# Supporting Information

**1. General Procedure**: All non-aqueous reactions were performed in flame-dried or oven dried round-bottomed flasks under an atmosphere of argon. Stainless steel syringes or cannula were used to transfer air- and moisture-sensitive liquids. Reaction temperatures were controlled using a thermocouple thermometer and analog hotplate stirrer. Reactions were conducted at room temperature (rt, approximately 23 °C) unless otherwise noted. Flash column chromatography was conducted using silica gel 230-400 mesh. Analytical thin-layer chromatography (TLC) was performed on E. Merck silica gel 60 F254 plates and visualized using UV, and potassium permanganate stain. Yields were reported as isolated, spectroscopically pure compounds.

**2. Materials**: Solvents were obtained from either an MBraun MB-SPS solvent system or freshly distilled (tetrahydrofuran was distilled from sodium-benzophenone; toluene was distilled from calcium hydride and used immediately; dimethyl sulfoxide was distilled from calcium hydride and stored over 4 Å molecular sieves). Commercial reagents were used as received. The molarity of *n*-butyllithium solutions was determined by titration using diphenylacetic acid as an indicator (average of three determinations).

**3. Instrumentation**: Semi-preparative reverse phase HPLC was conducted on a Waters HPLC system using a Phenomenex Luna 5 μm C18(2) 100A Axia 250 x 10.00 mm column or preparative reverse phase HPLC (Gilson) using a Phenomenex Luna column (100 Å, 50 x 21.20 mm, 5 μm C18) with UV/Vis detection. Infrared spectra were obtained as thin films on NaCl plates using a Thermo Electron IR100 series instrument and are reported in terms of frequency of absorption ($cm^{-1}$). $^{1}H$ NMR spectra were recorded on Bruker 400, 500, or 600 MHz spectrometers and are reported relative to deuterated solvent signals. Data for $^{1}H$ NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, app = apparent), coupling constants (Hz), and integration. $^{13}C$ NMR spectra were recorded on Bruker 100, 125, or 150 MHz spectrometers and are reported relative to deuterated solvent signals. LC/MS was conducted and recorded on an Agilent Technologies 6130 Quadrupole instrument. High-resolution mass spectra were obtained from the Department of Chemistry and Biochemistry, University of Notre Dame using either a JEOL AX505HA or JEOL LMS-GCmate mass spectrometer or by the Vanderbilt University Center for Neuroscience Drug Discovery (VCNDD) on a Micromass –Q-Tof API-US mass spectrometer.

## 4. Compound Preparation: Fermentation

### Apopotlidin A: Production and Isolation

**Plate Culture:** 20 microliters of glycerol stock of *Nocardiopsis* sp. FU40 was plated onto a Petri dish containing Bennett's medium and incubated upside down at 30 °C for 3 days. Dishes with spores were sealed with parafilm and stored at room temperature for up to 1 month for future use. The composition of Bennett's medium was as follows: yeast extract 1.0 g, beef extract 1.0 g, NZ amine A (casein digest) 2.0 g, glucose 10.0 g, agar 20.0 g per liter of deionized water. The pH of the medium was adjusted to 7.0 with 1 M HCl and 1 M NaOH. After autoclaving, the solution was cooled to room temperature and poured into sterile Petri dishes (30 mL/dish).

**Seed Culture:** The production fermentation was initiated by aseptically inoculating one full loop of mycelia grown on Bennett's agar plate into a sterile 50 mL Falcon tube containing 5 mL seed medium. The seed cultures were incubated for 4 days in a rotary shaker (170 rpm) at 30 °C. The composition of seed medium was as follows: soluble starch 10.0 g, molasses 10.0 g, peptone 10.0 g, beef extract 10.0 g per liter of deionized water. The pH of the medium was adjusted to 7.2 with 1 M HCl and 1 M NaOH. After autoclaving, the solution was cooled to room temperature and distributed into sterile 50 mL Falcon tubes (5 mL/tube). Typically, for compound production, 20 tubes of seed culture were used.

**Production Culture and Extraction:** Each 5 mL seed culture was poured into a 250 mL Erlenmeyer flask containing 50 mL production medium. In a typical production, 20 flasks were used to create a total of 1 L of production culture. The flasks were incubated for 6 days in a rotary shaker (170 rpm) at 30 °C. The composition of production medium was as follows: glycerol 20.0 g, molasses 10.0 g, casamino acids 5.0 g, peptone 1.0 g, calcium carbonate ($CaCO_3$) 4.0 g per liter of deionized water. Specifically, 200 mg of calcium carbonate was first distributed into each 250 mL Erlenmeyer flask. The remaining media components were dissolved in an appropriate volume of water and 50 mL of this solution was distributed into each Erlenmeyer flask. The pH of each flask was individually adjusted to 7.2 with 1 M HCl and 1 M NaOH. The resulting suspensions were autoclaved to produce the production media flasks. After incubation for 6 days, the cell bodies were scraped from each flask and transferred into 50 mL Falcon tubes. The resulting suspensions were centrifuged at 3750 rpm for 30 minutes. The

supernatants were decanted and combined into a separatory funnel and extracted with ethyl acetate (3 x 750 mL for 20 flasks). The combined ethyl acetate extracts were dried ($Na_2SO_4$) and concentrated under reduced pressure at 29 °C.

**Apoptolidin A/isoapoptolidin A Purification:** The resulting residue from concentration of ethyl acetate extracts were dissolved in 3 mL of DMSO per 1L culture and filtered through a 0.2 μm filter. The resulting DMSO solution was purified with preparative reverse phase HPLC (Gilson) with multiple 300 μL injections using a 4 minute gradient of 35-70% acetonitrile in water at a flow rate of 30 mL/min with UV detection at 220 nm and a Phenomenex Luna column (100 Å, 50 x 21.20 mm, 5 μm C18). Apoptolidin A eluted with RT = 2.45 min and isoapoptolidin A RT = 2.69 min. Fractions containing each product were combined and concentrated *in vacuo* (T < 31°) to remove acetonitrile. Remaining aqueous solution was frozen at -80 °C and lyopholized to yield a white solid. NMR spectral data of apoptolidin A and isoapoptolidin A matched the literature.[1-2]

**ApoGT2 Knockout:** The apramycin resistance gene *aac(3)IV* was amplified from plasmid pIJ773 using primers ApoGT2-red-F (5'-ATTTTCTCCCGATCCGATGGCGAAAGGCTCACGCGCGTGATTCCGGGGATCCGTCGACC-3') and ApoGT2-red-R(5'-GAGCTACCCCCCTGTGGCGGCTCCGGCCCGAAACCCTTATGTAGGCTGGAGCTGCTTC-3') with flanking regions appropriate for gene replacement.[3] The resulting 1.4 kb PCR products were transformed into *E. coli* BW25113/pIJ790 containing cosmid 14C7 from a genomic cosmid library preparation.[4] Apramycin resistant colonies were selected and cosmid DNA was purified from these colonies using a Qiagen Miniprep Kit (Catalog#27106). Gene replacements were confirmed by sequencing with the primers Del-up (5'-GGTCGACGGATCCCCGGAAT- 3') and Del-down (5'-GAAGCAGCTCCAGCCTACA-3'). The modified cosmids were passage through a methylation deficient *E. coli* host ET12567 (ATCC® BAA525™) and introduced into *Nocardiopsis sp*. FU40 by electroporation.

To prepare electro competent cells, 10 μL of a glycerol stock of *Nocardiopsis sp*. FU40 spores were inoculated into 10 mL of FU40 Seed Media (1% soluble starch, 1% molasses, 1% peptone, and 1% beef extract, pH 7.2) and cultured at 30 °C with shaking for 24 h. Aliquots of 1 mL of the seed culture was inoculated into 30 mL of CRM media (1% glucose, 10.3% sucrose, 1.012%

$MgCl_2 \cdot 6H_2O$, 1.5% Oxoid Tryptic Soy Broth, and 0.5% yeast extract)[5] and cultured with shaking for 18 h at 30 °C. Three such cultures were pooled together and the cells were harvest from this 90 mL pool by centrifugation at 10,000 rpm and washing with 100 mL ice-cold 10% sucrose then 50 mL of 15% ice-cold glycerol. The cells were re-suspended in 10 mL 15% glycerol containing 100 mg/mL lysozyme **(**Sigma L6876**)**, incubated at 37 °C for 30 min and washed twice by resuspension with 10 mL ice-cold 15% glycerol and centrifugation (10,000 rpm). The pellet was re-suspended in 2.5 mL of 30% PEG1000, 10% glycerol, 6.5% sucrose, aliquoted (50μL/tube), and stored until use at -80 °C. For electroporation, 5 μL of cosmid DNA was mixed gently with 50 μL of *Nocardiopsis sp*. FU40 electro competent cells and the mixture was transferred into an ice-cold electroporation cuvette (BioRad Gene Pulser, Xcell, 2 mm electrode gap) and exposed to a single electrical pulse (2.0 kV, 25 μF, 400 Ω, ~5 μs). Immediately following the discharge, the suspension was mixed with 0.75 mL ice-cold CRM media and incubated with shaking for 3 h at 30 °C. A 300 μL portion was spread on Nocardiopsis sp. FU40 Seed agar (1% soluble starch, 1% molasses, 1% peptone, 1% beef extract, and 2% agar, pH 7.2) containing 80 μg/mL apramycin and incubated at 30 °C. Apramycin resistant colonies generally appeared after 4 - 5 days.

Apramycin resistant colonies were picked from the plates and inoculated onto fresh Bennett's agar plates containing 80 μg/mL apramycin. One of the colonies, *Nocardiopsis sp*. FU40ΔApoGT2, was used for further studies. Spores from this strain were collected in 20% glycerol and stored at -80 °C. To confirm the targeted gene replacements, spores of the above strain were inoculated into 3 mL of Seed Media with 50 μg/mL apramycin added and cultured at 30 °C with shaking for 2 days. Genomic DNA was purified from the culture by using Wizard genomic DNA purification kit (Promega, Catalog #297885). PCR was performed to confirm the disruption using these genomic DNA samples as templates with primers AprF (5'-ATTCCGGGGATCCGTCGACC-3') and AprR (5'-TGTAGGCTGGAGCTGCTTC-3'). Additional confirmation of targeted deletion of *GT2* was confirmed by analysis of accumulated secondary metabolites in fermentations of *Nocardiopsis sp*. FU40ΔApoGT2 via HPLC/MS and NMR analysis (see below), which confirm that GT2 was functionally deleted with no observable polar effects on downstream genes comprising the apparent operon *ApoD1 – ApoM4*.

