman filter paper to remove DCU precipitate, and the filtrate was dried. The residue was suspended in 50 mL of anhydrous ether, filtered, and washed with an additional 75 mL of ether (3 × 25 mL). The material was dried, yielding the compound as a white powder (1.413 g, 74% yield). The material was stored at 4°C under desiccation and used without further purification. ^{1}H NMR ($CDCl_3$, 700 MHz) δ: 8.14 (d, J = 8.3 Hz,), 7.68 (t, J = 7.4 Hz), 7.52 (t, J = 8.3 Hz), 2.91 (bs); ^{13}C NMR ($CDCl_3$, 176 MHz) δ: 169.4, 162.0, 135.1, 130.7, 129.0, 128.7, 125.2, 25.8 ppm. These data are consistent with literature values.[2]

*Nonoic acid N-hydroxysuccinimide ester (**5**)*



Compound **5** was synthesized using modified literature methodology.[3] *N*-hydroxysuccinimide (0.7433, 6.4 mmol) was added to a 50 mL round bottom flask with a stir bar, and placed under an N_2 atmosphere. Nonoic acid (1.043 g, 6.3 mmol) was dissolved in 20 mL of DCM under an N_2 atmosphere and added, with gentle stirring, to the flask containing the *N*-hydroxysuccinimide and cooled to 0°C. A solution of DCC (2.59 g, 12.6 mmol) dissolved in 20 mL anhydrous CH_3CN was added drop-wise to the mixture with gentle stirring. Upon complete addition of the DCC, the reaction flask was removed from the ice bath and the reaction was allowed to proceed for 16 hours. The reaction mixture was filtered through #5 Whatman filter paper to remove DCU precipitate, and the filtrate was dried. The reaction mixture was brought up in minimal DCM (~ 2 mL) and loaded onto a 40 g pre-packed normal phase silica column (SiliCycle®), conditioned with 100% hexanes. Material was eluted with a flow rate of 25 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system, first washing with 1 CV of 100% hexanes followed by a linear increasing gradient of 0% ➔ 50% EtOAc in hexanes over 5 CV, followed finally by an isocratic gradient of 50% EtOAc in hexanes over 5 CV. Fractions were checked by TLC for purity and combined yielding the compound as a white powder (1.255 g, 78% yield). The pure material was stored at 4°C under desiccation and used without further purification. R_f: 0.67 (1:1 EtOAc:Hexanes), ^{1}H NMR ($CDCl_3$, 700 MHz) δ: 2.83 (bd, J = 13 Hz), 2.59 (t, J = 7.5 Hz), 1.74 (p, J = 7.5 Hz), 1.40 (p, J = 7.5 Hz), 1.33-1.22 (bm, 4 × CH_2), 0.87 (t, 7.0 J = Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.4, 168.9, 31.9, 31.1, 29.2, 29.1, 28.9, 25.7, 24.7, 22.8, 14.2 ppm. These data are consistent with literature values.[3]

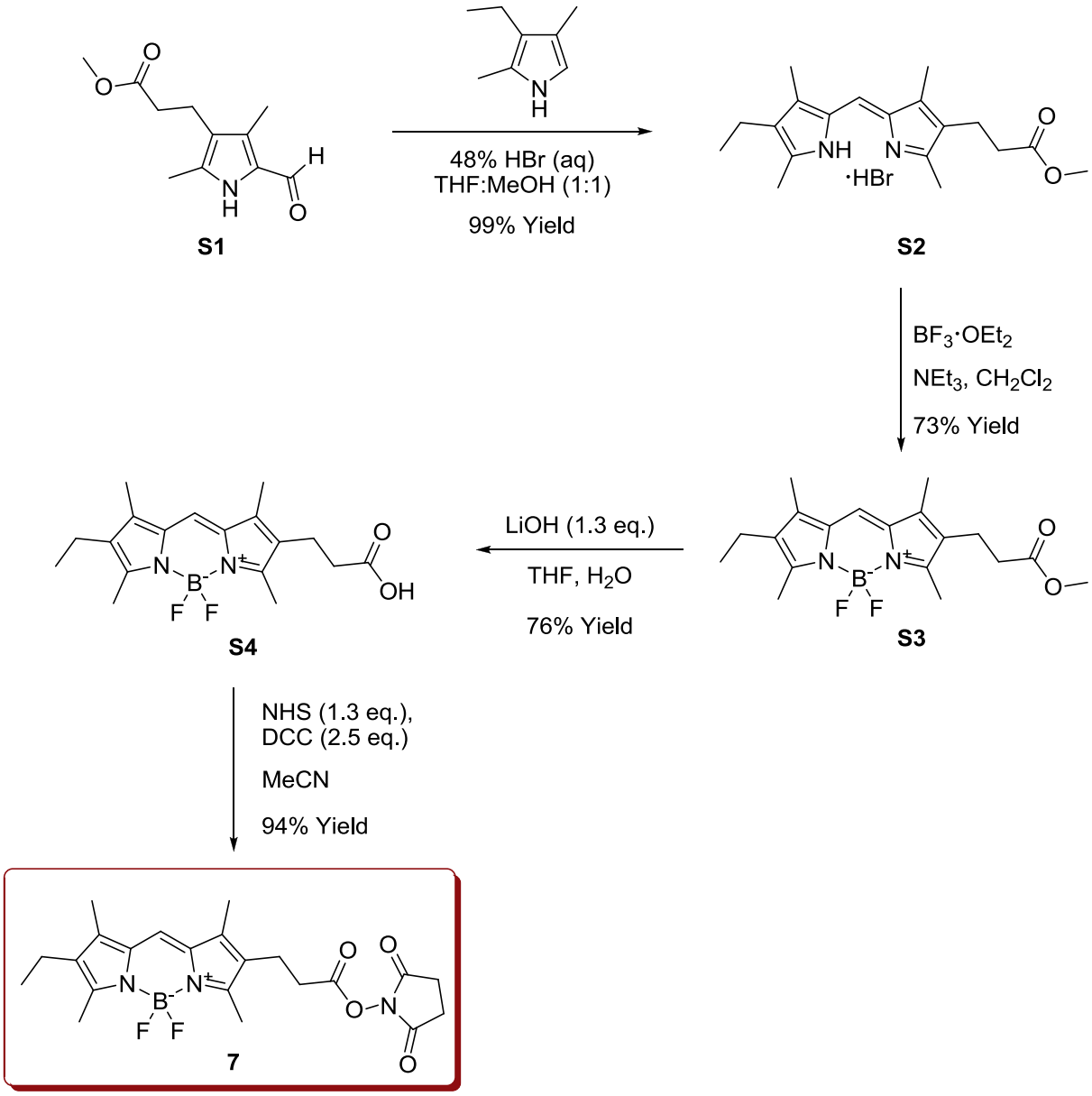
*SuO-Fmoc (**6**)*



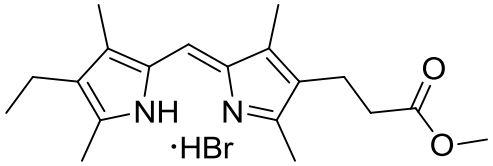
Compound **6** was synthesized using modified literature methodology.[4] *N*-hydroxysuccinimide (450 mg, 3.90 mmol) and diisopropylamine (550 µL, 4 mmol) were dissolved in anhydrous dichloromethane (5 mL) under N_2 at room temperature. The mixture was added drop wise to a solution of Fmoc chloride (1.007 g, 3.89 mmol) in anhydrous dichloromethane (5 mL) under N_2 at 0°C with stirring over a period of 5 minutes. Upon completion, the flask was allowed to warm to room temperature, and the reaction mixture was stirred under N_2 for 16 hours. The solution was filtered, and the precipitate was washed with 20 mL dichloromethane. The filtrate and washings were combined and washed with, in order, 15 mL of 10% (w/v) citric acid in distilled water, 15 mL of 10% sodium bicarbonate (w/v) in distilled water, and 3 × 15 mL of distilled water. The organic layer was dried with anhydrous magnesium sulfate and filtered. The dichloromethane was removed *in vacuo* to give compound **6** (1.115 g, 85% yield) as a light yellow powder. The pure material was stored at 4°C under desiccation and used without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78 (d, 2H, *J* = 7.6 Hz), 7.63 (d, 2H, *J* = 7.6 Hz), 7.44 (t, 2H, *J* =7.5 Hz), 7.35 (dt, 2H, *J* = 7.5 Hz, 1.0 Hz), 4.58 (d,2H, *J* = 7.5 Hz), 4.35 (t, 1H, *J* = 7.4 Hz), 2.81 (bs, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.6, 151.7, 142.6, 141.5, 128.4, 127.6, 125.4, 120.3, 73.1, 46.5, 25.6 ppm. These data are consistent with literature values.[4]

Synthesis of SuO-F-BODIPY (7)

Scheme S1. Synthetic Methodology Towards the Activated SuO-BODIPY (7).

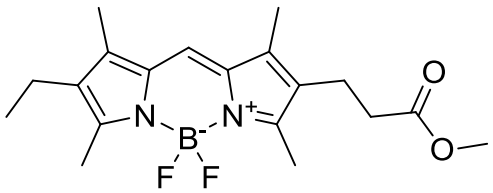


*(Z)-Methyl-3-(5-((4-ethyl-3,5-dimethyl-2H-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate hydrobromide (**S2·HBr**)*



A solution of aldehyde **S1**[5] (350 mg, 1.67 mmol) and kryptopyrrole (0.23 mL, 1.67 mmol) in THF (4 mL) and methanol (4 mL) was bubbled with nitrogen for 10 minutes, before adding concentrated HBr (48% solution in water, 3.67 mmol, 2.2 eq.) drop-wise, with stirring at room temperature under nitrogen for 3 hours. The reaction mixture was then concentrated and the residue was washed with diethyl ether and dried under vacuum to give the title compound (654 mg, 99% yield) as a brown solid. M.p. 123-128 °C. ^{1}H NMR (DMSO, 500 MHz) δ 12.16 (br s, 1H, NH), 12.12 (br s, 1H, NH), 7.32 (s, 1H, CH), 3.52 (s, 3H, OCH_3), 2.63 (t, 2H, *J* = 7.5 Hz, $CH_2CH_2CO_2Me$), 2.42-2.46 (m, 8H), 2.38 (q, 2H, *J* = 7.6 Hz, CH_2CH_3), 2.25 (s, 6H, 2 × CH_3), 0.97 (t, 3H, *J* = 7.6 Hz, CH_2CH_3) ppm; ^{13}C NMR (MeOD, 125 MHz) δ 174.8, 155.4, 154.2, 144.9, 132.7, 129.0, 128.6, 128.5, 121.93, 121.90, 52.3, 34.7, 20.3, 18.1, 14.8, 13.0, 12.9, 10.3, 10.1 ppm; LRMS: 315.2 $(M+H)^+$; HRMS: 315.2081 Found, 315.2067 calculated for $C_{19}H_{27}N_2O_2$.

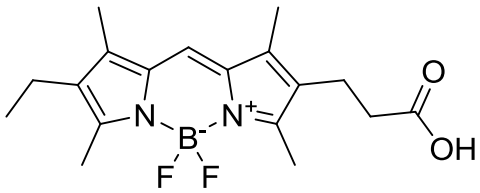
*4,4-Difluoro-1,3,5,7-tetramethyl-2-ethyl-6-(3-methylpropanoate)-8-H-4-bora-3a,4a-diaza-s-indacene (**S3**)*



Triethylamine (0.63 mL, 4.55 mmol) was added dropwise to a solution of **S2**·HBr (300 mg, 0.76 mmol) in dry dichloromethane (50 mL) under nitrogen, with stirring at room temperature for 10

minutes. Boron trifluoride diethyl etherate (0.84 mL, 6.83 mmol) was then added dropwise over 5 minutes and the resulting solution was stirred at room temperature, under nitrogen for 18 hours, before concentrating *in vacuo* and separating the residue between diethyl ether (50 mL) and 1 M aqueous HCl (50 mL). The aqueous phase was extracted with diethyl ether (3 × 50 mL) and the organic extracts were combined and washed with 1 M aqueous HCl (100 mL), brine (100 mL), dried over anhydrous magnesium sulfate, filtered through a short pad of silica, washing with diethyl ether, and concentrated *in vacuo* to give the crude product, which was purified using column chromatography on silica, eluting with 20% ethyl acetate in hexanes to give the title compound (268 mg, 73% yield) as a deep red solid. M.p. 115-117 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.96 (s, 1H, *meso*-H), 3.67 (s, 3H, OMe), 2.71 (t, 2H, *J* = 7.8 Hz, CH_2CH_2CO), 2.50 (s, 6H, 2 × CH_3), 2.44 (t, 2H, *J* = 7.8 Hz, CH_2CH_2CO), 2.38 (q, 2H, *J* = 7.5 Hz, CH_2CH_3), 2.18 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 1.06 (t, 3H, *J* = 7.5 Hz, CH_2CH_3); ^{11}B NMR ($CDCl_3$, 160 MHz) δ 0.78 (t, *J* = 34.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 156.1, 153.9, 137.5, 137.1, 133.0, 132.3, 132.2, 127.6, 119.0, 51.8, 34.4, 19.7, 17.4, 14.7, 12.8, 12.7, 9.7, 9.6 ppm; LRMS: 385.2 $(M+Na)^+$; HRMS: 385.1853 Found, 385.1869 Calculated for $C_{19}H_{25}N_2O_2BF_2Na$; ε_{529nm} = 87,400 (CH_2Cl_2).

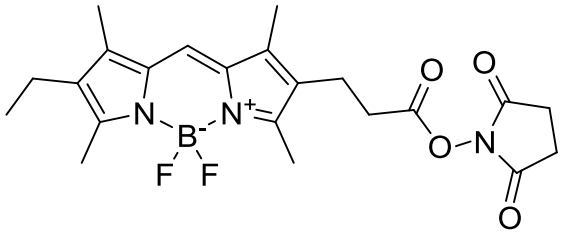
*4,4-Difluoro-1,3,5,7-tetramethyl-2-ethyl-6-(3-propanoic acid)-8-H-4-bora-3a,4a-diaza-s-indacene (**S4**)*



Lithium hydroxide monohydrate (22 mg, 0.516 mmol) was added to a solution of BODIPY methyl ester **S3** (170 mg, 0.469 mmol) in THF (15 mL) and water (15 mL), with stirring at 40 °C

for 2 hours. Further lithium hydroxide monohydrate (5 mg, 0.119 mmol) was then added, with continued stirring at 40°C for 1 hour, before adding 1 M aqueous HCl solution (30 mL) and stirring for 5 minutes before extracting with dichloromethane (4 × 40 mL). The organic extracts were combined and washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product, which was purified using column chromatography on silica, eluting with 0-2% methanol in dichloromethane to give the title compound (124 mg, 76% yield) as a red solid. M.p. 177-179 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.97 (s, 1H, *meso*-H), 2.72 (t, 2H, *J* = 7.8 Hz, $PyCH_2$), 2.51-2.48 (m, 8H, 2 × CH_3 + CH_2), 2.38 (q, 2H, *J* = 7.5 Hz, CH_2CH_3), 2.19 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 1.06 (t, 3H, *J* = 7.5 Hz, CH_2CH_3); ^{11}B NMR ($CDCl_3$, 160 MHz) δ 0.78 (t, *J* = 34.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.3, 156.3, 153.7, 137.6, 137.1, 133.1, 132.4, 132.1, 127.2, 119.1, 34.2, 19.4, 17.4, 14.7, 12.8, 12.7, 9.7, 9.6 ppm; LRMS: 347.2 $(M-H)^-$; HRMS: 347.1742 Found, 347.1748 Calculated for $C_{18}H_{22}N_2O_2BF_2$; ε_{528nm} = 84,300 (CH_2Cl_2).

*4,4-Difluoro-1,3,5,7-tetramethyl-2-ethyl-6-(3-propanoic acid)-8-H-4-bora-3a,4a-diaza-s-indacene N-hydroxysuccinimide ester (**7**)*



Compound **7** was synthesized using modified literature methodology for similar compounds.[6] An anhydrous solution of *N*-hydroxysuccinimide (45 mg, 0.388 mmol) in CH_3CN (5 mL) was added to a 100 mL round bottom flask containing an anhydrous solution of the **S4** (101 mg, 0.287 mmol) in CH_3CN (5 mL) with gentle stirring under an N_2 atmosphere at 0°C in an ice bath. An

anhydrous solution of DCC (146 mg, 0.708 mmols) in CH_3CN (10 mL) was then added drop-wise to the mixture. Upon the complete addition, the flask was taken from the ice bath and allowed to warm to room temperature with gentle stirring for 16 hours. The reaction mixture was filtered through a #5 Whatman filter paper disk to remove DCU and the filtrate was dried *in vacuo*. The crude reaction mixture was brought up in minimal DCM (~ 2 mL) and loaded onto a 25 g pre-packed normal phase silica column. Material was eluted with a flow rate of 25 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system, first washing with 1 CV of 100% DCM followed by a linear increasing gradient of 0% ➔ 20% EtOAc in DCM over 10 CV, followed finally by an isocratic gradient of 20% EtOAc in DCM over 5 CV. Fractions were checked by TLC for purity and combined yielding **7** (120 mg, 94% yield) as a solid red powder. TLC R_f = 0.8 (1:4 EtOAc:DCM); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.98 (s), 2.86 (bs), 2.84 (bs), 2.82 (t, *J* = 8.0 Hz), 2.72 (t, *J* = 8.0 Hz), 2.51 (s, 2 × CH_3), 2.38 (q, *J* = 7.6 Hz), 2.20 (s), 2.17 (s), 1.06 (t, 7.6 *J* = Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.2, 167.9, 157.0, 153.2, 137.9, 133.3, 132.7, 132.0, 126.0, 119.2, 31.4, 25.7, 19.4, 17.4, 14.6, 12.9, 12.7, 9.8, 9.6 ppm; ^{11}B NMR ($CDCl_3$, 160 MHz) δ 0.87 (t, *J* = 33.4 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -146.2 (q, *J* = 33.1 Hz) LRMS: 446.4 $(M+H)^+$; HRMS: 468.1882 Found, 468.1867 Calculated for $C_{22}H_{26}BF_2N_3NaO_4$.

Jadomycin Oct Amide Syntheses (1a-1f)

*Jadomycin Oct Phenoxyacetylamide (**1a**)*

Crude **1** (68.2 mg) was dissolved in 15 mL of a 1:1 mixture of CH_3CN:PBS buffer (pH 7.6), and added to compound **2** (63 mg, 0.253 mmol) in a 25 mL round bottom flask drop-wise with gentle stirring. The flask was corked and protected from light, and the reaction was allowed to proceed for 6 hours. The reaction mixture was diluted with 15 mL PBS, and extracted with dichloromethane (3 × 50 mL). By TLC analysis, the organic fractions contained the new purple compound of interest. The organic fractions were pooled and dried *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 3 mL) and filtered. Filtrate was applied to a 40 g silica column preconditioned with a 1:1 EtOAc:CH_3CN solution. Material was eluted with a flow rate of 35 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system comprised of solvent A (1:1 EtOAc:CH_3CN), and solvent B (5:5:1 EtOAc:CH_3CN:H_2O). To start, an initial isocratic gradient step using solvent A (1 CV) was performed. This was followed by a linear increasing gradient of 0% ➔ 100% B over 10 CV, followed finally by an isocratic gradient of 100% B over 7.5 CV. Fractions were checked by TLC for the presence of the compound of interest, combined, and dried yielding 15.2 mg of crude **1a**. Prep TLC was performed twice using the same 5:5:1 EtOAc:CH_3CN:H_2O solvent system yielding 12.6 mg. Final purification was accomplished by size exclusion chromatography (LH-20 resin). Material was eluted using 5:5:1 EtOAc:CH_3CN:H_2O and dried yielding **1a** as a reddish-purple solid (7.0 mg) as a mixture of diastereomers (M_j/M_n = 5/4) by NMR. TLC R_f: 0.46 (5:5:1 EtOAc:CH_3CN:H_2O); HPLC R_t = 9.73 min; NMR data to follow, for labeling scheme see **1a** NMR data table; UV-Vis (4×10^{-4} and 5×10^{-5} M, MeOH): λ_{max} (ε) = 312 (14820), 375 (2943), 523 (1875); LRMS (ESI^+): MS/MS (685) found 685 $[M+H]^+$, 555 $[M+H-digitoxose]^+$, 306

[M+H−digitoxose−$C_{13}H_{15}NO_4$]$^+$; HRMS (ESI$^+$): 685.2383 Found, 685.2392 Calculated for $C_{37}H_{37}N_2O_{11}$.

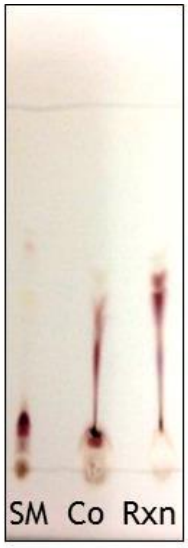


Figure S2. Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **1** starting material (SM), the reaction mixture of **1** and **2** after 6 hours (Rxn), and the co-spot of both (Co).

Table S1. Jadomycin Oct Phenoxyacetylamide (**1a**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.39	bs	54.5	1'	3b, 7b
2			176.8		
3a	5.58 (s)	s	92.6	None	1, 4, 7a, 7b, 13, 13a, 3'
3b			115.0		
4	6.74 (s)	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a
5			141.3		
5-CH_3	2.34 (s)	s	21.1	4, 6	4, 5, 6
6	6.82 (s)	s	120.6	4, 5-CH_3	3b, 5-CH_3, 7, 7a, 7b
7			154.8		
7-OH					
7a			114.4		
7b			130.2		
8			183.3		
8a			136.7		
9	7.82	d (7.6)	121.4	10	8, 8a, 10
10	7.71	t (8.0)	136.7	9, 11	8a, 9, 11, 12
11	7.52	d (8.2)	120.6	10	9, 10, 12, 13
12			155.8		
12a			120.6		
13			185.7		
13a			153.1		
1'	1.81, 2.01	bm	30.5	2'	none
2'	1.69, 1.74	bm	28.3	1', 3'	none
3'	3.90, 3.94	bm	54.5	2'	3a, 13a
4'			170.4		
4a'	4.51	s	68.3	none	4', 5'
5'			159.1		
5a'	6.92	d (8.2)	115.9	6'	5', 5a', 7'
6'	7.14	t (8.2)	130.6	5a', 7'	5', 6'
7'	6.84	t (7.3)	122.9	6'	5a'
1''	5.94	d (3.0)	96.3	2''	3'', 5''
2''	2.19	dd (15.2, 3.9)	36.3	1'', 3''	3'', 4''
2''	2.42	bt (13.2)	36.3	1'', 3''	3'', 4''
3''	4.08	m	68.2	2'', 4''	none
3''-OH					
4''	3.94	m	74.1	3'', 5''	none
4''-OH					
5''	3.28	obscured	66.6	4'', 5''-CH_3	none
5''-CH_3	1.22	d (6.8)	18.2	5''	4'', 5''

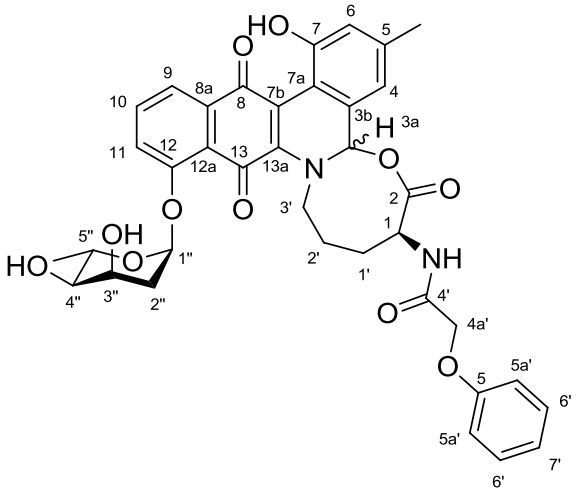
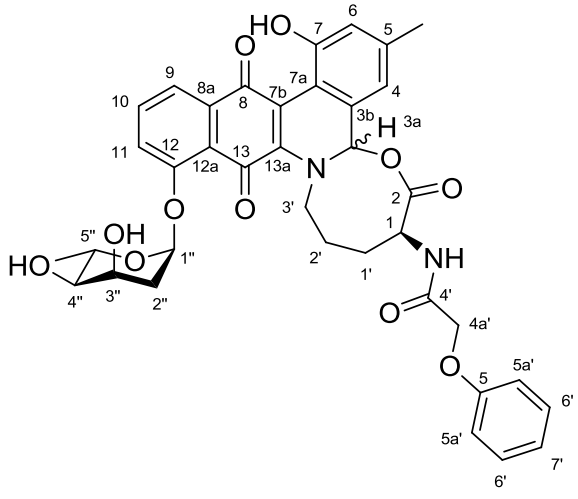


Table S2. Jadomycin Oct Phenoxyacetylamide (**1a**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.39 (bs)	bs	54.5	1'	3b, 7b
2			176.8		
3a	5.57 (s)	s	92.3	None	1, 4, 7a, 7b, 13, 13a, 3'
3b			118.9		
4	6.75 (s)	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a
5			141.7		
5-CH_3	2.34 (s)	s	21.1	4, 6	4, 5, 6
6	6.82 (s)	s	120.6	4, 5-CH_3	3b, 5-CH_3, 7, 7a, 7b
7			154.8		
7-OH					
7a			114.2		
7b			131.1		
8			184.0		
8a			136.7		
9	7.82	d (7.6)	121.4	10	8, 8a, 10
10	7.70	t (8.0)	136.7	9, 11	8a, 9, 11, 12
11	7.51	d (8.2)	121.3	10	9, 10, 12, 13
12			156.4		
12a			120.7		
13			184.1		
13a			151.3		
1'	1.74, 1.89	bm	30.8	2'	none
2'	1.69, 1.74	bm	28.2	1', 3'	none
3'	3.79, 4.29	bm	55.2	2'	3a, 13a
4'			170.5		
4a'	4.51	s	68.3	none	4', 5'
5'			159.1		
5a'	6.91	d (8.2)	115.9	6'	5', 5a', 7'
6'	7.16	t (8.2)	130.6	5a', 7'	5', 6'
7'	6.86	t (7.3)	122.8	6'	5a'
1''	5.89	d (3.0)	96.6	2''	3'', 5''
2''	2.19	dd (15.2, 3.9)	36.3	1'', 3''	3'', 4''
2''	2.42	bt (13.2)	36.3	1'', 3''	3'', 4''
3''	4.08	m	68.2	2'', 4''	none
3''-OH					
4''	3.9	m	74.1	3'', 5''	none
4''-OH					
5''	3.28	obscured	66.5	4'', 5''-CH_3	none
5''-CH_3	1.21	d (6.8)	18.2	5''	4'', 5''



*Jadomycin Oct Naphthoxyacetylamide (**1b**)*

Crude **1** (80 mg) was dissolved in 20 mL of a 1:1 mixture of CH_3CN:PBS buffer (pH 7.6), and added to solid compound **3** (92 mg, 0.308 mmol) in a 25 mL round bottom flask drop-wise with gentle stirring. The flask was corked and protected from light, and the reaction was allowed to proceed for 8 hours. The reaction mixture was diluted with 15 mL PBS, and extracted with dichloromethane (3 × 50 mL). The organic fractions were pooled and dried *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 4 mL) and filtered. Filtrate was then applied to a 40 g silica column preconditioned with a 1:1 EtOAc:CH_3CN solution. Material was eluted with a flow rate of 35 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system comprised of solvent A (1:1 EtOAc:CH_3CN), and solvent B (5:5:1 EtOAc:CH_3CN:H_2O). To start, an initial isocratic gradient step using solvent A (1 CV) was performed. This was followed by a linear increasing gradient of 0% ➔ 100% B over 10 CV, followed finally by an isocratic gradient of 100% B over 7.5 CV. Fractions were checked by TLC for the presence of the compound of interest, and combined and dried yielding 21 mg of crude **1b**. Prep TLC was then performed three times using the same 5:5:1 EtOAc:CH_3CN:H_2O solvent system yielding 14 mg. Final purification was accomplished by size exclusion chromatography (LH-20 resin). Material was dissolved and eluted using 5:5:1 EtOAc:CH_3CN:H_2O. This process was repeated twice more (total = 3 ×) and dried yielding **1b** as a reddish-purple solid (11 mg) as a mixture of diastereomers (M_j/M_n = 50/37) by NMR. TLC R_f: 0.45 (5:5:1 EtOAc:CH_3CN:H_2O), HPLC R_t = 9.92 min, NMR data to follow, for labeling scheme see **1b** NMR data table, UV-Vis (5.10×10^{-4} and 6.38×10^{-5} M, MeOH): λ_{max} (ε) = 312 (12774), 389 (2496), 520 (1727); LRMS (ESI^+): MS/MS (735) found 735 $[M+H]^+$, 605

[M+H−digitoxose]$^+$, 306 [M+H−digitoxose−$C_{17}H_{17}NO_4$]$^+$, HRMS (ESI$^+$): 735.2517 Found, 735.2548 Calculated for $C_{41}H_{39}N_2O_{11}$.

Figure S3. Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **1** starting material (SM), the reaction mixture of **1** and **3** after 8 hours (Rxn), and the co-spot of both (Co).

Table S3. Jadomycin Oct Naphthoxyacetylamide (**1b**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.38	bm	55.3	1'	2, 1', 2'
2			177.6		
3a	5.52	s	92.4	None	3b, 4, 7a, 13a, 3'
3b			130.1		
4	6.72	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a
5			141.2		
5-CH_3	2.34	s	21.1	4, 6	4, 5, 6
6	6.84	s	120.6	4, 5-CH_3	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.2		
7b*			127.9		
8			183.2		
8a*			127.3		
9	7.78	d (7.4)	121.3	10	8
10	7.66	Obscured	136.5	9, 11	Obscured
11	7.47	d (8.5)	120.4	10	12
12			155.7		
12a*			120.6		
13			185.3		
13a			153.0		
1'	1.81	bm	30.5	2'	1
1'	2.02	bm	30.5	2'	1, 2
2'	1.65	bm	28.1	1', 3'	None
2'	1.72	bm	28.1	1', 3'	None
3'	3.87	bm	54.6	2'	3a, 13a, 2'
3'	3.91	bm	54.6	2'	3a, 13a, 2'
4'			170.1		
4a'	4.65, 4.68	d (14.9)	68.4	4a'	4', 5'
5'			156.9		
6'	7.22	Obscured	108.7	None	13', 14'
7'			135.7		
8'	7.59-7.68	Obscured	130.7	9'	Obscured
9'	7.14-7.25	Obscured	124.9 or 127.4	8', 10'	Obscured
10'	7.14-7.26	Obscured	124.9 or 127.5	9', 11'	Obscured
11'	7.59-7.71	Obscured	128.6	10'	Obscured
12'			127.1		
13'	7.56	Obscured	130.7	14'	7', 11'
14'	7.19	Obscured	119.5	13'	12', 13'
1''	5.89	d (3.1)	96.3	2''	3''
2''	2.19	bm	36.4	1'', 3''	None
2''	2.37	dd (15.1, 2.6)	36.4	1'', 3''	None
3''	4.07	bd (3.1)	68.0	2'', 4''	None
3''-OH					
4''	3.27	dd (9.9, 3.2)	74.2	3'', 5''	None
4''-OH					
5''	3.96	dq (12.1, 5.3)	66.6	4'', 5''-CH_3	None
5''-CH_3	1.24	d (6.2)	18.3	5''	4'', 5''

(*) ^{13}C shifts were assigned by ^{13}C-NMR only and may be interchanged

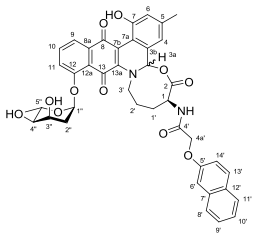
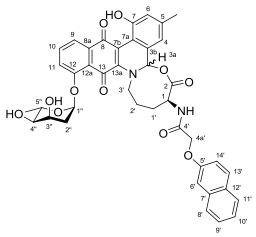


Table S4. Jadomycin Oct Naphthoxyacetylamide (**1b**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.38	bm	55.1	1'	2, 1', 2'
2			177.6		
3a	5.54	s	92.2	None	3b, 4, 7a, 13a, 3'
3b			130.8		
4	6.72	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a
5			141.6		
5-CH_3	2.34	s	21.1	4, 6	4, 5, 6
6	6.82	s	120.6	4, 5-CH_3	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.2		
7b*			127.8		
8			183.6		
8a*			127.5		
9	7.78	d (7.4)	121.5	10	8
10	7.66	Obscured	136.4	9, 11	Obscured
11	7.44	d (8.5)	121.3	10	12
12			155.7		
12a*			120.6		
13			183.9		
13a			151.5		
1'	1.9	bm	30.8	2'	None
1'	1.73	bm	30.8	2'	None
2'	1.65	bm	28.2	1', 3'	None
2'	1.72	bm	28.2	1', 3'	None
3'	3.7	bm	55.3	2'	None
3'	4.2	bm	55.3	2'	None
4'			170.2		
4a'	4.55, 4.68	d (14.9)	68.2	4a'	4', 5'
5'			157		
6'	7.14	Obscured	108.5	None	13', 14'
7'			135.7		
8'	7.59-7.68	Obscured	130.7	9'	Obscured
9'	7.14-7.25	Obscured	124.9 or 127.4	8', 10'	Obscured
10'	7.14-7.26	Obscured	124.9 or 127.5	9', 11'	Obscured
11'	7.59-7.71	Obscured	128.5	10'	Obscured
12'			127.1		
13'	7.66	Obscured	130.6	14'	7', 11'
14'	7.19	Obscured	119.5	13'	12', 13'
1''	5.85	d (3.1)	96.5	2''	3''
2''	2.17	bm	36.3	1'', 3''	None
2''	2.42	dd (15.1, 2.6)	36.3	1'', 3''	None
3''	4.09	bd (3.1)	68	2'', 4''	None
3''-OH					
4''	3.24	dd (9.9, 3.2)	74.2	3'', 5''	None
4''-OH					
5''	3.87	Obscured	66.5	4'', 5''-CH_3	None
5''-CH_3	1.19	d (6.2)	18.2	5''	4'', 5''

(*) ^{13}C shifts were assigned by ^{13}C-NMR only and may be interchanged



*Jadomycin Oct Benzoylamide (**1c**)*

Crude **1** (82 mg) was dissolved in 20 mL of a 1:1 mixture of CH_3CN:PBS buffer (pH 7.6), and added to a 50 mL round bottom flask containing **4** (67 mg, 0.306 mmol) drop-wise with gentle stirring. The flask was corked and protected from light, and the reaction was allowed to proceed for 16 hours. After 16 hours the reaction had not proceeded to completion as observed by the presence of **1** by TLC analysis. Another 36 mg (0.164 mmol) of **4** was dissolved in 5 mL of a 1:1 (CH_3CN:PBS) solution and added to the reaction flask. The reaction was allowed to proceed for an additional 2 hours until complete. The reaction mixture was diluted with 25 mL PBS, and extracted with dichloromethane (3 × 50 mL). The organic fractions were pooled and dried *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 3 mL) and filtered. Filtrate was then applied to a 40 g silica column preconditioned with a 1:1 EtOAc:CH_3CN solution. Material was eluted with a flow rate of 35 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system comprised of solvent A (1:1 EtOAc:CH_3CN), and solvent B (5:5:1 EtOAc:CH_3CN:H_2O). To start, an initial isocratic gradient step using solvent A (1 CV) was performed. This was followed by a linear increasing gradient of 0% ➔ 100% B over 10 CV, followed finally by an isocratic gradient of 100% B over 7.5 CV. Fractions were checked by TLC for the presence of the compound of interest, and combined and dried yielding 21 mg of crude **1c**. Prep TLC was then performed twice using the same 5:5:1 EtOAc:CH_3CN:H_2O solvent system yielding 15 mg. Final purification was accomplished by size exclusion chromatography (LH-20 resin). Material was eluted using 5:5:1 EtOAc:CH_3CN:H_2O and dried yielding **1c** as a purple solid (13 mg) as a mixture of diastereomers (M_j/M_n = 100/81) by NMR. TLC R_f: 0.43 (5:5:1 EtOAc:CH_3CN:H_2O), HPLC R_t = 9.13 min, NMR data to follow, for labeling scheme see **1c** NMR data table, UV-Vis (4.58×10^{-4} 5.73×10^{-5} M, MeOH): λ_{max} (ε) = 300 (13000), 388

(2647), 527 (1798); LRMS (ESI^+): MS/MS (655) found 655 $[M+H]^+$, 525 $[M+H-digitoxose]^+$, 306 $[M+H-digitoxose-C_{12}H_{13}NO_3]^+$, HRMS ($ESI^+$): 707.2202 Found, 707.2211 Calculated for $C_{37}H_{36}N_2NaO_{11}$.

Figure S4. Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **1** starting material (SM), the reaction mixture of **1** and **4** after 18 hours (Rxn), and the co-spot of both (Co).