**Apopotlidin H: Production and Isolation**

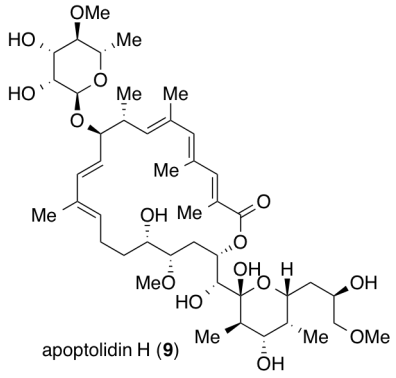
**Plate Culture:** 20 microliters of glycerol stock of *Nocardiopsis* sp. FU40ΔApoGT2 (also labeled as FU40Δ412) was plated onto a Petri dish containing apramycin dosed Bennett's medium and incubated upside down at 30 °C for 3 days. Dishes with spores were sealed with parafilm and stored at room temperature for up to 1 month for future use. The composition of apramycin dosed Bennett's medium was as follows: yeast extract 1.0 g, beef extract 1.0 g, NZ amine A (casein digest) 2.0 g, glucose 10.0 g, agar 20.0 g per liter of deionized water. The pH of the medium was adjusted to 7.0 with 1 M HCl and 1 M NaOH. After autoclaving, the solution was cooled to near room temperature, 80 mg apramycin was added per liter and the resulting solution was poured into sterile Petri dishes (30 mL/dish).

**Seed Culture:** The production fermentation was initiated by aseptically inoculating one full loop of mycelia grown on Bennett's agar plate into a sterile 50 mL Falcon tube containing 5 mL seed medium. The seed cultures were incubated for 4 days in a rotary shaker (170 rpm) at 30 °C. The composition of seed medium was as follows: soluble starch 10.0 g, molasses 10.0 g, peptone 10.0 g, beef extract 10.0 g per liter of deionized water. The pH of the medium was adjusted to 7.2 with 1 M HCl and 1 M NaOH. After autoclaving, the solution was cooled to room temperature, 80 mg apramycin was added per liter and the resulting solution was distributed into sterile 50 mL Falcon tubes (5 mL/tube). Typically, for compound production, 20 tubes of seed culture were used.

**Production Culture and Extraction:** Production culture and extraction procedure was identical to that used for apoptolidin A. No apramycin is added to the production media.

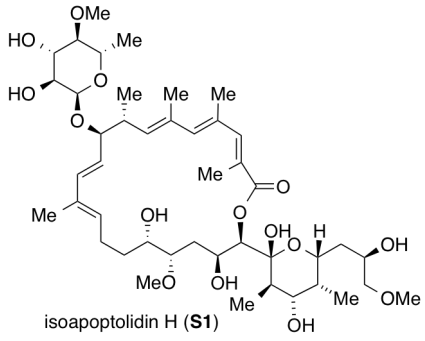
**Apoptolidin H/isoapoptolidin H Purification:** The resulting residue from concentration of ethyl acetate extracts were dissolved in 3 mL of DMSO per 1L culture and filtered through a 0.2 μm filter. The resulting DMSO solution was purified with preparative reverse phase HPLC (Gilson) with multiple 300 μL injections using a 4 minute gradient of 30-60% acetonitrile in water at a flow-rate of 30 mL/min with UV detection at 220 nm and a Phenomenex Luna column (100 Å, 50 x 21.20 mm, 5 μm C18). Apoptolidin H eluted with RT = 2.63 min and isoapoptolidin H RT = 3.13 min. Fractions containing each product were combined into a round bottomed flask and concentrated (T < 31°) to remove acetonitrile. Resulting aqueous solution was frozen at -80 °C and water was removed by lyophilization to yield white solids.

## 5. Compound Preparation: Chemical Synthesis



apoptolidin H (**9**)

**Apoptolidin H (3):** $^{1}$H NMR (600 MHz, $CD_3OD$): δ 7.36 (s, 1H), 6.19 (s, 1H), 6.18 (d, *J* = 15.7 Hz, 1H), 5.69 (dd, *J* = 2.2, 9.1 Hz, 1H), 5.30 (d, *J* = 11.4 Hz, 1H), 5.23 (dd, *J* = 9.1, 9.1, 1H), 5.21 (d, *J* = 9.0 Hz, 1H), 4.82 (d, *J* = 3.8 Hz, 1H), 4.09 (ddd, *J* = 2.1, 3.1, 7.9 Hz, 1H), 3.83 (dd, *J* = 9.2, 9.2 Hz, 1H), 3.75 (ddd, *J* = 4.8, 6.3, 11.1 Hz, 2H), 3.72 (dd, *J* = 9.2, 9.2 Hz, 1H), 3.58 (s, 3H), 3.54 (m, 2H), 3.44 (m, 1H), 3.40 (dd, *J* = 3.8, 9.8 Hz, 1H), 3.36 (s, 3H), 3.29 (s, 3H), 3.21 (dd, *J* = 4.7, 9.4 Hz, 1H), 3.17 (dd, *J* = 6.3, 9.4 Hz, 1H), 2.70-2.80 (m, 2H), 2.72 (dd, *J* = 29.1, 9.1, 1H), 2.46 (m, 1H), 2.18 (s, 3H), 2.16 (m, 1H), 2.12 (s, 3H), 2.05 (m, 1H), 2.04 (m, 2H), 1.93 (s, 3H), 1.76 (m, 1H), 1.74 (m, 1H), 1.68 (s, 3H), 1.58 (ddd, *J* = 2.6, 2.7, 5.4, 8.9 Hz, 1H), 1.48-1.55 (m, 1H), 1.37-1.45 (m, 1H), 1.29 (m, 1H), 1.26 (d, *J* = 6.4 Hz, 3H), 1.13 (d, *J* = 6.7, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); $^{13}$C NMR (150 MHz, $CD_3OD$): δ 172.6, 149.0, 147.1, 142.9, 141.2, 134.8, 133.4, 133.3, 133.2, 126.4, 123.9, 101.2, 96.0, 87.5, 84.1, 83.8, 78.6,75.4, 74.9, 74.5, 73.7, 73.6, 72.3, 69.1, 68.2, 68.1, 61.4, 61.0, 59.4, 40.7, 38.9, 38.5, 38.4, 36.5, 36.3, 24.6, 18.3(2), 18.2(6), 17.8, 16.5, 14.0, 12.2, 12.0, 5.3; HRMS (ESI-TOF MS) *m/z* 863.4769 (M+Na)+ calculated for $C_{44}H_{72}NaO_{15}$, measured 863.4766.

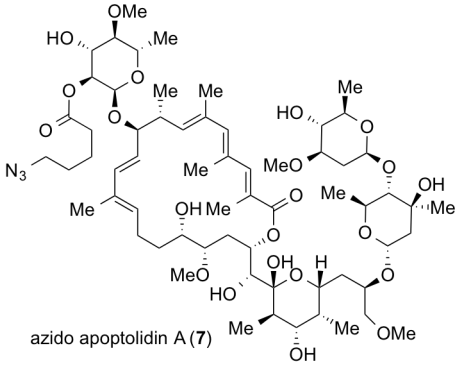


isoapoptolidin H (**S1**)

**Isoapoptolidin H (S1):** $^{1}$H NMR (600 MHz, $CD_3OD$): δ 7.34 (s, 1H), 6.04 (d, *J* = 15.8, 1H), 5.97 (s, 1H), 5.47 (dd, *J* = 6.5, 8.8 Hz, 1H), 5.26 (dd, *J* = 8.8, 15.8 Hz, 1H), 5.07 (d, *J* = 10.4 Hz, 1H), 4.94 (d, *J* = 2.4 Hz, 1H), 4.80 (d, *J* = 3.8 Hz, 1H) 4.45 (ddd, *J* = 2.0, 2.0, 10.0 Hz, 1H), 4.28 (ddd, *J* = 2.0, 2.0, 10.0 Hz, 1H), 3.86 (m, 1H), 3.78 (m, 1H), 3.76 (m, 1H), 3.75 (m, 1H), 3.73 (m, 1H), 3.58 (s, 3H), 3.49 (s, 3H), 3.47 (m, 1H), 3.40 (m,1H), 3.38 (s, 3H), 3.37 (m, 1H), 3.35 (m, 1H), 2.75 (m, 1H), 2.72 (dd, *J* = 9.2, 9.2 H, 1H), 2..48 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 1.94 (m, 1H), 1.6-1.8 (m, 4H), 1.73 (s, 3H), 1.71 (s, 3H), 1.42 (m, 1H), 1.39 (m, 3H), 1.25 (d, *J* = 6.2 Hz, 3H), 1.17 (d, *J* = 6.5 Hz, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); $^{13}$C NMR (150 MHz, $CD_3OD$): δ 170.3, 147.1, 143.5, 141.0, 136.9, 134.2, 133.6, 132.8, 132.7(9), 125.8, 124.9,

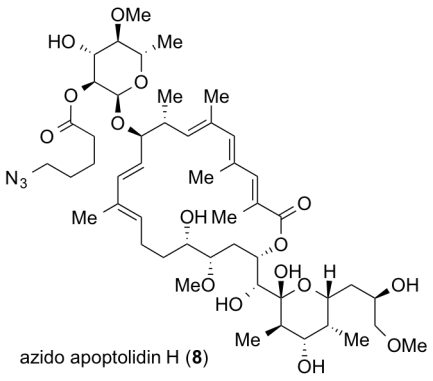
102.9, 96.2, 87.5, 84.0, 82.2, 78.4, 75.3, 74.9, 74.1, 73.7, 73.3, 69.0, 68.1(6), 68.0(9), 67.8, 61.0, 60.0, 59.4, 40.7, 39.4, 38.0, 36.6, 36.0, 33.7, 25.4, 18.4, 18.3, 17.9, 17.6, 13.4, 12.1, 12.0, 5.3; HRMS (ESI-TOF MS) *m/z* 863.4769 (M+Na)+ calculated for $C_{44}H_{72}NaO_{15}$, measured 863.4764.