Table S5. Jadomycin Oct Benzoylamide (**1c**) $3a_{Mj}$ diastereomer NMR data table.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.5	bs	55.1	1'	None
2			177.3		
3a	5.61	s	92.7	None	3b, 4, 7b, 13a, 3'
3b			130.1		
4	6.75	s	120.4	5-CH_3, 6	3a, 3b, 5-CH_3, 6, 7a
5			141.4		
5-CH_3	2.33	s	21.1	4, 6	4, 5, 6
6	6.81	s	120.6	4, 5-CH3	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.3		
7b			136.7		
8			183.4		
8a			136.7		
9	7.79	d (7.5)	121.3	10	8, 11
10	7.70	t (8.1)	136.6	9, 11	8a, 9, 11, 12
11	7.49	Obscured	120.6	10	9, 12, 12a, 13
12			155.7		
12a			121.6		
13			185.8		
13a			153.3		
1'	1.86	Broad Multiplet	30.7	1, 1', 2'	None
1'	2.05	Broad Multiplet	30.7	1, 1', 2'	None
2'	1.87	Broad Multiplet	28.9	1', 2', 3'	1
2'	1.95	Broad Multiplet	28.9	1', 2', 3'	1
3'	4.08-4.14	Broad Multiplet	54.4	2', 3'	3a, 13a, 1', 2'
3'	4.08-4.15	Broad Multiplet	54.4	2', 3'	3a, 13a, 1', 2'
4'			169.6		
4a'			135.7		
5'	7.83	d (7.4)	128.4	6'	4', 5', 7'
6'	7.44	Obscured	129.6	5', 7'	4a', 5', 6'
7'	7.51	Obscured	132.7	6'	5'
1''	5.89	d (3.1)	96.3	2''	3'', 5'', 12
2''	2.18 (bm)	Broad Multiplet	36.3	1'', 3''	1'', 3'', 4''
2''	2.34 (bm)	Broad Multiplet	36.3	1'', 3''	1'', 3'', 4''
3''	4.03	bd (2.7)	68.4	2'', 4''	none
3''-OH					
4''	3.26	t (2.4)	74.1	3'', 5''	5'', 5''-CH_3
4''-OH					
5''	3.89	bm	66.5	4'', 5''-CH_3	1'', 3'', 4''
5''-CH3	1.19	d (6.0)	18.2	5''	4'', 5''

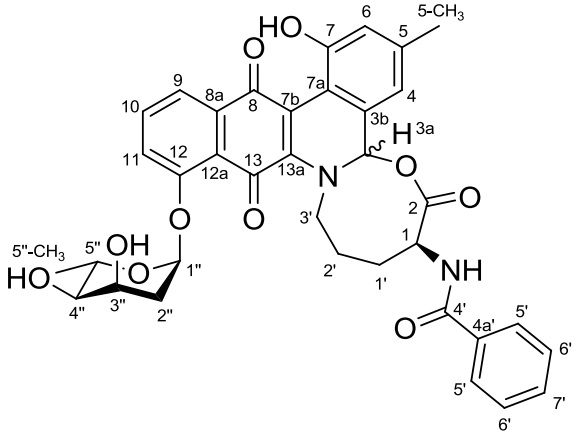
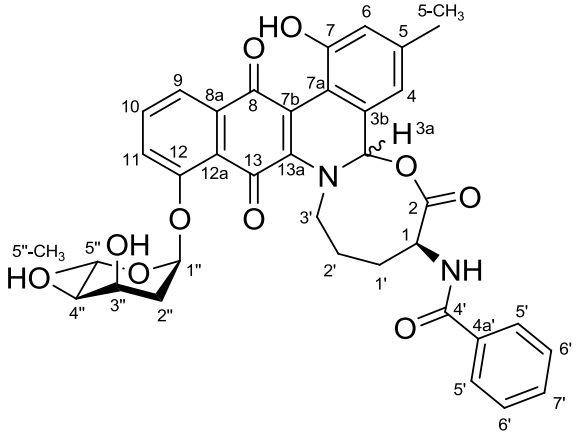


Table S6. Jadomycin Oct Benzoylamide (**1c**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.5	bs	55.1	1'	None
2			177.3		
3a	5.59	s	92.2	None	3b, 4, 7b, 13a, 3'
3b			131.0		
4	6.68	s	120.3	5-CH_3, 6	3a, 3b, 5-CH_3, 6, 7a
5			141.7		
5-CH_3	2.28	s	21.1	4, 6	4, 5, 6
6	6.79	s	120.6	4, 5-CH3	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.1		
7b			136.7		
8			184.1		
8a			136.7		
9	7.80	d (7.5)	121.3	10	8, 11
10	7.70	t (8.1)	136.6	9, 10	8a, 9, 11, 12
11	7.49	Obscured	120.6	10	9, 12, 12a, 13
12			156.3		
12a			121.6		
13			184.1		
13a			151.5		
1'	1.79	Broad Multiplet	30.7	1, 1', 2'	None
1'	2.00	Broad Multiplet	30.7	1, 1', 2'	None
2'	1.79	Broad Multiplet	28.5	1', 2', 3'	None
2'	1.90	Broad Multiplet	28.5	1', 2', 3'	None
3'	3.88-3.91	Obscured	54.8	2', 3'	3a, 13a, 1'
3'	4.33-4.37	Broad Multiplet	54.8	2', 3'	3a, 13a, 1'
4'			169.6		
4a'			135.7		
5'	7.85	d (7.4)	128.4	6'	4', 5', 7'
6'	7.44	Obscured	129.6	5', 7'	4a', 5', 6'
7'	7.51	Obscured	132.7	6'	5'
1''	5.90	d (3.1)	96.5	2''	3'', 5'', 12
2''	2.16 (bm)	Broad Multiplet	36.3	1'', 3''	1'', 3'', 4''
2''	2.37 (bm)	Broad Multiplet	36.3	1'', 3''	1'', 3'', 4''
3''	4.05	bd (2.7)	68.1	2'', 4''	none
3''-OH					
4''	3.25	t (2.4)	74.1	3'', 5''	5'', 5''-CH_3
4''-OH					
5''	3.89	bm	66.5	4'', 5''-CH_3	1'', 3'', 4''
5''-CH3	1.21	d (6.0)	18.2	5''	4'', 5''



*Jadomycin Oct Nonoylamide (**1d**)*

Crude **1** (98.7 mg) was dissolved in 20 mL of a 1:1 mixture of CH_3CN:PBS buffer (pH 7.6), and added to a 50 mL round bottom flask containing **5** (103 mg, 0.40 mmol) with gentle stirring at room temperature. The flask was corked and protected from light, and the reaction was allowed to proceed for 7 hours. The reaction mixture was diluted with 20 mL PBS, and extracted with dichloromethane (3 × 50 mL). The organic fractions were pooled and dried *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 4 mL) and filtered. Filtrate was then applied to a 40 g silica column preconditioned with a 1:1 EtOAc:CH_3CN solution. Material was eluted with a flow rate of 35 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system comprised of solvent A (1:1 EtOAc:CH_3CN), and solvent B (5:5:1 EtOAc:CH_3CN:H_2O). To start, an initial isocratic gradient step using solvent A (1 CV) was performed. This was followed by a linear increasing gradient of 0% ➔ 100% B over 10 CV, followed finally by an isocratic gradient of 100% B over 7.5 CV. Fractions were checked by TLC for the presence of the compound of interest, combined, and dried yielding 28 mg of crude **1d**. Prep TLC was then performed three times using the same 5:5:1 EtOAc:CH_3CN:H_2O solvent system yielding 13 mg. Final purification was accomplished by size exclusion chromatography (LH-20 resin). Material was dissolved and eluted using 5:5:1 EtOAc:CH_3CN:H_2O, this process was then repeated a second time. Compound **1d** was then dried *in vacuo*, re-dissolved in 10 mL of 1:1 H_2O:CH_3CN, and extracted with 3 × 10 mL of hexanes. The H_2O:CH_3CN layer was dried yielding **1d** as a purple solid (9 mg) as a mixture of diastereomers (M_j/M_n = 100/81) by NMR. TLC R_f: 0.52 (5:5:1 EtOAc:CH_3CN:H_2O); HPLC R_t = 6.95 and 7.19 min; NMR data to follow, for labeling scheme see **1d** NMR data table; UV-Vis (4.7×10^{-4} and 5.8×10^{-5} M, MeOH): λ_{max} (ε) = 312 (11745), 383 (2440), 527 (1474); LRMS (ESI^+): MS/MS (691) found 691 $[M+H]^+$,

561 [M+H−digitoxose]$^+$, 306 [M+H−digitoxose−$C_{14}H_{25}NO_3$]$^+$; HRMS (ESI$^+$): Found 691.3194, Calculated 691.3225 for $C_{41}H_{39}N_2O_{11}$.

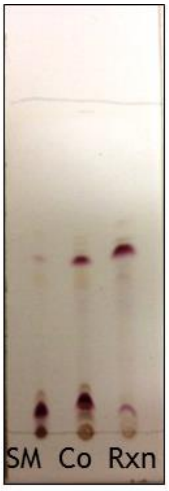


Figure S5. Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **1** starting material (SM), the reaction mixture of **1** and **5** after 7 hours (Rxn), and the co-spot of both (Co).

Table S7. Jadomycin Oct Nonoylamide (**1d**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.33	bs	54.3	None	None
2			Unobserved		
3a	5.6	s	92.5	None	4, 7a, 7b, 13a, 3'
3b			115.4		
4	6.76	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a, 7b
5			141.4		
5-CH_3	2.36	s	21.1	4, 6	4, 5, 6
6	6.82	s	120.6	5-CH_3, 6	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.3		
7b			130.3		
8			183.4		
8a			136.7		
9	7.82	d (7.5)	121.4	10	8, 10, 11
10	7.72	t (8.1)	136.7	9, 11	8a, 9, 11, 12
11	7.55	d (8.4)	120.6	10	9, 10, 12, 12a, 13
12			155.9		
12a			120.5		
13			185.6		
13a			152.8		
1'	1.80, 1.86	bm	30.4	1', 1', 2'	Not assignable
2'	1.73, 1.93	bm	28.6	1', 3'	Not assignable
3'	3.82, 4.22	bm	54.6	2'	Not assignable
4'			176.1		
4a'	2.23	bm	37.2	5'	4'
5'	1.58	bm	27.1	4a', 6'	4'
6'	1.2-1.7	bm	23.7, 30.4, 33.0	Obscured	Obscured
7'	1.2-1.7	bm	23.7, 30.4, 33.1	Obscured	Obscured
8'	1.2-1.7	bm	23.7, 30.4, 33.2	Obscured	Obscured
9'	1.2-1.7	bm	23.7, 30.4, 33.3	Obscured	Obscured
10'	1.2-1.7	bm	23.7, 30.4, 33.4	Obscured	Obscured
11'	0.84	t (7.0)	14.4	10'	Alkyl chain
1''	5.99	bs	96.2	2''	3'', 5''
2''	2.41	bm	36.3	1'', 3''	None
2''	2.23	bm	36.3	1'', 3''	None
3''	4.11	bm	68.2	2'', 4''	None
3''-OH					
4''	3.3	Obscured	74.1	3'', 5''	None
4''-OH					
5''	3.95	bm	66.6	4'', 5''-CH_3	None
5''-CH_3	1.22	d (5.8)	18.2	5''	None

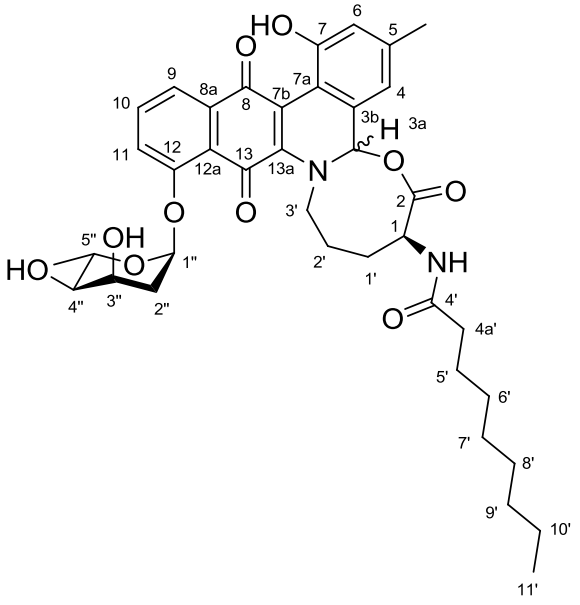
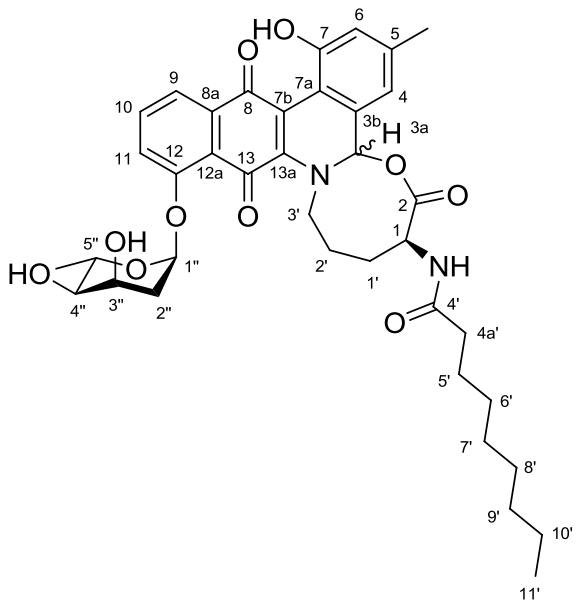


Table S8. Jadomycin Oct Nonoylamide (**1d**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.33	bs	54.3	None	None
2			Unobserved		
3a	5.6	s	92.1	None	4, 7a, 7b, 13a, 3'
3b			116.3		
4	6.76	s	120.3	5-CH_3, 6	3a, 5-CH_3, 6, 7a, 7b
5			141.5		
5-CH_3	2.36	s	21.1	4, 6	4, 5, 6
6	6.82	s	120.6	5-CH_3, 6	4, 5-CH_3, 6, 7, 7a
7			154.8		
7-OH					
7a			114.3		
7b			130.7		
8			183.7		
8a			136.7		
9	7.82 (d, 7.5)	d (7.5)	121.4	10	8, 10, 11
10	7.72 (t, 8.1)	t (8.1)	136.7	9, 11	8a, 9, 11, 12
11	7.54 (d, 8.4)	d (8.4)	120.6	10	9, 10, 12, 12a, 13
12			156.3		
12a			120.5		
13			184.1		
13a			152.1		
1'	1.73, 1.90	bm	30.5	1, 1', 2'	Not assignable
2'	1.67, 1.87	bm	28.3	1', 3'	Not assignable
3'	3.94, 4.16	bm	55.1	2'	Not assignable
4'			176.1		
4a'	2.23	bm	37.2	5'	4'
5'	1.58	bm	27.1	4a', 6'	4'
6'	1.2-1.7	bm	23.7, 30.4, 33.0	Obscured	Obscured
7'	1.2-1.7	bm	23.7, 30.4, 33.1	Obscured	Obscured
8'	1.2-1.7	bm	23.7, 30.4, 33.2	Obscured	Obscured
9'	1.2-1.7	bm	23.7, 30.4, 33.3	Obscured	Obscured
10'	1.2-1.7	bm	23.7, 30.4, 33.4	Obscured	Obscured
11'	0.81	t (7.0)	14.4	10'	Alkyl chain
1''	5.98	bs	96.3	2''	3'', 5''
2''	2.41	bm	36.3	1'', 3''	None
2''	2.23	bm	36.3	1'', 3''	None
3''	4.11	bm	68.9	2'', 4''	None
3''-OH					
4''	3.3	Obscured	74.1	3'', 5''	None
4''-OH					
5''	3.9	bm	66.6	4'', 5''-CH_3	None
5''-CH_3	1.22	d (5.8)	18.2	5''	None



*Jadomycin Oct Fmoc amide (**1e**)*

Crude **1** (171 mg) was dissolved in 20 mL of CH_3CN:PBS (1:1) and added to a 50 mL round bottom flask containing **6** (215 mg, 637 µmol) and stirred gently at room temperature. The reaction was determined to be complete after 3 hours by TLC analysis. The reaction was diluted with 10 mL PBS and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were combined and dried *in vacuo*, giving an impure reddish-purple solid (120 mg). The material was brought up in minimal (5:5:1) EtOAc:CH_3CN:PBS (pH 7.6) solvent and loaded onto an LH-20 column and eluted using the same solvent system at room temperature, collecting ~3 mL fractions. To each fraction, ~3 mL of PBS was added, resulting in the EtOAc to separate from the rest of the solution. All color was contained within the EtOAc layer. The fractions were concentrated down and analyzed by HPLC. Pure fractions were combined and dried. Impure fractions containing the compound of interest were pooled and dried *in vacuo*, and the process was repeated 4 more times (5 × LH-20 columns total). Pure material was brought up in ethyl acetate, filtered to remove any residual salt, and dried down yielding **1e** as a purple solid (3.2 mg) as a mixture of diastereomers (M_j/M_n = 4/3) by NMR. TLC R_f = 0.64 (5:5:1 EtOAc:CH_3CN:H_2O); HPLC R_t = 11.18 min & 11.63 min; NMR data to follow, for labeling scheme see **1e** NMR data table; UV-Vis (5.8×10^{-6} M and 7.3×10^{-8} M, MeOH): λ_{max} (ε) = 320 (12570), 386 (2638), 530 (1660); LRMS (ESI^+): Q1 found ; LC-MS/MS of 773 found 773 $[M+H]^+$, 643 $[M+H-L-digitoxose]^+$, 306 $[M+H-C_{26}H_{29}NO_7]^+$; HRMS (ESI-) for $C_{45}H_{43}N_2O_{12}$ $[M-H+MeOH]^-$: Found = 803.2838; Calculated = 803.2821 for $C_{45}H_{43}N_2O_{12}$.

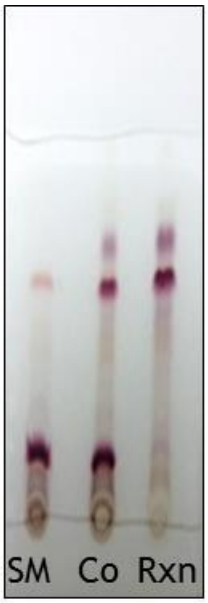


Figure S6. Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **1** starting material (SM), the reaction mixture of **1** and **6** after 3 hours (Rxn), and the co-spot of both (Co).

Table S9. Jadomycin Oct Fmoc amide (**1e**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.07	Obscured	57.3	1'	2, 1', 2'
2			178.6		
3a	5.61	s	92.6	None	3b, 4, 7a, 13, 13a, 3'
3b			130.3		
4	6.74	s	120.4	5-CH_3, 6	3a, 3b, 5-CH3, 6, 7, 7a
5			141.3		
5-CH_3	2.33	s	21.1	4, 6	4, 5, 6
6	6.83	s	120.6	4, 5-CH_3	4, 5-CH3, 7, 7a
7			154.8		
7-OH					
7a			114.3		
7b			136.6		
8			183.4		
8a			136.6		
9	7.8	d (7.2)	121.3	10	8, 10, 11, 12a
10	7.67	Obscured	136.6	9, 11	8a, 9, 11, 12, 12a
11	7.45	d (8.5)	120.8	10	9, 10, 11, 13
12			156.4		
12a			120.8		
13			185.6		
13a			152.9		
1'	1.83	Broad Multiplet	28.6	2'	None Observed
1'	1.83	Broad Multiplet	28.6	2'	None Observed
2'	1.74	Broad Multiplet	30.9	1', 3'	None Observed
2'	2.00	Broad Multiplet	30.9	1', 3'	None Observed
3'	4.01	Obscured	54.8	2'	3a, 13a, 1', 2'
3'	4.07	Obscured	54.8	2'	3a, 13a, 1', 2'
4'			158		
5'	4.21	Obscured	67.8	6'	4', 6', 7'
5'	4.34	dd (9.9, 6.9)	67.8	6'	4', 6', 7'
6'	4.2	Obscured	48.4	5'	5', 7', 12'
7'			145.5		
8'	7.59	d (7.4)	126.3	9'	6', 10', 12'
9'	7.21	Obscured	128.1	8', 10'	7', 11'
10'	7.31	Obscured	128.7	9', 11'	8', 12'
11'	7.71	d (7.6)	121	10'	7', 8', 9', 12'
12'			142.5		
1''	5.87	d, 3.2	96.4	2''	12, 3'', 5''
2''	2.15	dt (11.2, 3.6)	36.3	1'', 3''	1'', 3'', 4''
2''	2.36	Obscured	36.3	1'', 3''	1'', 3'', 4''
3''	4.06	Obscured	68.2	2'', 4''	Obs
3''-OH					
4''	3.25	dd (7.4, 3.4)	74.2	3'', 5''	5'', 5''-CH3
4''-OH					
5''	3.87	Obscured	66.6	4'', 5''-CH_3	5''-CH_3
5''-CH_3	1.19	d (6.2)	18.2	5''	4'', 5''

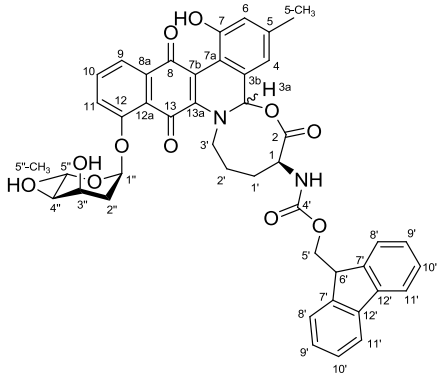
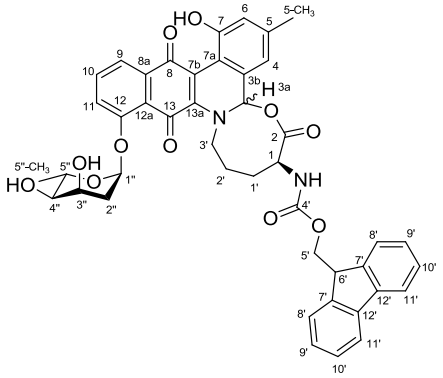


Table S10. Jadomycin Oct Fmoc amide (**1e**) $3a_{Mn}$ diastereomer NMR data table.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.02	Obscured	57.1	1'	2, 1', 2'
2			178.6		
3a	5.58	s	92.2	None	3b, 4, 7a, 13, 13a, 3'
3b			130.9		
4	6.73	s	120.4	5-CH_3, 6	3a, 3b, 5-CH3, 6, 7, 7a
5			141.6		
5-CH_3	2.3	s	21.1	4, 6	4, 5, 6
6	6.81	s	120.5	4, 5-CH_3	4, 5-CH3, 7, 7a
7			154.8		
7-OH					
7a			114.2		
7b			136.6		
8			183.9		
8a			136.6		
9	7.79	d (7.2)	121.5	10	8, 10, 11, 12a
10	7.67	Obscured	136.6	9, 11	8a, 9, 11, 12, 12a
11	7.47	d (8.5)	121.3	10	9, 10, 11, 13
12			155.9		
12a			120.8		
13			183.9		
13a			151.7		
1'	1.73	Broad Multiplet	28.2	2'	None Observed
1'	1.73	Broad Multiplet	28.2	2'	None Observed
2'	1.7	Broad Multiplet	31.2	1', 3'	None Observed
2'	1.86	Broad Multiplet	31.2	1', 3'	None Observed
3'	3.78	Broad Multiplet	55.5	2'	3a, 13a, 1', 2'
3'	4.23	Obscured	55.5	2'	3a, 13a, 1', 2'
4'			158.1		
5'	4.11 (obs)	Obscured	67.4	6'	4', 6', 7'
5'	4.45	dd (10.7, 6.9)	67.4	6'	4', 6', 7'
6'	4.13 (obs)	Obscured	48.5	5'	5', 7', 12'
7'			145.2		
8'	7.63	Obscured	126.3	9'	6', 10', 12'
9'	7.19	Obscured	128.1	8', 10'	7', 11'
10'	7.27	Obscured	128.7	9', 11'	8', 12'
11'	7.66	Obscured	121	10'	7', 8', 9', 12'
12'			142.5		
1''	5.82	d (3.2)	96.6	2''	12, 3'', 5''
2''	2.14	dt (11.2, 3.6)	36.3	1'', 3''	1'', 3'', 4''
2''	2.36	Obscured	36.3	1'', 3''	1'', 3'', 4''
3''	4.06	Obscured	68	2'', 4''	Obs
3''-OH					
4''	3.23	dd (7.4, 3.4)	74.1	3'', 5''	5'', 5''-CH3
4''-OH					
5''	3.82	Obscured	66.6	4'', 5''-CH_3	5''-CH_3
5''-CH_3	1.12	d (6.2)	18.2	5''	4'', 5''



*Jadomycin Oct BODIPY amide (**1f**)*

Compound **7** (35 mg, 0.80 mmol) was dissolved in 10 mL CH_3CN with gentle stirring. Crude **1** (42 mg) was brought up in 10 mL PBS buffer (pH 7.6), and added to the solution of **7** drop-wise. The reaction flask was protected from light and the reaction was allowed to proceed for 5 hours. After 5 hours the reaction had not proceeded to completion as observed by the presence of **1** by TLC analysis. Another 16 mg (0.035 mmol) of **7** was dissolved in 5 mL of a 1:1 (CH_3CN:PBS) solution and added to the reaction flask. The reaction was allowed to proceed for an additional 2 hours until complete. The reaction mixture was diluted with 25 mL PBS, and extracted with dichloromethane (3 × 50 mL). The organic fractions were pooled and dried *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 5 mL) and filtered. Filtrate was then applied to a 40 g silica column preconditioned with a 1:1 EtOAc:CH_3CN solution. Material was eluted with a flow rate of 30 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system comprised of solvent A (1:1 EtOAc:CH_3CN), and solvent B (5:5:1 EtOAc:CH_3CN:H_2O). To start, an initial isocratic gradient of 100% A (1 CV) was performed. This was followed by a linear increasing gradient of 0% ➔ 100% B over 10 CV, followed by an isocratic gradient of 100% B over 5 CV. Fractions were checked by TLC for the presence of the compound of interest, and combined and dried yielding crude **1f (**22 mg). Prep TLC was then performed using the same 5:5:1 EtOAc:CH_3CN:H_2O solvent system yielding 11 mg of partially pure **1f**. Final purification was accomplished by size exclusion chromatography (LH-20 resin). Material was eluted using 5:5:1 EtOAc:CH_3CN:H_2O and dried yielding 6 mg of **1f** as a solid pink-red compound. TLC R_f: 0.41 (5:5:1 EtOAc:CH_3CN:H_2O); HPLC R_t = 12.3 min; NMR data to follow, for labeling scheme see **1f** NMR data table; UV-Vis (7.8×10^{-6} and 1.95×10^{-6} M, MeOH): λ_{max} (ε) = 285 (13128), 319 (14333), 377 (8038), 495 (23487), 526 (49538); LRMS

(ESI$^+$): MS/MS (881) found 881 $[M+H]^+$, 751 [M+H−digitoxose]$^+$, 306 [M+H−digitoxose−$C_{23}H_{30}BF_2N_3O_3$]$^+$; HRMS (ESI$^+$): Found 881.3698 $[M+H]^+$, Calculated 881.3739 $[M+H]^+$ for $C_{47}H_{52}BF_2N_4O_{10}$.

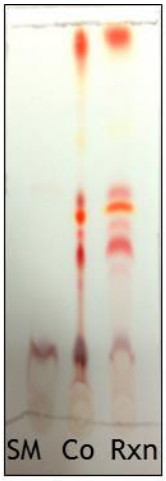


Figure S7. Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **1** starting material (SM), the reaction mixture of **1** and **7** after 7 hours (Rxn), and the co-spot of both (Co).

Table S11. Jadomycin Oct BODIPY amide (**1f**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.28	bs	55.2	1'	2, 1', 2', 4'
2			178.1		
3a	5.47	s	91.7	None	3b, 4, 7a, 13a, 3'
3b			129.9		
4	6.68	s	120.4	5-CH3, 6	3a, 5, 5-CH_3, 6, 7a
5			141.1		
5-CH_3	2.35	s	21.1	4, 6	4, 5, 6
6	6.80	s	120.7	4, 5-CH3	4, 5-CH_3, 6, 7, 7a
7			154.8		
7-OH					
7a			114.4		
7b			120.5		
8			183.0		
8a			137.0		
9	7.77	d (7.6)	121.5	10	8, 8a, 11
10	7.67	t (8.2)	136.5	9, 11	8a, 12
11	7.49	d (8.6)	120.4	10	9, 12, 13
12			155.9		
12a			120.7		
13			183.1		
13a			153.2		
1'	1.71, 1.83	m	30.8	1, 2'	None
2'	1.60, 1.84	m	27.1	1', 3'	None
3'	3.60, 3.85	m	55.0	2'	3a, 1', 2'
4'			174.9		
4a'	2.36, 2.60	m	37.0	5a'	4', 5a', 5b'
5a'	2.66, 2.81	m	21.1	4a'	4', 4a', 5b' 6', 11b'
5b'			129.3		
6'			155.1		
6'-CH_3	2.39	s	12.8	None	5b', 6'
7'			156.0		
7'-CH_3	2.17	s	12.4	None	7', 8'
8'			132.9		
8'-CH_2	2. 28, 2.35	obscured	18.0	8'-CH3	7', 8', 8'-CH_3, 9a'
8'-CH_3	1.01	t (7.5)	14.8	8'-CH2	8', 8'-CH_2
9a'			138.6		
9a'-CH_3	1.88	s	9.2	None	8', 9a', 9b'
9b'			133.9		
10'	6.99	s	120.5	None	9a', 9b', 11a'
11a'			133.8		
11b'			139.3		
11b'-CH_3	2.21	s	9.9	None	5b', 11a', 11b'
1''	6.06	d (2.5)	95.7	2''	3'', 5''
2''	2.24	obscured	36.1	1'', 2'', 3''	1'', 3'', 4''
2''	2.46	obscured	36.1	1'', 2'', 3''	1'', 3'', 4''
3''	4.13	q (3.1)	68.2	2'', 4''	None
3''-OH					
4''	3.23	m	74.4	3'', 5''	None
4''-OH					
5''	3.81	m	66.7	4'', 5''-CH3	None
5''-CH_3	1.18	d (6.2)	18.3	5''	4'', 5''

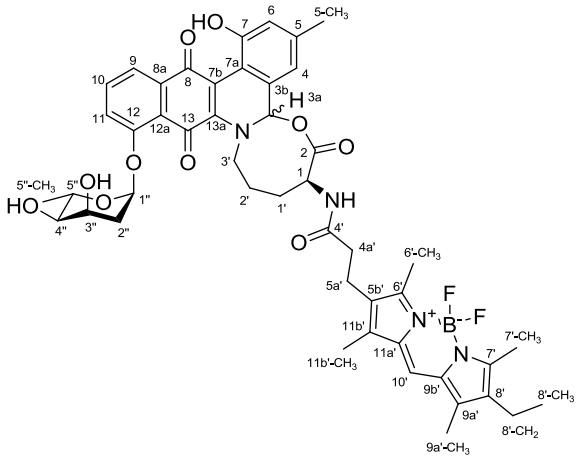
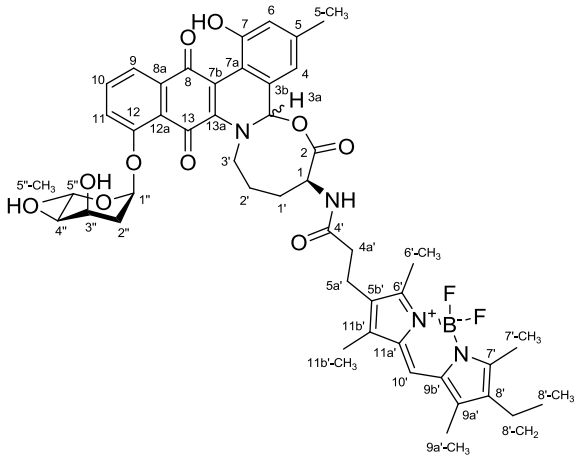


Table S12. Jadomycin Oct BODIPY amide (**1f**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	4.29	bs	55.4	1'	1', 2, 2'
2			177.8		
3a	5.49	s	92.3	None	3b, 4, 7a, 13a, 3'
3b			130.3		
4	6.73	s	120.4	5-CH3, 6	3a, 5, 5-CH_3, 6, 7a
5			141.4		
5-CH_3	2.34	s	21.1	4, 6	4, 5, 6
6	6.81	s	120.7	4, 5-CH3	4, 5-CH_3, 6, 7, 7a
7			154.9		
7-OH					
7a			114.1		
7b			120.5		
8			183.9		
8a			136.4		
9	7.73	d (7.6)	121.3	10	8, 11
10	7.65	t (8.2)	136.4	9, 11	8a, 12
11	7.47	d (8.6)	120.4	10	9, 12, 13
12			155.9		
12a			120.7		
13			184.7		
13a			152.1		
1'	1.71, 1.83	m	30.4	1, 2'	None
2'	1.60, 1.84	m	27.1	1', 3'	None
3'	3.60, 3.85	m	55.0	2'	3a, 1', 2'
4'			174.8		
4a'	2.36, 2.60	m	37.4	5a'	4', 5a', 5b'
5a'	2.66, 2.81	m	21.4	4a'	4', 4a', 5b' 6', 11b'
5b'			129.7		
6'			155.3		
6'-CH_3	2.44	s	12.8	None	5b', 6'
7'			155.9		
7'-CH_3	2.30	s	12.6	None	7', 8'
8'			133.0		
8'-CH_2	2. 28, 2.35	obscured	18.0	8'-CH3	7', 8', 8'-CH_3, 9a'
8'-CH_3	0.97	t (7.5)	14.8	8'-CH2	8', 8'-CH_2
9a'			138.9		
9a'-CH_3	2.05	s	9.3	None	8', 9a', 9b'
9b'			134.0		
10'	7.16	s	120.9	None	9b', 11a'
11a'			133.8		
11b'			139.3		
11b'-CH_3	2.18	s	9.7	None	5b', 11a', 11b'
1''	5.95	d (2.5)	96.0	2''	3'', 5''
2''	2.18	obscured	36.3	1'', 2'', 3''	None
2''	2.36	obscured	36.3	1'', 2'', 3''	None
3''	4.08	q (3.1)	68.3	2'', 4''	None
3''-OH					
4''	3.25	m	74.2	3'', 5''	None
4''-OH					
5''	3.87	m	66.5	4'', 5''-CH3	None
5''-CH_3	1.18	d (6.2)	18.2	5''	4'', 5''



Jadomycin Oct (1) Purification and Characterization

Growth and crude purification of **1** was accomplished as previously described. Crude aqueous extract containing **1** (208 mg) was dissolved in minimal methanol, filtered, absorbed onto Isolute® HM-N, and dried *in vacuo*. The isolute containing crude **1** was then applied to an 80 g silica column preconditioned with dichloromethane. Material was eluted with a flow rate of 50 mL/min collecting 9 mL fractions. Purification was accomplished using a gradient system comprised of solvent A (dichloromethane), and solvent B (methanol). To start, an initial isocratic gradient step using solvent A (1 CV) was performed. This was followed by a linear increasing gradient of 0% ➔ 100% B over 10 CV. Fractions were checked by TLC for the presence of the compound of interest, combined and dried, yielding 46 mg of crude **1**. Prep TLC was then performed three times, first using a 9:1 (dichloromethane:methanol) solvent system, followed by two more prep TLCs using the 5:5:1 (EtOAc:CH_3CN:H_2O) solvent system yielding 12 mg. Final purification was accomplished by size exclusion chromatography (LH-20 resin). Material was eluted using 100% methanol. The compound was then dried *in vacuo*, yielding **1** as a purple solid (3 mg) as a mixture of diastereomers (M_j/M_n = 100/82) by NMR. Compound **1** proved to be very unstable, with significant breakdown occurring over time (within hours). TLC R_f: 0.074 (5:5:1 EtOAc:CH_3CN:H_2O); HPLC R_t = 6.47 and 6.76 min (broad peaks); NMR data to follow, for labeling scheme see **1** NMR data table; UV-Vis (8.17×10^{-4} and 1.02×10^{-4} M, MeOH): λ_{max} (ε) = 319 (8941), 388 (1850), 541 (1218) ; LRMS (ESI+): MS/MS (551) found 551 $[M+H]^+$, 436 $[M+H-C_5H_9NO_2]^+$, 306 $[M+H-\text{digitoxose}-C_{12}H_{13}NO_3]^+$; HRMS (ESI+): found 573.1834, calculated 573.1844 for $C_{37}H_{36}N_2NaO_{11}$.

Table S13. Jadomycin Oct (**1**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	3.78	bm	55.1	1'	None
2			Unobserved		
3a	5.66	s	92.1	None	3b, 4, 7a, 13a, 3'
3b			129.8		
4	6.77	d (1.5)	120.4	5-CH_3, 6	3a, 5-CH3, 6, 7a
5			141.5		
5-CH_3	2.36	s	20.9	None	4, 5, 6
6	6.83	d (1.5)	120.6	4, 5-CH_3	4, 5-CH3, 7, 7a
7			154.6		
7-OH					
7a			114.3		
7b			Unobserved		
8			183.1		
8a			136.2		
9	7.83	dd (7.6, 0.9)	121.3	10	8, 11
10	7.73	t (8.1)	135.3	9, 11	8a, 12
11	7.55	bd (7.6)	120.5	10	9, 12a
12			155.4		
12a			120.1		
13			Unobserved		
13a			153.0		
1'	1.82-2.03	bm	28.9	1, 1', 2'	None
2'	1.82-2.04	bm	26.5	1', 2' 3'	None
3'	3.80, 4.20	dd (10.7, 3.6)	55.2	2', 3'	None
1''	6.03	d (3.1)	96.0	2''	3'', 5''
2''	2.23	m	36.0	1'', 3''	None
2''	2.39	dd (obscured)	36.0	1'', 3''	None
3''	4.10	m (obscured)	68.5	2'', 4''	None
3''-OH					
4''	3.33	Obscured	73.8	3'', 5''	None
4''-OH					
5''	3.92	m	66.5	4'', 5''-CH_3	None
5''-CH_3	1.21	d (6.2)	18.3	5''	4'', 5''

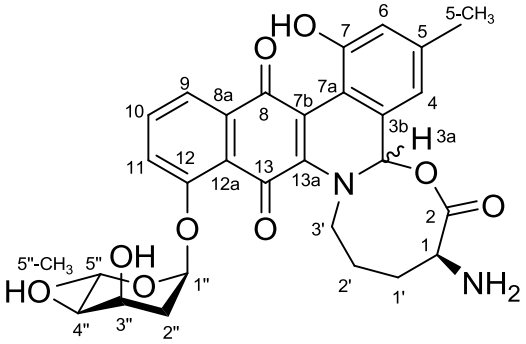
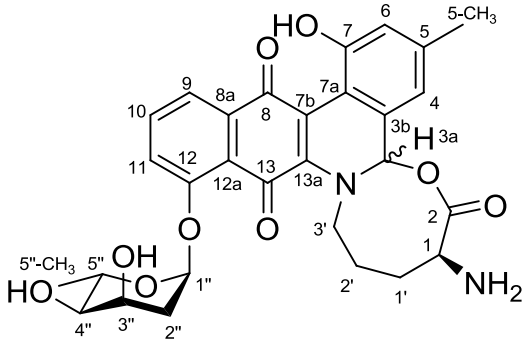


Table S14. Jadomycin Oct (**1**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	3.72	bm	55.4	1'	None
2			Unobserved		
3a	5.55	s	92.5	None	3b, 4, 7a, 13a, 3'
3b			130.6		
4	6.77	d (1.5)	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a
5			141.5		
5-CH_3	2.36	s	20.9	None	4, 5, 6
6	6.84	d (1.5)	120.6	4, 5-CH_3	4, 5-CH_3, 7
7			Unobserved		
7-OH					
7a			113.8		
7b			Unobserved		
8			184.2		
8a			136.2		
9	7.84	dd (7.6, 0.9)	121.3	10	8, 11
10	7.72	t (8.1)	135.3	9, 11	8a, 12
11	7.54	bd (7.6)	120.5	10	9, 12a
12			155.4		
12a			120.1		
13			Unobserved		
13a			149.7		
1'	1.82-2.03	bm	28.9	1, 1', 2'	None
2'	1.82-2.04	bm	26.5	1', 2' 3'	None
3'	3.70, 4.12	Obscured	54.4	2', 3'	None
1''	5.98	d (3.1)	95.8	2''	3'', 5''
2''	2.23	m	36.0	1'', 3''	None
2''	2.39	Obscured	36.0	1'', 3''	None
3''	4.12	m (obscured)	68.5	2'', 4''	None
3''-OH					
4''	3.3	Obscured	74.0	3'', 5''	None
4''-OH					
5''	3.86	m	66.4	4'', 5''-CH_3	None
5''-CH_3	1.2	d (6.2)	18.3	5''	4'', 5''



Deprotection of 1e

As a potential method for enhanced isolation and purification of **1**, a removable fluorenylmethyloxycarbonyl (Fmoc) protecting group was introduced in the case of **1e**. We had anticipated **1e** would prove more stable compared to **1** allowing for easier purification in higher yields. Counterintuitively, the stability of **1e** was poor and purification was challenging with a great deal of material degrading during this process. Nevertheless, **1e** was treated with 1,8-diazabicycloundec-7-ene (DBU) and SiliaBond® Piperazine in a buffered organic solvent system (Scheme 2). This was followed by workup providing the starting material **1** (1.3 mg, 61% yield). The identity of **1** was confirmed by HPLC, LC-MS/MS, and TLC.

Compound **1e** (3 mg, 3.9 μmol) was dissolved in 1 mL (5:5:1) EtOAc:CH_3CN:PBS (pH 7.6). The solution of **1e** was added to a vial containing 51 mg of Si-PPZ resin (46 μmol, 11.6 equivalents) with gentle stirring. To this solution 500 μL of a 1.5% (v/v) 1,8-Diazabicycloundec-7-ene solution (45 μmol, 11 equivalents) in 5:5:1 (EtOAc:CH_3CN:PBS) was added. The reaction flask was protected from light and the reaction was allowed to proceed for 3 min. The reaction mixture was centrifuged at 13000 RPM (4°C) for 5 min, and the supernatant was removed from the Si-PPZ. The resin was washed twice with 0.5 mL of 5:5:1 (EtOAc:CH_3CN:PBS) and centrifuged after each wash to ensure removal of all resin. Supernatant fractions were pooled (~ 2.5 mL), filtered and loaded onto an LH-20 resin size exclusion column preconditioned in 5:5:1 (EtOAc:CH_3CN:H_2O). Elution was accomplished using the same 5:5:1 (EtOAc:CH_3CN:H_2O) solvent system. Fractions were checked by TLC and HPLC for the presence of the compound of **1**, combined and dried yielding **1 (**1.3 mg, 61% yield). R_f: 0.074 (5:5:1 EtOAc:CH_3CN:H_2O);

HPLC R_t = 6.47 and 6.76 min (broad peaks); LRMS (ESI+): MS/MS (551) found 551 $[M+H]^+$, 436 $[M+H-C_5H_9NO_2]^+$, 306 $[M+H-\text{digitoxose}-C_{12}H_{13}NO_3]^+$.