**5. Compound preparation: chemical synthesis**




**Azido Apoptolidin A (7)** To a solution of 5-azidopentanoic acid (7.6 mg, 0.053 mmol) in dichloromethane (4.0 mL) at 0 °C was added bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBrop, 25 mg, 0.053 mmol) and diisopropylethyl amine (31 $\mu$L, 0.177 mmol) the resulting solution was stirred at 0 °C for 10 min. Apoptolidin A (20 mg, 0.018 mmol) was added to the resulting solution followed by one small crystal of 4-dimethylaminopyridine (DMAP). The resulting solution was warmed to room temperature and maintained at that temperature for 16 h. Methanol (100 $\mu$L) was added to the reaction mixture and then concentrated. The resulting residue was diluted in EtOAc (20 mL) and washed with 1 N HCl (5 mL). The aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic extracts were washed with $NaHCO_3$ (1 x 5mL) and brine (1 x 5 mL) and dried ($Na_2SO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in 1.2 mL of DMSO, filtered through a 0.2 $\mu$m polytetrafluoroethylene PTFE syringe tip filter and purified by preparative reversed phase HPLC with a 4 minute gradient from 45% to 75% MeCN in $H_2O$ in four 300 $\mu$L injections. Fractions containing desired product (r.t. 2.68 min) were combined and concentrated to remove acetonitrile only (T < 31°C) and the resulting aqueous solutions were frozen at -80 °C. Water was removed by lyophilization to afford 7.0 mg (31%) of **7** as a white solid: IR (neat) 3436, 2930, 2098, 1668, 1381, 1256 $cm^{-1}$; $^1H$ NMR (600 MHz, $CD_3OD$) δ 7.38 (s, 1H), 6.19 (s, 1H), 6.15 (d, *J* = 15.7 Hz, 1H), 5.65 (dd, *J* = 7.9, 8.0 Hz, 1H), 5.29 (d, *J* = 11.4 Hz, 1H), 5.21 (d, *J* = 10.0 Hz, 1H), 5.05 (dd, *J* = 8.9, 15.8 Hz, 1H), 4.98 (d, *J* = 3.8 Hz, 1H), 4.94 (d, *J* = 4.1 Hz, 1H), 4.82 (d, *J* = 1.7 Hz, 1H), 4.56 (dd, J= 3.9, 10.1 Hz, 1H), 3.95 (m, 1H), 3.91 (dd, J= 9.1, 9.9 Hz, 1H), 3.83 (dd, *J* = 8.9, 8.9 Hz, 1H), 3.78 (dd, *J* = 6.3, 9.5 Hz, 1H), 3.71 (dd, *J* = 4.6, 11.1 Hz, 1H), 3.67 (dd, *J* = 6.2, 9.7 Hz, 1H), 3.60 (s, 3H), 3.53 (d, *J* = 1.3 H z, 1H), 3.45 (m, 1H), 3.43 (s, 3H), 3.41 (m, 1H), 3.37 (s, 3H), 3.34 (m, 1H), 3.33 (m, 5H), 3.27 (s,

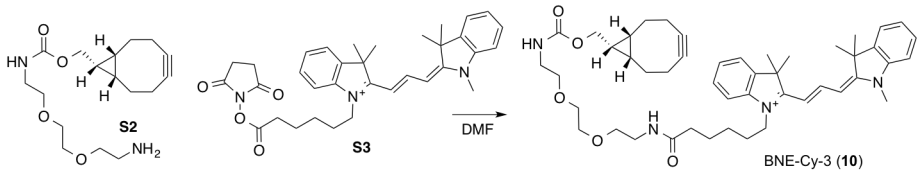
3H), 3.21 (dd, *J* = 6.2, 9.2 Hz, 1H), 3.17 (m, 1H), 2.97 (dd, *J* = 8.9, 9.0 Hz, 1H), 2.82 (dd, *J* = 9.2, 9.2 Hz, 1H), 2.72 (dd, *J* = 4.6, 9.8 Hz, 1H), 2.67 (m, 1H), 2.40-2.48 (m, 4H), 2.19 (s, 3H), 2.16 (m, 1H), 2.11 (s, 3H), 2.08 (m, 1H), 2.05 (m, 1H), 1.95 (s, 3H), 1.92 (d, J= 12.7 Hz, 1H), 1.80 (dd, *J* = 4.4, 13.5 Hz, 1H), 1.74 (m, 1H), 1.73 (m, 1H), 1.69 (m, 2H), 1.66 (s, 3H), 1.65 (m, 2H), 1.57 (m, 1H), 1.48 (m, 2H), 1.32 (s, 3H), 1.30 (m, 2H), 1.29 (m, 1H), 1.25-1.30 (m, 12H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.13 (d, *J* = 6.6 Hz, 3H), 1.02 (d, J= 6.7 Hz, 3H), 0.89 (d, J= 6.9 Hz, 3H); $^{13}$C NMR (150 MHz, $CD_3OD$) δ; 174.5, 172.7, 149.3, 146.9, 142.7, 141.3, 134.6, 133.9, 133.7, 133.1, 125.5, 123.8, 101.9, 101.3, 99.5, 93.6, 87.4, 87.4, 85.9, 84.4, 83.9, 82.0, 77.1, 76.9, 76.8, 75.4, 75.1, 74.6, 73.9, 73.2, 73.0, 72.5, 72.3, 69.4, 68.2, 67.4, 61.3, 61.3, 59.5, 57.3, 52.2, 45.5, 40.6, 39.1, 38.4, 37.2, 36.4, 34.4, 33.1, 30.8, 29.4, 24.7, 23.3, 22.8, 18.9, 18.3, 18.2, 18.1, 17.9, 16.5, 14.2, 12.2, 12.1, 5.2; HRMS (ESI-TOF MS) m/z 1276.6925 (M+Na)+ calculated for $C_{63}H_{103}N_3NaO_{22}$, measured 1276.6940.



azido apoptolidin H (**8**)

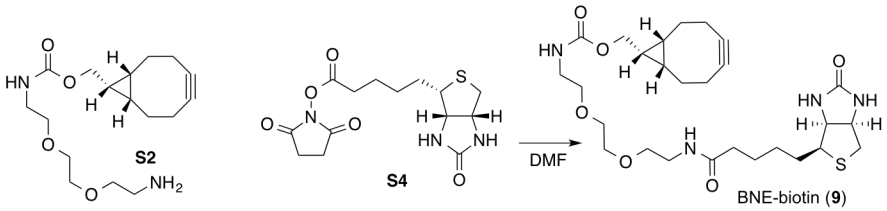
**Azido Apoptolidin H (8)** To a solution of 5-azidopentanoic acid (10 mg, 0.071 mmol) in dichloromethane (5.0 mL) at 0 °C was added PyBrop (33 mg, 0.071 mmol) and diisopropylethyl amine (41 $\mu$L, 0.238 mmol) the resulting solution was stirred at 0 °C for 10 min. Apoptolidin H (20 mg, 0.024 mmol) was added to the resulting solution followed by one small crystal of DMAP. The resulting solution was warmed to room temperature and maintained at that temperature for 16 h. Methanol (100 $\mu$L) was added to the reaction mixture and then concentrated. The resulting residue was diluted in EtOAc (20 mL) and washed with 1 N HCl (5 mL). The aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic extracts were washed with $NaHCO_3$ (1x 5mL) and brine (1 x 5 mL) and dried ($Na_2SO_4$) and concentrated in vacuo. The resulting residue was dissolved in 1.2 mL of DMSO, filtered through a 0.2 $\mu$m polytetrafluoroethylene PTFE syringe tip filter and purified by preparative reversed phase HPLC with a 4 minute gradient from 35% to 75% MeCN in $H_2O$ (r.t. of desired product 3.23 min) in four 300 $\mu$L injections. Fractions containing desired product were combined and concentrated to remove acetonitrile only (T < 31°C) and the resulting aqueous solution was frozen at -80 °C. Water was removed by lyophilization to afford 5.5 mg

(24%) of **8** as a white solid: IR (neat) 3425, 2928, 2098, 1669, 1385, 1257 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$) δ 7.28 (s, 1H), 6.15 (s, 1H), 6.11 (d, *J* = 15.7 Hz, 1H), 5.61 (dd, *J* = 6.3, 9.8 Hz, 1H), 5.25 (d, *J* = 5.25 Hz, 1H), 5.16 (d, *J* = 10.0 Hz, 1H), 5.00 (d, *J* = 3.5 Hz, 1H), 4.99 (dd, *J* = 9.6, 15.2 Hz, 1H), 4.71 (dd. *J* = 3.8, 10.2 Hz, 1H), 4.19 (d, *J* = 8.5 Hz, 1H), 4.00 (dd, *J* = 9.5, 9.5 Hz, 1H), 3.86 (dd, *J* = 4.7, 10.7 Hz, 1H), 3.79 (m, 1H), 3.77 (m, 1H), 3.61 (s, 3H), 3.56 (m, 1H), 3.51 (m, 1H), 3.47 (m, 1H), 3.37 (s, 3H), 3.31 (s, 3H), 3.30 (m, 2H), 3.22 (dd, *J* = 3.3, 9.3 Hz, 1H), 3.11 (dd, *J* = 7.7, 9.1 Hz, 1H), 2.86 (dd, *J* = 9.3, 9.3 Hz, 1H), 2.74 (dd, *J* = 5.8, 9.6 Hz, 1H), 2.62 (m, 1H), 2.41 (m, 2H), 2.15 (s, 3H), 2.10 (s, 3H), 2.01 (m, 1H) 1.90 (s, 3H), 1.82 (m, 1H), 1.72 (m, 2H), 1.64 (m, 3H), 1.63 (s, 3H), 1.43 (m, 2H), 1.32 (d, *J* = 6.24 Hz, 3H), 1.25 (m, 2H) 1.12 (d, *J* = 6.4 Hz, 3H), 1.05 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); $^{13}C$ NMR (150 MHz, $CDCl_3$) δ;172.8, 171.7, 148.1, 146.6, 141.6, 140.1, 133.3, 133.2, 132.3, 132.2, 124.7, 122.7, 99.9, 92.8, 86.2, 83.9, 82.4, 73.6, 71.4, 67.5, 67.1, 61.2, 61.0, 59.2, 51.2, 38.9, 37.9, 37.0, 36.4, 35.9, 34.7, 33.7, 33.6, 32.1, 29.8, 29.7, 29.5, 28.3, 24.6, 22.8, 22.3, 18.2, 18.0, 17.4, 16.3, 14.3, 13.7, 12.1, 5.0; HRMS (ESI-TOF MS) m/z 988.5353 (M+Na)+ calculated for $C_{49}H_{79}N_3NaO_{16}$, measured 988.5381.

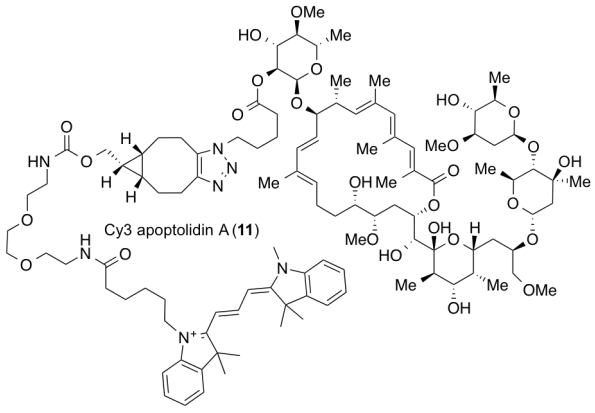



**BNE-Cy-3 (10)** To a solution of amine **S2**[6] (24.0 mg, 0.074 mmol) in DMF (1.0 mL) was added Cy3 NHS **S3** (Lumiprobe, Catalog #21020) as a solution in DMF (0.5 mL). After six hours, the DMF was removed on high vacuum overnight. The resulting purple residue was dissolved in methanol (1.0 mL) and purified on semipreperative reverse phase HPLC (30 minute gradient: 35:65 strong:weak buffer to 100% strong buffer with strong buffer composed of 5% aqueous MeCN and 10 mM $NH_4OAc$ and weak buffer 95% aqueous MeCN and 10 mM $NH_4OAc$). Fractions containing the desired product (r.t. 22.50 min) were combined and concentrated *in vacuo* to remove acetonitrile (T<31°C). The remaining aqueous solution was frozen at -80 °C and lyopholized to yield 4.3 mg (63%) of bicyclononyne Cy-3 conjugate **10** as a deep red solid: $^1H$ NMR (600 MHz, $CD_3OD$) δ 8.55 (t, *J* = 13.5, 1H) 7.55 (d, *J* = 7.5, 2H), 7.45 (m, 2H), 7.36 (dd, *J* = 4.1, 8.0, 2H), 7.32 (m, 2H), 6.45 (dd, *J* = 8.4, 13.5 Hz, 2H), 4.15 (dd, *J* = 7.5, 7.5 Hz, 2H), 4.11 (d, *J* = 8.1 Hz, 2H), 3.69 (s, 3H), 3.59 (s, 4H), 3.52 (t, *J* = 5.6 Hz, 4H), 3.34 (t, *J* = 5.6 Hz, 2H), 3.26 (t, *J* = 3.3 Hz, 2H), 2.23 (t, *J* = 7.3 Hz, 2H), 2.1-2.25 (m, 4H),

1.85 (m, 2H), 1.77 (s, 12 H), 1.71 (m, 2H) 1.58 (m, 2H), 1.51 (m, 2H), 1.33 (m, 1H), 1.29 (s, 4H) 0.91 (m, 2H); $^{13}$C NMR (150 MHz, $CD_3OD$) δ 179.2, 176.7, 176.0, 175.9, 159.2, 152.1, 144.1, 143.4, 142.2, 142.1, 130.0, 129.9, 126.8, 126.8, 123.5, 123.4, 112.5, 112.3, 103.8, 103.6, 99.5, 71.3, 71.3, 71.0, 70.6, 63.7, 50.7, 50.6, 45.1, 41.6, 40.3, 36.6, 31.8, 30.1, 28.3, 28.2, 28.1, 27.3, 26.5, 23.4, 21.9, 21.4, 18.9; HRMS (ESI-TOF MS) m/z 763.4798 (M-Cl)+ calculated for $C_{47}H_{63}N_4O_5$, measured 763.4794.

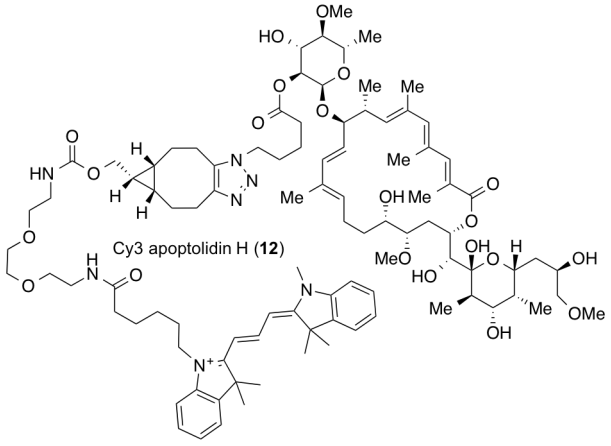



**BNE-Biotin (9)** To a solution of amine **S2**[6] (34.0 mg, 0.105 mmol) in DMF (1.0 mL) was added Biotin NHS **S4** (71 mg, 0.210 mmol). After 16 hours, the DMF was removed on high vacuum. Flash column chromatography (gradient: dichloromethane to 80:18:2. dichloromethane:MeOH:$NH_4OH$) yielded 35.0 mg (61%) of bicyclononyne biotin conjugate **9** as a pale yellow oil: $^1$H NMR (400 MHz, $CDCl_3$) δ 6.66 (s (br), 2H), 5.77 (s(br), 1H), 5.40 (s (br), 1H), 4.48 (t, *J* = 6.0 Hz, 1H), 4.29 (t, *J* = 5.9 Hz, 1H), 4.13 (d, *J* = 7.8 Hz, 2H) 3.59 (s (br), 4H), 3.55 (m, 4H), 3.43 (m, 3H), 3.35 (m, 2H), 3.12 (dd, *J* = 6.9, 11.6 Hz, 1H), 2.88 (dd, *J* = 4.7, 12.7 Hz, 1H), 2.72 (d, *J* = 12.8 Hz, 1H), 2.11-2.34 (m, 6H), 1.48-1.76 (m, 6H), 1.28-1.48 (m, 4H), 1.23 (s, 1H), 0.92 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 173.3, 164.1, 156.8, 98.7, 70.0, 62.7, 61.7, 60.1, 55.6, 40.7, 40.1, 39.0, 35.9, 33.2, 29.6, 29.0, 28.2, 28.0, 25.5, 22.8, 21.3, 20.0, 17.7; HRMS (ESI-TOF MS) m/z 551.2903 (M+H)+ calculated for $C_{27}H_{43}N_4O_6$, measured 551.2900.



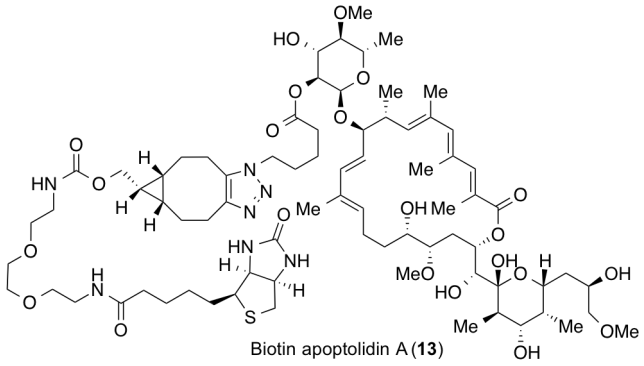

**Cy3 Apoptolidin A (11)** To a solution of BNE-Cy-3 (**10**) (2.6 mg, 0.0033 mmol) in methanol (0.5 mL) was added azido apoptolidin A (**7**) (2.05 mg, 0.0016 mmol) as a solution in methanol (0.5 mL). The resulting solution was stirred at 37 °C for 4 hours, removed from the bath and concentrated *in vacuo*. The resulting residue was dissolved in methanol (0.5 mL) and

purified on semipreperative reverse phase HPLC (30 minute gradient from 35% strong to 100% strong buffer in weak buffer where strong is 5% aqueous MeCN and 10 mM $NH_4OAc$ and weak buffer is 95% aqueous MeCN and 10 mM $NH_4OAc$). Fractions containing the desired product (RT = 28.10 min) were combined and concentrated *in vacuo* (T<31°C). The resulting aqueous solution was frozen at -80°C and lyopholized to yield 1.29 mg (39%) of Cy3 apoptolidin A (**11**) as a deep red solid: $^1$H NMR (600 MHz, $CD_3OD$) δ 8.55 (t, *J* = 13.5, 1H) 7.55 (d, *J* = 7.5, 2H), 7.45 (m, 2H), 7.36 (dd, *J* = 3.6, 8.0, 2H), 7.32 (m, 3H), 6.44 (dd, *J* = 8.7, 13.4 Hz, 2H), 6.17 (s, 1H), 6.12 (d, *J* = 15.7 Hz, 1H), 5.62 (m, 1H), 5.4 (m, 1H), 5.34 (m, 1H), 5.29 (d, *J* = 11.3 Hz, 1H), 5.19 (d, *J* = 10.0 Hz, 1H), 5.00 (m, 1H), 4.9 (m, 2H), 4.82 (m, 1H), 4.54 (m, 1H), 4.30 (dd, *J* = 7.1, 7.1 Hz, 2H), 4.15 (dd, *J* = 7.5, 7.5 Hz, 2H), 4.11 (m, 2H), 3.95 (m, 2H), 3.89 (m, 1H), 3.80 (m, 2H), 3.69 (s, 3H), 3.65 (m, 2H), 3.60 (s(br), 4H), 3.58 (m, 3H), 2.52 (dd, *J* = 5.5, 5.5 Hz, 4H), 3.42 (s, 3H), 3.41 (m, 2H), 3.35 (m, 4H), 3.26 (m, 2H), 3.14-3.21 (m, 3H), 2.97 (dd, *J* = 9.0, 9.0 Hz, 1H), 2.67-2.88 (m, 5H), 2.63 (m, 1H), 2.29-2.49 (m, 7H), 2.23 (t, *J* = 7.3 Hz, 2H), 2.11-2.27 (m, 8H), 2.00-2.11 (m, 8H), 1.9-1.98 (m, 5H), 1.85 (m, 6H), 1.77 (s, 12H), 1.71 (m, 2H), 1.54-1.67 (m, 6H) 1.51 (m, 4H), 1.19-1.36 (m, 13 H), 1.11 (d, *J* = 6.6 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.97-1.07 (m, 2H), 0.90 (d, *J* = 7.2 Hz 3H), 0.83-0.93 (m, 1H). HRMS (ESI-TOF MS) m/z 2017.1832 (M-Cl)+ calculated for $C_{110}H_{166}N_7O_{27}$, measured 2017.1821.