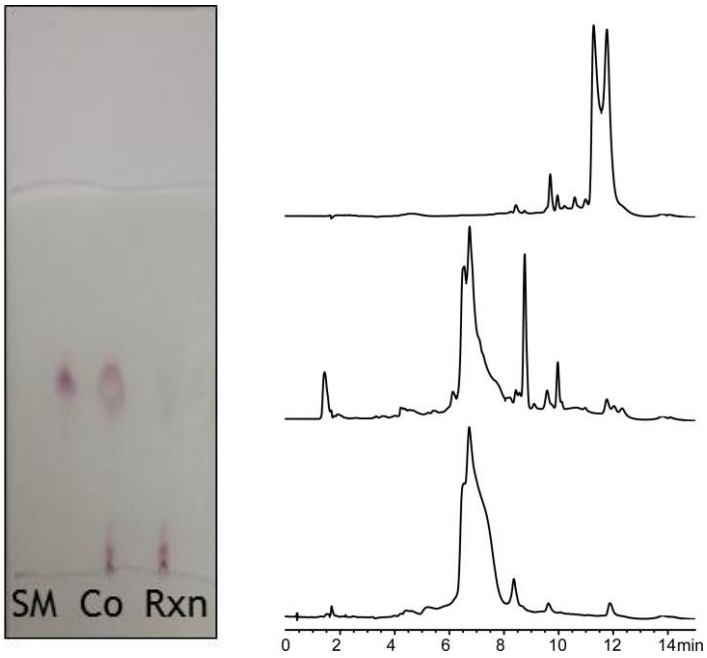


Figure S8. (Left) Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of **1e** starting material (SM), the reaction mixture after 2 minutes (Rxn), and the co-spot of both (Co); and HPLC comparison of starting material **1e** (top), reaction mixture after 3 min (middle) showing the formation of **1**, and the purified reaction mixture containing **1** (bottom).

Jadomycin AVA (8) Purification and Characterization

Crude **8** (16.4 mg) was purified using prep TLC using 15% MeOH in DCM as the elution system yielding 15.2 mg. This material was further purified using prep TLC using 1:1 EtOAc:MeCN followed by 5:5:1 EtOAc:MeCN:H_2O as the elution system, with drying in between, yielding 5.2 mg. Final purification was accomplished using prep TLC using 15% MeOH in DCM as the elution system yielding **8** as a reddish-purple solid (2 mg) as a mixture of diastereomers (Mj/Mn = 100:67) by NMR. TLC R_f: 0.54 (10% MeOH in DCM); HPLC R_t = 8.57 min; NMR data to follow, for labeling scheme see **8** NMR data table; UV-Vis (3×10^{-4} and 3×10^{-5} M, MeOH): λ_{max} (ε) = 311 (10087), 522 (1167); LRMS (ESI+): MS/MS (536) found 536 $[M+H]^+$, 406 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-digitoxose-C5H10O2}]^+$; HRMS (ESI+): 558.1753 found, 558.1735 calculated for $C_{29}H_{29}NNaO_9$.

Table S15. Jadomycin AVA (**8**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	2.16 - 2.21	obscured	38.2	1'	2
2			182.0		
3a	5.64	s	92.6		3', 4, 7a, 7b, 13, 13a
3b					
4	6.77	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a, 7b
5			141.3		
5-CH_3	2.36	s	21.1	4, 6	4, 5, 6
6	6.82	s	120.6	4, 5-CH_3	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.3		
7b			129.9		
8			183.0		
8a			136.8		
9	7.81	d(7.5)	121.3	10	8, 8a, 10, 11
10	7.72	t(8.1)	136.7	9, 11	8a, 9, 11, 12
11	7.54	d(8.1)	120.6	10	9, 10, 12, 12a, 13
12			155.8		
12a			121.6		
13			185.8		
13a			154.0		
1'	1.16 - 1.68	bm	24.5	1, 2'	1, 2, 2', 3'
2'	1.72	obscured	32.2	1', 3'	1, 1', 3'
3'	3.98, 4.11	obscured	54.8	2', 3'	1', 2', 3a, 13a
1''	5.95	d(2.9)	96.5	2''	2'', 3'', 5'', 12
2''	2.22	bm	36.4	1'', 2'', 3''	
2''	2.41	bm	36.4	1'', 2'', 3''	
3''	4.08	obscured	68.4	5''-CH_3, 2'', 4', 5''	
3''OH					
4''	3.28	obscured	74.1	3'', 5''	5'', 5''-CH_3
4''-OH					
5''	3.93	bm	66.4	4'', 3'', 5''-CH_3	
5''-CH_3	1.22	d(6.2)	18.2	3'', 5''	4'', 5''
MeOH	3.31		49.0		

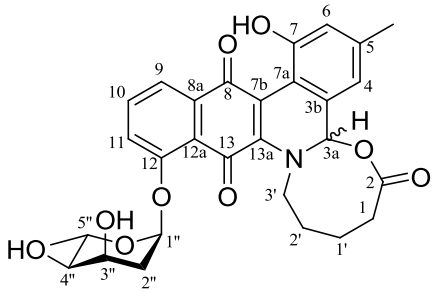
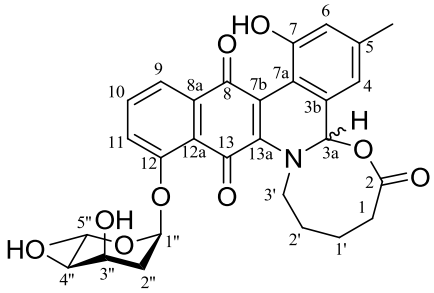


Table S16. Jadomycin AVA (**8**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	2.16 - 2.21	obscured	38.2	1'	2
2			182.0		
3a	5.61	s	92.2		3', 4, 7a, 7b, 13, 13a
3b					
4	6.78	s	120.4	5-CH_3, 6	3a, 5-CH_3, 6, 7a, 7b
5			141.7		
5-CH_3	2.36	s	20.9	4, 6	4, 5, 6
6	6.82	s	120.6	4, 5-CH_3	4, 5-CH_3, 7, 7a
7			154.8		
7-OH					
7a			114.2		
7b			131.3		
8			184.0		
8a			136.8		
9	7.85	d(7.5)	121.6	10	8, 8a, 10, 11
10	7.72	t(8.1)	136.7	9, 11	8a, 9, 11, 12
11	7.56	d(8.1)	121.3	10	9, 10, 12, 12a, 13
12			156.4		
12a			121.0		
13			184.2		
13a			151.3		
1'	1.16 - 1.68	bm	24.5	1, 2'	1, 2, 2', 3'
2'	1.68 - 1.86	obscured	32.0	1', 3'	1, 1', 3'
3'	3.80, 4.36	bm	55.6	2', 3'	1', 2', 3a, 13a
1''	5.93	d(2.9)	96.8	2''	2'', 3'', 5'', 12
2''	2.22	bm	36.4	1'', 2'', 3''	
2''	2.41	bm	36.4	1'', 2'', 3''	
3''	4.08	obscured	68.1	5''-CH_3, 2'', 4', 5''	
3''OH					
4''	3.28	obscured	74.1	3'', 5''	5'', 5''-CH_3
4''-OH					
5''	3.93	bm	66.5	4'', 3'', 5''-CH_3	
5''-CH_3	1.22	d(6.2)	18.2	3'', 5''	4'', 5''
MeOH	3.31		49.0		



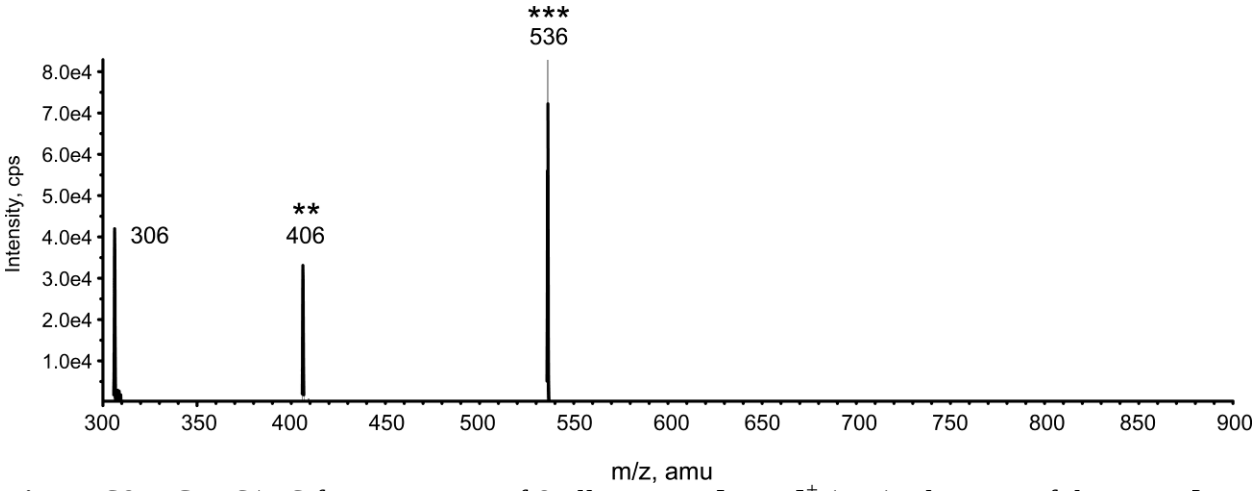


Figure S9. LC-MS/MS fragmentation of **8**, illustrating $[M+H]^+$ (***), cleavage of the sugar [M + H – digitoxose]$^+$ (**) and the amino acid groups [M + H – digitoxose – R]$^+$ (*m*/*z* 306).

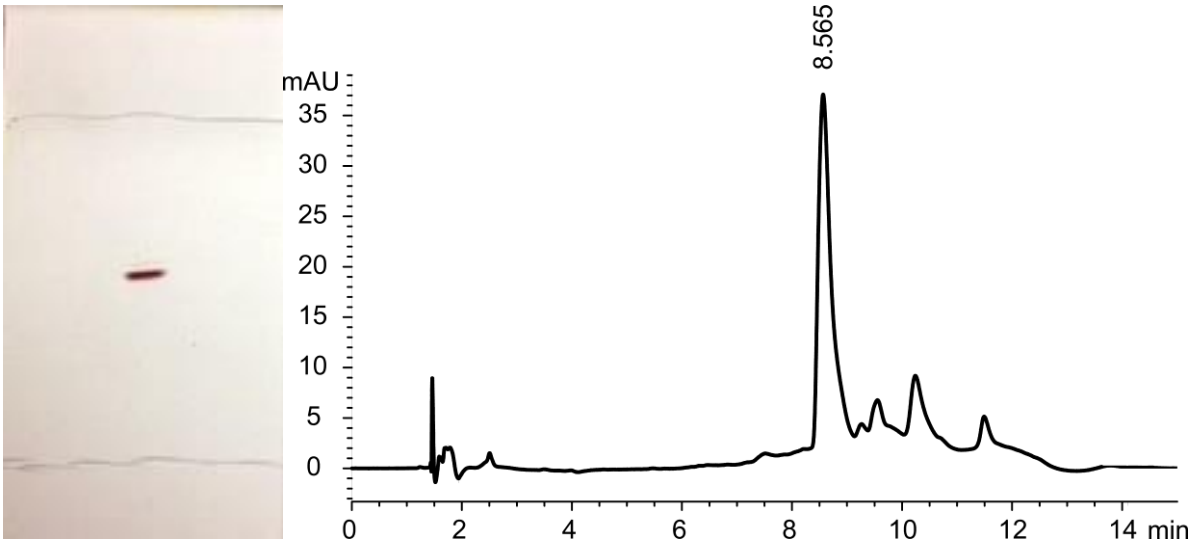


Figure S10. (Left) Normal phase silica TLC (9:1, DCM:MeOH) of purified **8**; and (right) HPLC of purified **8** showing separation of diastereomers. Ratio of diastereomers by HPLC matches ratio by NMR.

Jadomycin DOct (9) Crude Isolation

Due to a lack of material (5 mg crude extract), purification of compound **9** was not attempted. LC-MS/MS performed on the crude extract used for further derivatization showed the same fragmentation pattern observed for compound **1**, suggesting similar chemical properties (Figure S11). For crude extraction procedure see: *Bacteria Maintenance and Growth: Jadomycin Oct (**1**), Jadomycin AVA (**8**) and Jadomycin DOct (**9**) Production*

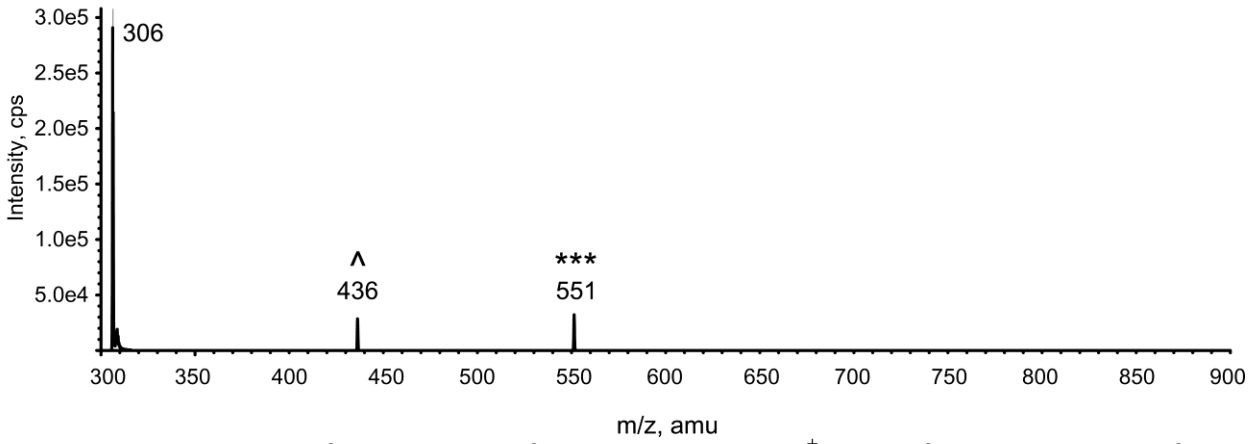


Figure S11. LC-MS/MS fragmentation of **9** illustrating $[M+H]^+$ (***),of amino acid group first $[M+H–R]^+$ (^) and cleavage of the sugar $[M + H – digitoxose – R]^+$ (*m*/*z* 306).

Jadomycin DOct Amide Synthesis 9a

*Jadomycin DOct Phenoxyacetylamide (**9a**)*

Crude **9** (5 mg) was dissolved in 5 mL of a 1:1 mixture of CH_3CN:PBS buffer (pH 7.6), and added to solid compound **2** (9 mg, 18 μmol) in a 25 mL round bottom flask drop-wise with gentle stirring. The flask was corked and protected from light, and the reaction was allowed to proceed for 2 hours. After 2 hours the reaction had not proceeded to completion as observed by the presence of **9** by TLC analysis. Another 5 mg (9 μmol) of **2** was dissolved in 1 mL of a 1:1 (CH_3CN:PBS) solution and added to the reaction flask. The reaction was allowed to proceed for an additional 2 hours until complete. The reaction mixture was dried *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 1 mL), loaded onto prep TLC plate a developed 3× using a 5:5:1 EtOAc:CH_3CN:H_2O solvent system. The band of interest was scraped from the plate and the compound was eluted with 100% methanol yielding >1 mg of the purple solid **9a** as as a mixture of diastereomers (M_j/M_n = 100:65) by NMR. TLC R_f: 0.36 (5:5:1 EtOAc:CH_3CN:H_2O), HPLC R_t = 8.90 min; LRMS (ESI^+): MS/MS (685) found 685 $[M+H]^+$, 555 $[M+H-\text{digitoxose}]^+$, 306 $[M+H-\text{digitoxose}-C_{13}H_{15}NO_4]^+$.

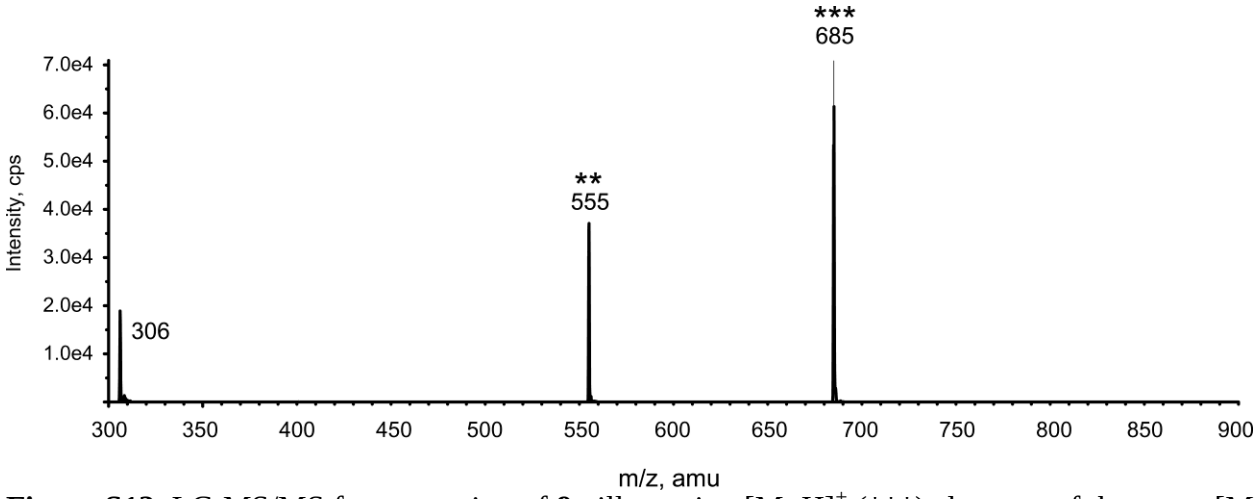


Figure S12. LC-MS/MS fragmentation of **9a** illustrating $[M+H]^+$ (***),cleavage of the sugar $[M + H - \text{digitoxose}]^+$ (**) and the amino acid groups $[M + H - \text{digitoxose} - R]^+$ (*m*/*z* 306).

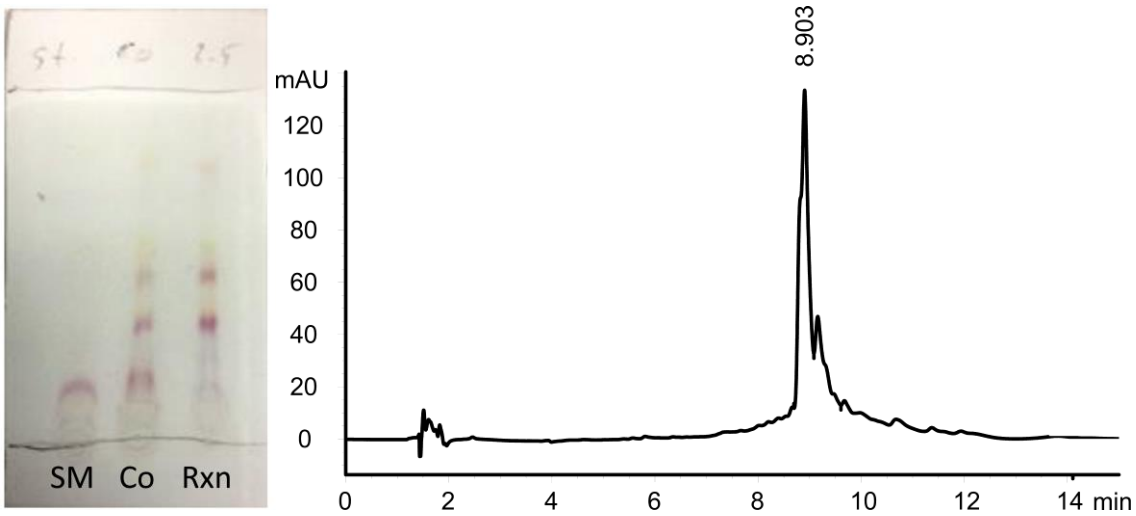


Figure S13. (Left) Normal phase silica TLC (5:5:1, EtOAc:CH_3CN:H_2O) comparison of crude **9** starting material (SM), the reaction mixture of **9** and **2** after 2.5 hours (Rxn), and the co-spot of both (Co); and (right) HPLC of purified **9a**.

Biological Activity Evaluation

Photo-mediated DNA cleavage assays

Supercoiled plasmid (form I) was prepared by transformation of NovaBlue cells (Novagen) followed by purification using the QIAprep Spin miniprep kit (Qiagen) to yield approximately 30 μg of plasmid DNA per 20 mL culture. Jadomycins **1a-1d** were dissolved in 99% ethanol, and subsequent dilutions were made with distilled water, where the final assay tubes contained <1% ethanol. Reaction mixtures (20 μL total volume) were prepared in 0.5 mL sterile microcentrifuge tubes. Transformed pUC19 plasmid (final concentration 130 ng, or 20 μM bases, >95% form I) was delivered to the assay tubes as a solution in 10 mM Tris-Cl (pH 8.5) and diluted with Tris (pH 7.4, final concentration 5 mM) and NaCl (final concentration 50 mM). Solutions of jadomycins were added to give the final concentrations (10, 25, 50, 75, 100 μM) and the reaction mixtures were diluted to a final volume of 20 μL with ultrapure water. Sample tubes were kept at 37°C in the dark, or irradiated with white light in a photoreactor (Luzchem LZC-4X, 7.72 mW cm^{-2}) for 30 min in order to yield an energy density of approximately 14 J cm^{-2}. After treatment, all samples (dark and light) were quenched by the addition of 4 μL gel loading buffer (0.025% bromophenol blue, 40% glycerol), loaded onto 1% agarose gels cast with 1 × TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.2) containing ethidium bromide (0.75 μg mL^{-1}) and electrophoresed for 30 min at 80 V cm^{-1} in 1 × TAE. The bands were visualized with UV-transillumination (UVP transilluminator) and processed using the Gel Doc-It Imaging System (UVP).

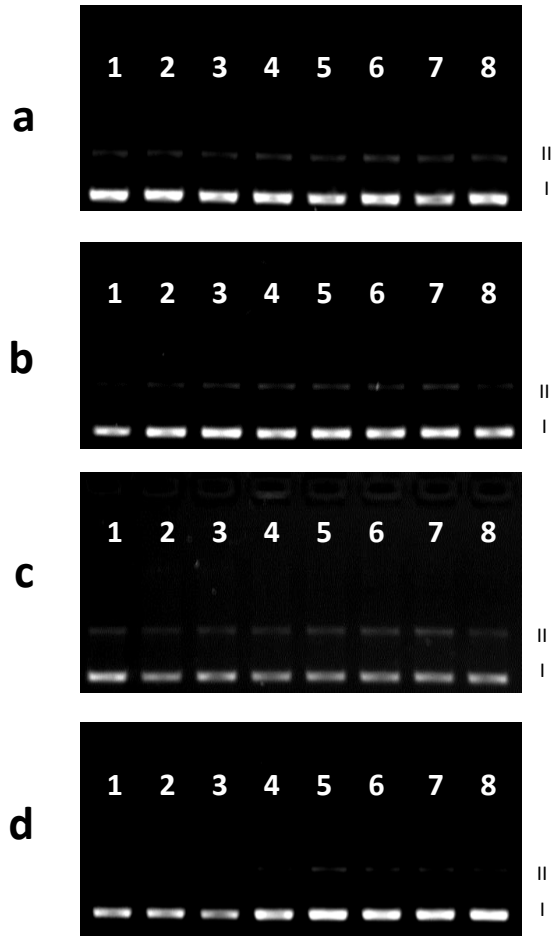


Figure S14. Photo-mediated DNA cleavage assay with jadomycins **1a**-**1d**. Gel electrophoresis analysis of pUC19 DNA (20 μM bases) in a photocleavage assay, performed in 1% agarose gel, 1X TAE, 8V cm^{-1}, 30 min photoreactor with vis bulbs (14 J cm^{-2}), in the presence of jadomycin compounds **1a** (a), **1b** (b), **1c** (c), **1d** (d). Lane 1, DNA alone dark; lane 2-7 different concentrations of jadomycin compounds: (2) 0 μM, hν; (3) 10 μM, hν; (4) 25 μM, hν; (5) 50 μM, hν; (6) 75 μM, hν; (7) 100 μM, hν; lane (8) 100 μM, dark. Form I (supercoiled) and form II (nicked) plasmid DNA are indicated.

Copper-mediated DNA cleavage assays

Plasmid DNA cleavage assays were prepared as above for compounds **1a - 1d** at 100 μM, in 20 μL reaction volumes, with and without the addition of 100 μM cupric acetate. The reaction tubes were incubated at 37°C for 24 hr. The samples were then quenched by the addition of 4 μL gel loading buffer (0.025% bromophenol blue, 40% glycerol), loaded onto 1% agarose gels cast with 1 × TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.2) containing ethidium bromide

(0.75 μg mL^{-1}) and electrophoresed for 30 min at 80 V cm^{-1} in 1 × TAE. The bands were visualized with UV-transillumination (UVP transilluminator) and processed using the Gel Doc-It Imaging System (UVP).

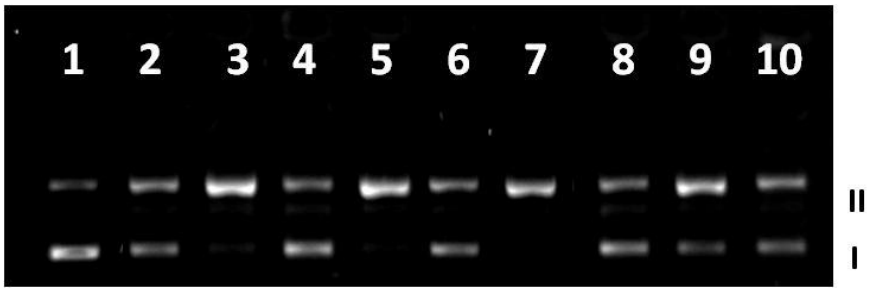


Figure S15. Copper-mediated DNA cleavage by jadomycins **1a**-**1d**. Gel electrophoresis analysis of pUC19 DNA (20 μM bases) in a DNA cleavage assay, performed in 1% agarose gel containing 0.75 μg mL^{-1} ethidium bromide, 1 × TAE, 8 V cm^{-1}, overnight dark incubation, in the presence of 4 jadomycin compounds with/without cupric acetate. Lane 1, DNA alone ; Lane 2, **1a**, 100 μM; Lane 3, **1a** and Cu^{2+}, 100 μM; Lane 4, **1b**, 100 μM; Lane 5, **1b** and Cu^{2+}, 100 μM; Lane 6, **1c**, 100 μM; Lane 7, **1c** and Cu^{2+}, 100 μM; Lane 8, **1d**, 100 μM; Lane 9, **1d** and Cu^{2+}, 100 μM; Lane 10, Cu^{2+} only, 100 μM. Form I (supercoiled) and form II (nicked) plasmid DNA are indicated.

Bacterial culture

Using aseptic technique, a vial of *Streptococcus mutans* (*S. mutans* Clarke, NCTC 10449) was propagated by transferring half of the freeze-dried pellet, using a sterile loop, to a culture tube containing 2 mL Brain Heart Infusion medium (BHI, Oxoid), and gently mixed by swirling. The tube was capped loosely and placed in a 37°C incubator for 24 hr. The following day, 10 serial dilutions were made (10^{-1} to 10^{-8}), then 0.1 mL aliquots from each dilution were spread on to fresh BHI agar plates (3.8% BHI) using sterile loops, allowed to dry with lids askew, then placed upside down in 37°C incubator overnight. Purity of the colony growth was verified, and then the bacterial culture tube was subcultured by centrifuging (5000 rpm, 5 min), carefully pouring off the supernatant to waste, and replacing with 5 mL fresh media. A frozen stock of *S. mutans* was prepared by transferring 500 μL aliquots of *S. mutans* culture to sterile 1.5 mL microcentrifuge

tubes containing 500 µL sterile 70% glycerol/water. The tubes were mixed by vortexing briefly and subsequently stored in a -80°C freezer. Primary growth colony plates were prepared by transferring 50 µL frozen *S. mutans* to a sterile microcentrifuge tube containing 500 µL tryptic soy broth (TSB, Fluka 22092) and the solution was mixed well by vortexing. An aliquot of 50 µL was applied, using a quadrant streak method, to a TSA plate (3% TSB in agar), allowed to dry with the lid askew, and incubated overnight. The following morning the purities of the primary growth cultures were verified, then 1-2 colonies were transferred to a sterile microfuge tube containing 500 µL TSB and mixed well by pipetting up and down and vortexing. A secondary growth colony plate was prepared by transferring 50 µL of this mixture to a warmed TSA plate, quadrant streaked, allowed to dry as before, then incubated overnight. The next morning the purities of the secondary growth cultures were again verified and subsequently used for agar well diffusion assays. All experiments using *S. mutans* were made from freshly made (less than one week old) secondary growth colony plates.

Agar well diffusion assays

The agar well diffusion method was used as an antibacterial screening test for jadomycins **1a-1d**. Under aseptic conditions, an inoculum of *S. mutans* was prepared by transferring colonies from a secondary growth plate to a sterile 15 mL conical tube (VWR, Canada) containing 5 mL sterile distilled water and the contents were mixed well by vortexing. A turbidity matching a McFarland barium sulfate standard 3 (approximately 9×10^8 CFU mL^{-1}) was made. A 500 µL aliquot of the inoculum was applied to each of 4 TSA plates and was spread evenly, using sterile loops, to allow uniform growth of a bacterial lawn. The plates were given time to completely dry, with lids askew, for about 30 min. Using sterile glass Pasteur pipettes, bored holes of about 6 cm were made in 4 quadrants of each TSA plate. Ethanolic stock solutions of jadomycins (5

mM) were diluted to 300 µM in water (final concentration of ethanol 6%) and 50 µL aliquots were added to duplicate wells along with a 6% ethanol solvent control, for dark and light treatments. The dark plates were kept in a dark drawer, covered in foil, and the light plates were irradiated with white light in a photoreactor (Luzchem LZC-4X, 7.72 mW cm^{-2}) for 60 min in order to yield an energy density of approximately 28 J cm^{-2}. The bacterial growth inhibition zones were measured in mm using vernier calipers (Bel-Art, USA).

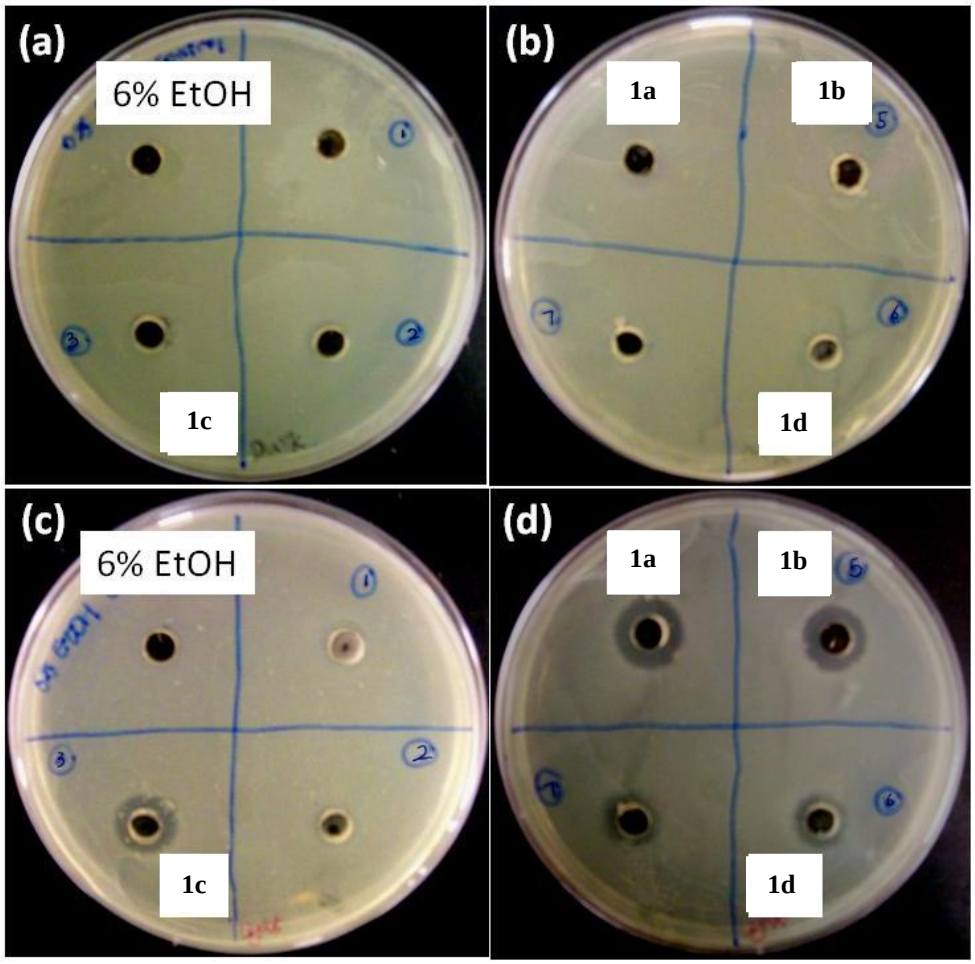


Figure S16. *Streptococcus mutans* growth plates and agar well diffusion inhibition zones from jadomycins **1a**-**1d** (300 µM), in the dark (a, b) and light (c, d).

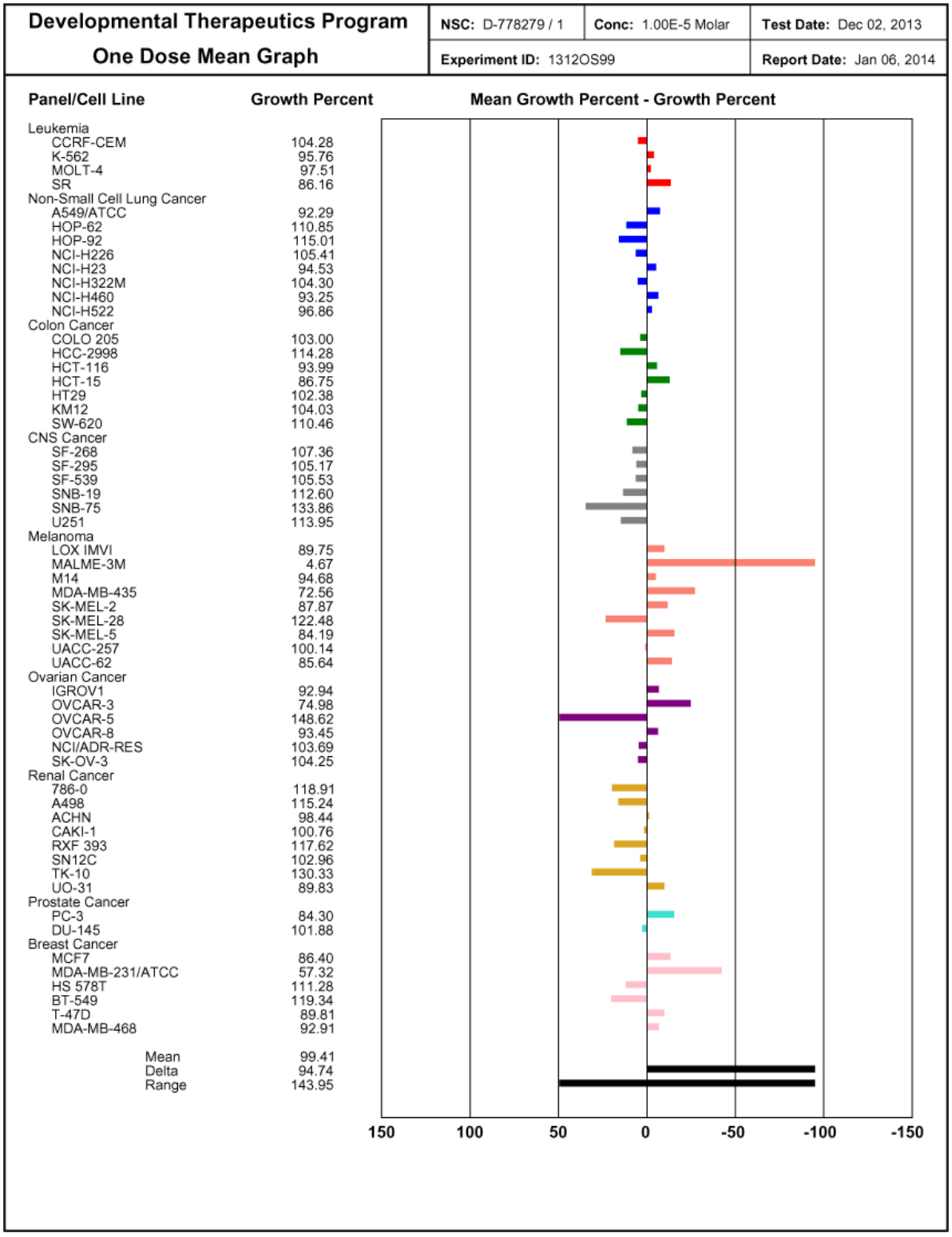
Table S17. Agar Well Diffusion Tabulated Results for Bacterial Growth Inhibition by Jadomycins 1a-1d.

Jadomycin	Dark Inhibition Zone (mm)	Light Inhibition Zone (mm)
1a	6.0	11.3
1b	6.0	10.5
1c	6.0	10.0
1d	6.0	7.3
Control (6% EtOH)	6.0	6.0

Inhibition zones (mm) given as the diameter of the inhibition halo, including the 6 mm sample well

National Cancer Institute (NCI) 60 DTP Human Tumor Cell Line Screen anticancer activity

Table S18. One Dose NCI-60 Tumor Cell Line Screen of 1a (1 × 10^{-5} M).



Developmental Therapeutics Program
One Dose Mean Graph

NSC: D-778279 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Dec 02, 2013
Experiment ID: 1312OS99 | **Report Date:** Jan 06, 2014

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	104.28
K-562	95.76
MOLT-4	97.51
SR	86.16
Non-Small Cell Lung Cancer	
A549/ATCC	92.29
HOP-62	110.85
HOP-92	115.01
NCI-H226	105.41
NCI-H23	94.53
NCI-H322M	104.30
NCI-H460	93.25
NCI-H522	96.86
Colon Cancer	
COLO 205	103.00
HCC-2998	114.28
HCT-116	93.99
HCT-15	86.75
HT29	102.38
KM12	104.03
SW-620	110.46
CNS Cancer	
SF-268	107.36
SF-295	105.17
SF-539	105.53
SNB-19	112.60
SNB-75	133.86
U251	113.95
Melanoma	
LOX IMVI	89.75
MALME-3M	4.67
M14	94.68
MDA-MB-435	72.56
SK-MEL-2	87.87
SK-MEL-28	122.48
SK-MEL-5	84.19
UACC-257	100.14
UACC-62	85.64
Ovarian Cancer	
IGROV1	92.94
OVCAR-3	74.98
OVCAR-5	148.62
OVCAR-8	93.45
NCI/ADR-RES	103.69
SK-OV-3	104.25
Renal Cancer	
786-0	118.91
A498	115.24
ACHN	98.44
CAKI-1	100.76
RXF 393	117.62
SN12C	102.96
TK-10	130.33
UO-31	89.83
Prostate Cancer	
PC-3	84.30
DU-145	101.88
Breast Cancer	
MCF7	86.40
MDA-MB-231/ATCC	57.32
HS 578T	111.28
BT-549	119.34
T-47D	89.81
MDA-MB-468	92.91
Mean	99.41
Delta	94.74
Range	143.95

Mean Growth Percent - Growth Percent

150 100 50 0 -50 -100 -150

Table S19. One Dose NCI-60 Tumor Cell Line Screen of 1c (1×10^{-5} M).