**Cy3 Apoptolidin H (12)** To a solution of BNE-Cy-3 (**10**) (2.03 mg, 0.0025 mmol) in methanol (0.5 mL) was added azido apoptolidin H (**8**) (2.45 mg, 0.0025 mmol) as a solution in 0.5 mL methanol. The resulting solution was stirred at 37 °C for 4 hours, removed from the bath and concentrated. The resulting residue was dissolved in methanol (0.5 mL) and purified by semipreperative reverse phase HPLC (30 minute gradient from 35% strong to 100% strong buffer in weak buffer where strong is 5% aqueous MeCN and 10 mM $NH_4OAc$ and weak buffer is 95% aqueous MeCN and 10 mM $NH_4OAc$). Fractions containing the desired product (r.t. 27.35 min) were combined and concentrated *in vacuo* (T<31°C). The resulting aqueous solution was frozen at -80 °C and

lyophilized to yield 1.38 mg (32%) of Cy3 apoptolidin H (**12**) as a deep red solid: $^1$H NMR (600 MHz, $CD_3OD$) δ 8.55 (t, *J* = 13.5, 1H) 7.55 (d, *J* = 7.4, 2H), 7.45 (m, 2H), 7.36 (dd, *J* = 3.5, 8.0, 2H), 7.32 (m, 3H), 6.44 (dd, *J* = 8.6, 13.5 Hz, 2H), 6.17 (s, 1H), 6.12 (d, *J* = 15.7 Hz, 1H), 5.64 (m, 1H), 5.57 (m, 1H), 5.40 (m, 1H), 5.30 (d, *J* = 11.2 Hz, 1H), 5.19 (d, *J* = 10.0 Hz, 1H), 5.00 (ddd, *J* = 8.2, 8.2, 16.0 Hz, 1H), 4.95 (m, 1H), 4.54 (ddd, *J* = 2.0, 3.8, 10.2 Hz, 1H), 4.51 (m, 1H), 4.30 (dd, *J* = 7.2, 7.2 Hz, 2H), 4.15 (dd, *J* = 7.5, 7.5 Hz, 2H) 4.00-4.10 (m, 3H), 3.96 (dd, *J* = 9.5, 9.5 Hz, 1H), 3.98 (m, 1H), 3.81 (dd, *J* = 8.9, 8.9 Hz, 1H), 3.72-3.78 (m, 2H), 3.69 (s, 3H), 3.65 (m, 1H), 3.60 (m, 4H), 3.54 (m, 1H), 3.52 (t, *J* = 5.6 Hz, 4H), 3.42 (m, 2H), 3.25-3.72 (m, 2H), 3.24-3.29 (m, 2H), 3.14-3.22 (m, 3H), 3.02 (m, 2H), 3.69-3.87 (m, 3H), 2.63 (m, 1H), 2.29-2.50 (m, 4H), 2.24 (t, *J* = 7.3 Hz, 4H), 2.12-2.20 (m, 4H), 1.97-2.08 (m, 4H), 1.89-1.95 (m, 2H), 1.86 (m, 4H), 1.74 (s, 12H), 1.71 (m, 4H), 1.55-1.68 (m, 6H), 1.50 (m, 5H), 1.22-1.35 (m, 10H), 1.11 (d, *J* = 6.6 Hz, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.97-1.07 (m, 2H),0.83-0.93 (m, 4H). HRMS (ESI-TOF MS) m/z 1729.0259 (M-Cl)+ calculated for $C_{96}H_{142}N_7O_{21}$, measured 1729.0250.



Biotin apoptolidin A (**13**)

**Biotin Apoptolidin A** (**13**) To a solution of BNE-biotin (**9**) (2.6 mg, 0.0033 mmol) in methanol (0.5 mL) was added azido apoptolidin A (**7**) (2.05 mg, 0.0016 mmol) as a solution in methanol (0.5 mL). The resulting solution was stirred at 37 °C for 14 hours, removed from the bath and concentrated. The resulting residue was dissolved in methanol (0.5 mL) and purified by semipreperative reverse phase HPLC (30 minute gradient from 35% strong to 100% strong buffer in weak buffer where strong is 5% aqueous MeCN and 10 mM $NH_4OAc$ and weak buffer is 95% aqueous MeCN and 10 mM $NH_4OAc$). Fractions containing the desired product (r.t. 10.5 min) were combined and concentrated *in vacuo* to remove acetonitrile (T<31°C). The resulting aqueous solution was frozen at -80 °C and lyophilized to yield 2.63 mg (29%) of biotin apoptolidin A (**13**) as a white solid: $^1$H NMR (600 MHz, $CD_3OD$) δ 3.73 (s, 1H), 6.18 (s, 1H), 6.13 (d, *J* = 15.8 Hz, 1H), 5.64 (dd, *J* = 7.9, 7.9 Hz, 1H) 5.29 (d, *J* = 11.3 Hz, 1H), 5.20 (d, *J* = 10.1 Hz, 1H), 5.00 (m, 1H), 4.94 (m, 3H), 4.83 (dd, *J* = 1.8, 9.9 Hz, 1H), 4.54 (m, 2H), 4.49 (dd, *J* = 4.9, 7.8 Hz, 1H), 4.31 (m,

5H), 4.17 (m, 3H), 3.95 (m, 1H), 3.90 (m, 3H), 3.81 (dd, *J* = 8.8, 8.8 Hz, 1H), 3.78 (dd, *J* = 6.3, 9.4 Hz, 1H), 3.71 (dd, *J* = 4.6, 11.0 Hz, 1H), 3.67 (dd, *J* = 6.3, 9.7 Hz, 1H), 3.65-3.86 (m, 3H), 3.62 (s(br), 4H), 3.59 (s, 3H), 3.58 (m, 1H), 3.52-3.57 (m, 5H), 3.42 (s, 3H), 3.41 (m, 3H), 3.37 (m, 3H), 3.36 (s, 3H), 3.32 (m, 1H), 3.27 (s, 3H), 3.21 (dd, *J* = 6.0, 9.2 Hz, 1H), 3.17 (m, 2H), 3.06 (m, 1H), 2.97 (dd, *J* = 9.0, 9.0 Hz, 1H), 2.92 (dd, *J* = 5.0, 12.8 Hz, 1H), 2.81 (dd, *J* = 9.3, 9.3 Hz, 1H), 2.71 (m, 2H), 2.64 (m, 1H), 2.43 (m, 4H), 2.21 (m, 6H), 2.18 (s, 3H), 2.14 (m, 1H), 2.10 (s, 3H), 1.97-2.08 (m, 4H), 1.92 (m, 1H), 1.89 (s, 3H), 1.50-1.82 (m, 18H), 1.32 (s, 3H), 1.28-1.48 (m, 9 H), 1.25-128 (m, 13H), 1.22 (d, *J* = 6.3 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1,02 (d, *J* = 6.7 Hz, 3H), 0.90 (m, 2H), 0.89 (d, *J* = 6.9 Hz, 3H); HRMS (ESI-TOF MS) m/z 1804.9937 (M+H)+ calculated for $C_{90}H_{146}N_7O_{28}S$, measured 1804.9946.

**6. Cytotoxicity Assay**

**MTT Cell Viability Assay for Cell Density Requirement:** Low passage (P#<25) H292 human lung carcinoma cells (obtained from the American Type Culture Collection, ATCC) were plated at 5, 10, 15, 20 or 25 thousand cells per well in 96-well plates in 100 μL of RMPI 1640 medium containing 10% fetal bovine serum and 100 IU penicillin and 100 mg/mL streptomycin and incubated for 16 hours to attach. Apoptolidin A was dissolved in DMSO at1 μM, 10 μM, 100 μM and 1 mM. The resulting DMSO stock solutions were diluted in complete RPMI medium 1000:1 to yield medium solutions containing 1 nM, 10 nM, 100 nM, and 1 μM apoptolidin A or DMSO to give a final DMSO concentration of 0.1%. Media was removed from each well by aspiration and replaced with media containing DMSO vehicle or apoptolidin A for a total of n=4 wells per cell density and concentration. Cells were incubated for four days (96 hours). The media from each well was then aspirated and replaced with 100 μL of complete RPMI medium containing 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 0.5 mg/mL and returned to the incubator at 37 °C for two hours. Media from ach well was aspirated and replaced with 100 μL DMSO. Absorbance was measured at 560 nM using a GloMax Multiplate reader (Promega, Madison, WI, USA). Blank absorbance from the average of 8 wells treated with MTT in cell-free medium was subtracted from each value. The percent cell viability of each well was calculated as the fraction of the average absorbance of DMSO control treated cells at each condition.

**Standard MTT Cell Viability Assay:** Low passage (P#<25) H292 human lung carcinoma cells

were plated at 25,000 cells per well in 96-well plates in 100 μL of RMPI 1640 medium containing 10% fetal bovine serum and 100 IU penicillin and 100 mg/mL streptomycin and incubated for 16 hours to attach. Compounds used for testing were dissolved in DMSO at various concentrations from 1 μM to 10 mM. The resulting DMSO stock solutions were diluted in complete RPMI medium 1000:1 to yield medium solutions containing 1 nM to 10 μM of the respective compound to be assayed and a uniform DMSO concentration of 0.1%. Media was removed from each well by aspiration and replaced with media containing DMSO vehicle or compound for a total of n=8 wells per concentration. Cells were incubated for four days (96 hours). The media from each well was then aspirated and replaced with 100 μL of complete RPMI medium containing MTT at 0.5 mg/mL and returned to the incubator at 37 °C for two hours. Media from each well was aspirated and replaced with 100 μL DMSO. Absorbance was measured at 560 nM using a GloMax Multiplate reader (Promega, Madison, WI, USA). Blank absorbance from the average of 8 wells treated with MTT in cell-free medium was subtracted from each value. The percent cell viability of each well was calculated as the fraction of the average absorbance of DMSO control treated cells. Data was plotted GraphPad Prism 5 and fitted with a non-linear regression curve. Effective concentration 50 ($EC_{50}$) was estimated graphically as the concentration at which 50 % of control formazan product absorbance was detected.

**Effect of Culture Media on Toxicity of Apoptolidin A:** H292 cells (ATCC, mycoplasma negative on 05/17/10 by pcr assay) were plated at a density of 500 per well in 96 well plates and treated for 7 days with apoptolidin A, from 3-30 nM. Cell viability was detected by loading the cells with Calcein-AM reagent for 30 min at 37°C, followed by measurement of Calcein Fluorescence (485 nm excitation, 520 nm emission). Glucose concentrations of media types are shown in parenthesis MEM (1.0 g/L), DMEM (4.5 g/L), and RPMI 1640 (2.0 g/L).