Developmental Therapeutics Program	NSC: D-778280 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 02, 2013
One Dose Mean Graph	Experiment ID: 1312OS99		Report Date: Jan 06, 2014

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	101.13
K-562	116.48
MOLT-4	114.45
SR	95.66
Non-Small Cell Lung Cancer	
A549/ATCC	94.08
HOP-62	107.67
HOP-92	135.53
NCI-H226	104.56
NCI-H23	96.57
NCI-H322M	101.27
NCI-H460	104.71
NCI-H522	98.62
Colon Cancer	
COLO 205	101.89
HCC-2998	118.68
HCT-116	95.72
HCT-15	95.18
HT29	104.92
KM12	108.45
SW-620	112.89
CNS Cancer	
SF-268	107.10
SF-295	106.26
SF-539	103.09
SNB-19	105.54
SNB-75	128.48
U251	110.16
Melanoma	
LOX IMVI	97.85
MALME-3M	104.50
M14	100.35
MDA-MB-435	111.30
SK-MEL-2	96.94
SK-MEL-28	125.89
SK-MEL-5	97.48
UACC-257	101.58
UACC-62	103.45
Ovarian Cancer	
IGROV1	97.44
OVCAR-3	99.76
OVCAR-5	141.04
OVCAR-8	95.47
NCI/ADR-RES	101.55
SK-OV-3	101.44
Renal Cancer	
786-0	111.12
A498	131.29
ACHN	98.54
CAKI-1	99.83
RXF 393	114.02
SN12C	105.84
TK-10	122.85
UO-31	92.69
Prostate Cancer	
PC-3	96.59
DU-145	124.35
Breast Cancer	
MCF7	99.96
MDA-MB-231/ATCC	77.30
HS 578T	113.00
BT-549	100.84
T-47D	90.32
MDA-MB-468	103.54
Mean	105.84
Delta	28.54
Range	63.74

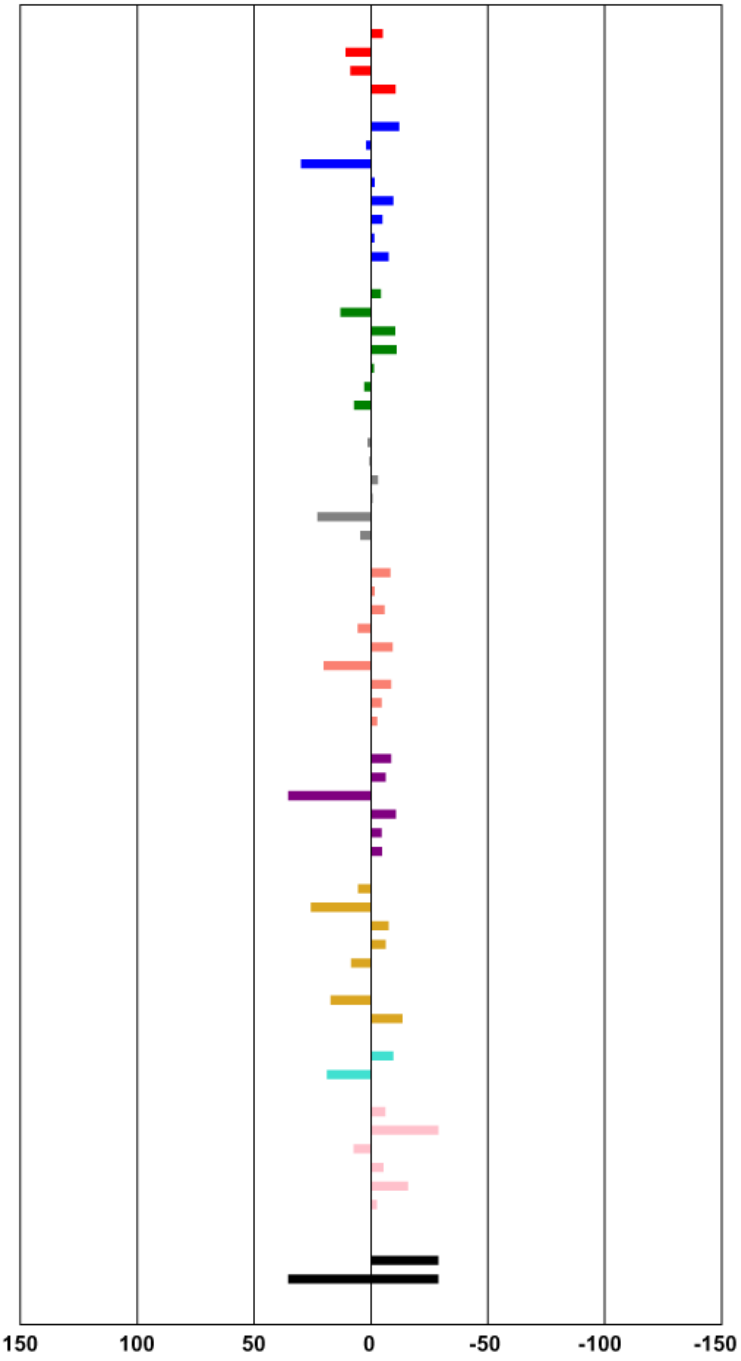


Table S20. One Dose NCI-60 Tumor Cell Line Screen of 1d (1×10^{-5} M).

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-778281 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 02, 2013
Experiment ID: 1312OS99		Report Date: Jan 06, 2014

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	102.00
HL-60(TB)	82.80
K-562	84.60
MOLT-4	80.99
SR	98.41
Non-Small Cell Lung Cancer	
A549/ATCC	96.07
HOP-62	126.61
HOP-92	127.94
NCI-H226	101.39
NCI-H23	104.23
NCI-H322M	105.41
NCI-H460	98.48
NCI-H522	101.06
Colon Cancer	
COLO 205	111.83
HCC-2998	119.74
HCT-116	103.14
HCT-15	93.57
HT29	107.60
KM12	112.79
SW-620	110.28
CNS Cancer	
SF-268	112.53
SF-295	106.98
SF-539	102.99
SNB-19	108.89
SNB-75	125.47
U251	107.65
Melanoma	
LOX IMVI	94.19
MALME-3M	60.67
M14	99.81
MDA-MB-435	98.73
SK-MEL-2	99.32
SK-MEL-28	121.73
SK-MEL-5	89.53
UACC-257	105.97
UACC-62	95.57
Ovarian Cancer	
IGROV1	90.74
OVCAR-3	94.78
OVCAR-5	139.89
OVCAR-8	96.23
NCI/ADR-RES	103.58
SK-OV-3	118.09
Renal Cancer	
786-0	128.11
A498	124.99
ACHN	97.38
CAKI-1	105.33
RXF 393	126.57
SN12C	100.98
TK-10	137.31
UO-31	92.07
Prostate Cancer	
PC-3	90.00
DU-145	116.37
Breast Cancer	
MCF7	95.53
MDA-MB-231/ATCC	68.14
HS 578T	112.67
BT-549	126.63
T-47D	89.17
MDA-MB-468	93.50
Mean	104.33
Delta	43.66
Range	79.22

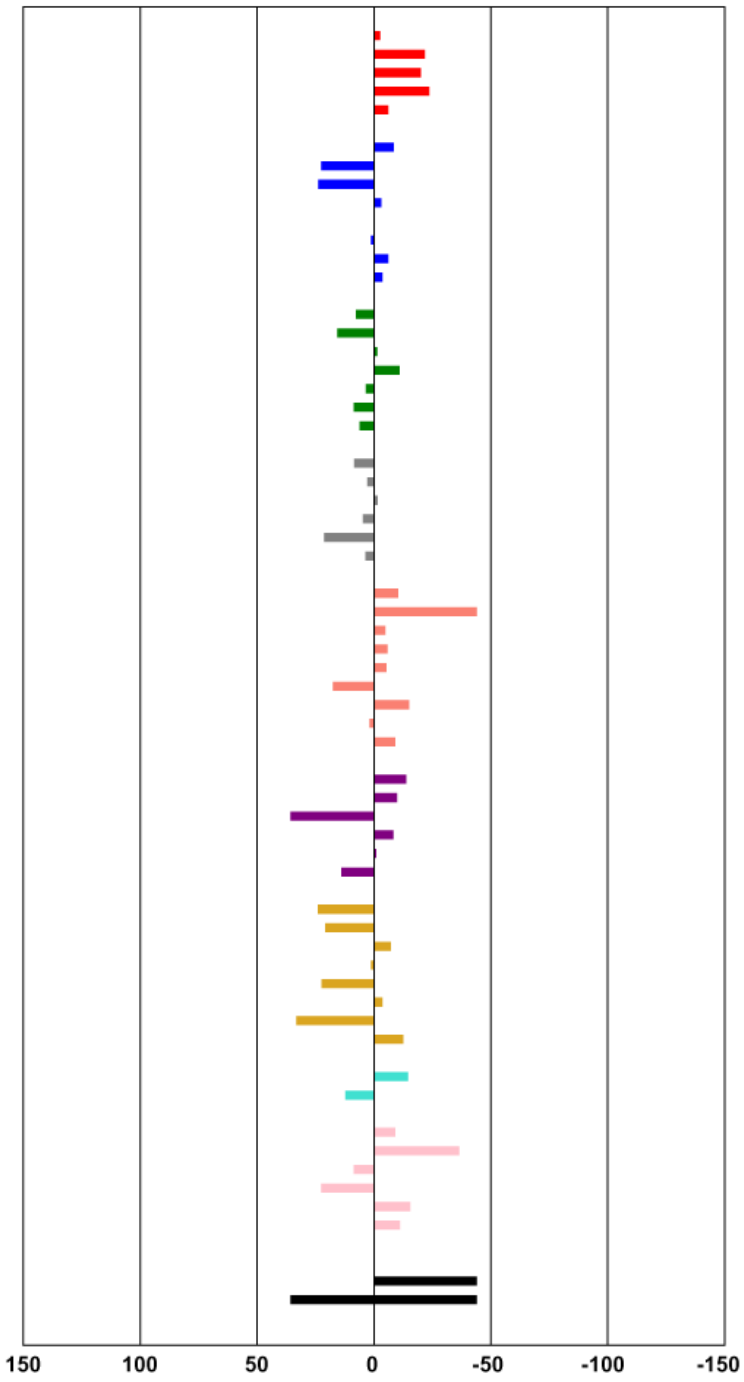
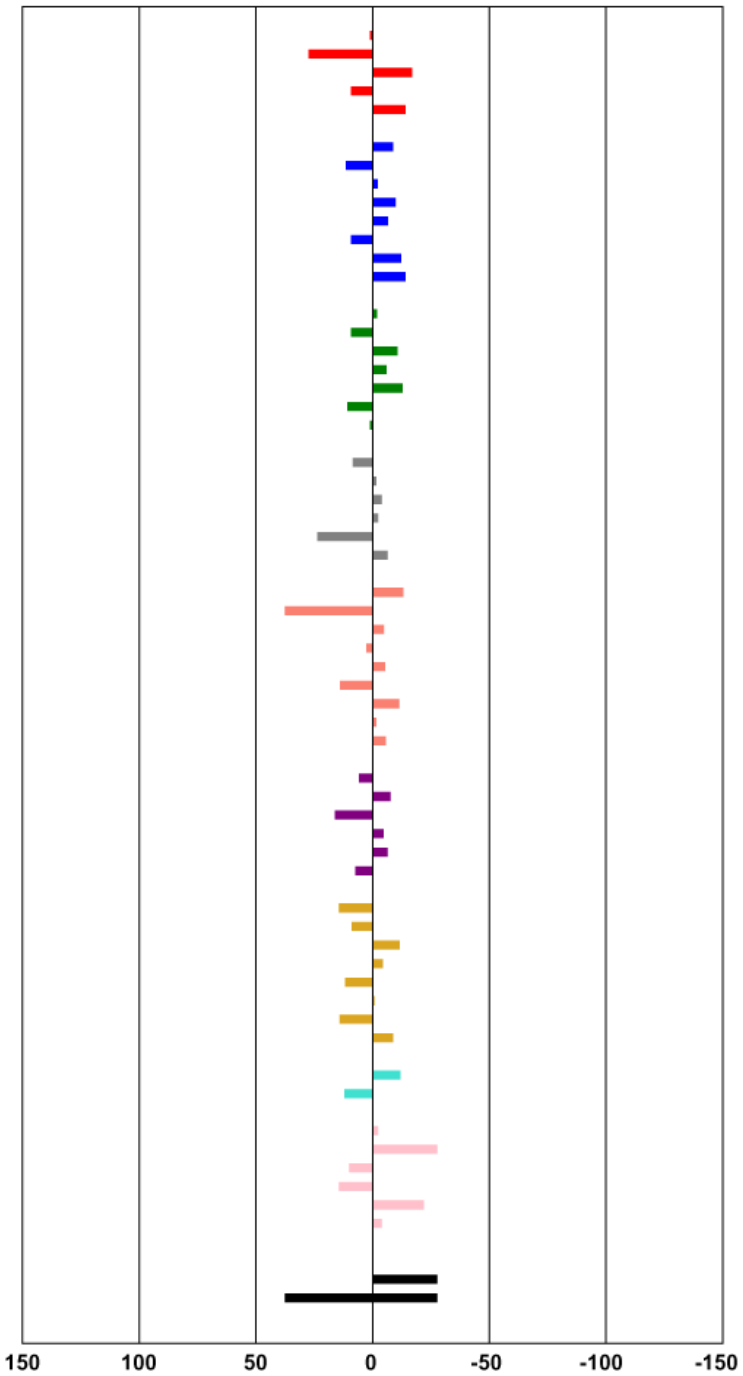


Table S21. One Dose NCI-60 Tumor Cell Line Screen of 1f (1×10^{-5} M).

Developmental Therapeutics Program	NSC: D-778282 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 02, 2013
One Dose Mean Graph	Experiment ID: 1312OS99		Report Date: Jan 06, 2014

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	110.34
HL-60(TB)	136.54
K-562	92.79
MOLT-4	118.45
SR	95.65
Non-Small Cell Lung Cancer	
A549/ATCC	100.85
HOP-62	120.61
HOP-92	107.44
NCI-H226	99.73
NCI-H23	103.13
NCI-H322M	118.48
NCI-H460	97.49
NCI-H522	95.52
Colon Cancer	
COLO 205	107.80
HCC-2998	118.38
HCT-116	99.05
HCT-15	103.78
HT29	96.80
KM12	119.92
SW-620	110.30
CNS Cancer	
SF-268	117.70
SF-295	108.03
SF-539	105.75
SNB-19	107.32
SNB-75	132.76
U251	103.20
Melanoma	
LOX IMVI	96.48
MALME-3M	146.70
M14	104.88
MDA-MB-435	111.79
SK-MEL-2	104.41
SK-MEL-28	123.10
SK-MEL-5	98.19
UACC-257	108.10
UACC-62	103.97
Ovarian Cancer	
IGROV1	114.92
OVCAR-3	102.06
OVCAR-5	125.36
OVCAR-8	104.93
NCI/ADR-RES	103.18
SK-OV-3	116.59
Renal Cancer	
786-0	123.62
A498	118.11
ACHN	98.05
CAKI-1	105.25
RXF 393	120.93
SN12C	108.95
TK-10	123.30
UO-31	100.97
Prostate Cancer	
PC-3	97.81
DU-145	121.27
Breast Cancer	
MCF7	107.36
MDA-MB-231/ATCC	81.88
HS 578T	119.29
BT-549	123.55
T-47D	87.81
MDA-MB-468	105.78
Mean	109.41
Delta	27.53
Range	64.82



NMR Spectra of Compounds 1a-1f and 1

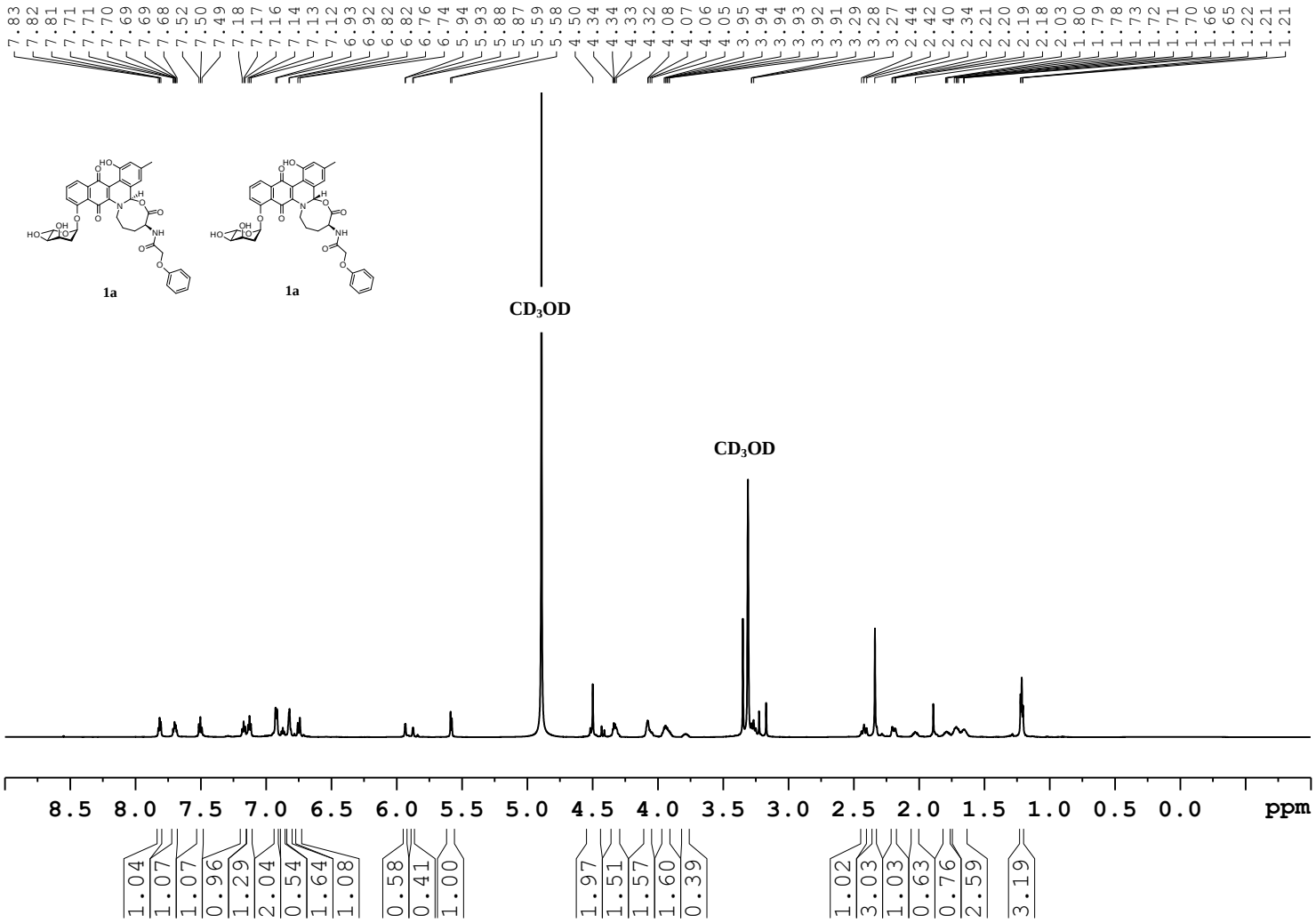


Figure S17. ^{1}H-NMR spectrum of **1a** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz).

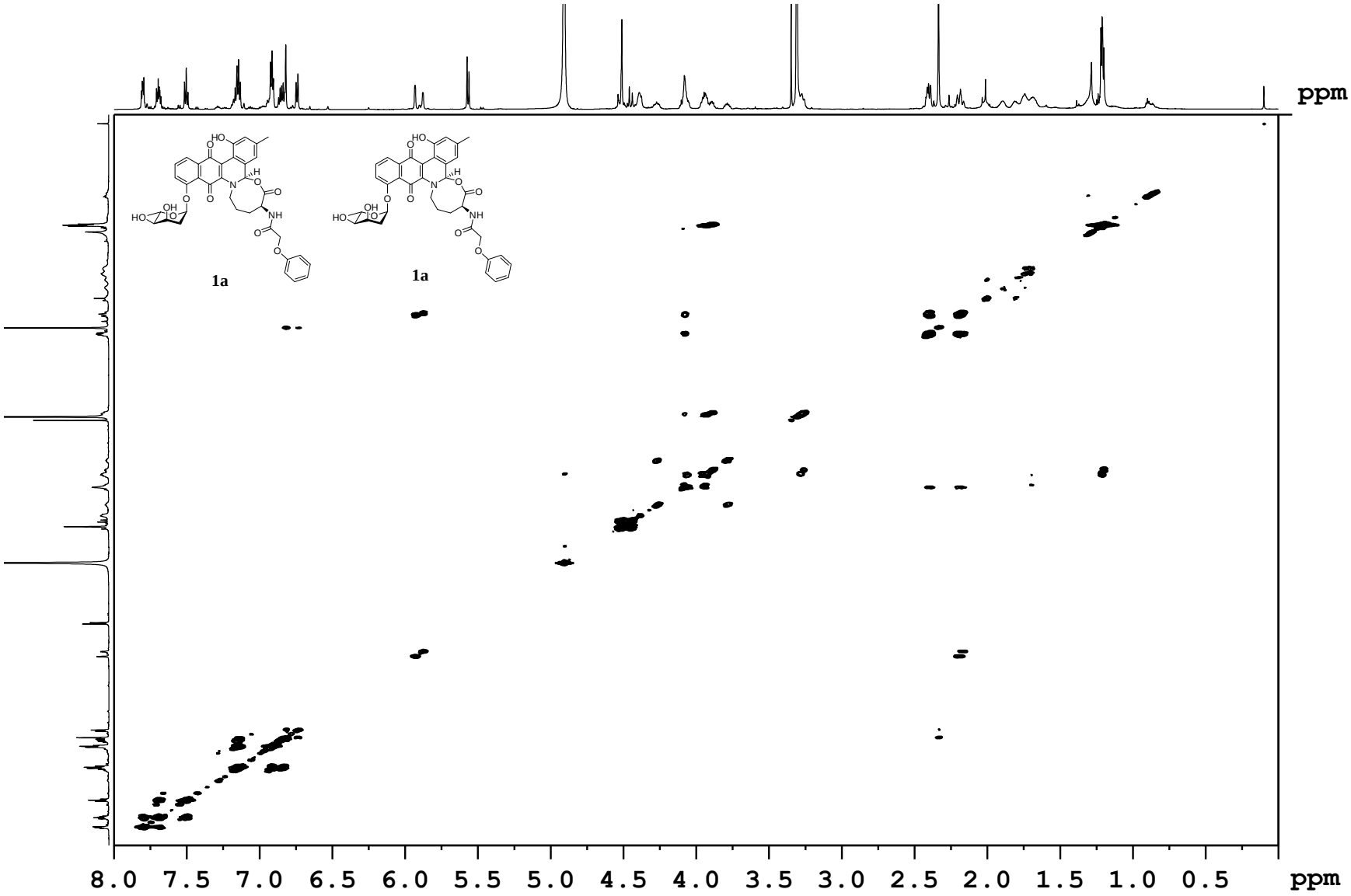


Figure S18. COSY (^{1}H-^{1}H) spectrum of **1a** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

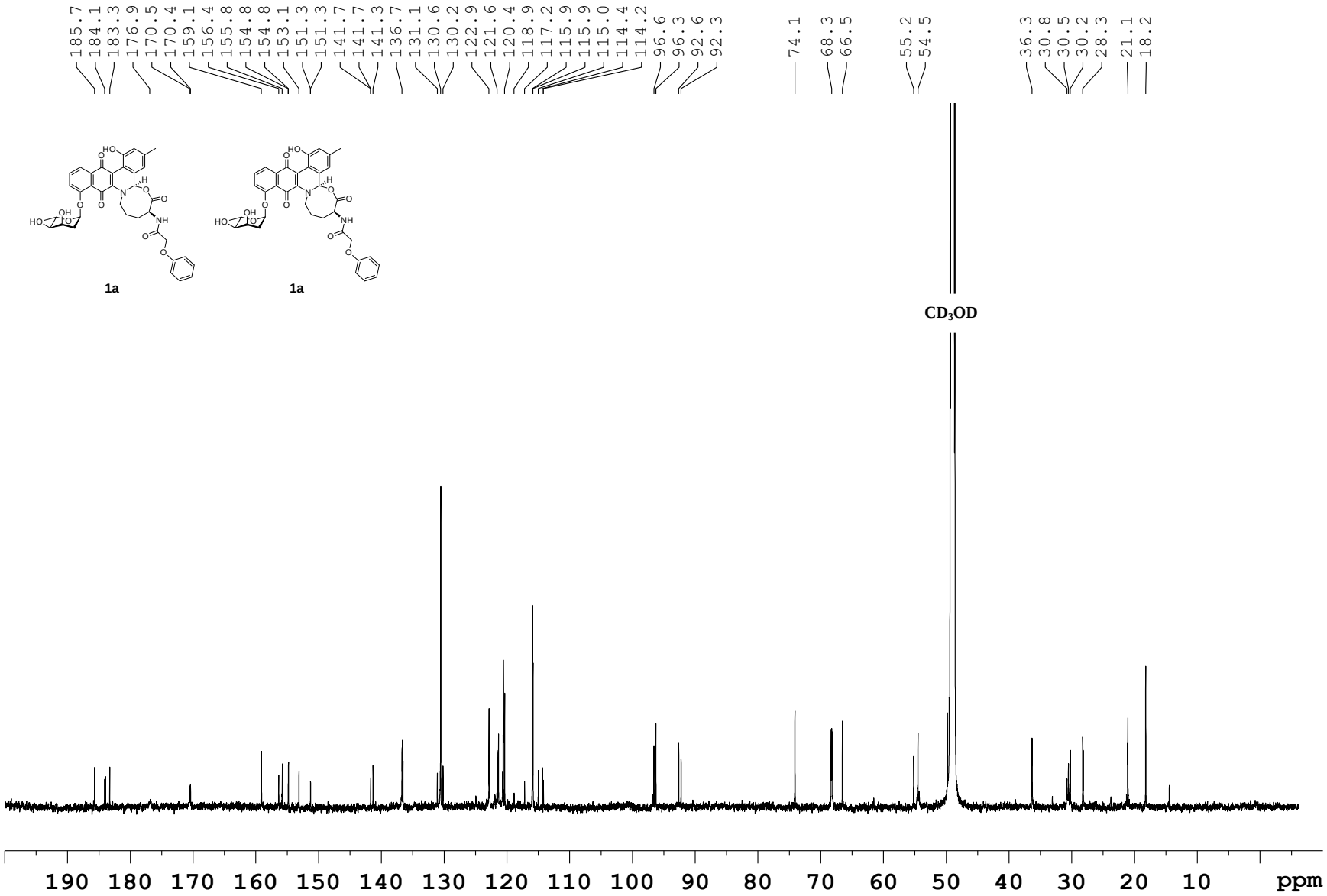


Figure S19. ^{13}C-NMR spectrum of **1a** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

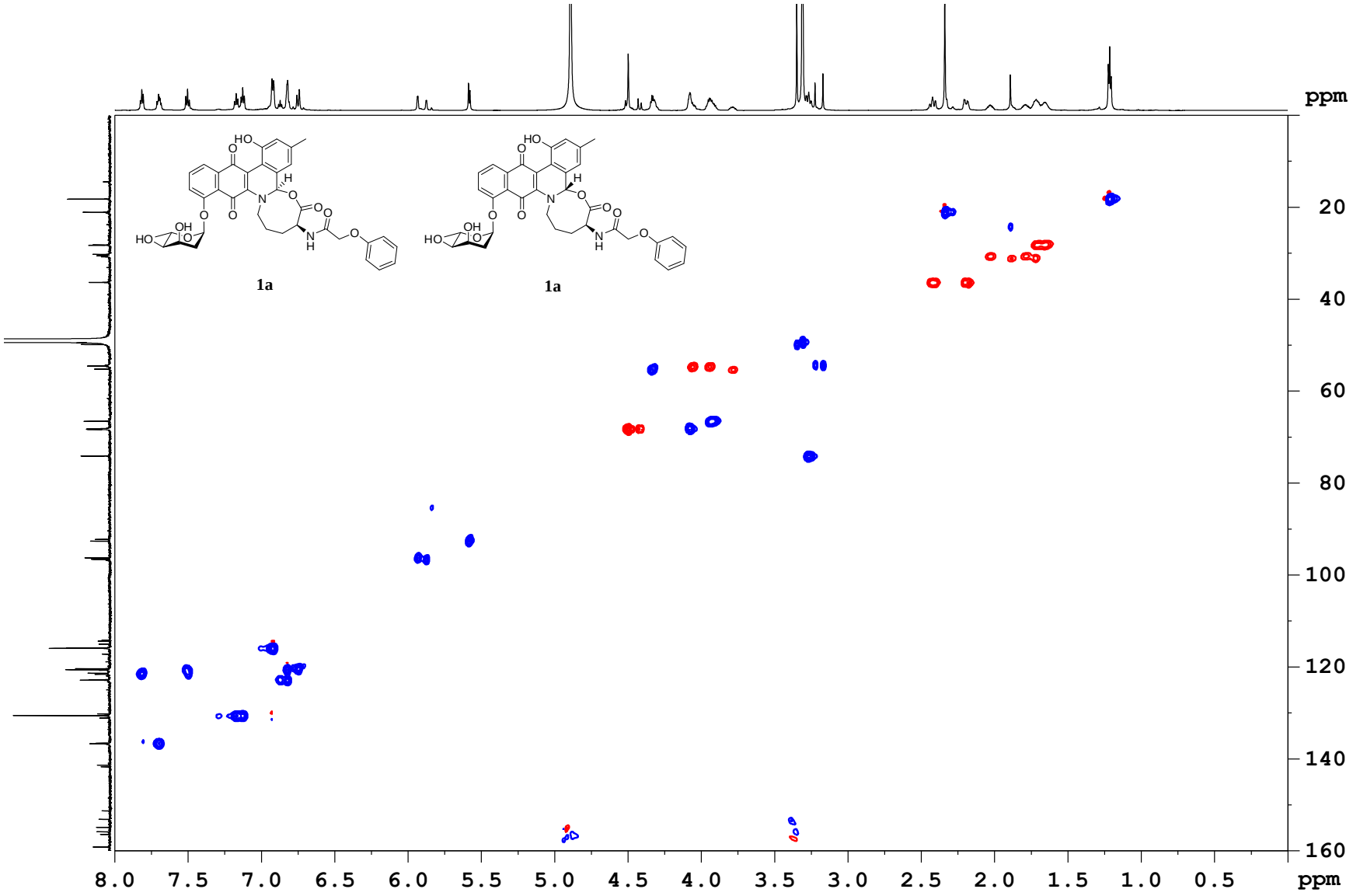


Figure S20. Edited-HSQC (^{1}H-^{13}C) spectrum of **1a** (diastereomeric mixture) in MeOD-d_4.

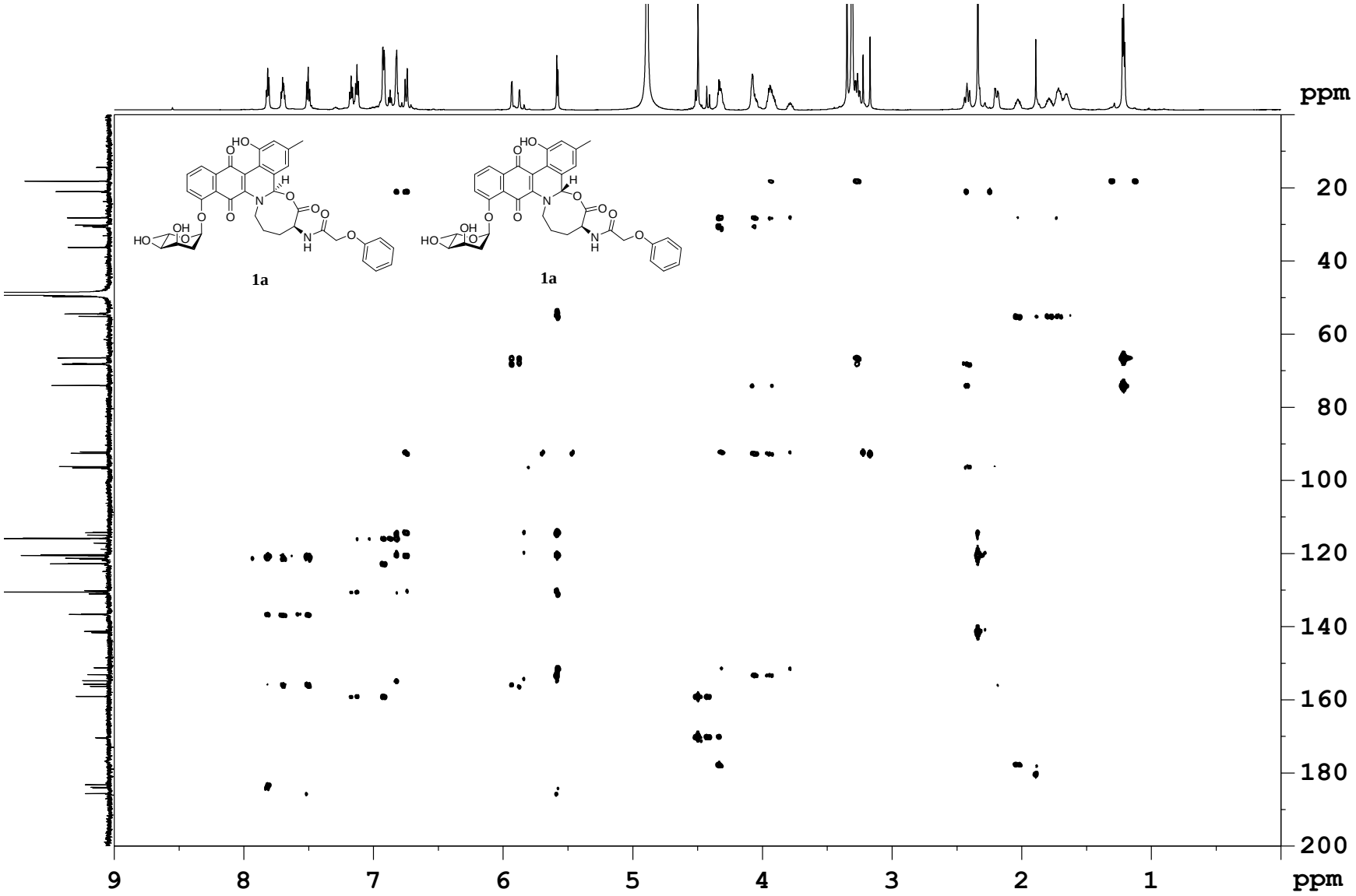


Figure S21. HMBC (^{1}H-^{13}C) spectrum of **1a** (diastereomeric mixture) in MeOD-d_4

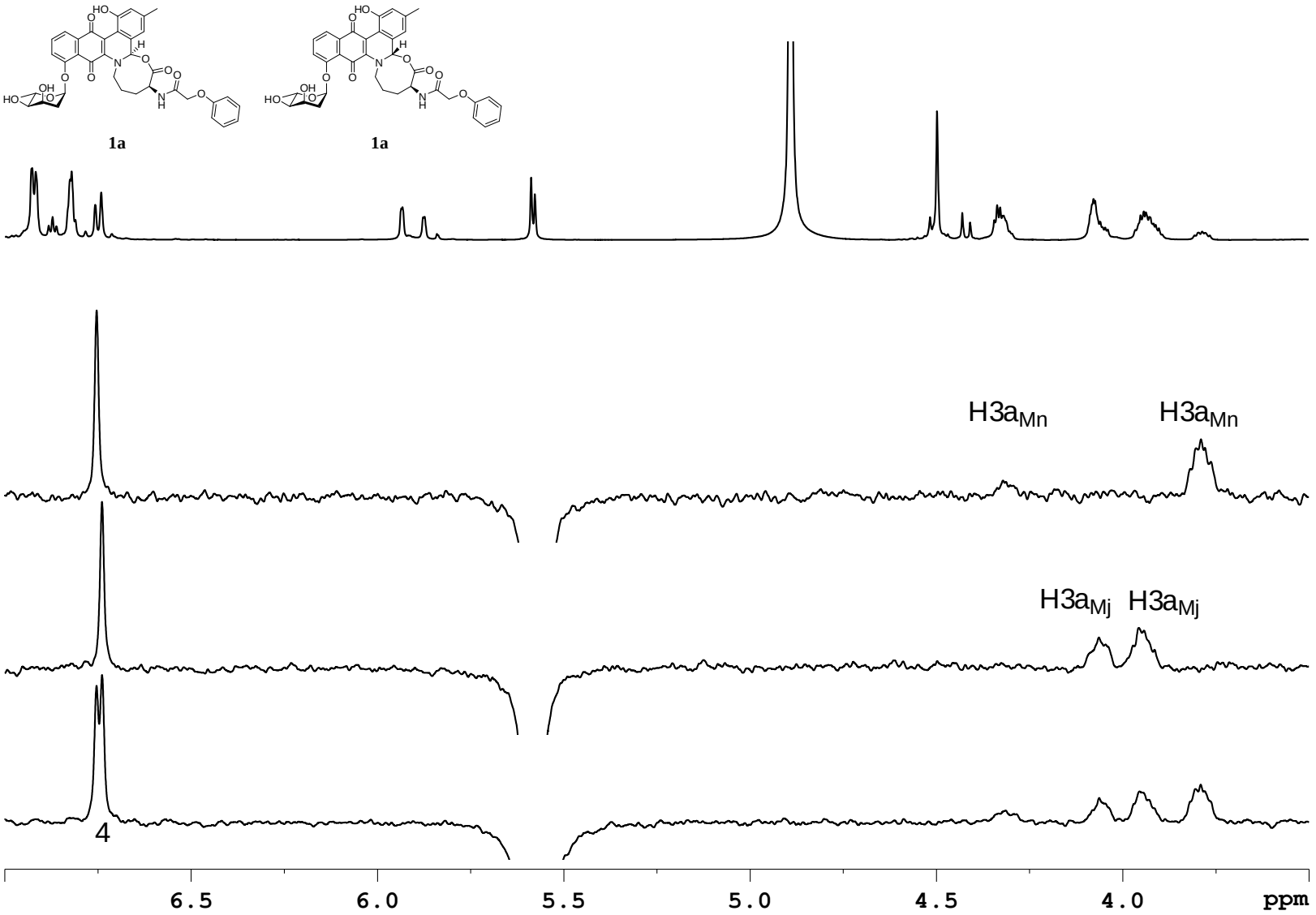


Figure S22. Overlay of ^{1}H-NMR spectrum of **1a** (top) with ROESY (500 MHz) showing irradiation of H3a$_{Mn}$ (2nd from top), H3a$_{Mj}$ (2nd from bottom), and both H3a$_{Mn}$ and H3a$_{Mj}$ simultaneously, in MeOD-d$_4$.

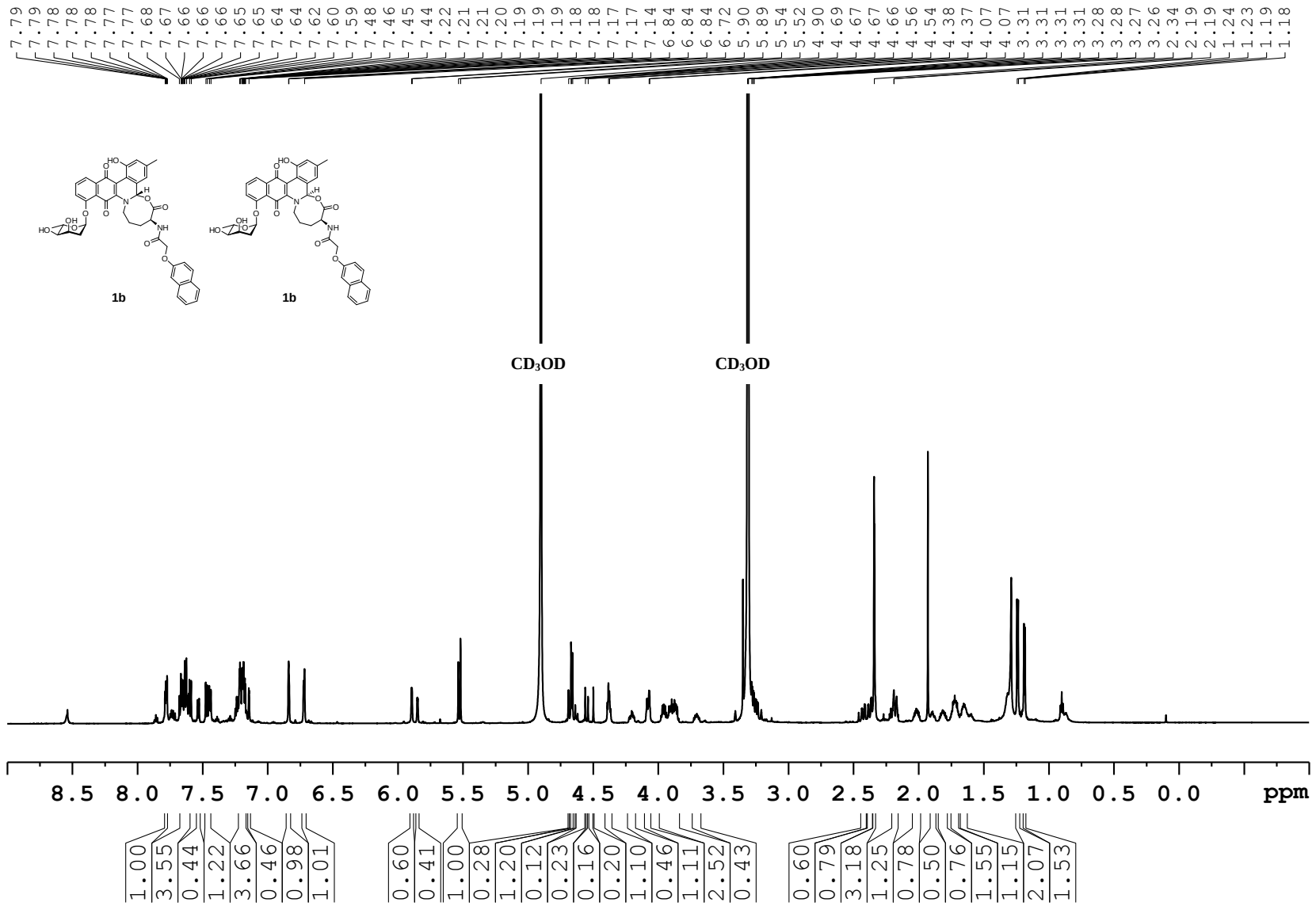
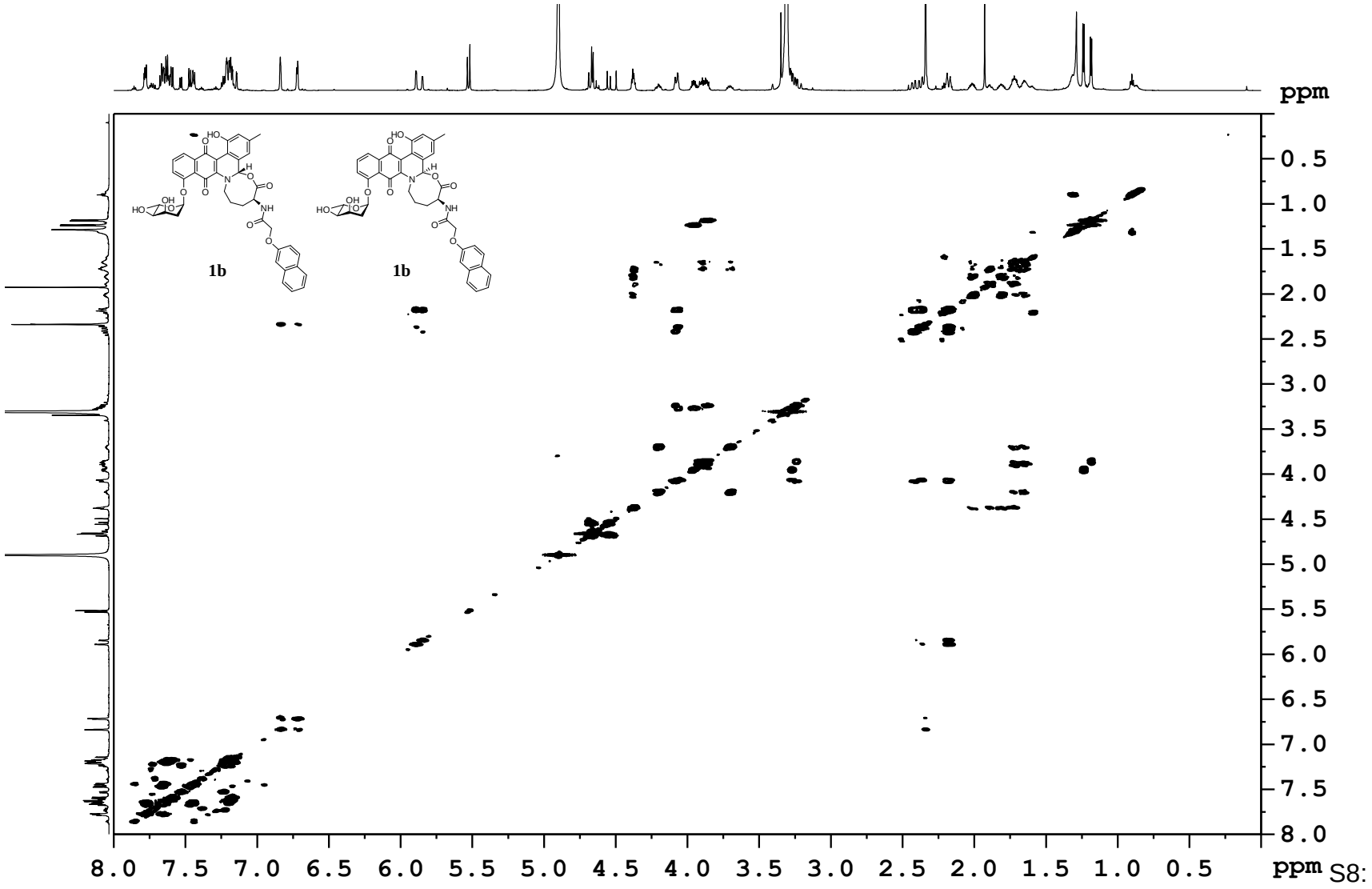


Figure S23. ^{1}H-NMR spectrum of **1b** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz).



S8:

Figure S24: COSY (^{1}H-^{1}H) spectrum of **1b** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

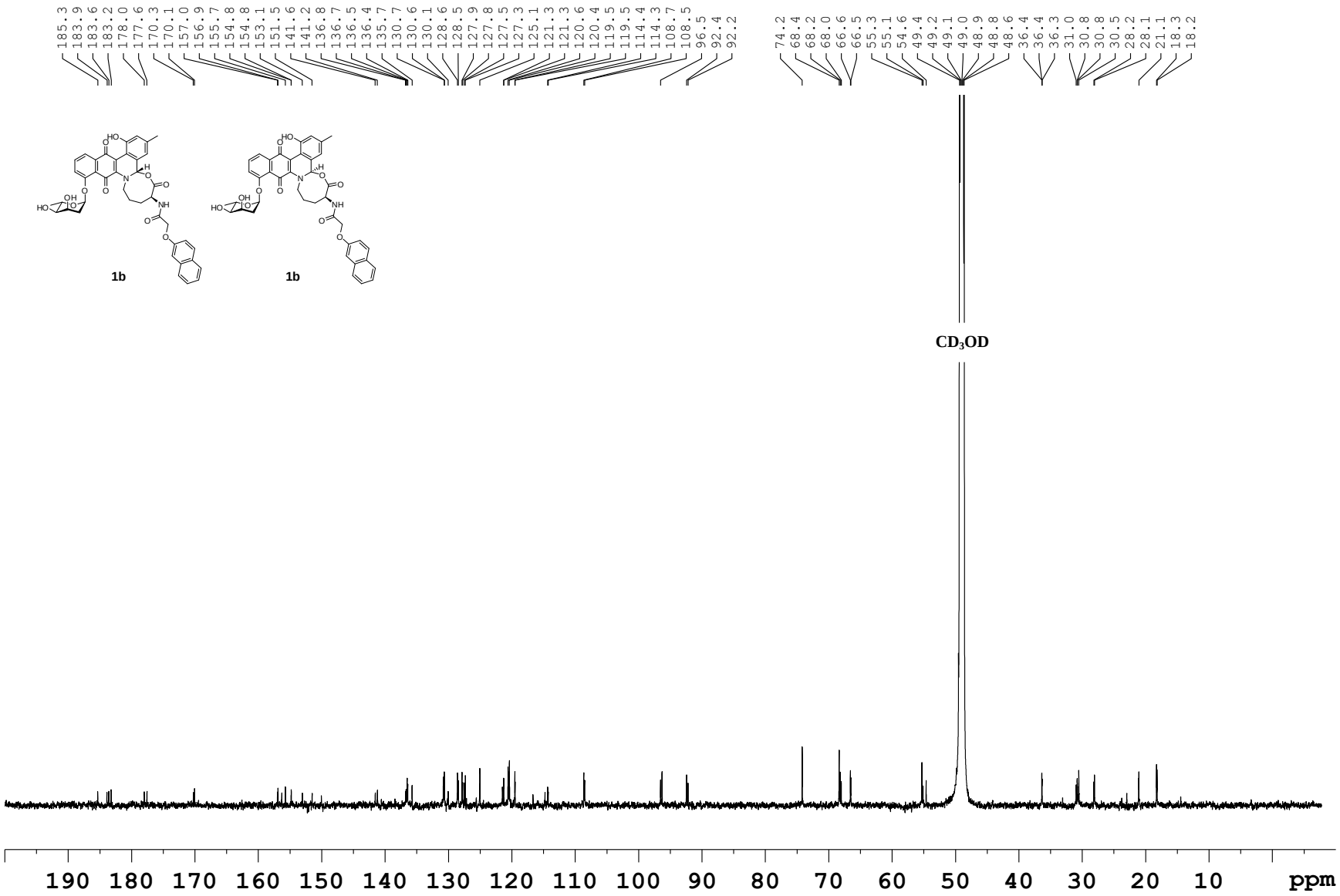


Figure S25. ^{13}C-NMR spectrum of **1b** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

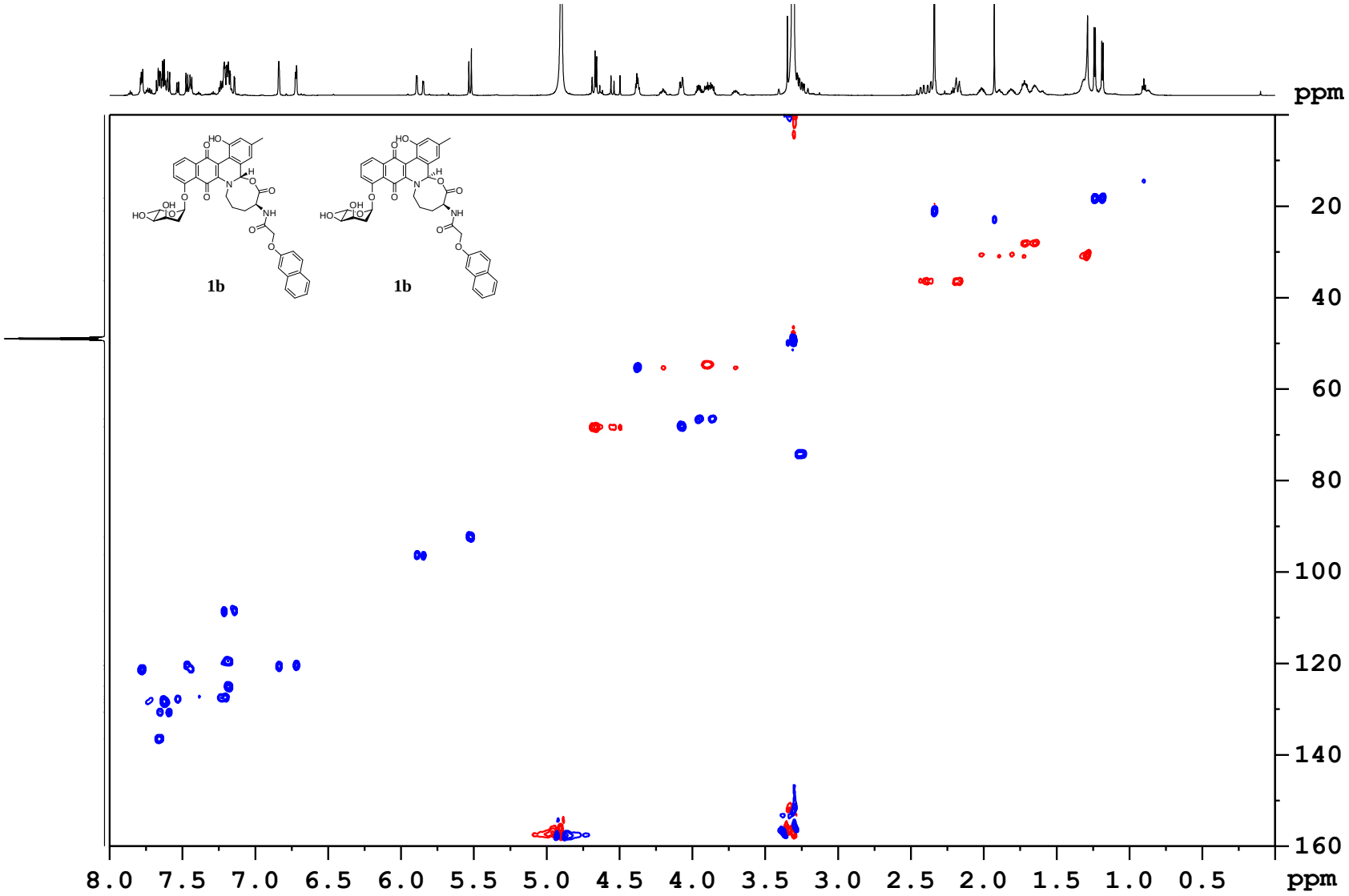


Figure S26. Edited-HSQC (^{1}H-^{13}C) spectrum of **1b** (diastereomeric mixture) in MeOD-d_4.

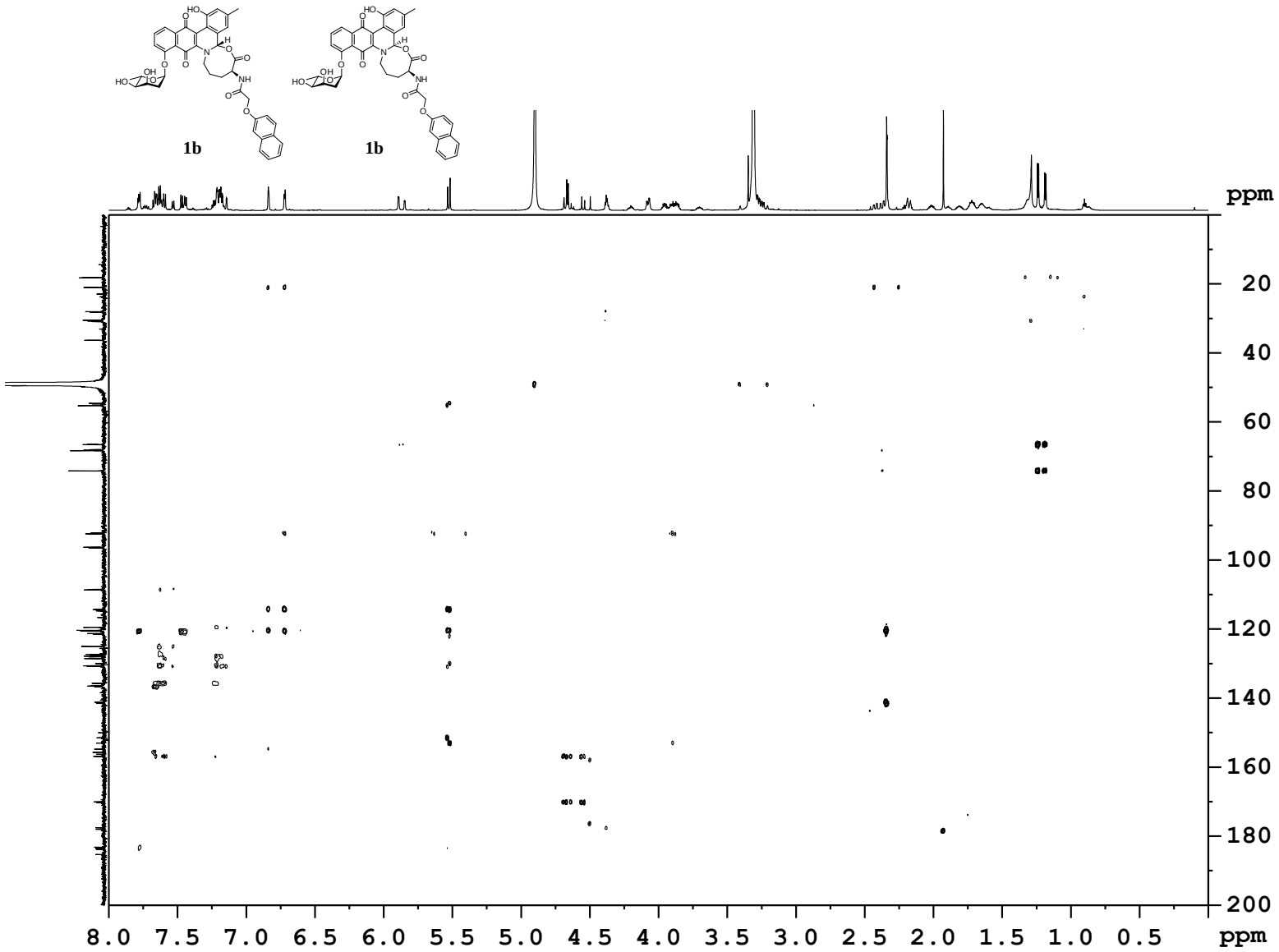


Figure S27. HMBC (^{1}H-^{13}C) spectrum of **1b** (diastereomeric mixture) in MeOD-d_4.

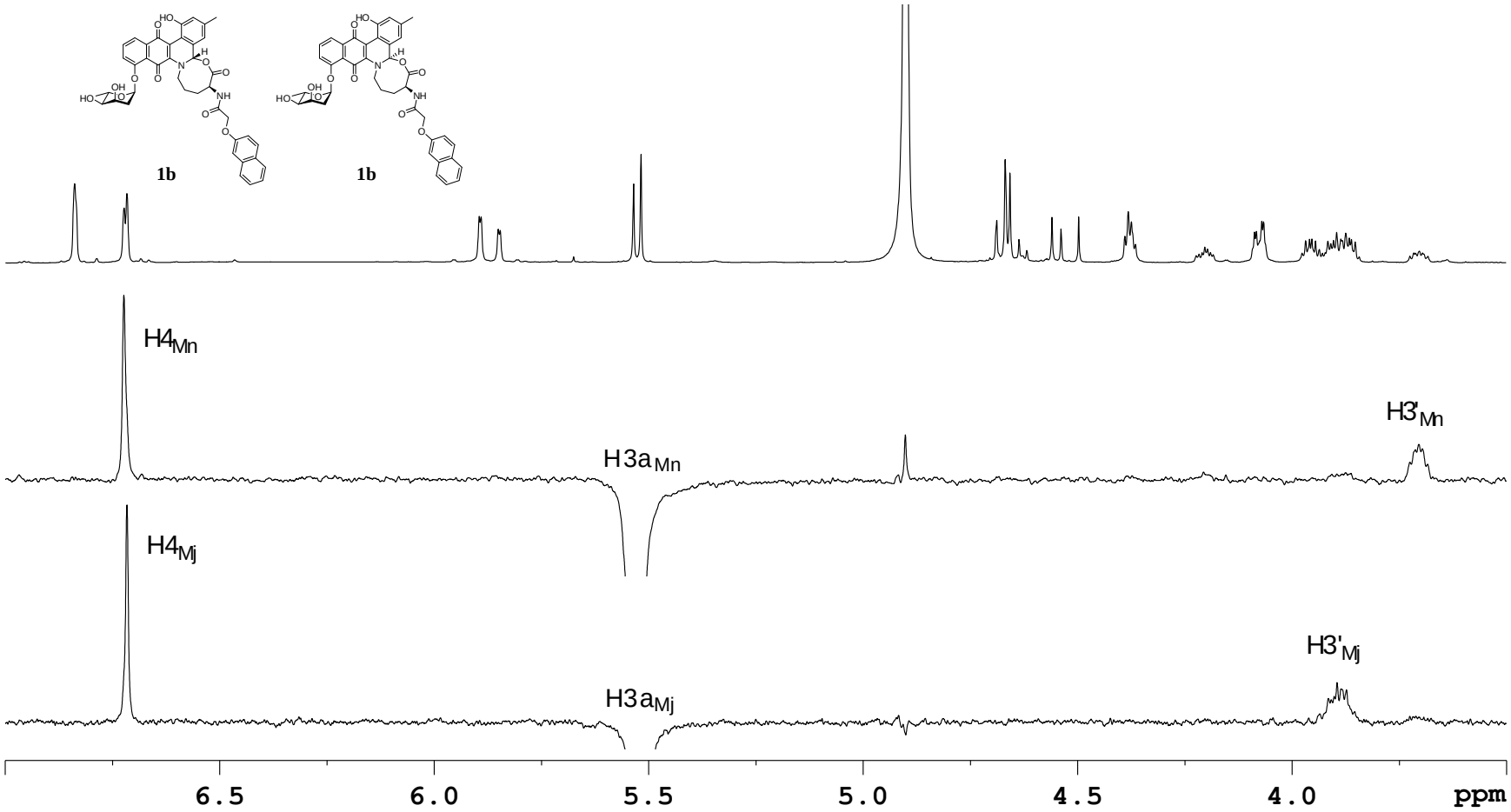


Figure S28. Overlay of ^{1}H-NMR spectrum of **1b** (top) with ROESY (700 MHz) showing irradiation of $H3a_{Mn}$ (middle), and $H3a_{Mj}$ (bottom) in MeOD-d_4.

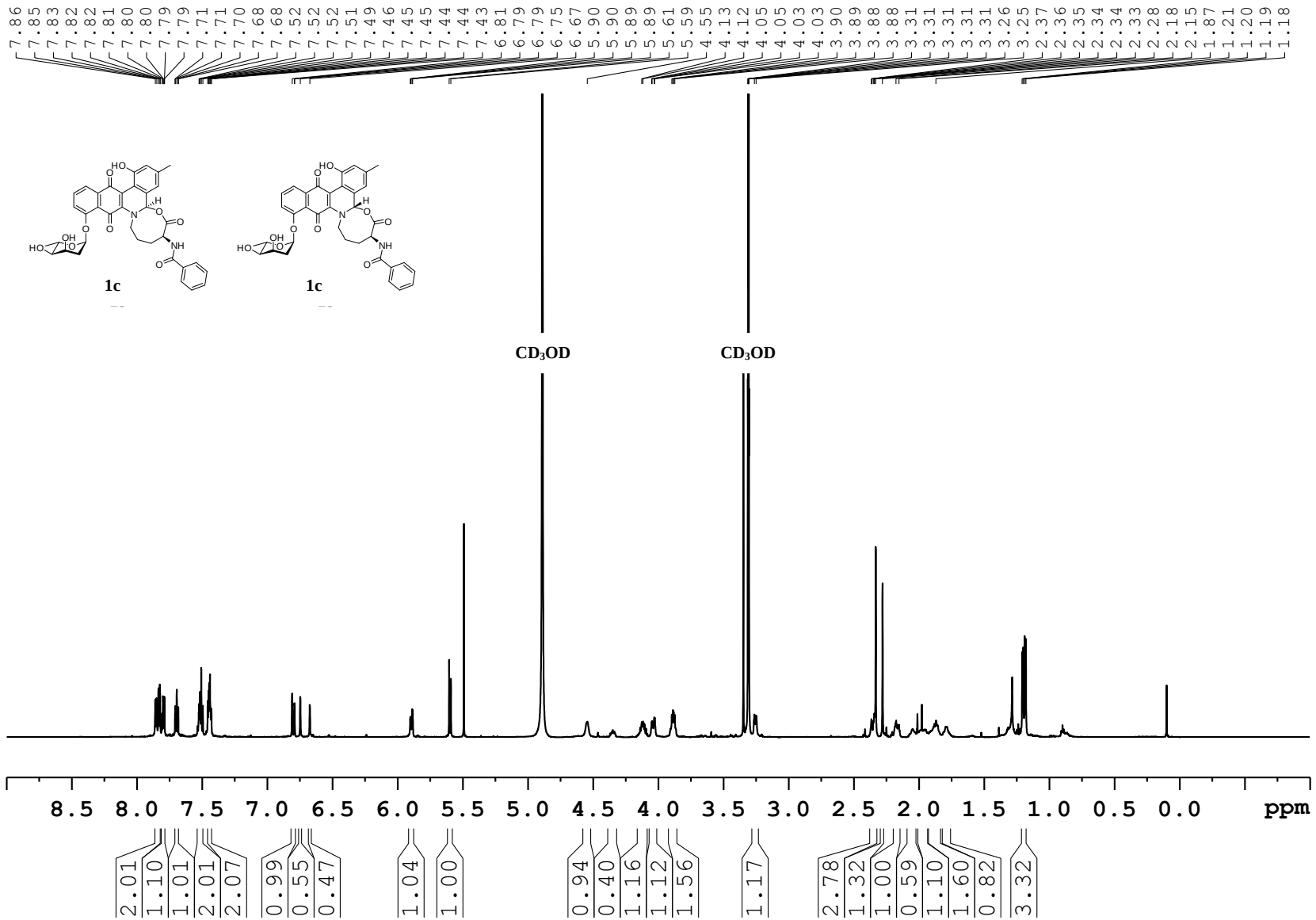


Figure S29. ^{1}H-NMR spectrum of **1c** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz).

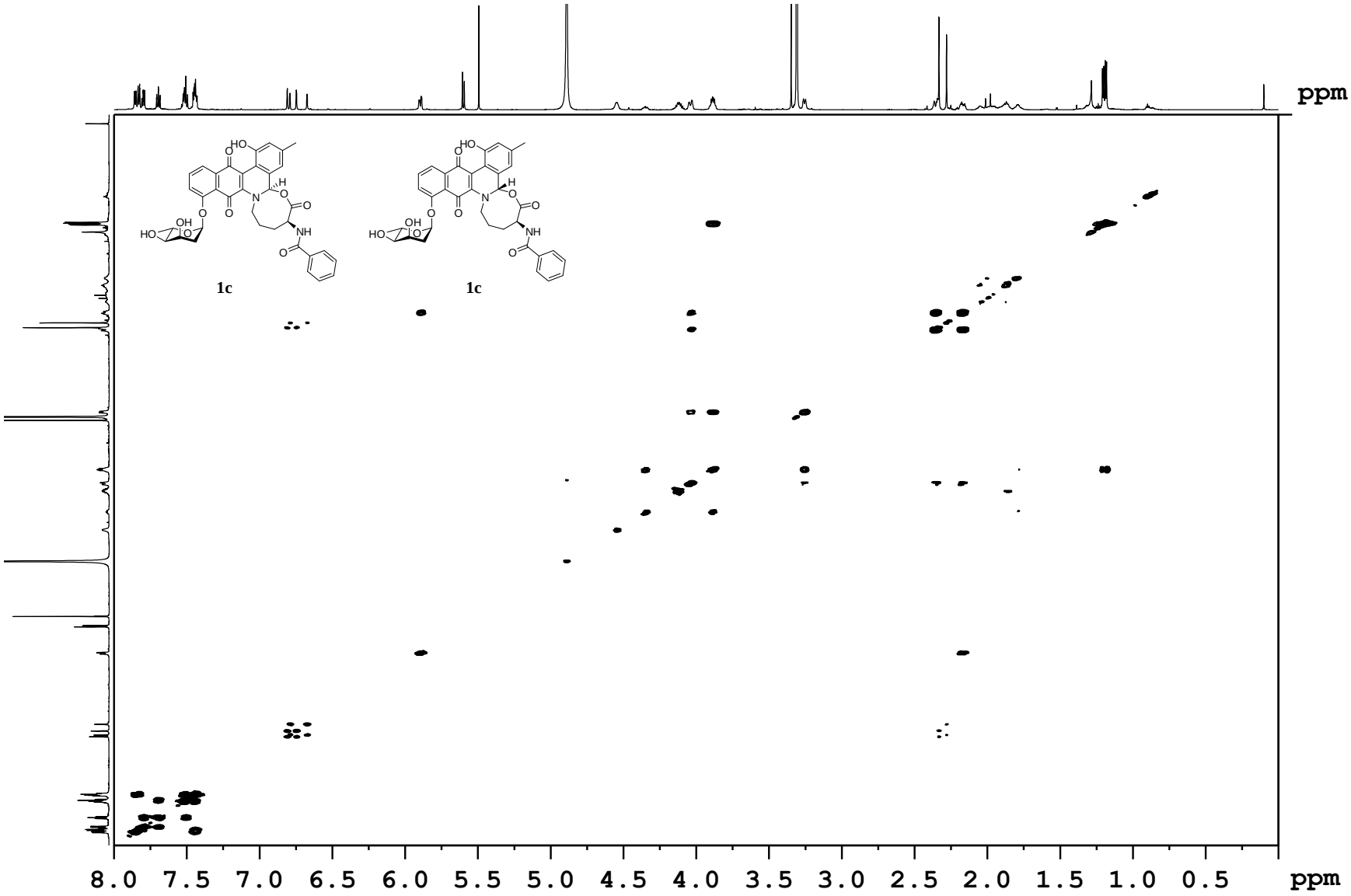


Figure S30. COSY (^{1}H-^{1}H) spectrum of **1c** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

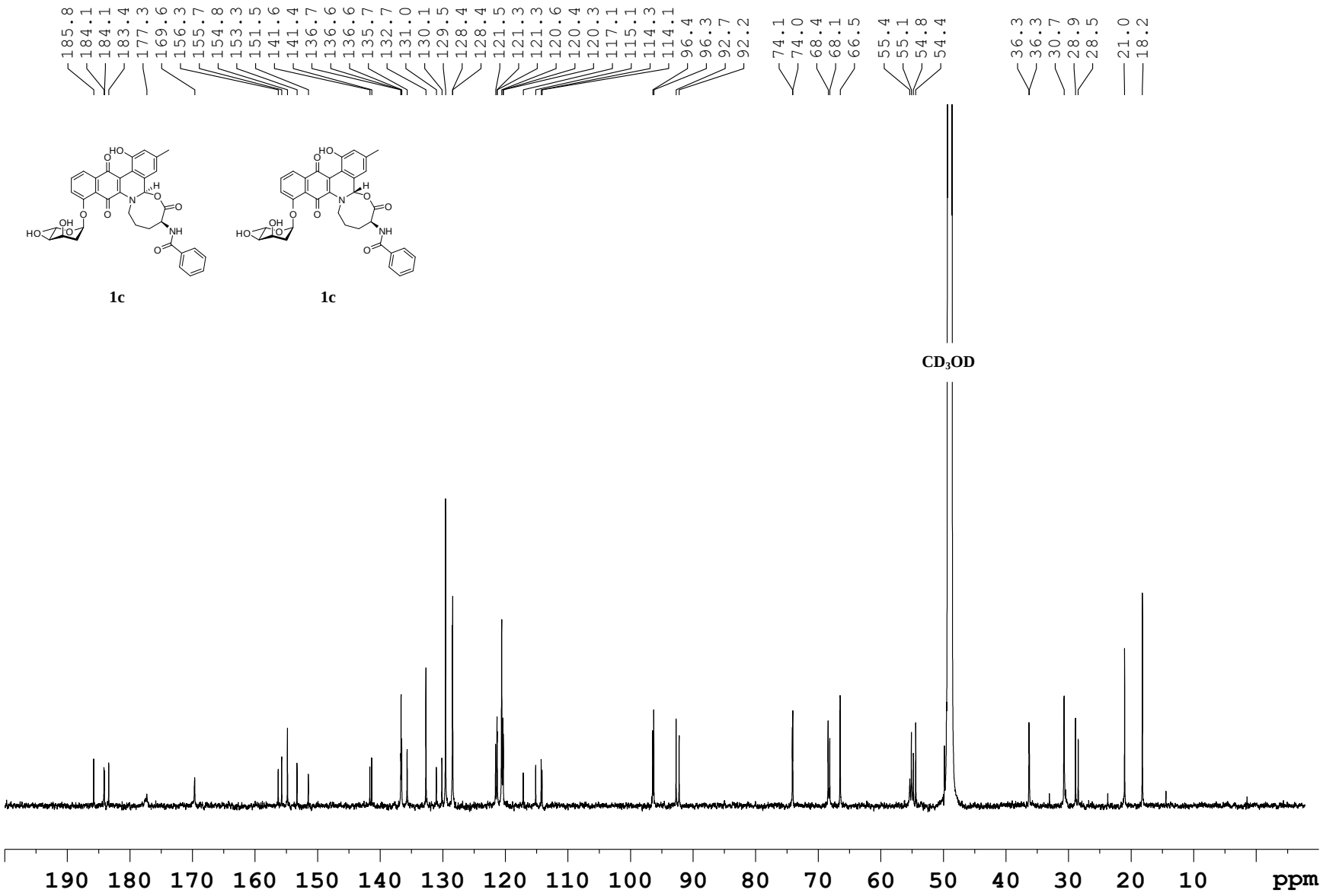


Figure S31. ^{13}C-NMR spectrum of **1c** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

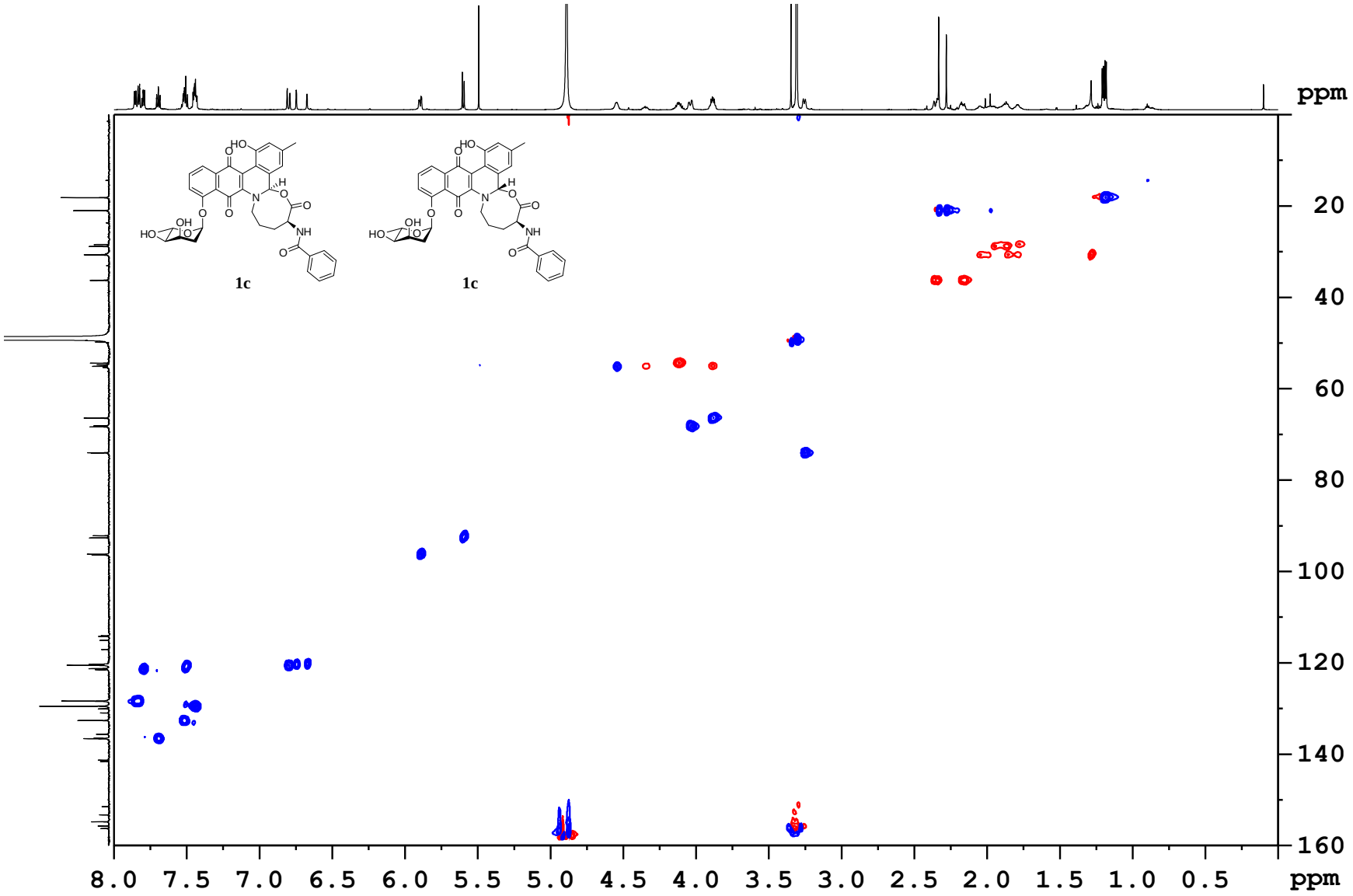


Figure S32. Edited-HSQC (^{1}H-^{13}C) spectrum of **1c** (diastereomeric mixture) in MeOD-d_4.