**7. $F_OF_1$-ATPase Inhibition Assay** A single colony of DBY7286 (mat A, ura-/-) was inoculated into a pre-culture (50 mL in a 250 mL shake flask) of semisynthetic media (3 g yeast extract, 0.5 g glucose, 0.5 g $CaCl_2 \cdot H_2O$, 0.5 g NaCl, 0.6 g $MgCl_2 \cdot H_2O$, 0.1 g $KH_2PO_4$, 0.1 g $NH_4Cl$, 22 mL 90% DL-lactic acid, 8 g NaOH, and 1L ddI water, pH = 5.5), and incubated with orbital shaking for 15 h in flasks at 30 °C. Semisynthetic media (5 x 1 L of in 3 L Fernbach Flasks) were inoculated with 1% of preculture and incubated at 30 °C for 16 hrs to an $OD_{600}$ of 3.

Cells were collected by centrifugation at 2000 x g for 15 min. Supernatant was removed and the combined cell pellets were resuspended in 150 mL of distilled $H_2O$. The suspension was then transferred to pre-weighed centrifuge bottles and centrifuged at 2000 x g for 5 minutes. After decanting the supernatant, the wet weight of the cell pellet was determined. The 3 – 4 g pellet was then resuspended in 25 mL of 0.1 M Tris, 10 mM dithiothreitol, pH 9.4, and incubated for 15 minutes in a 30 °C water bath. The cells were then centrifuged at 2000 x g, resuspended in 20 mL of buffer A (1.2 M sorbitol, 20 mM $KH_2PO_4$, pH 7.4), and converted to spheroplasts by incubation with Zymolyase (2.5 mg/g cell pellet) for 30 min at 30 °C. Spheroplasts were collected by centrifugation at 4000 x g, washed, and resuspended twice in 20 mL cold buffer A. Washed spheroplasts were resuspended in 50 mL cold buffer B (0.6 M sorbitol, 20 mM K+MES, 0.5 mM PMSF, pH6.0), homogenized using a Dounce homogenizer, diluted to 125 mL with buffer B, and centrifuged at 1500 x g for 5 minutes. The supernatants were retained and the pellets resuspended in 50 mL buffer B, homogenized and centrifuged at 1500 x g for 5 minutes. The pellets were then discarded, the supernatant suspensions were pooled, and centrifugation at 12000 x g for 10 minutes. The resulting supernatant was removed and the pellets were resuspended in 60 mL buffer B without PMSF with a homogenizer and centrifuged at 1500 x g for 5 minutes. The supernatant suspensions were then centrifuged at 12000 x g for 10 minutes. The mitochondria containing pellets were resuspended in 1 mL buffer B and total mitochondrial protein was determined with a BCA protein assay kit (Thermo, Inc). Samples were adjusted to desired protein concentration with buffer C (0.6 M sorbitol, 20 mM K+HEPES, pH 7.4)

To measure inhibition of ATPase activity 20 $\mu$g of mitochondrial protein was added to 100 uL of a solution of 50 mM Tris (pH 8.0), 3.3 mM $MgCl_2$, 2 $\mu$g/mL Antimycin, 5 u/mL lactate dehydrogenase, 3 u/mL pyruvate kinase, and 0.3 mM NADH, at 25 °C. The ATP consumption assay was initiated by addition of 1 mM ATP and 1 mM phosphoenol pyruvate, and rate of oxidation of NADH was monitored by following the decrease in adsorption at 350 nM over 10 minutes.

## 8. Imaging Experiments

**Confocal Microscopy Studies:** Low passage (P < 25) H292 human lung carcinoma cells were plated in MaTek dishes at 15% confluence in 2.0 mL of RMPI 1640 medium containing 10% fetal bovine serum and allowed to attach and grow for 40 hours. 200 μM stock solutions of Cy3 apoptolin A (**17**), Cy3 apoptolidin H (**18**), or BME-Cy3 (**15**) in DMSO was prepared. Each dish was treated by the following protocol. Media was removed by aspiration and replaced with 2.0 mL serum free RPMI 1640 media. 2.0 μL of the appropriate DMSO stock solution was added to the dish and cells were returned to the incubator for 15 min. Media containing fluorophores was removed by aspiration followed by a wash with serum free RPMI 1640 media (3 x 2.0 mL) followed by a final addition of 2.0 mL of serum free media. After incubation for 30 min, media was removed and replaced by a freshly sonicated (important for mitotracker solubility) 30 nM solution of Mitotracker Green FM. After an additional incubation of 30 min in the incubator, media was removed by aspiration, (PBS, 2 x 2.0 mL) and replaced with 2.0 mL PBS. Each dish was then imaged at ten random fields by confocal microscopy. Confocal microscopy was performed on a LSM780 (Zeiss) using a c- Apochromat 40x 1.2 W Corr M27 oil immersion objective. Cy3 fluorescence was excited using 488 nm laser (2%) and emission was measured with a 492-542 nm bandpass. Mitotracker Green FM fluorescence was excited with a 488 nm laser (2%) and emission as measured with a bandpass of 552-683 nm. All images were acquired using 512x512, 0.14 μm diameter pixels, a 12.6 μs pixel dwell time, 12-bit gray levels and a 2.4 μm optical section. Each compound was tested in 3 dishes of cells. Pearson's coefficients were calculated using the JACoP plugin[7] for ImageJ[8] 1.46r software for each field from each dishes and are reported as the average of the 30 calculations.

**Table S1. $^{1}H$ and $^{13}C$ NMR shift assignments for apoptolidin H (3) and isoapoptolidin H (S1).**

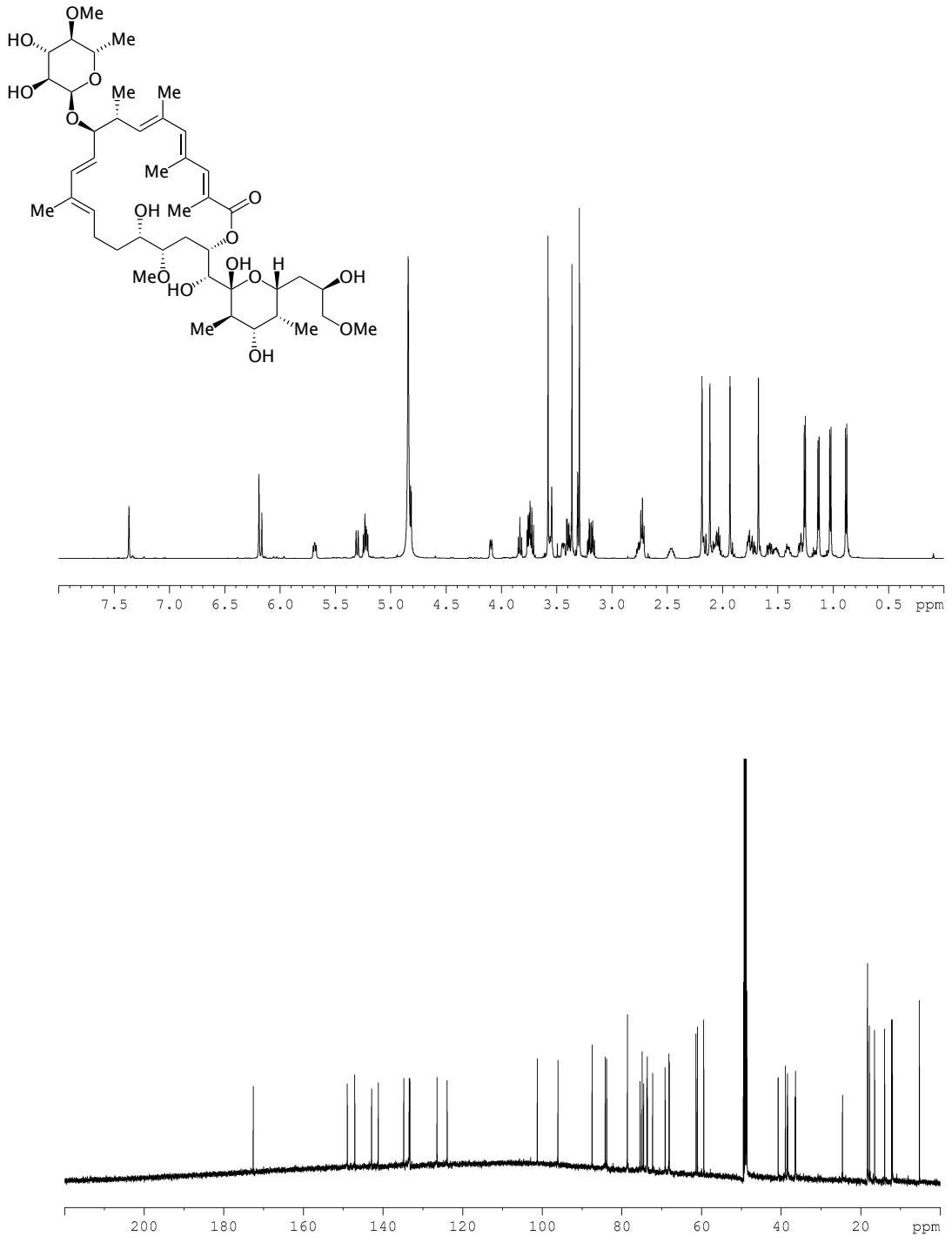
| | $\delta_C$ | | | $\delta_C$ | | | $\delta_C$ | | | $\delta_C$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C# | **3** | **S1** | C# | **3** | **S1** | C# | **3** | **S1** | C# | **3** | **S1** |
| **1** | 172.5 | 170.3 | **12** | 134.8 | 134.2 | **23** | 73.7 | 73.3 | **22-Me** | 12.2 | 12.0 |
| **2** | 123.9 | 124.9 | **13** | 133.3 | 133.6 | **24** | 40.7 | 40.7 | **24-Me** | 5.3 | 5.3 |
| **3** | 149.0 | 147.1 | **14** | 24.6 | 25.4 | **25** | 69.1 | 69.0 | **17-OMe** | 61.4 | 60.0 |
| **4** | 133.4 | 132.7 | **15** | 36.5 | 33.7 | **26** | 38.4 | 38.0 | **28-OMe** | 59.4 | 59.4 |
| **5** | 147.1 | 143.5 | **16** | 74.5 | 68.1 | **27** | 68.1 | 74.1 | **1'** | 96.0 | 96.2 |
| **6** | 133.2 | 132.8 | **17** | 83.8 | 82.2 | **28** | 78.6 | 78.4 | **2'** | 73.9 | 73.7 |
| **7** | 142.9 | 136.9 | **18** | 38.5 | 36.0 | **2-Me** | 14.0 | 13.4 | **3'** | 74.9 | 74.9 |
| **8** | 38.9 | 39.4 | **19** | 72.3 | 67.8 | **4-Me** | 17.8 | 17.9 | **4'** | 87.5 | 87.5 |
| **9** | 84.1 | 84.0 | **20** | 75.4 | 75.3 | **6-Me** | 16.5 | 17.6 | **5'** | 68.2 | 68.2 |
| **10** | 126.4 | 125.8 | **21** | 101.2 | 102.9 | **8-Me** | 18.2 | 18.4 | **6'** | 18.3 | 18.3 |
| **11** | 141.2 | 141.0 | **22** | 36.3 | 36.6 | **12-Me** | 12.0 | 12.1 | **4'-OMe** | 61.0 | 61.0 |
| | $\delta_H$ | | | $\delta_H$ | | | $\delta_H$ | | | $\delta_H$ | |
| C# | **3** | **S1** | C#. | **3** | **S1** | C# | **3** | **S1** | C# | **3** | **S1** |
| **1** | -- | -- | **12** | -- | -- | **23** | 2.04 | 3.76 | **22-Me** | 1.03 | 1.05 |
| **2** | -- | -- | **13** | 5.68 | 5.47 | **24** | 1.76 | 1.78 | **24-Me** | 0.88 | 0.86 |
| **3** | 7.36 | 7.34 | **14** | 2.47,2.05 | 2.49,1.94 | **25** | 4.09 | 4.28 | **17-OMe** | 3.36 | 3.50 |
| **4** | -- | -- | **15** | 1.53,1.41 | 1.39 | **26** | 1.58,1.29 | 1.78,1.41 | **28-OMe** | 3.29 | 3.38 |
| **5** | 6.19 | 5.97 | **16** | 3.44 | 3.86 | **27** | 3.55 | 3.47 | **1'** | 4.82 | 4.80 |
| **6** | -- | -- | **17** | 2.72 | 3.36 | **28** | 3.21,3.17 | 3.38 | **2'** | 3.39 | 3.39 |
| **7** | 5.23 | 5.08 | **18** | 2.15,1.72 | 1.68,1.39 | **2-Me** | 2.11 | 2.06 | **3'** | 3.72 | 3.73 |
| **8** | 2.75 | 2.75 | **19** | 5.31 | 4.45 | **4-Me** | 2.19 | 2.09 | **4'** | 2.71 | 2.72 |
| **9** | 3.83 | 3.78 | **20** | 3.54 | 4.94 | **6-Me** | 1.93 | 1.73 | **5'** | 3.74 | 3.75 |
| **10** | 5.22 | 5.26 | **21** | -- | -- | **8-Me** | 1.13 | 1.16 | **6'** | 1.26 | 1.25 |
| **11** | 6.18 | 6.05 | **22** | 2.04 | 1.78 | **12-Me** | 1.68 | 1.71 | **4'-OMe** | 3.58 | 3.58 |