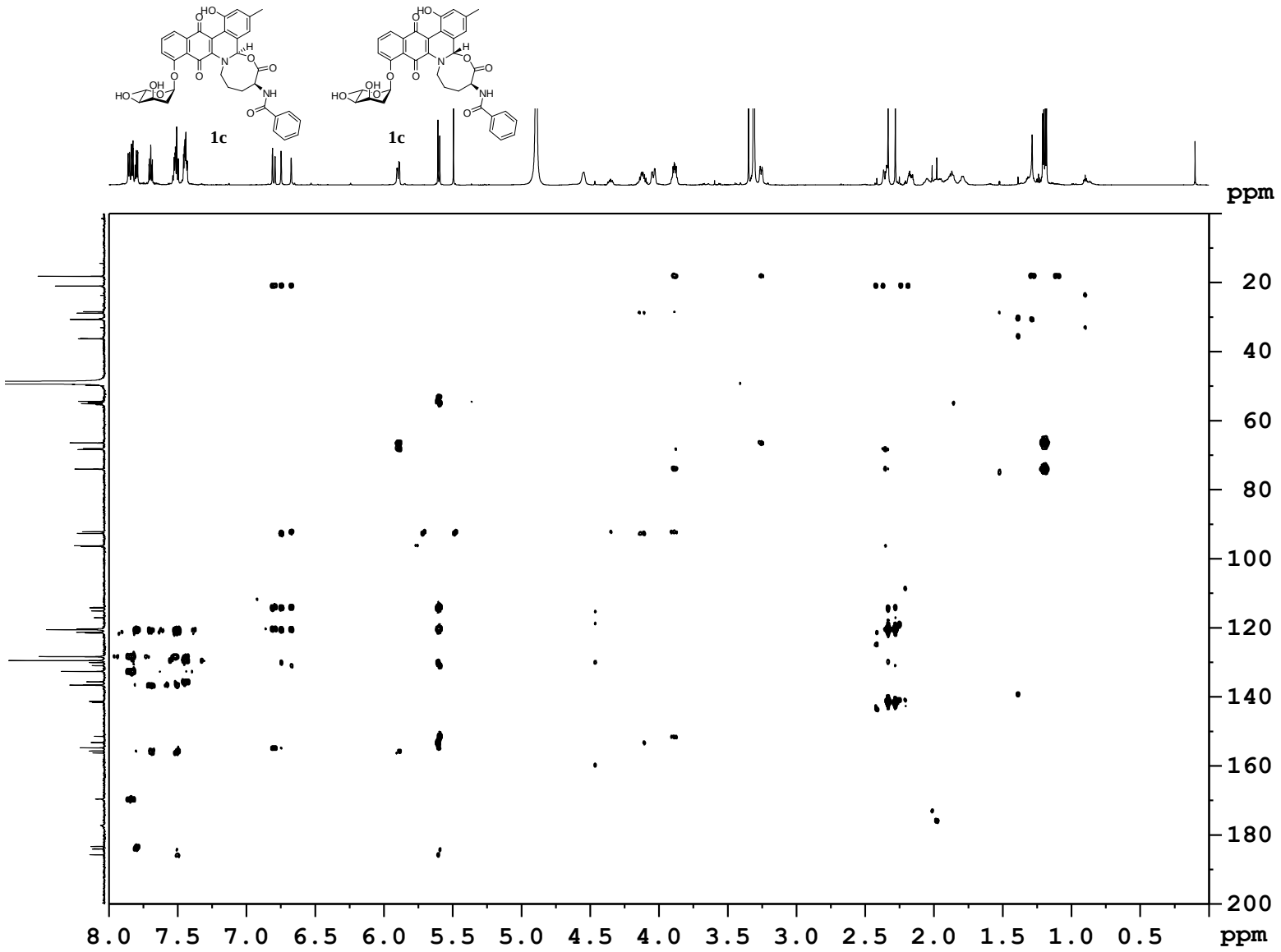


Figure S33. HMBC (^{1}H-^{13}C) spectrum of **1c** (diastereomeric mixture) in MeOD-d_4.

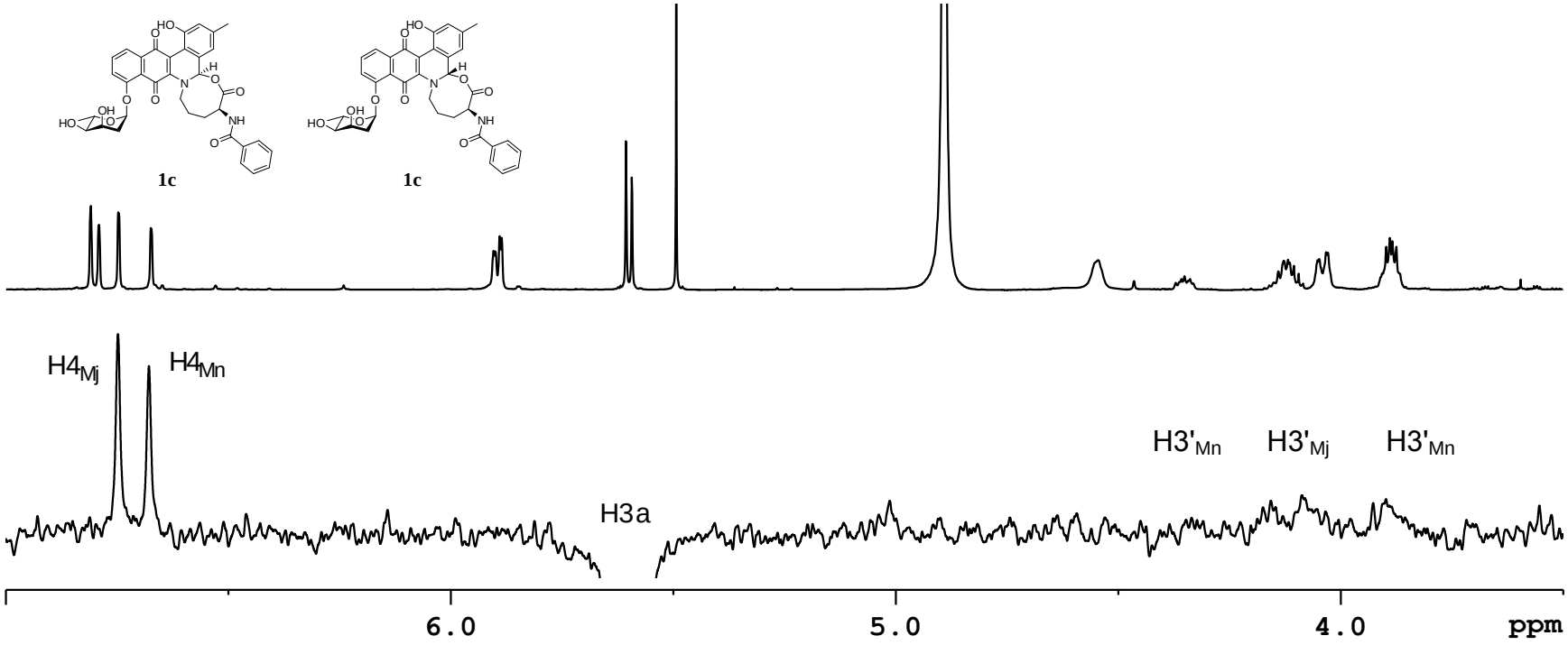


Figure S34. Overlay of ^{1}H-NMR spectrum of **1c** (top) with ROESY (500 MHz) showing irradiation of both $H3a_{Mn}$ and $H3a_{Mj}$ simultaneously (bottom), in MeOD-d_4.

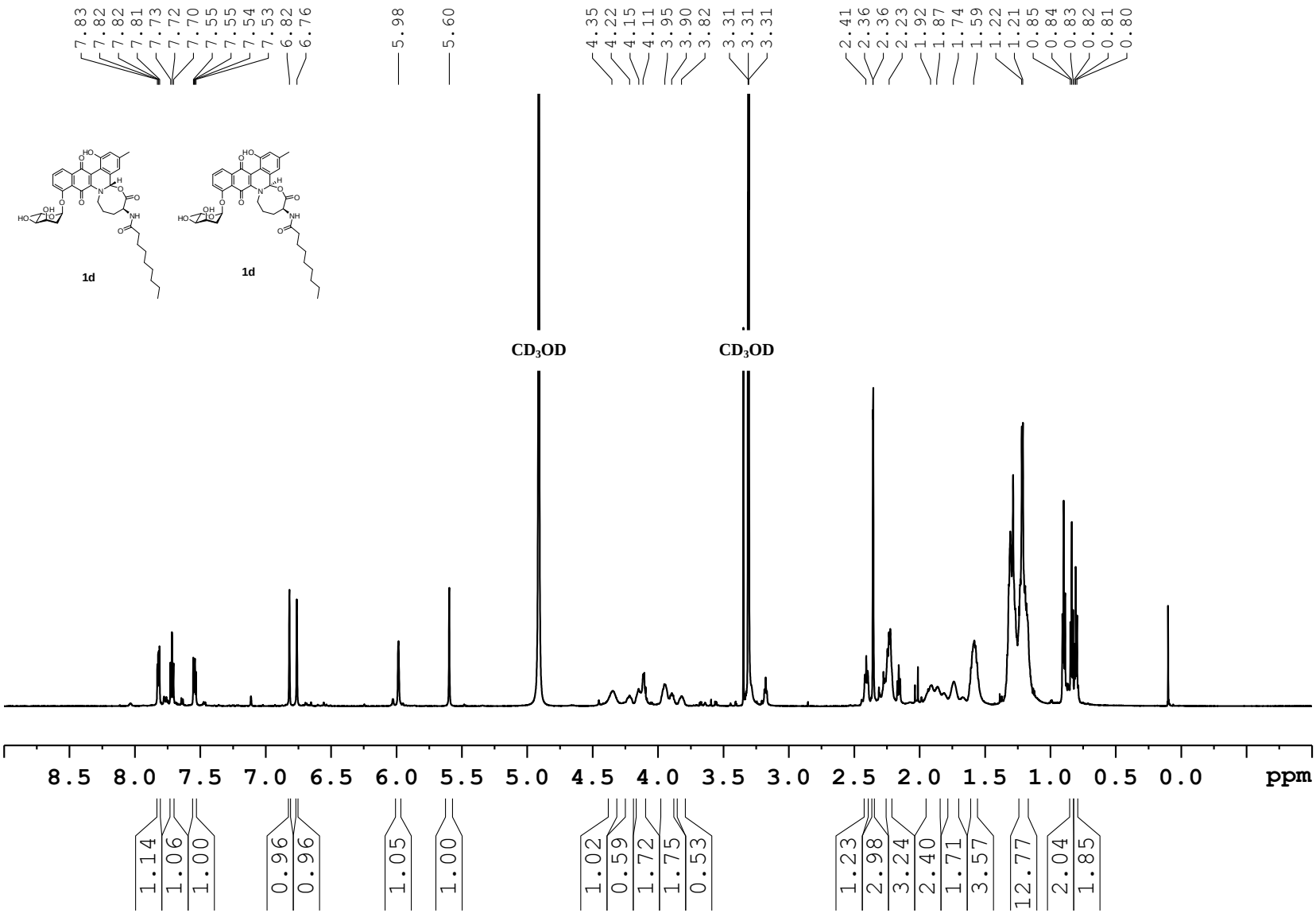


Figure S35. ^{1}H-NMR spectrum of **1d** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz).

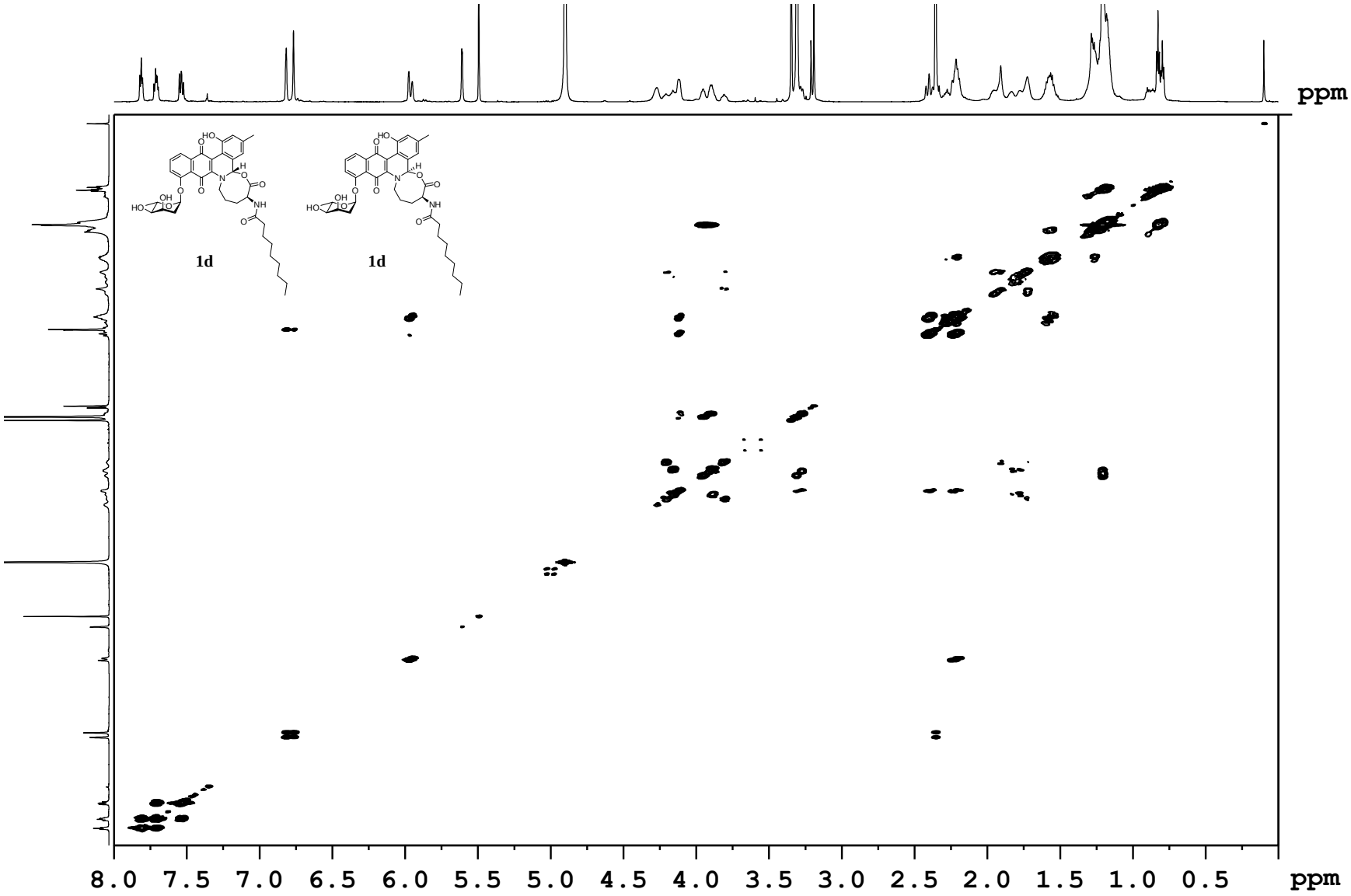


Figure S36. COSY (^{1}H-^{1}H) spectrum of **1d** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

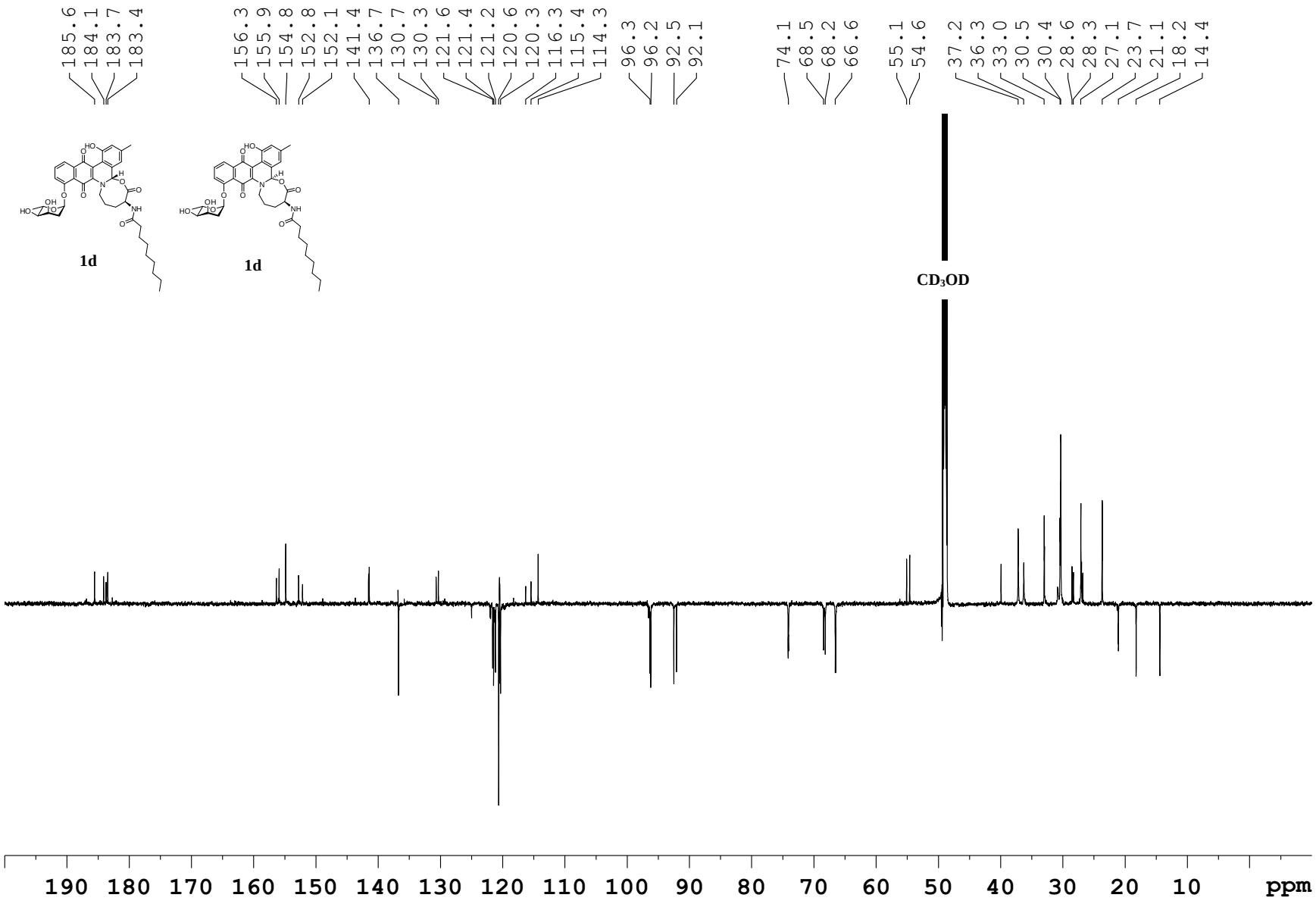


Figure S37. DEPTQ spectrum of **1d** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

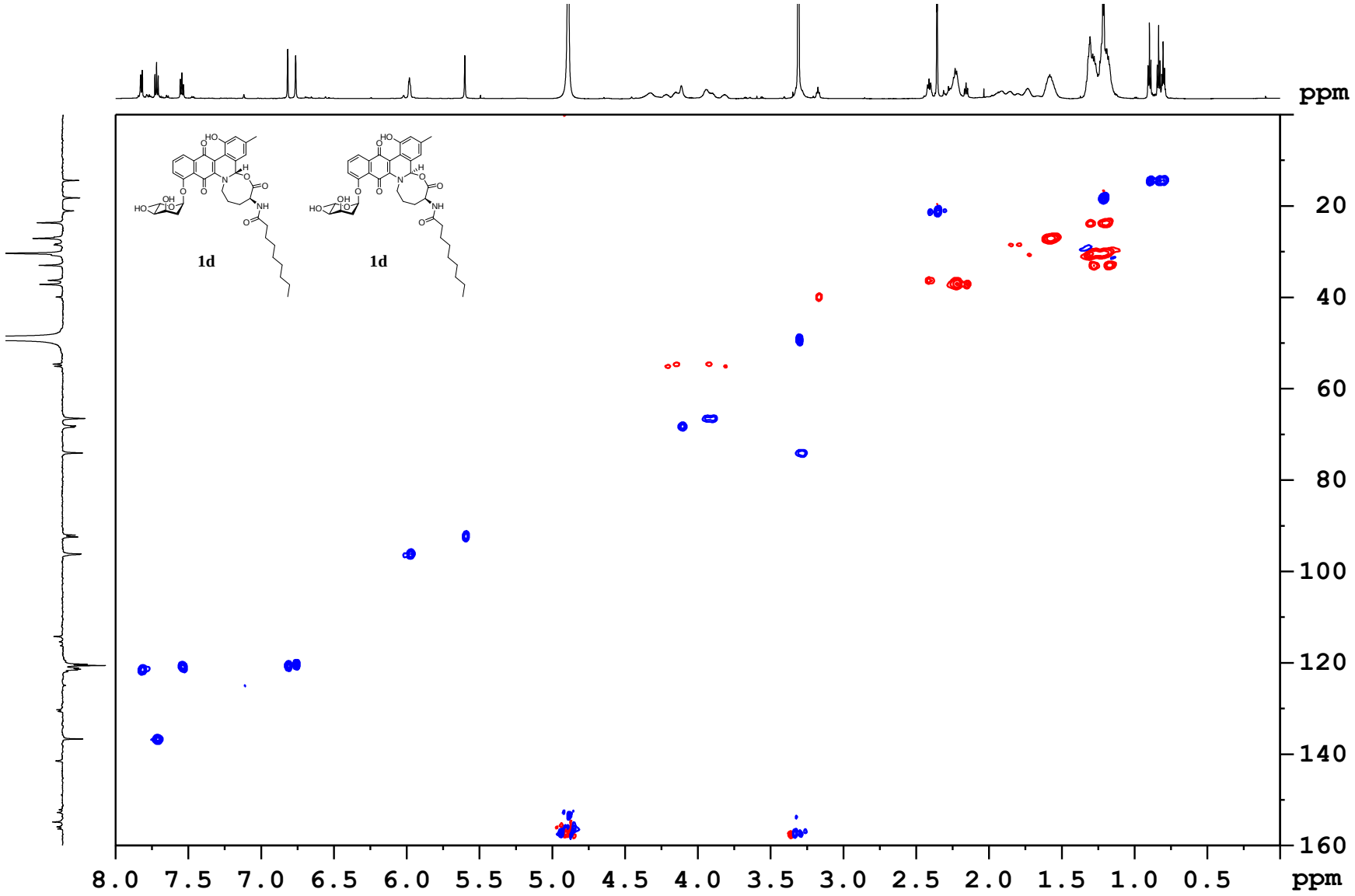


Figure S38. Edited-HSQC (^{1}H-^{13}C) spectrum of **1d** (diastereomeric mixture) in MeOD-d_4.

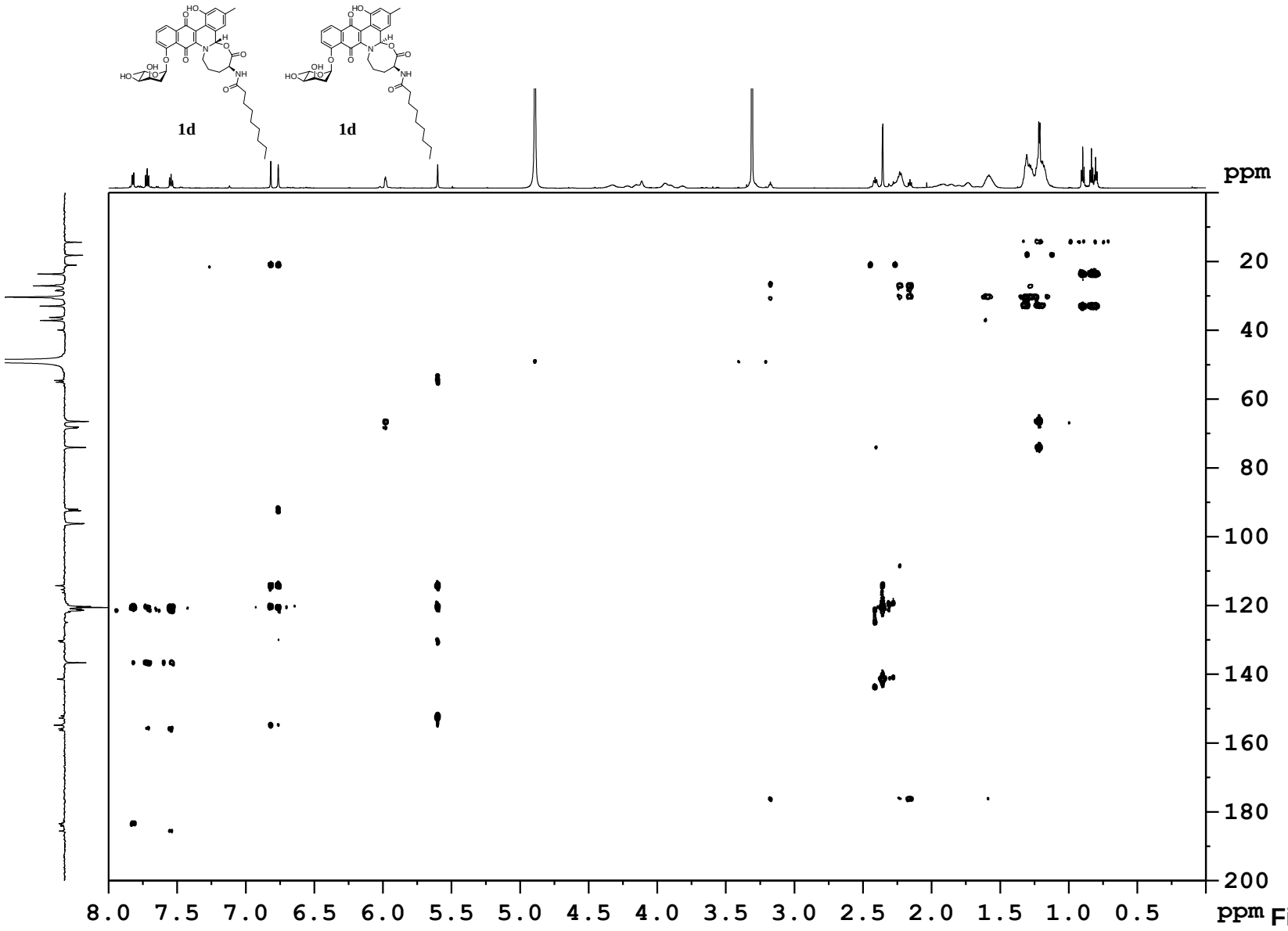


Figure S39. HMBC (^{1}H-^{13}C) spectrum of **1d** (diastereomeric mixture) in MeOD-d_4.

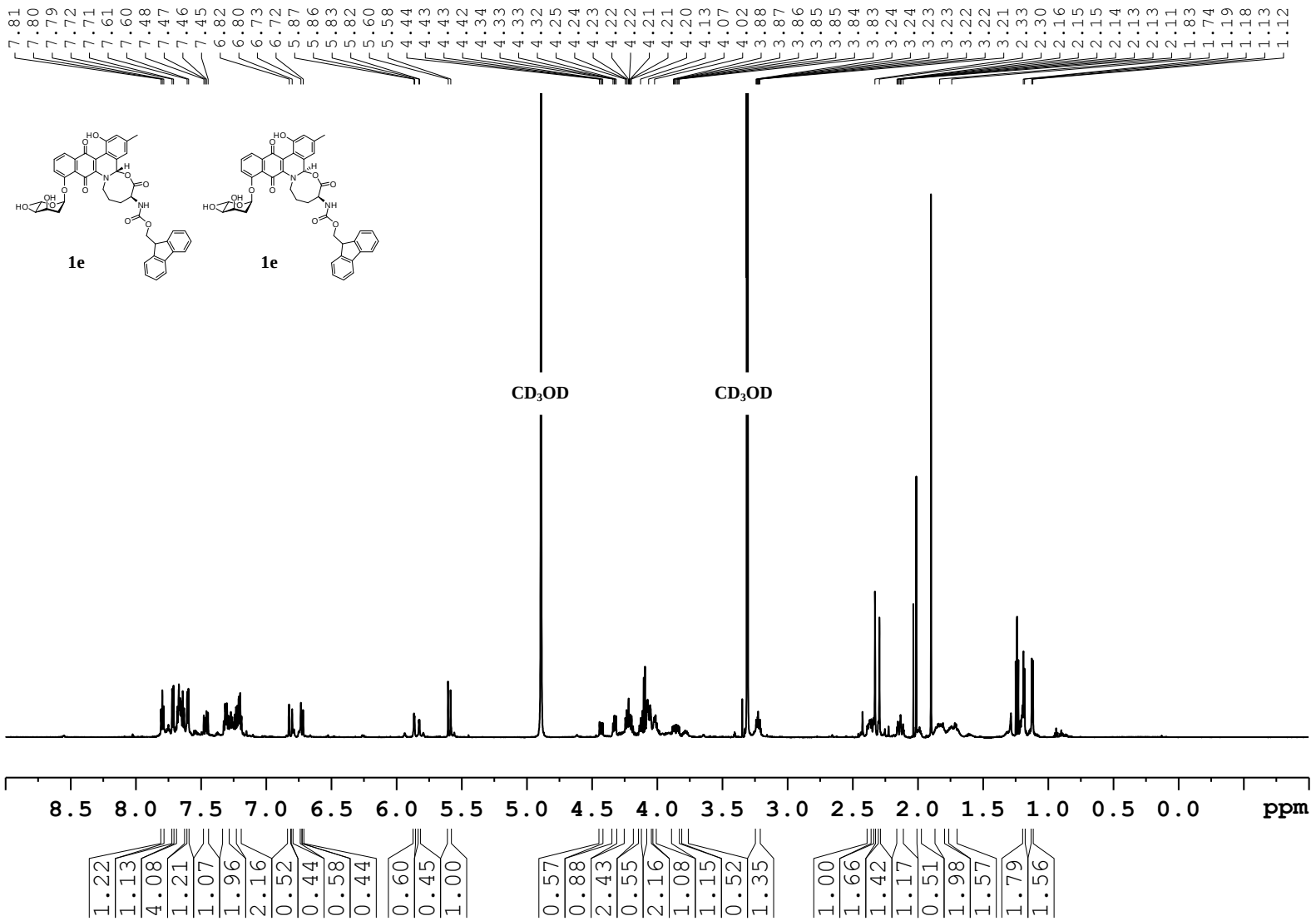


Figure S40. ^{1}H-NMR spectrum of **1e** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz)
Note: Compound prone to breakdown over time in NMR tube/solvent.

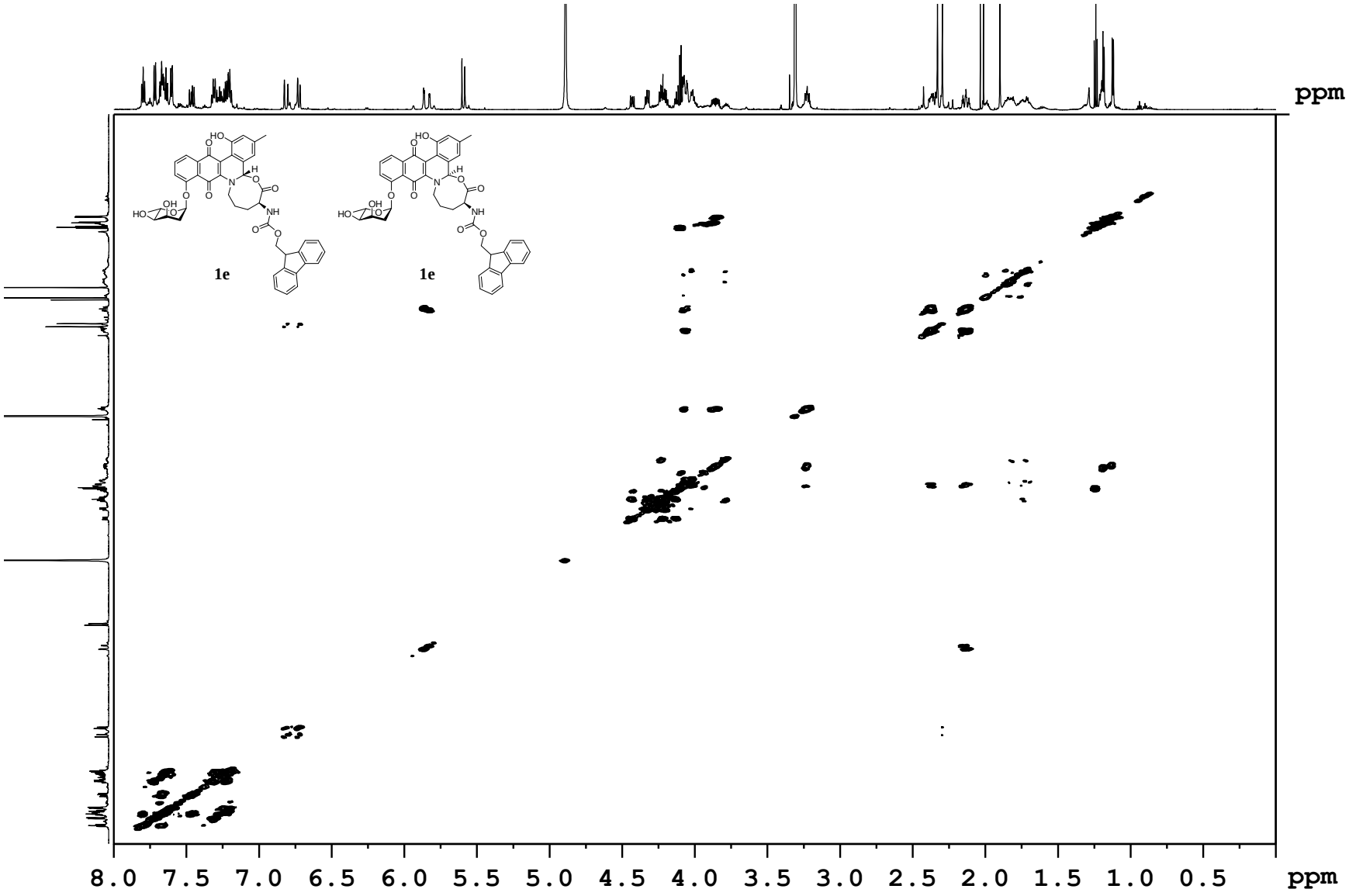


Figure S41. COSY (^{1}H-^{1}H) spectrum of **1e** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

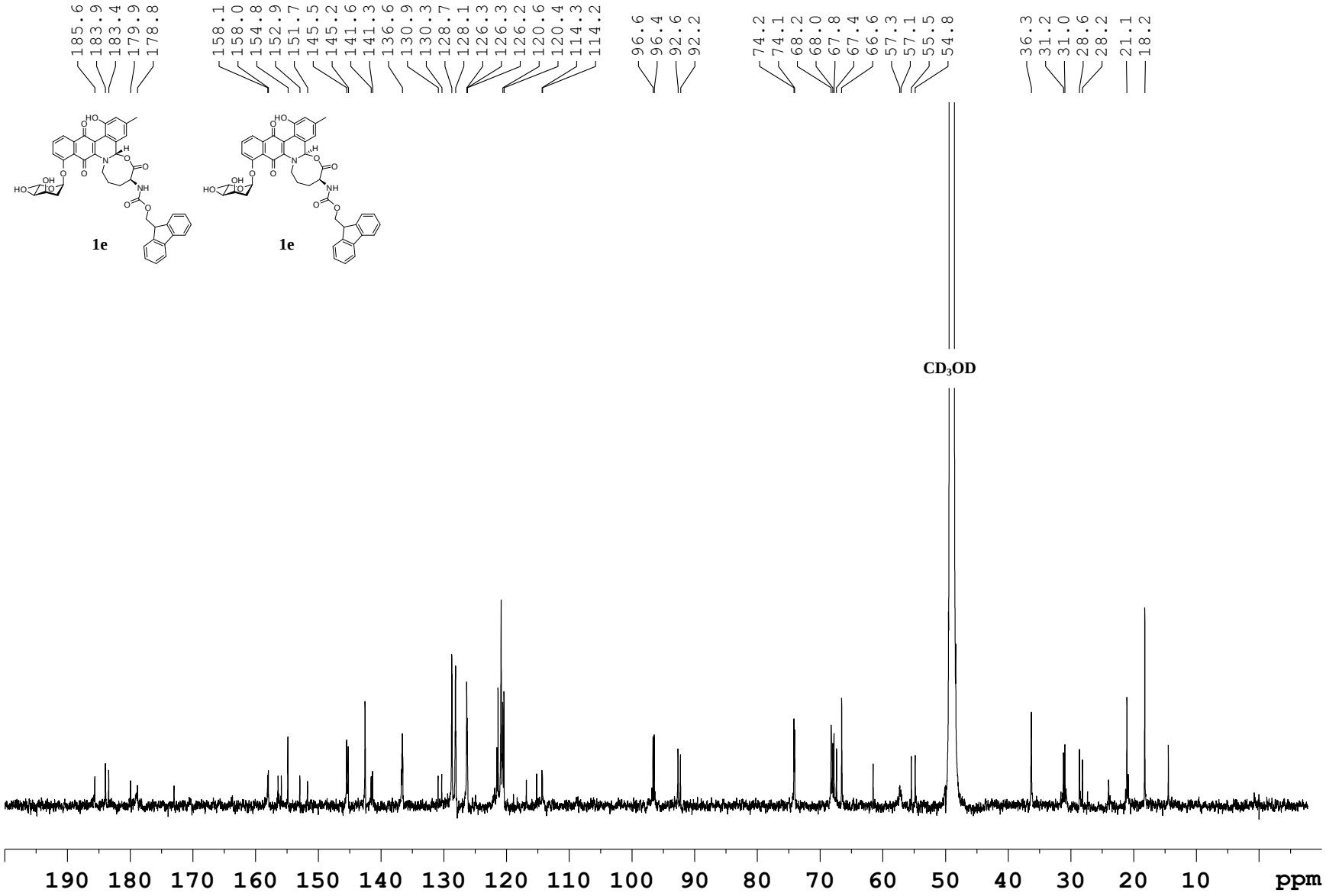


Figure S42. ^{13}C-NMR spectrum of **1e** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

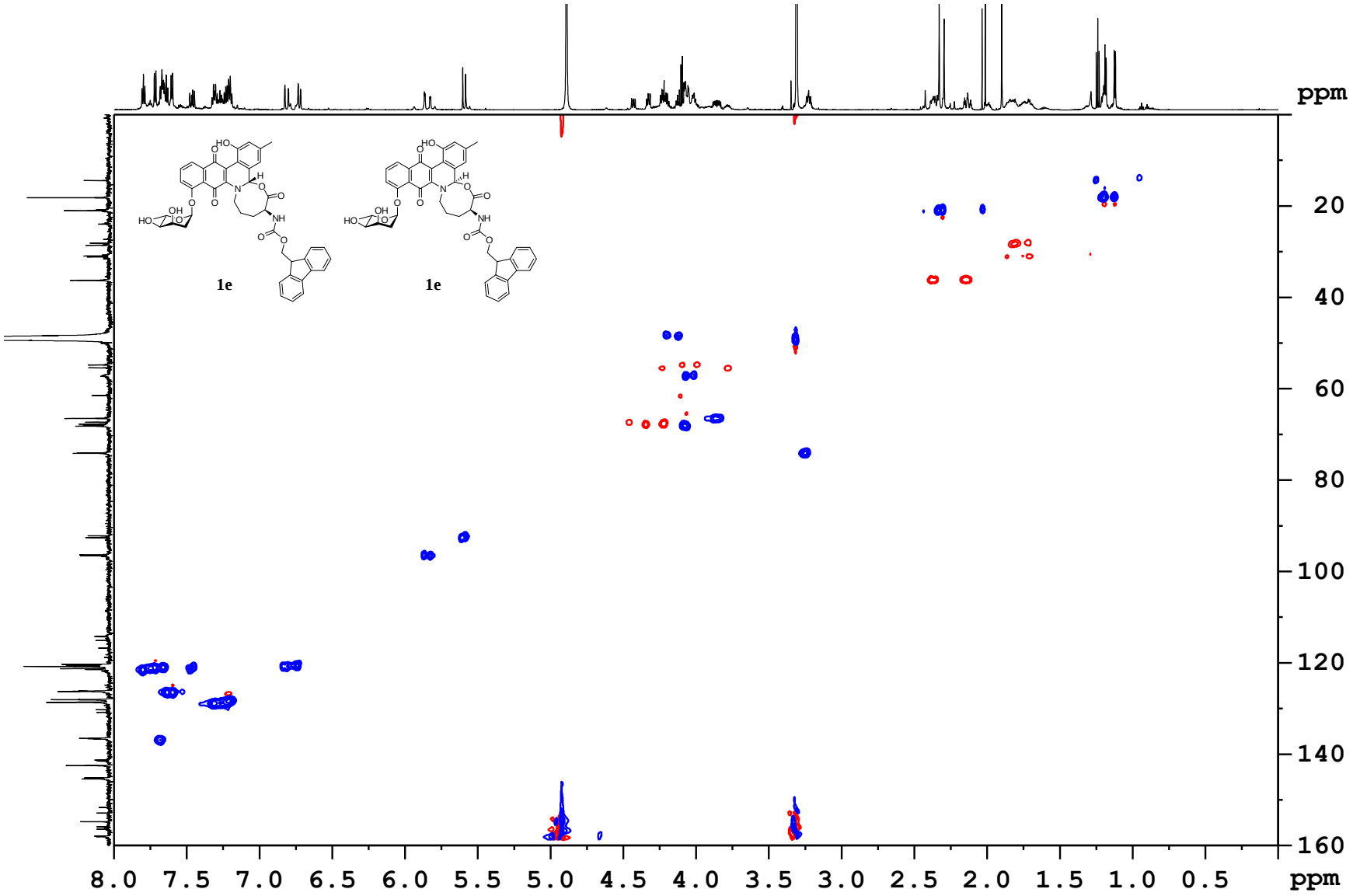


Figure S43. Edited-HSQC (^{1}H-^{13}C) spectrum of **1e** (diastereomeric mixture) in MeOD-d_4.