**Table S2. $^{13}$C NMR shift assignments for apoptolidin H (9) and synthetic apoptolidin H from Koert et.al.[a]**

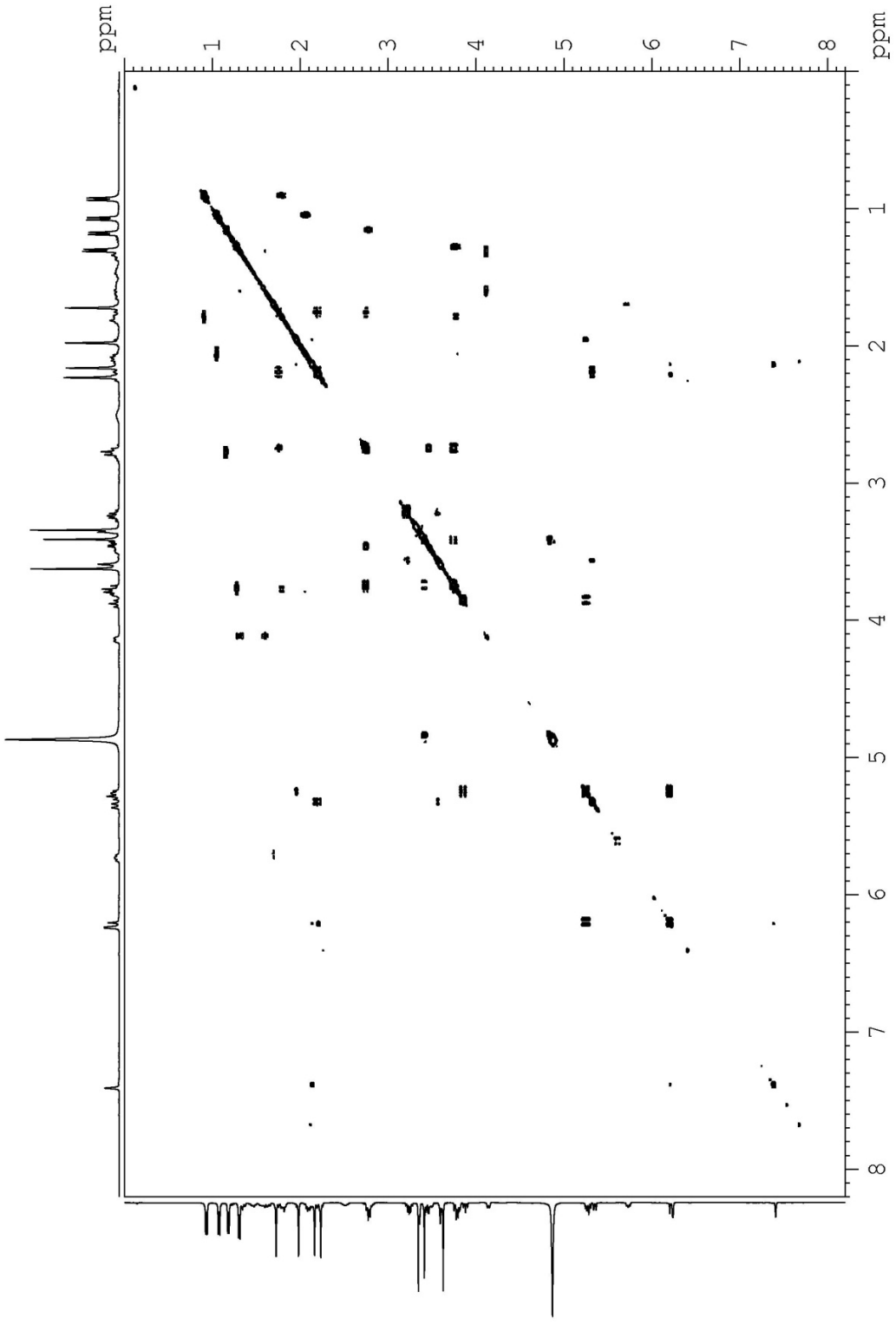
| | $\delta_C$ | | | $\delta_C$ | | | $\delta_C$ | | | $\delta_C$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C# | **9** | **Koert** | C# | **9** | **Koert** | C# | **9** | **Koert** | C# | **9** | **Koert** |
| **1** | 172.5 | 172.6 | **12** | 134.8 | 134.8 | **23** | 73.7 | 73.7 | **22-Me** | 12.2 | 12.2 |
| **2** | 123.9 | 124.0 | **13** | 133.3 | 133.3 | **24** | 40.7 | 40.8 | **24-Me** | 5.3 | 5.3 |
| **3** | 149.0 | 148.9 | **14** | 24.6 | 24.6 | **25** | 69.1 | 69.2 | **17-OMe** | 61.4 | 61.3 |
| **4** | 133.4 | 133.4 | **15** | 36.5 | 36.4 | **26** | 38.4 | 38.4 | **28-OMe** | 59.4 | 59.4 |
| **5** | 147.1 | 147.0 | **16** | 74.5 | 74.6 | **27** | 68.1 | 68.1 | **1'** | 96.0 | 96.1 |
| **6** | 133.2 | 132.2 | **17** | 83.8 | 83.8 | **28** | 78.6 | 78.7 | **2'** | 73.9 | 73.8 |
| **7** | 142.9 | 142.7 | **18** | 38.5 | 38.5 | **2-Me** | 14.0 | 14.0 | **3'** | 74.9 | 75.0 |
| **8** | 38.9 | 39.0 | **19** | 72.3 | 72.4 | **4-Me** | 17.8 | 17.8 | **4'** | 87.5 | 87.5 |
| **9** | 84.1 | 84.3 | **20** | 75.4 | 75.5 | **6-Me** | 16.5 | 16.5 | **5'** | 68.2 | 68.2 |
| **10** | 126.4 | 125.4 | **21** | 101.2 | 101.3 | **8-Me** | 18.2 | 18.2 | **6'** | 18.3 | 18.3 |
| **11** | 141.2 | 141.2 | **22** | 36.3 | 36.4 | **12-Me** | 12.0 | 12.0 | **4'-OMe** | 61.0 | 60.9 |

[a]H. Wehlan, M. Dauber, M. T. M. Fernaud, J. Schuppan, S. Keiper, R. Mahrwald, M.-E. J. Garcia, U. Koert, *Chem. Eur. J.* **2006**, *12*, 7378-7397