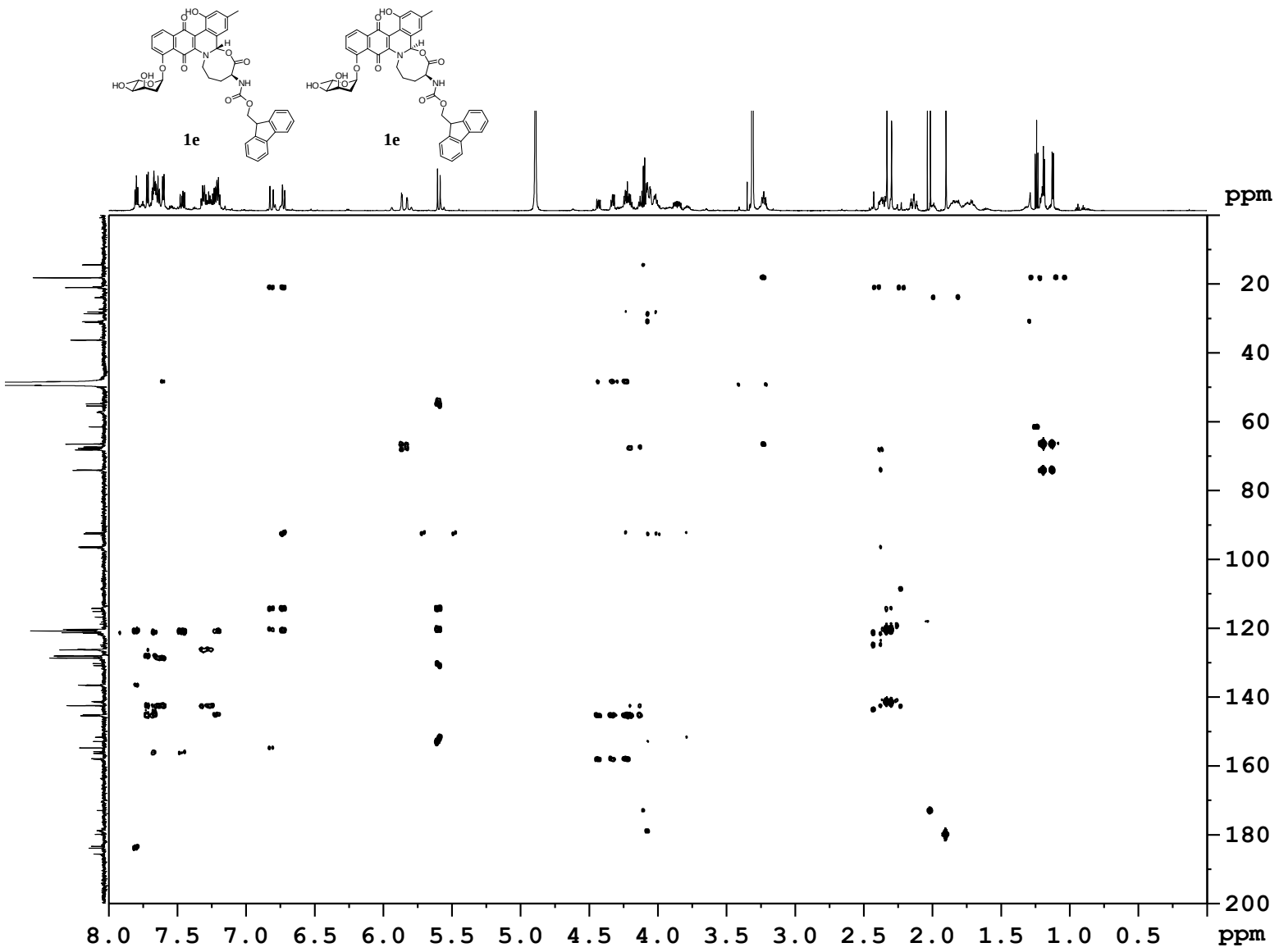


Figure S44. HMBC (^{1}H-^{13}C) spectrum of **1e** (diastereomeric mixture) in MeOD-d_4.

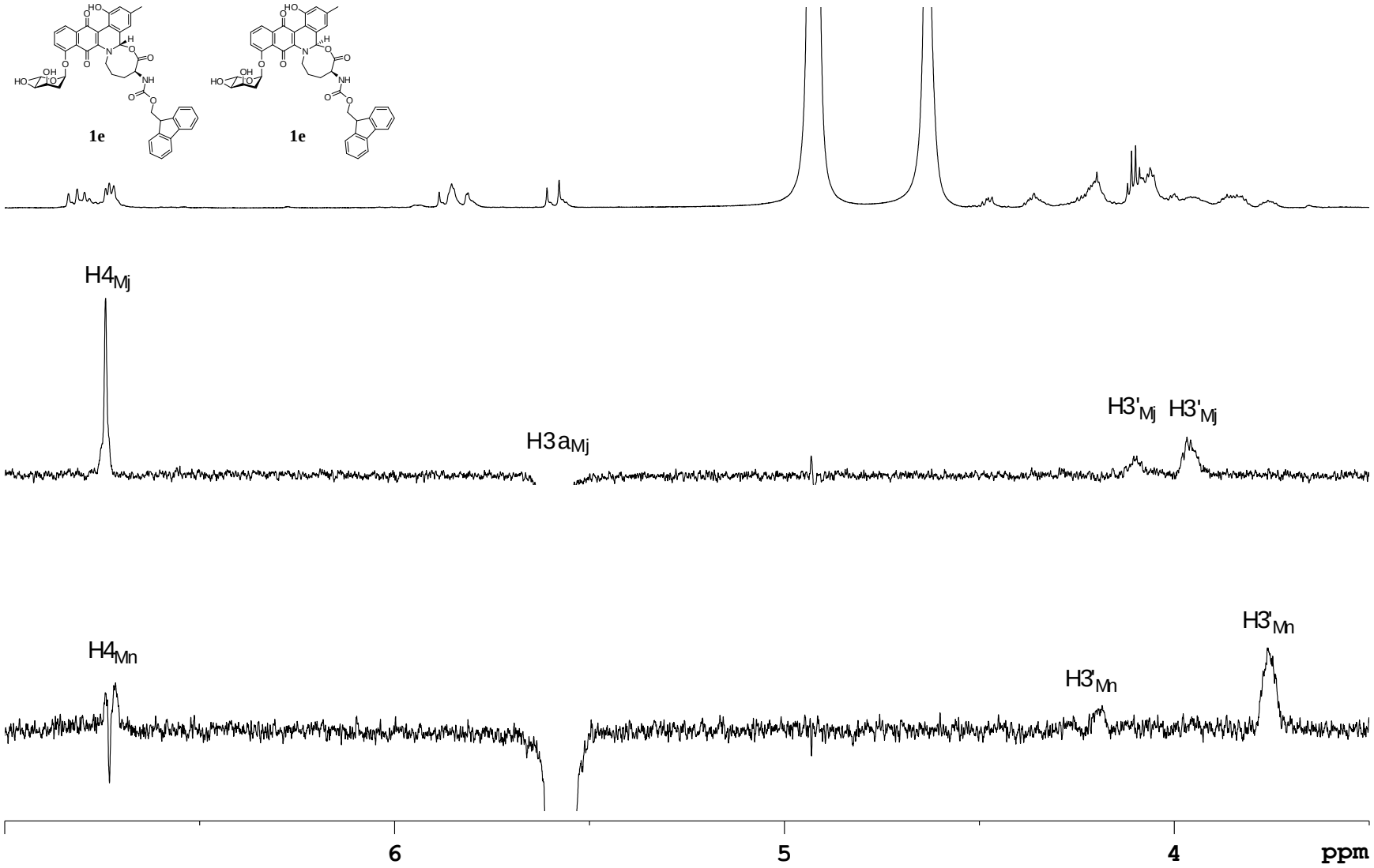


Figure S45. Overlay of ^{1}H-NMR spectrum of **1e** (top) with ROESY (500 MHz) showing irradiation of $H3a_{Mj}$ (middle), and $H3a_{Mn}$ (bottom) in MeOD-d_4.

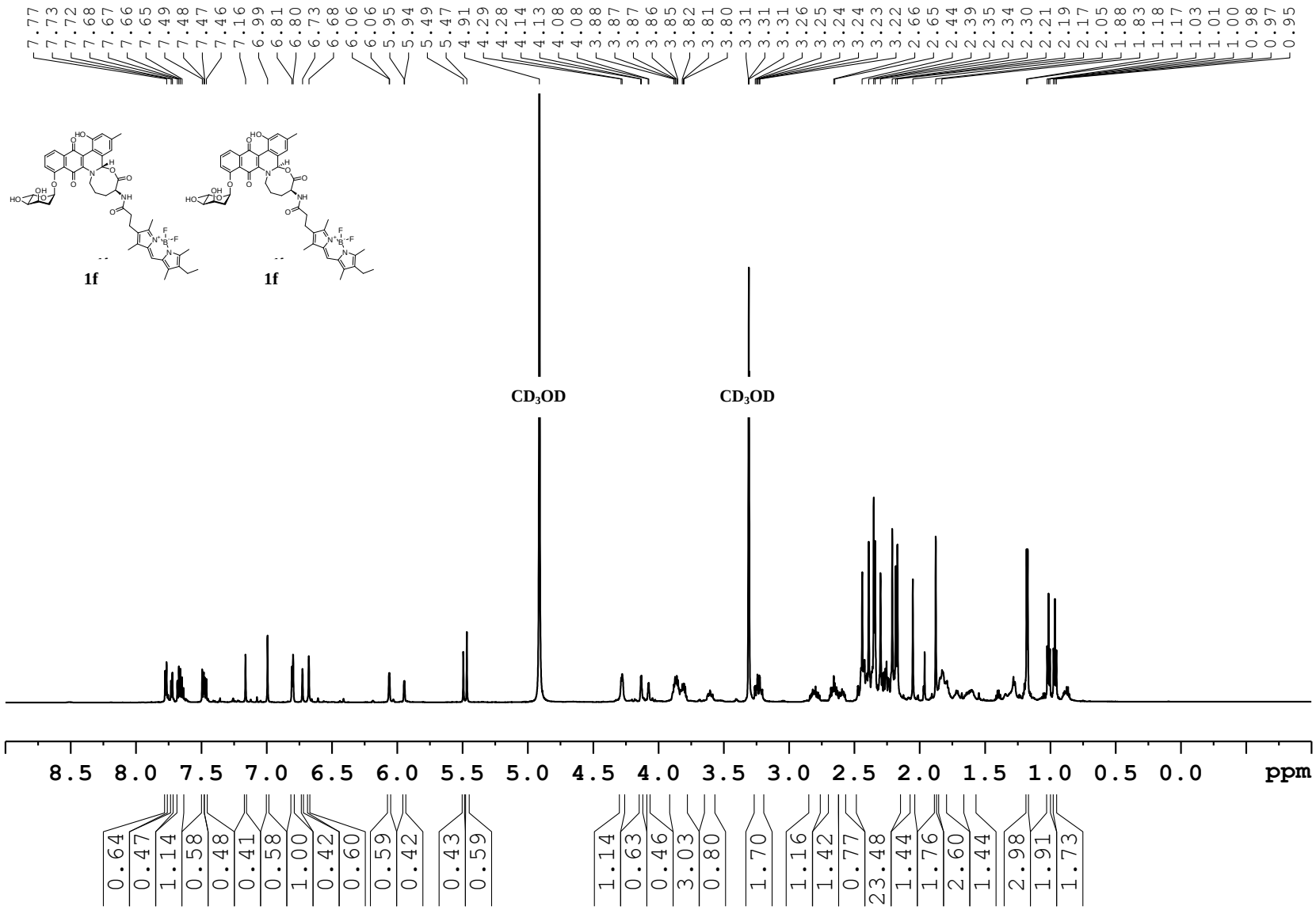


Figure S46. ^{1}H-NMR spectrum of **1f** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz).

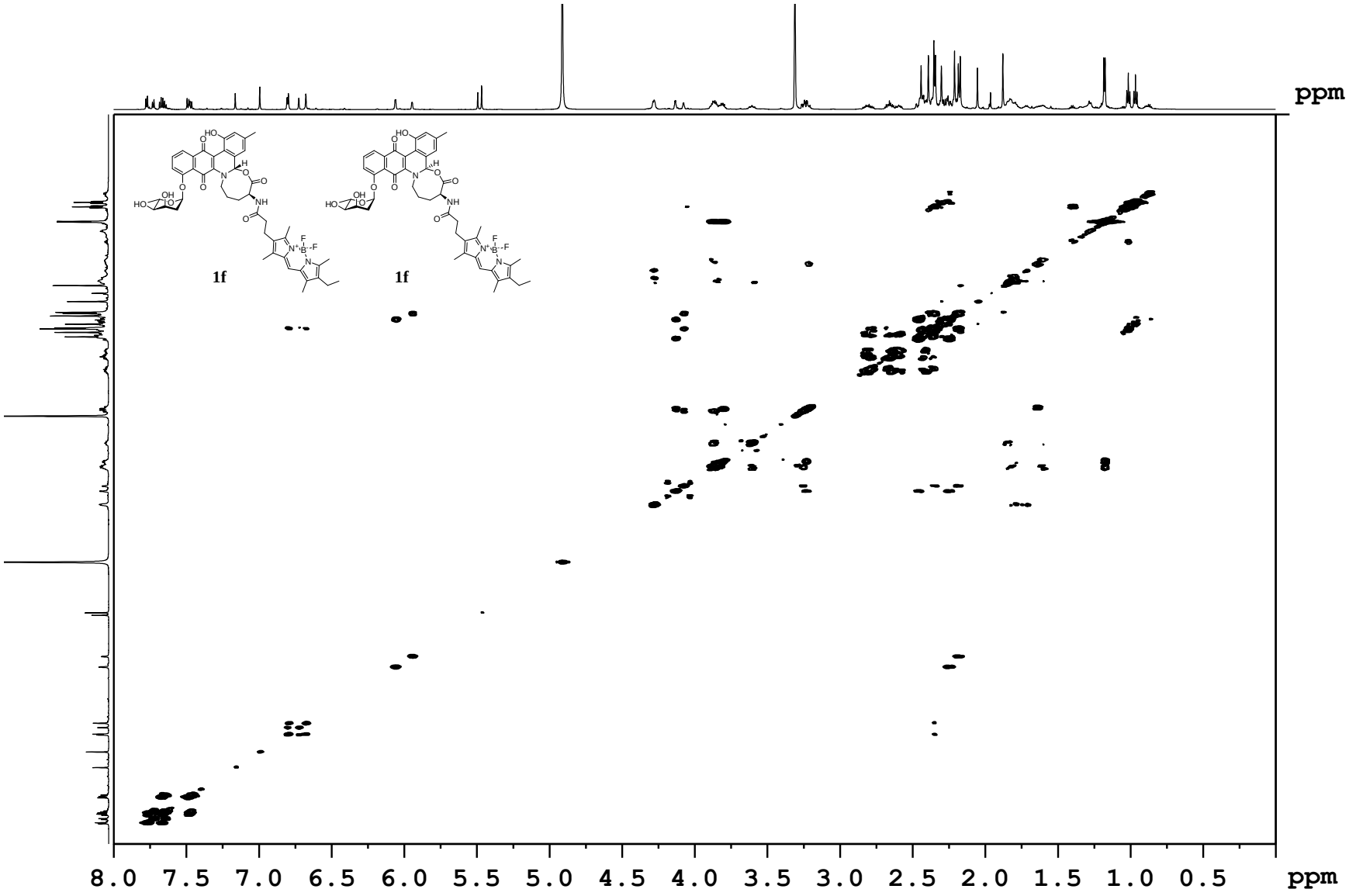


Figure S47. COSY (^{1}H-^{1}H) spectrum of **1f** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

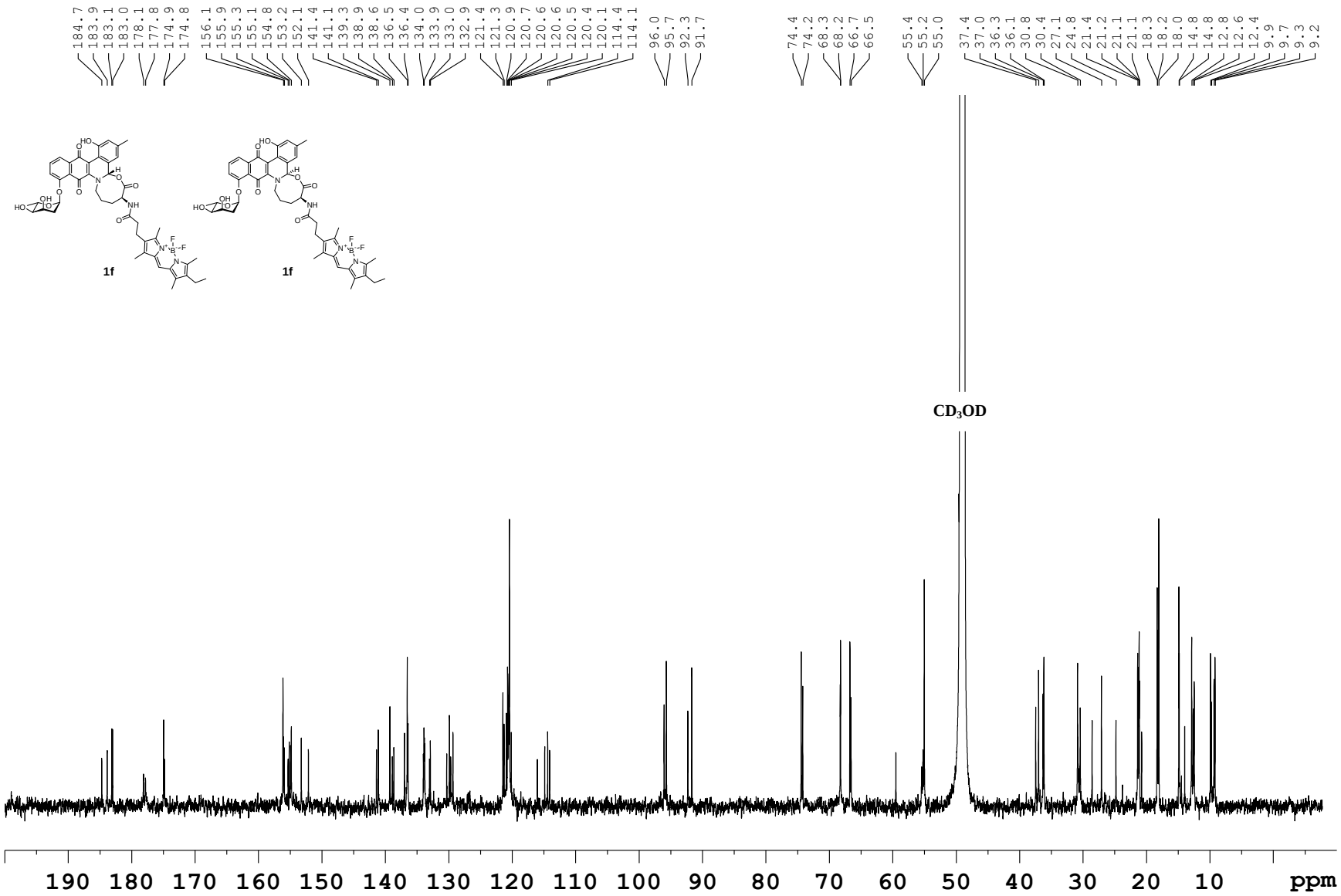


Figure S48. ^{13}C-NMR spectrum of **1f** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

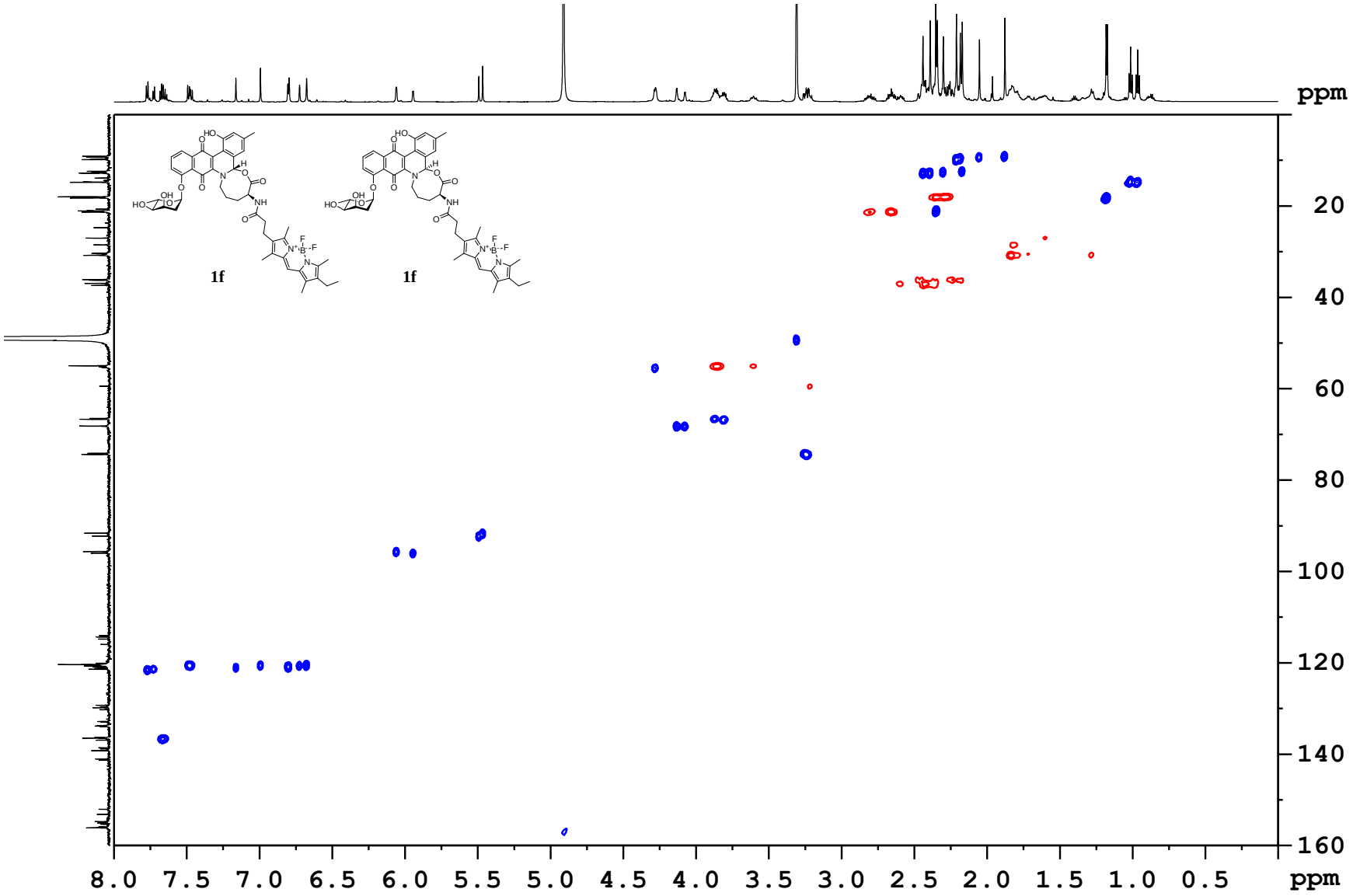


Figure S49. Edited-HSQC (^{1}H-^{13}C) spectrum of **1f** (diastereomeric mixture) in MeOD-d_4.

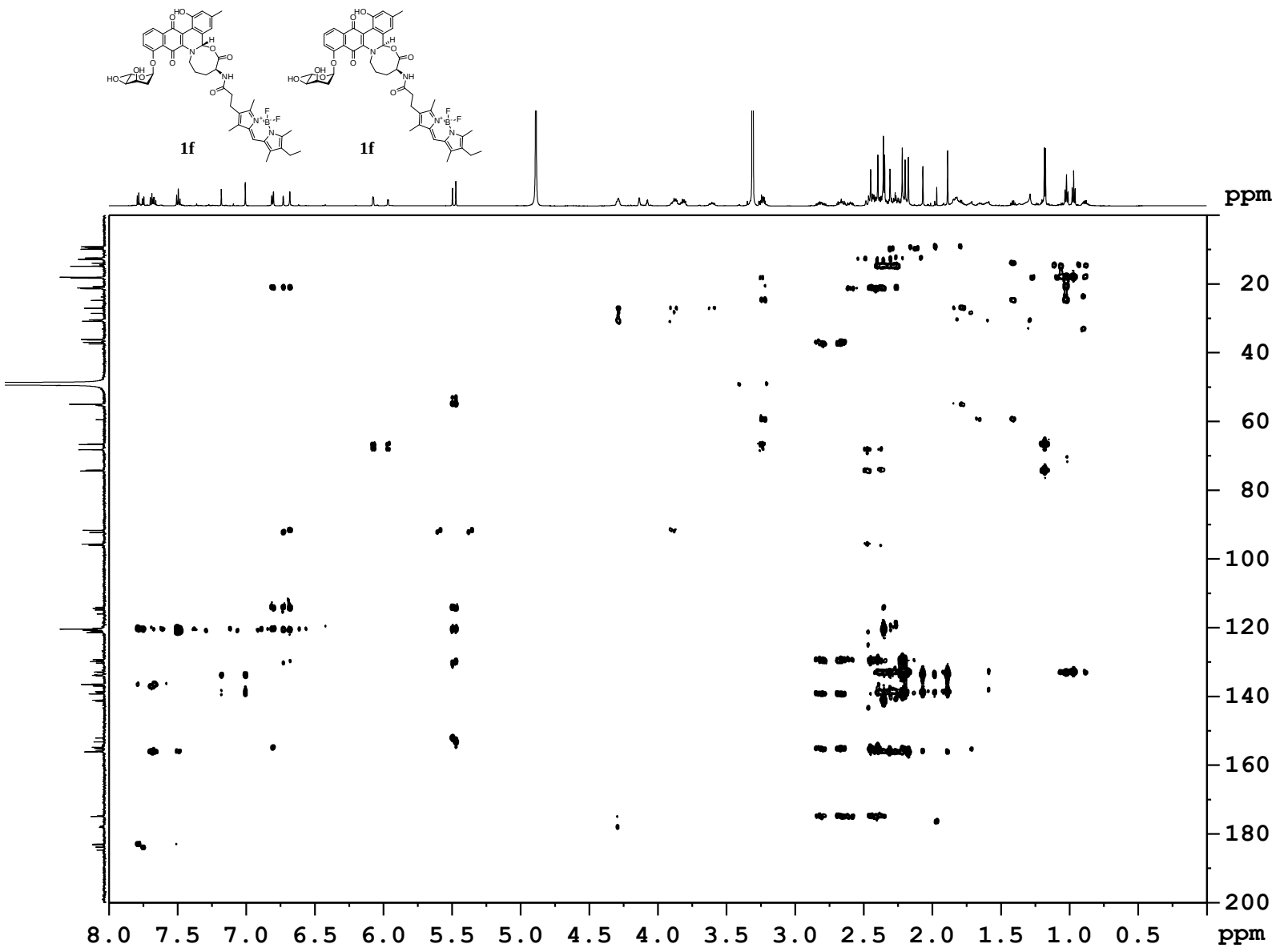


Figure S50. HMBC (^{1}H-^{13}C) spectrum of **1f** (diastereomeric mixture) in MeOD-d_4.

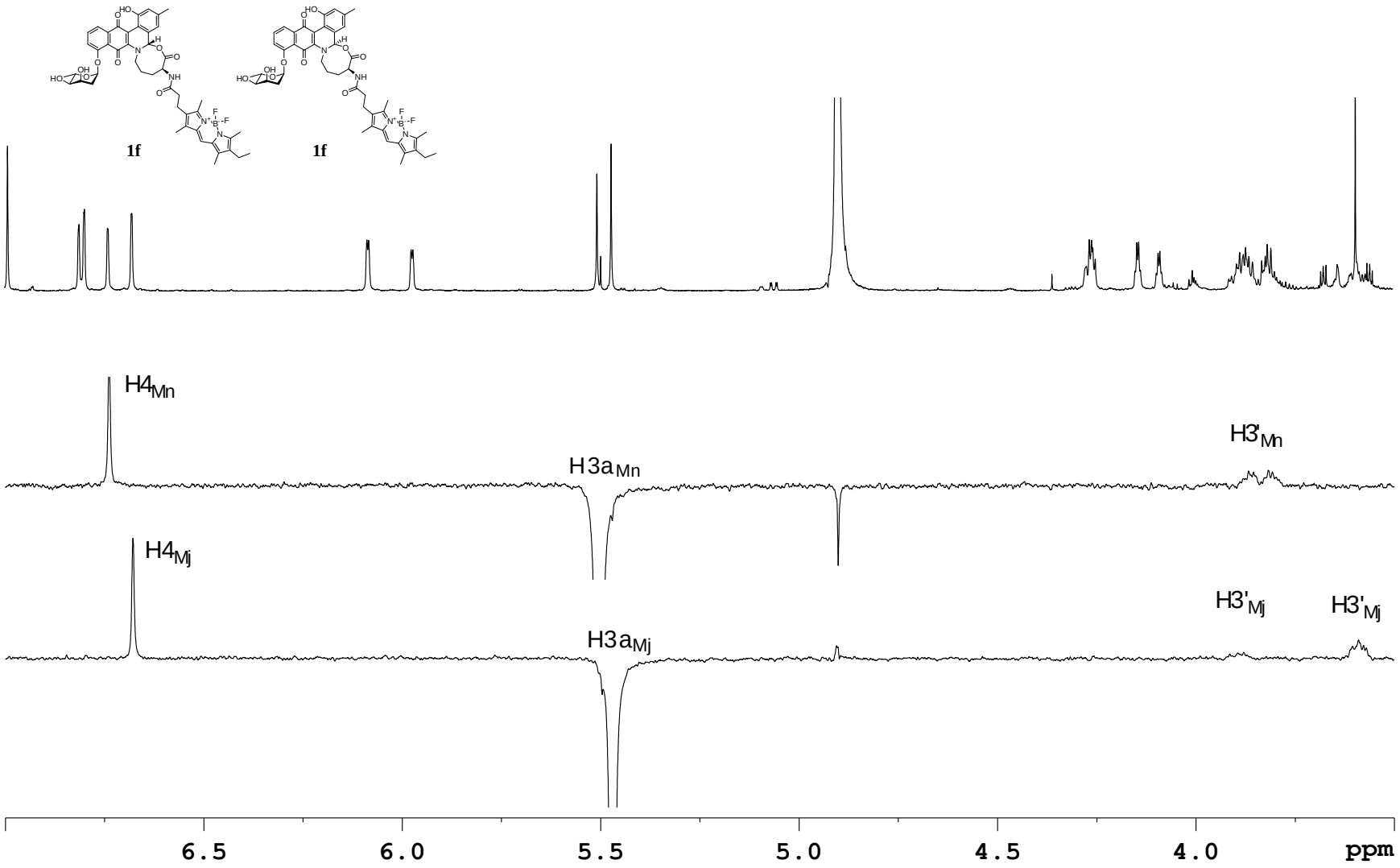


Figure S51. Overlay of ^{1}H-NMR spectrum of **1f** (top) with ROESY (700 MHz) showing irradiation of $H3a_{Mn}$ (middle), and $H3a_{Mj}$ (bottom) in MeOD-d_4.

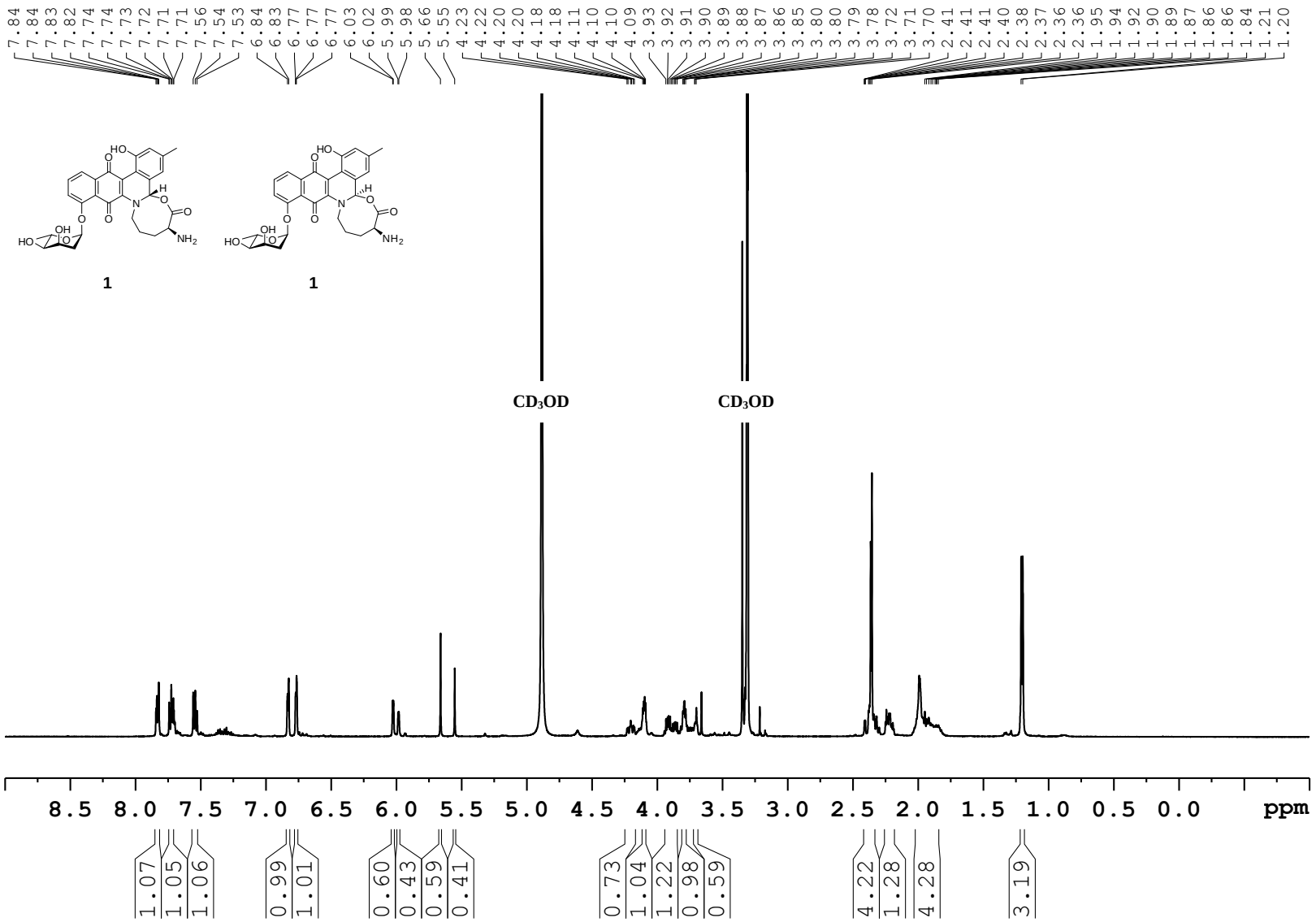


Figure S52. ^{1}H-NMR spectrum of **1** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 700 MHz). Note: Compound prone to breakdown over time in NMR tube/solvent.

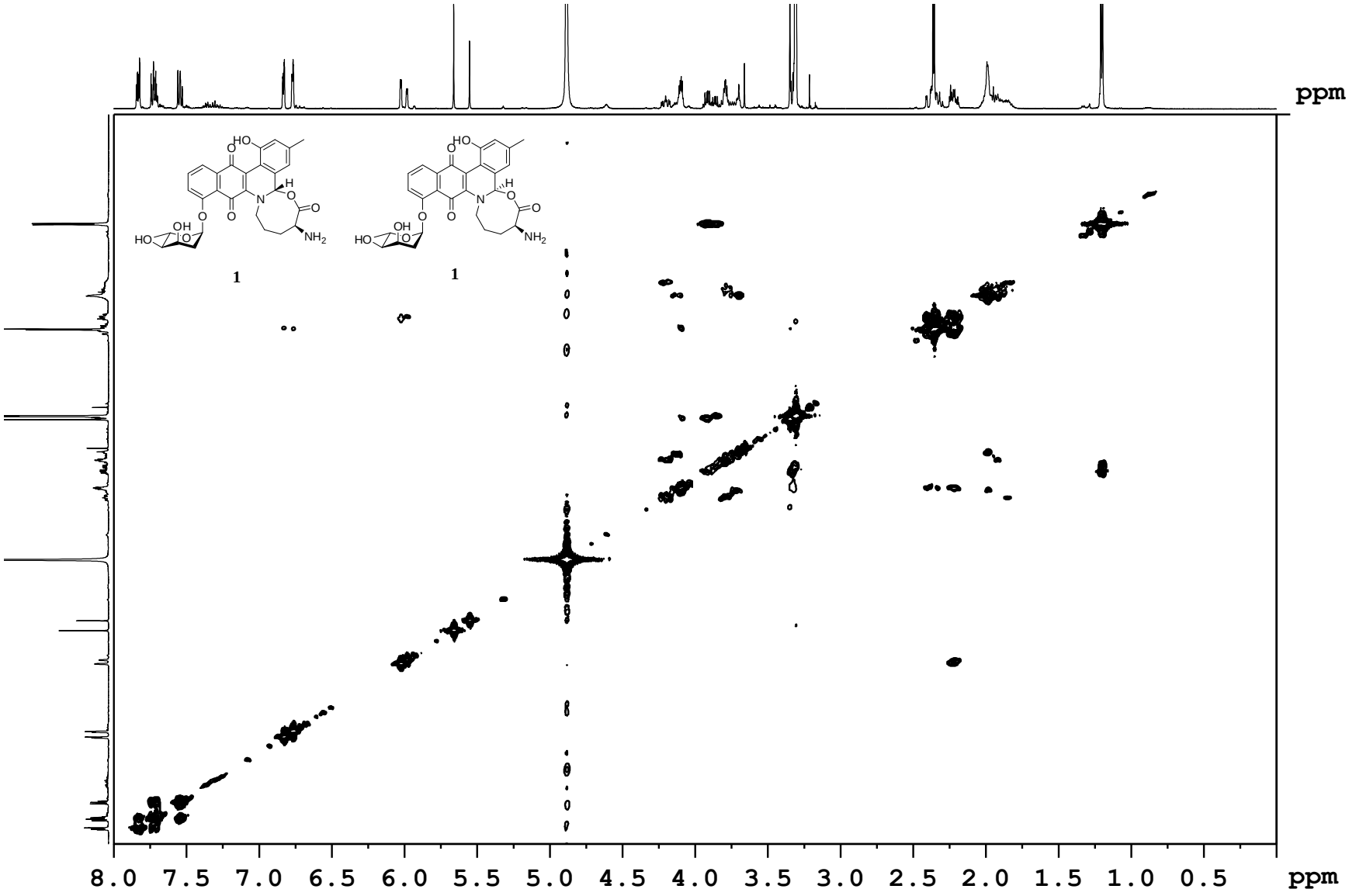


Figure S53. COSY (^{1}H-^{1}H) spectrum of **1** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

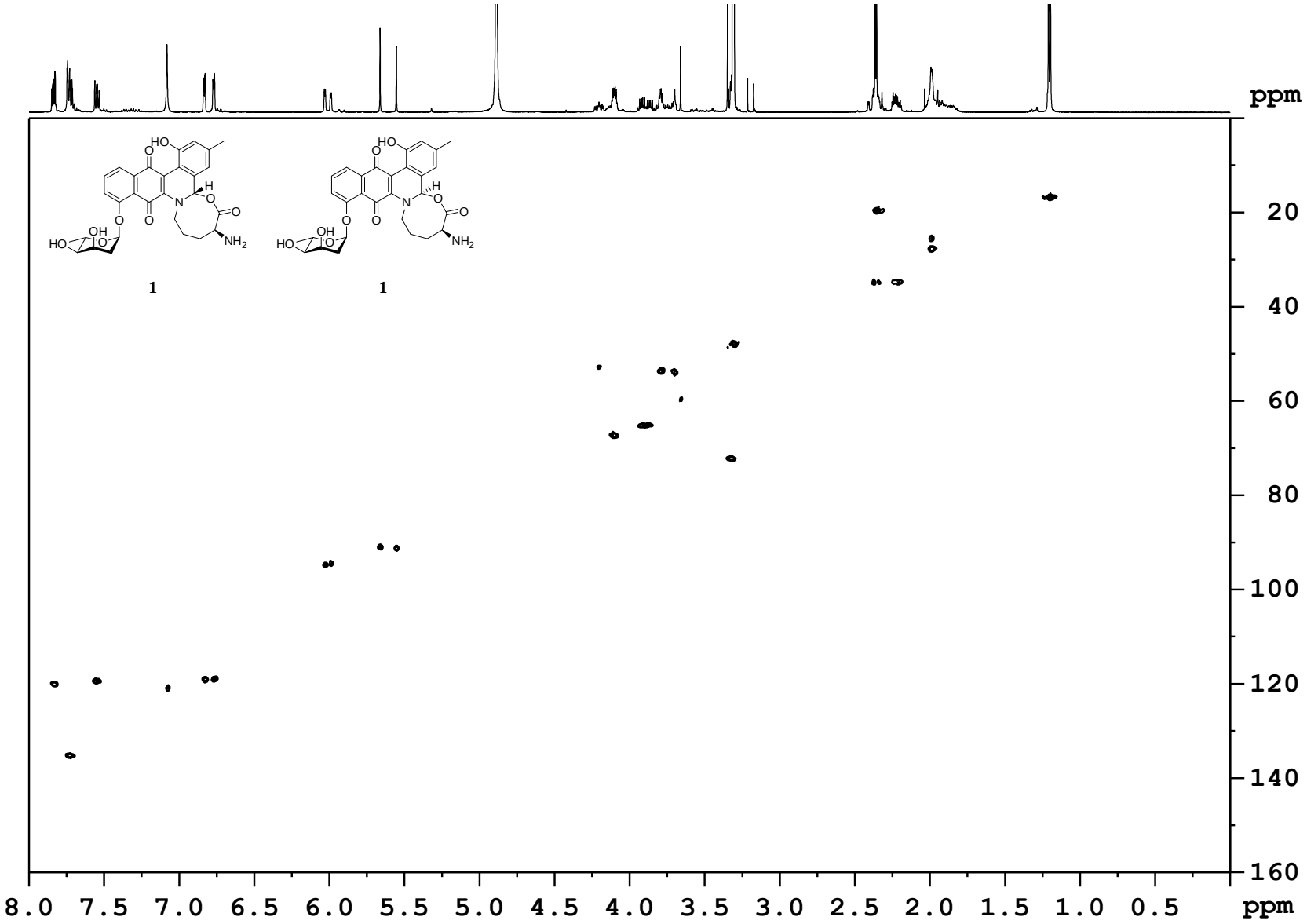


Figure S54. HSQC (^{1}H-^{13}C) spectrum of **1** (diastereomeric mixture) in MeOD-d_4.

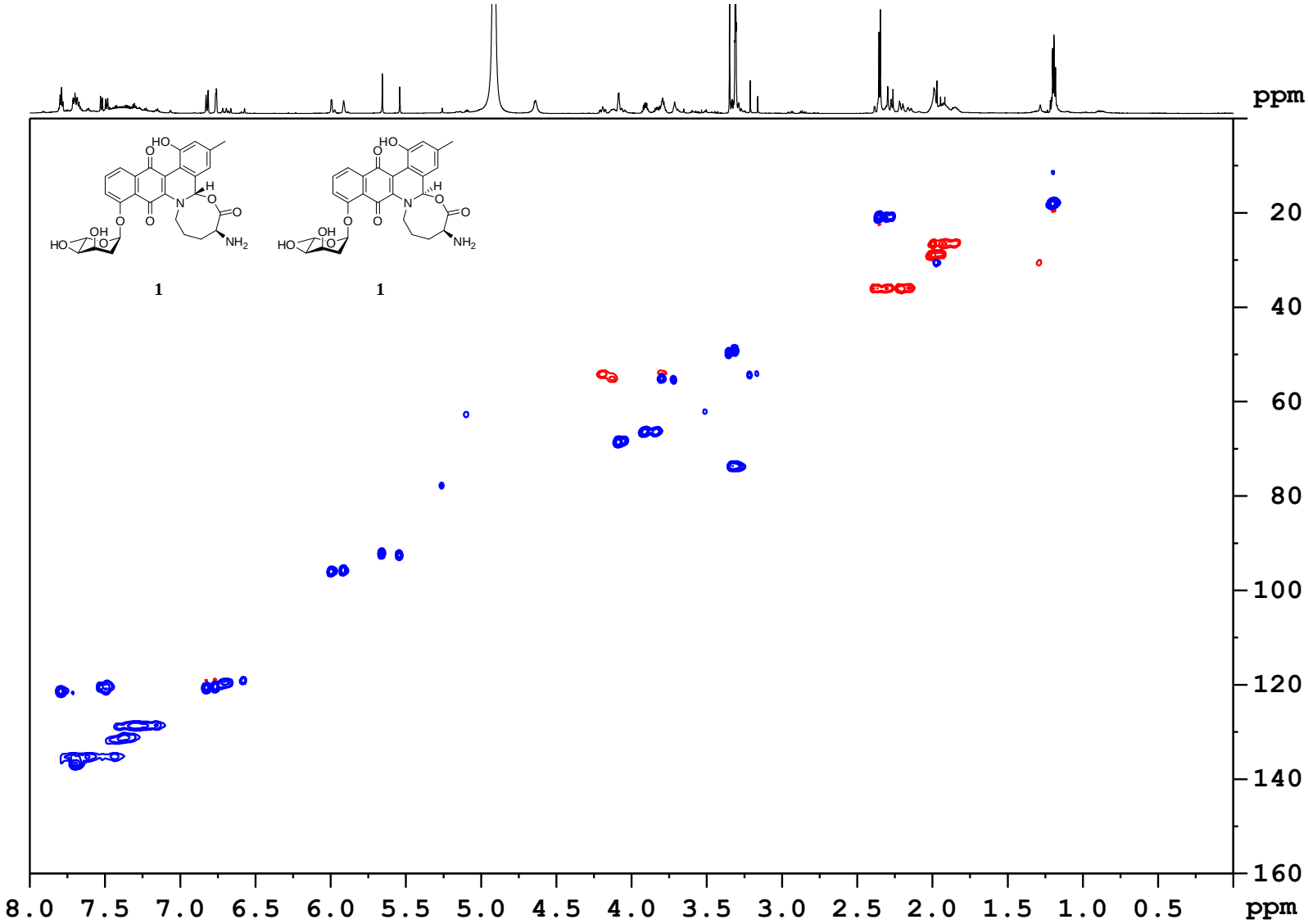


Figure S55. Edited-HSQC (^{1}H-^{13}C) spectrum of **1** (diastereomeric mixture) in MeOD-d_4. Breakdown of **3** overtime in the NMR tube is observed in the aromatic region as a broad set of peaks between 7.1-7.5 ppm.

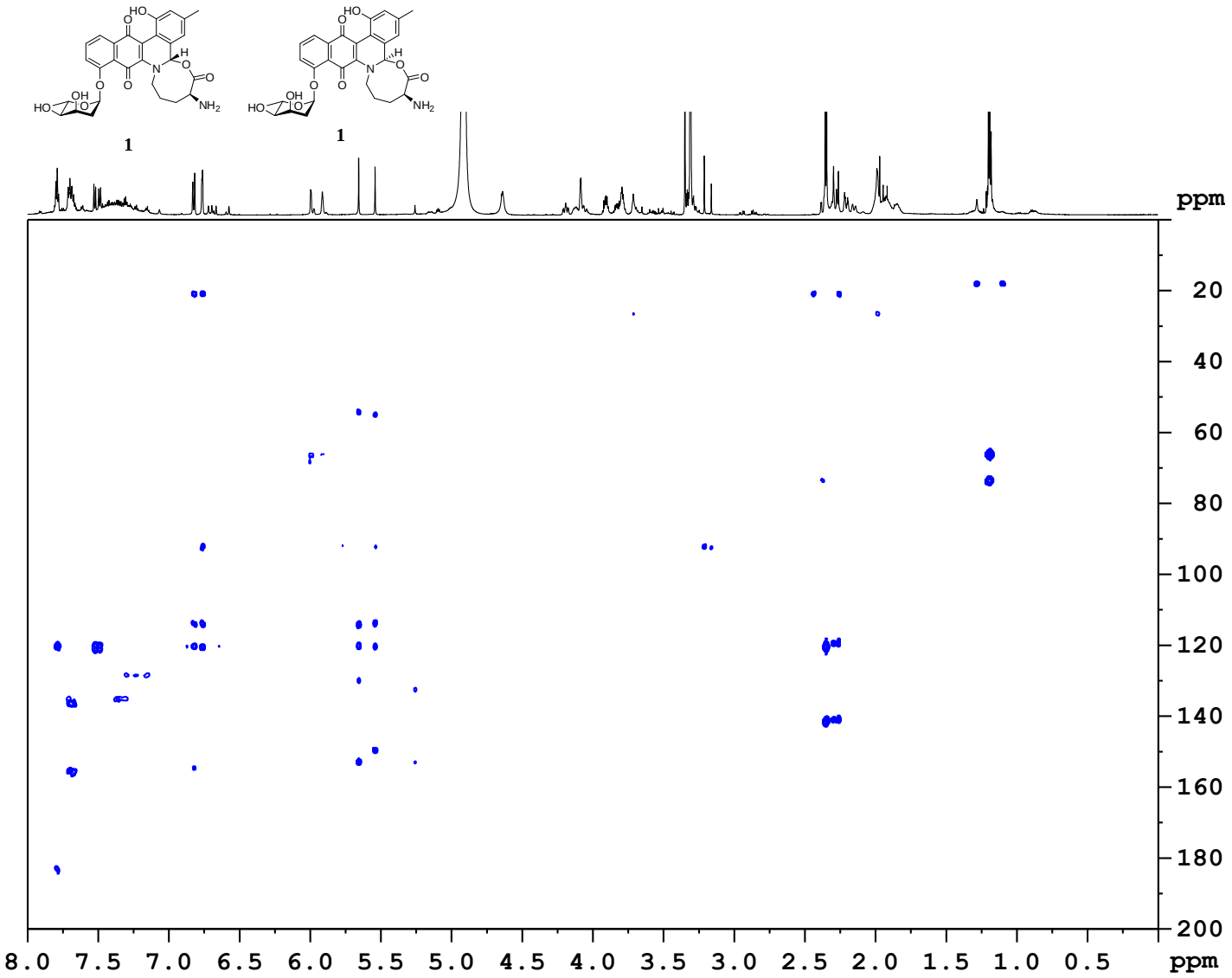


Figure S56. HMBC (^{1}H-^{13}C) spectrum of **1** (diastereomeric mixture) in MeOD-d_4. Breakdown of **3** overtime in the NMR tube is observed in the aromatic region as a broad set of peaks between 7.1-7.5 ppm.

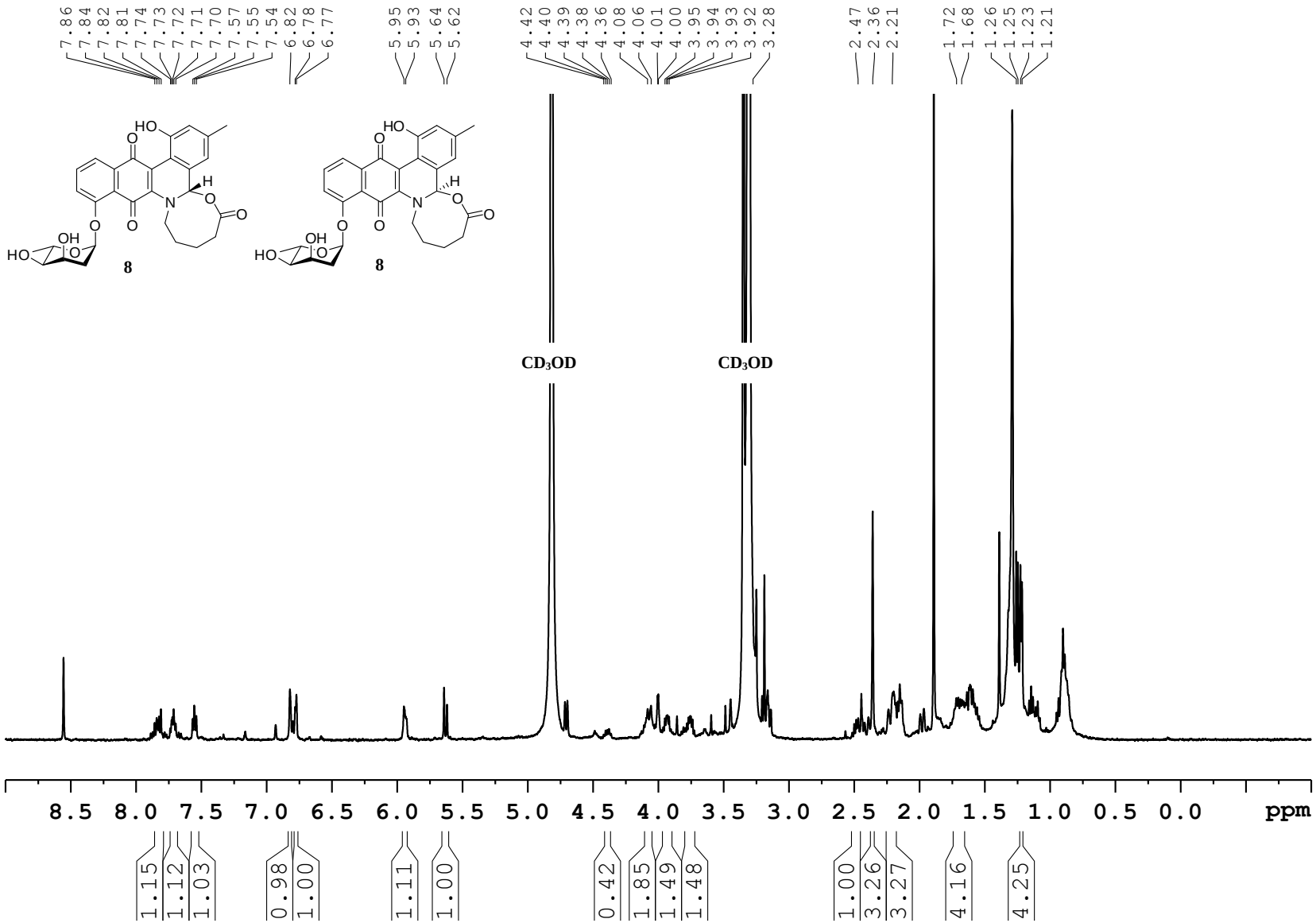


Figure S57. ^{1}H-NMR spectrum of **8** (diastereomeric mixture) in MeOD-d_4 (^{1}H: 500 MHz).

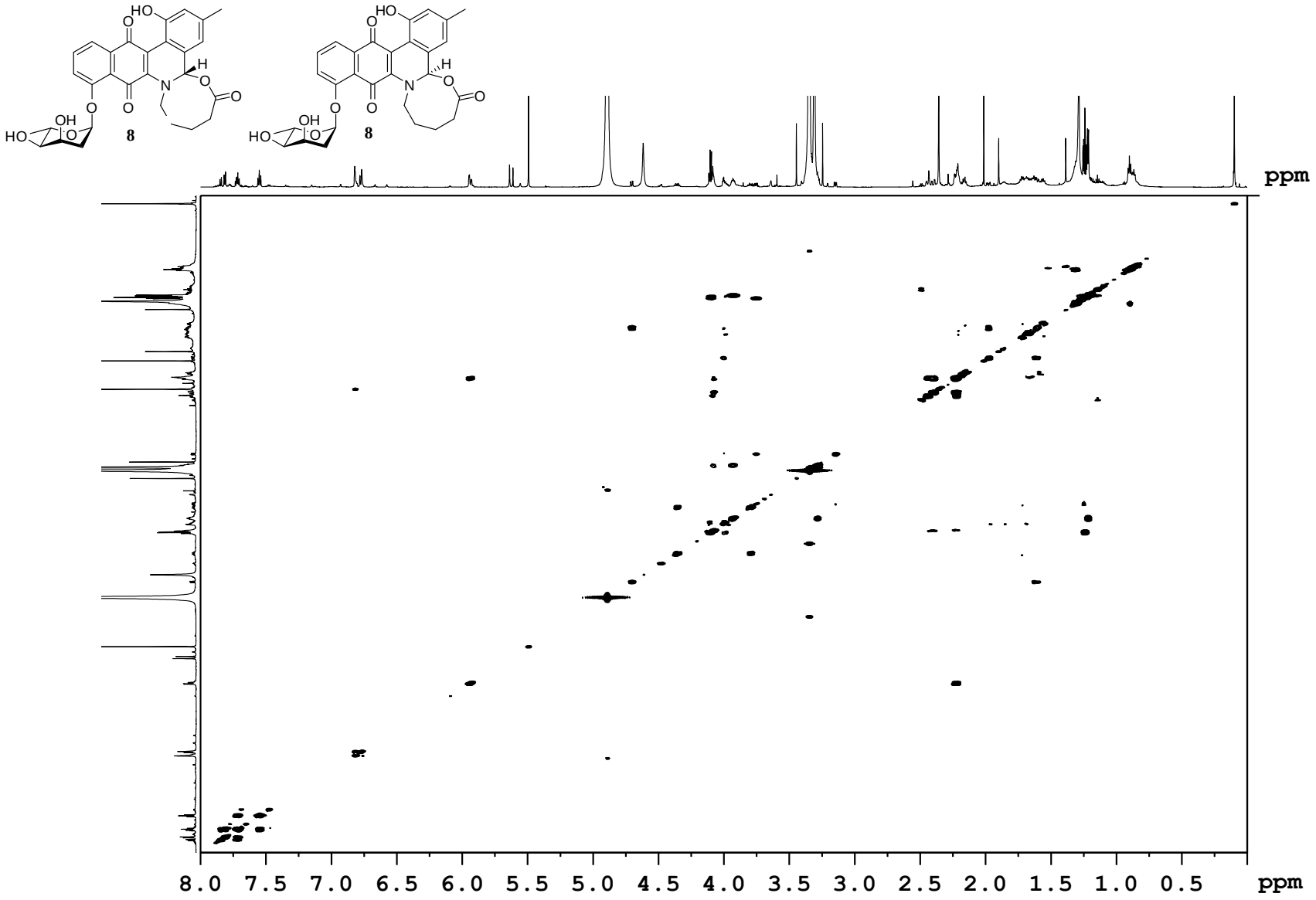


Figure S58. COSY (^{1}H-^{1}H) spectrum of **8** (diastereomeric mixture) in MeOD-d_4 (700 MHz).

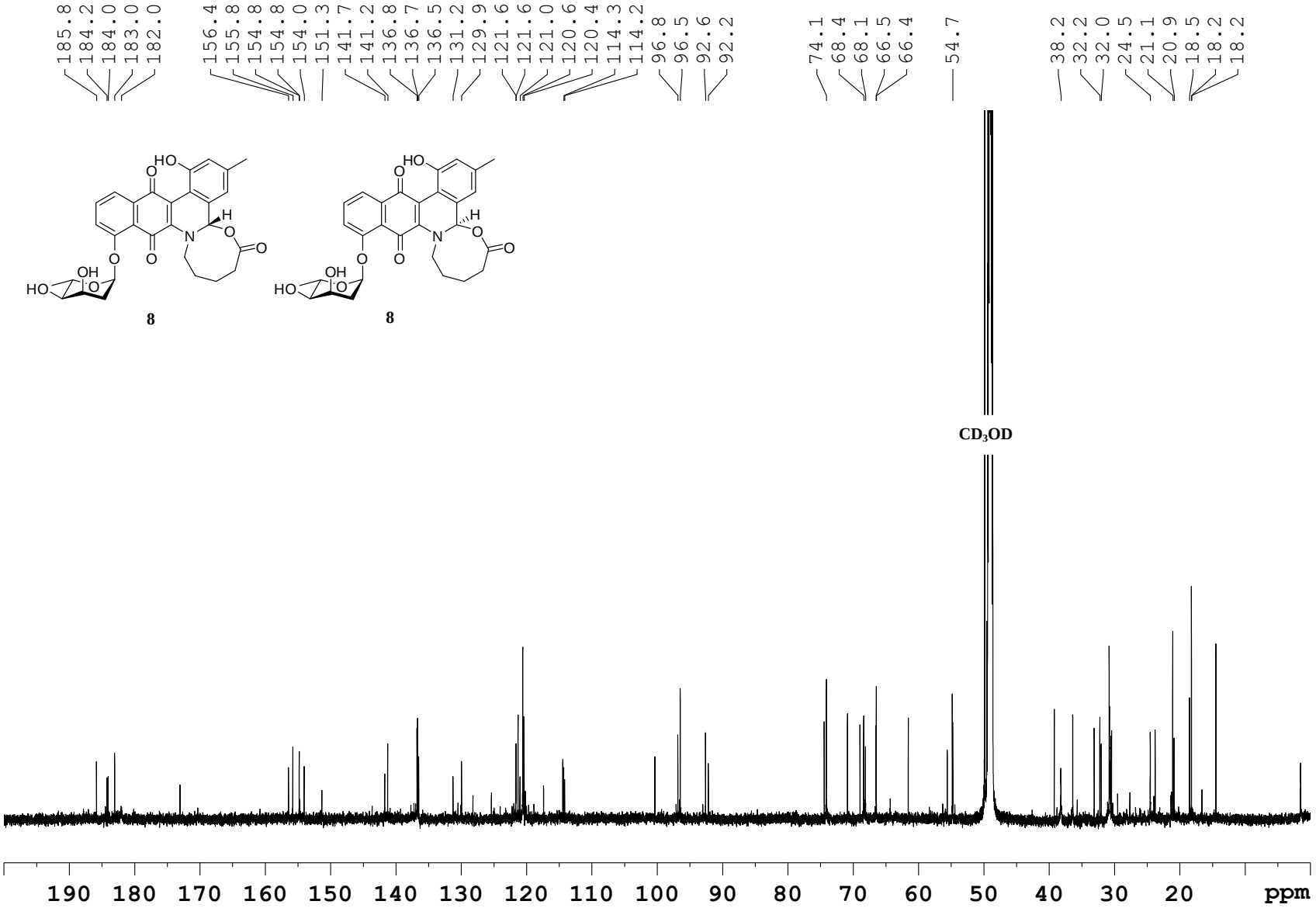


Figure S59. ^{13}C-NMR spectrum of **8** (diastereomeric mixture) in MeOD-d_4 (^{13}C: 176 MHz).

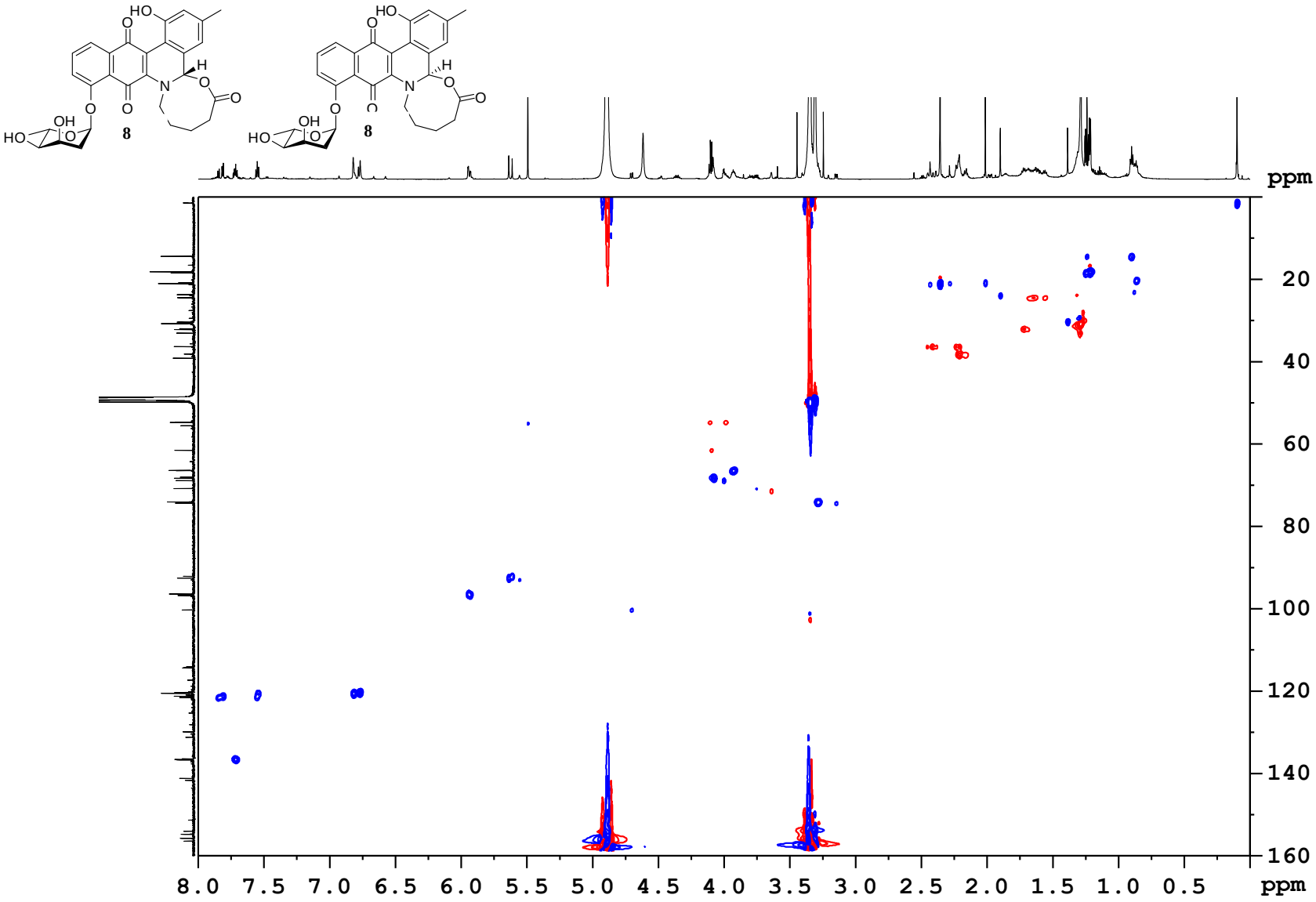


Figure S60. Edited-HSQC (^{1}H-^{13}C) spectrum of **8** (diastereomeric mixture) in MeOD-d_4.

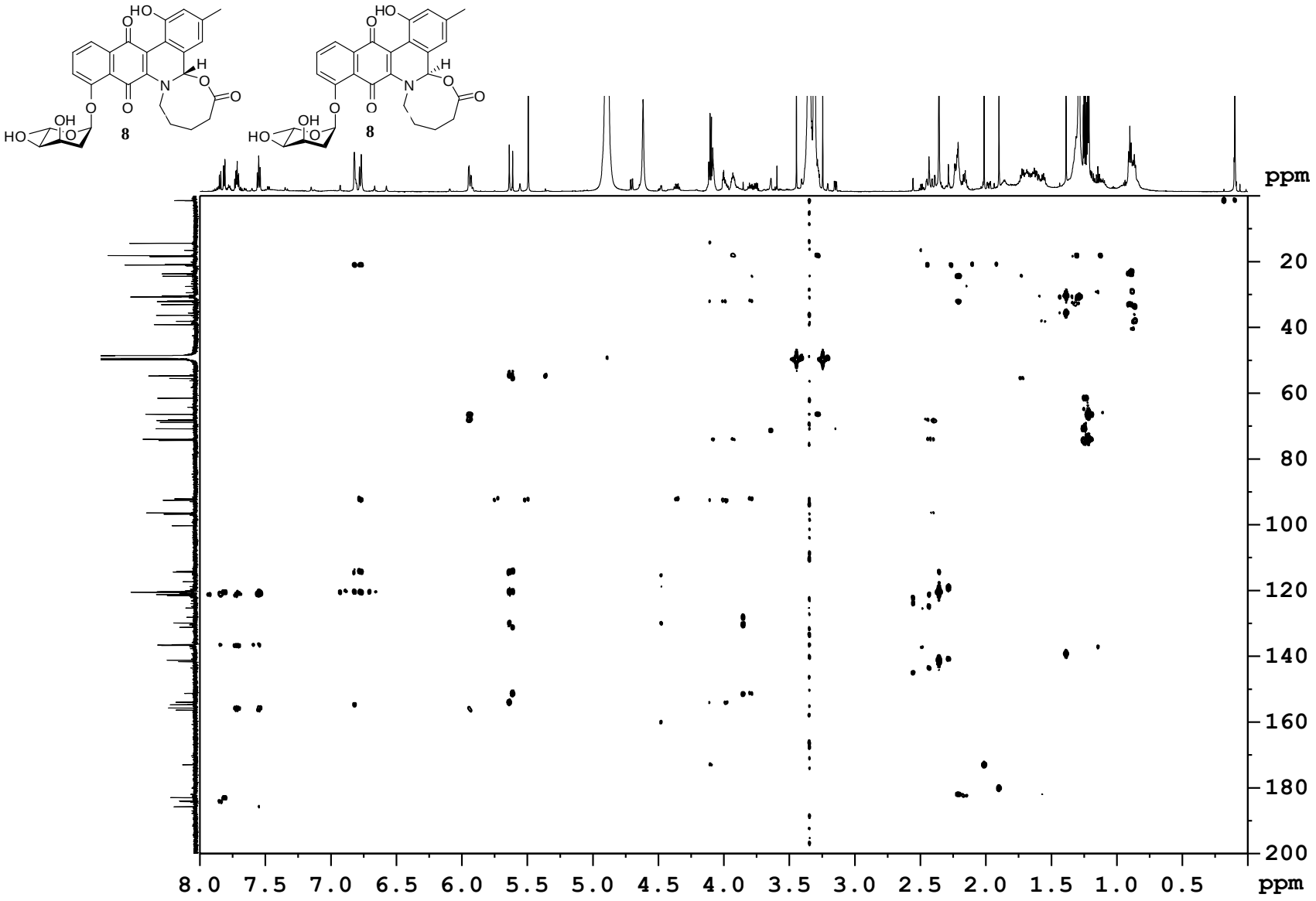


Figure S61. HMBC (^{1}H-^{13}C) spectrum of **8** (diastereomeric mixture) in MeOD-d_4.

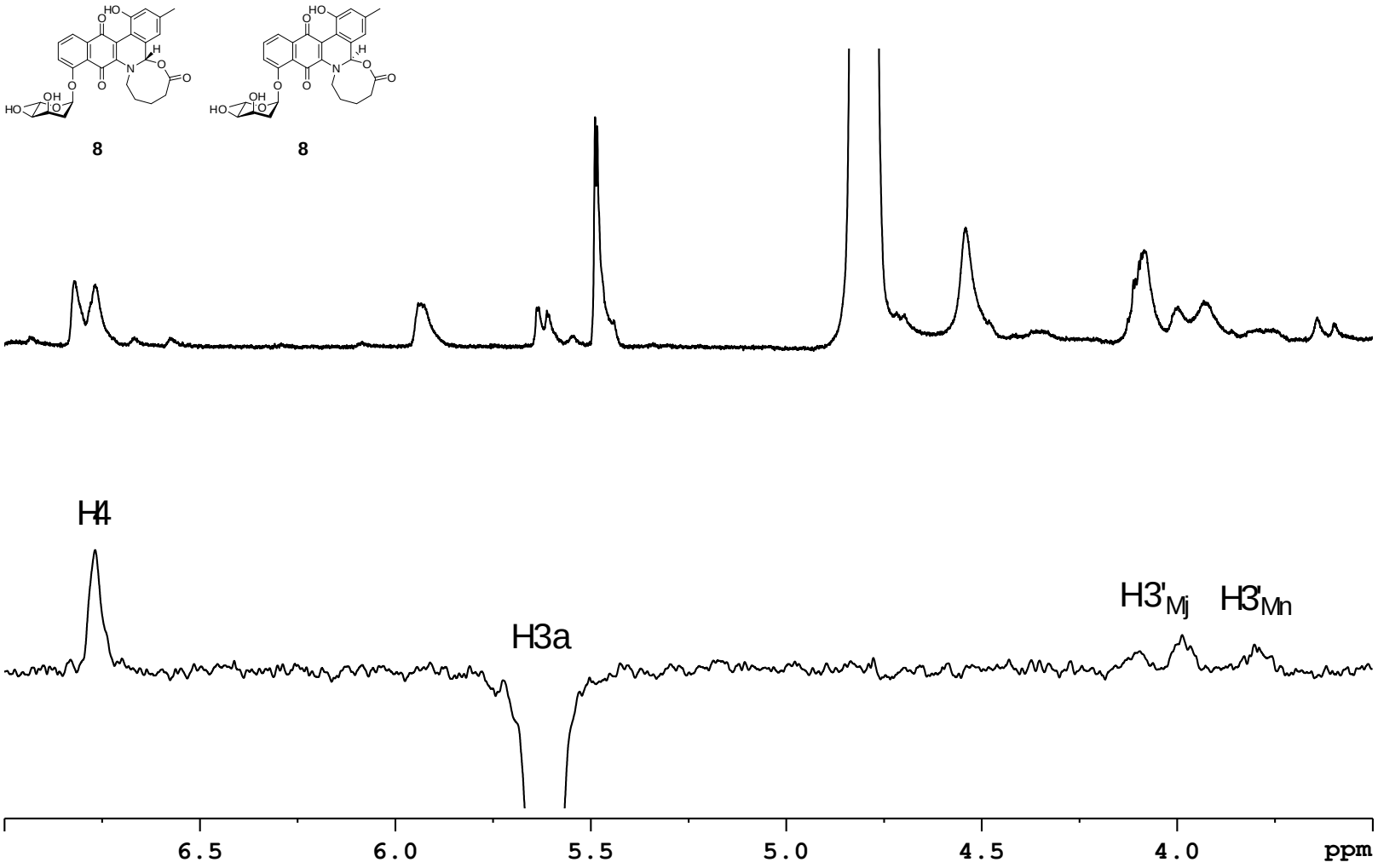


Figure S62. Overlay of ^{1}H-NMR spectrum of **8** (top) with NOESY (500 MHz) showing irradiation of both $H3a_{Mn}$ and $H3a_{Mj}$ simultaneously (bottom), in MeOD-d_4.

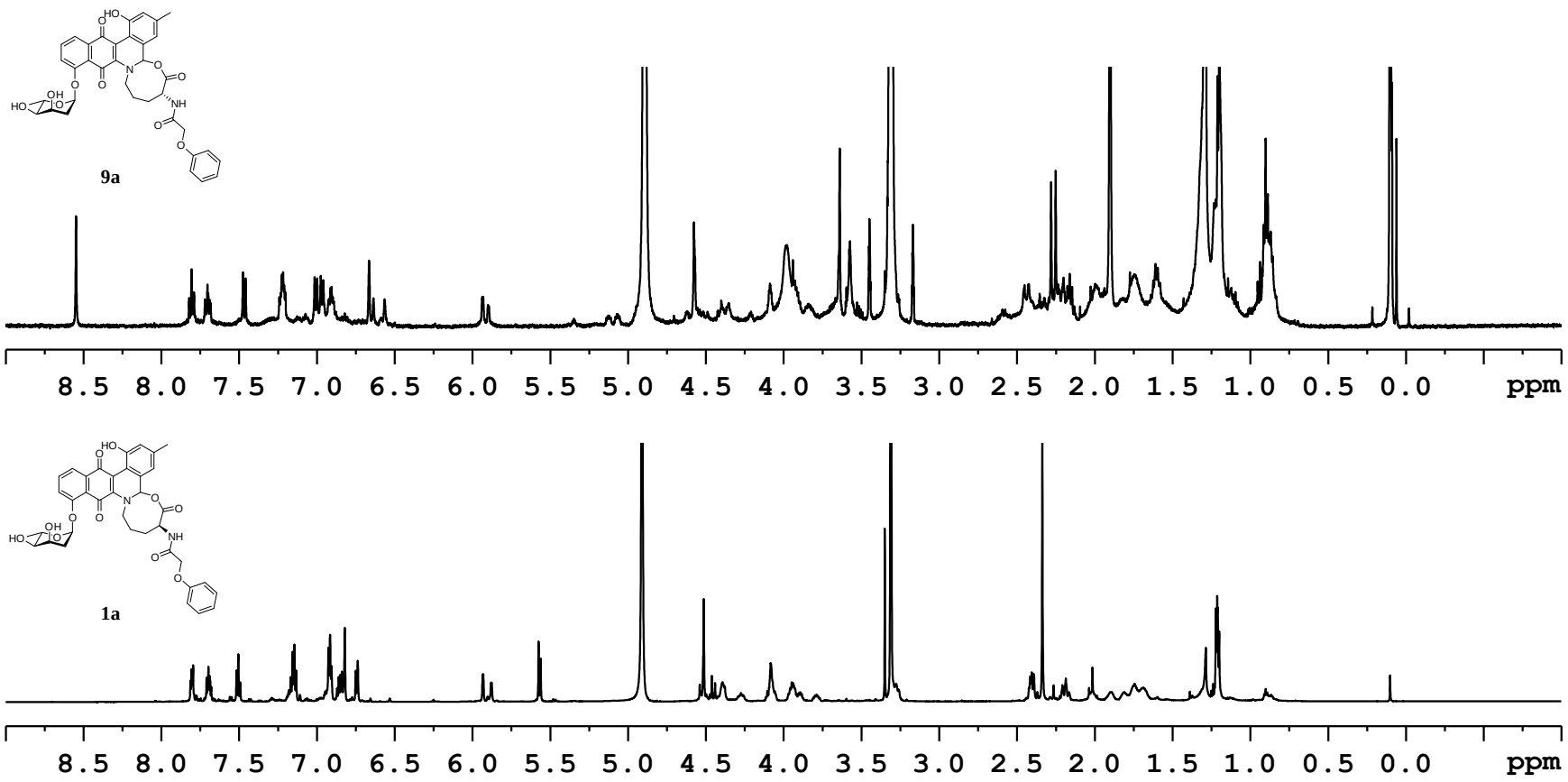


Figure S63. Overlaid ^{1}H-NMR of **9a**, (top) and **1a** (bottom). Compound **9a** was isolated in < 1 mg yield and was unable to be purified further.

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