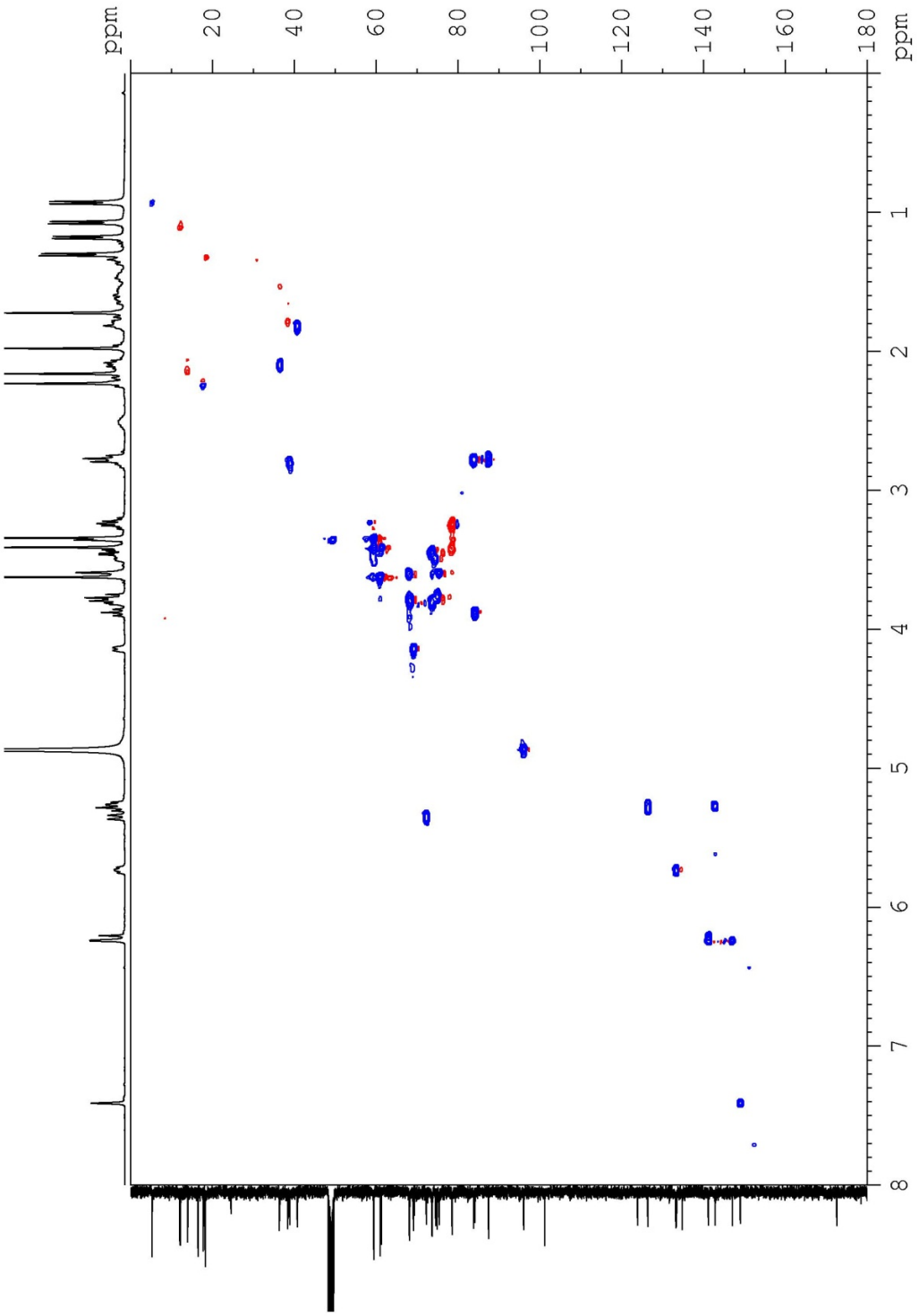
## 9. Copy of $^{1}H$, $^{13}C$ NMR and Spectra



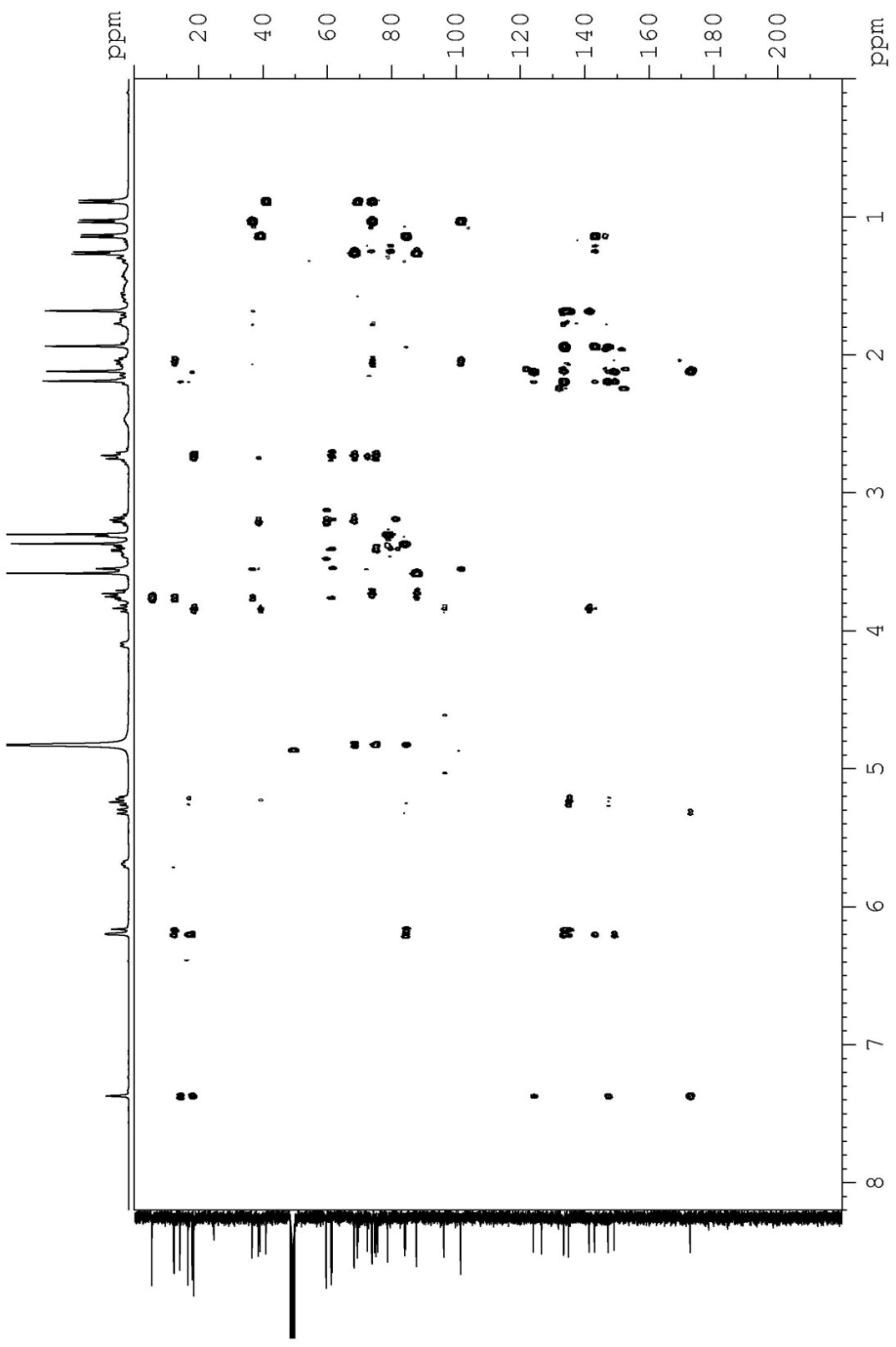

The 600 MHz $^{1}H$ and 150 MHz $^{13}C$ NMR spectra of apoptolidin H (**3**) in $CD_3OD$.



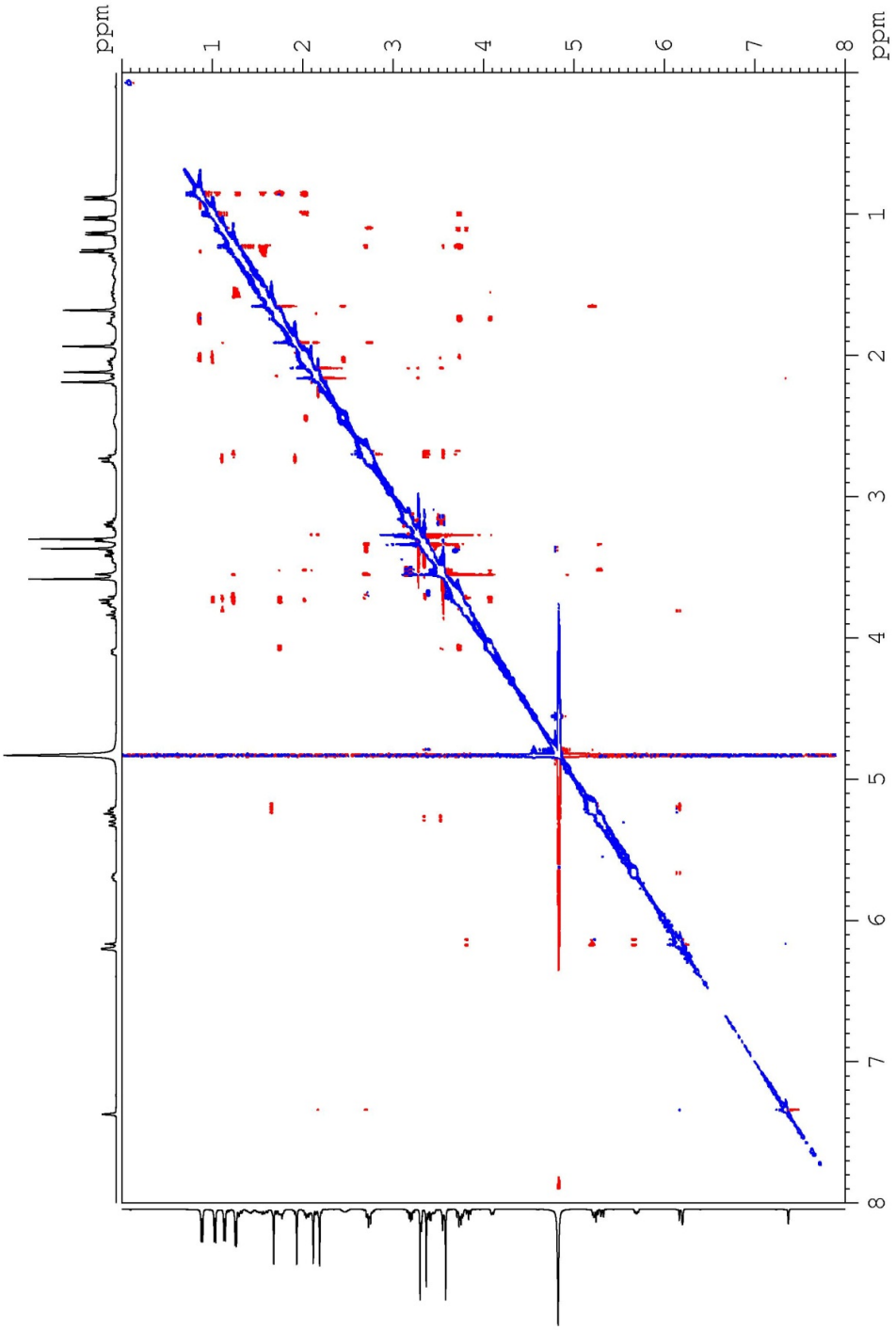

The 400 MHz COSY NMR spectra of apoptolidin H (**3**) in $CD_3OD$.



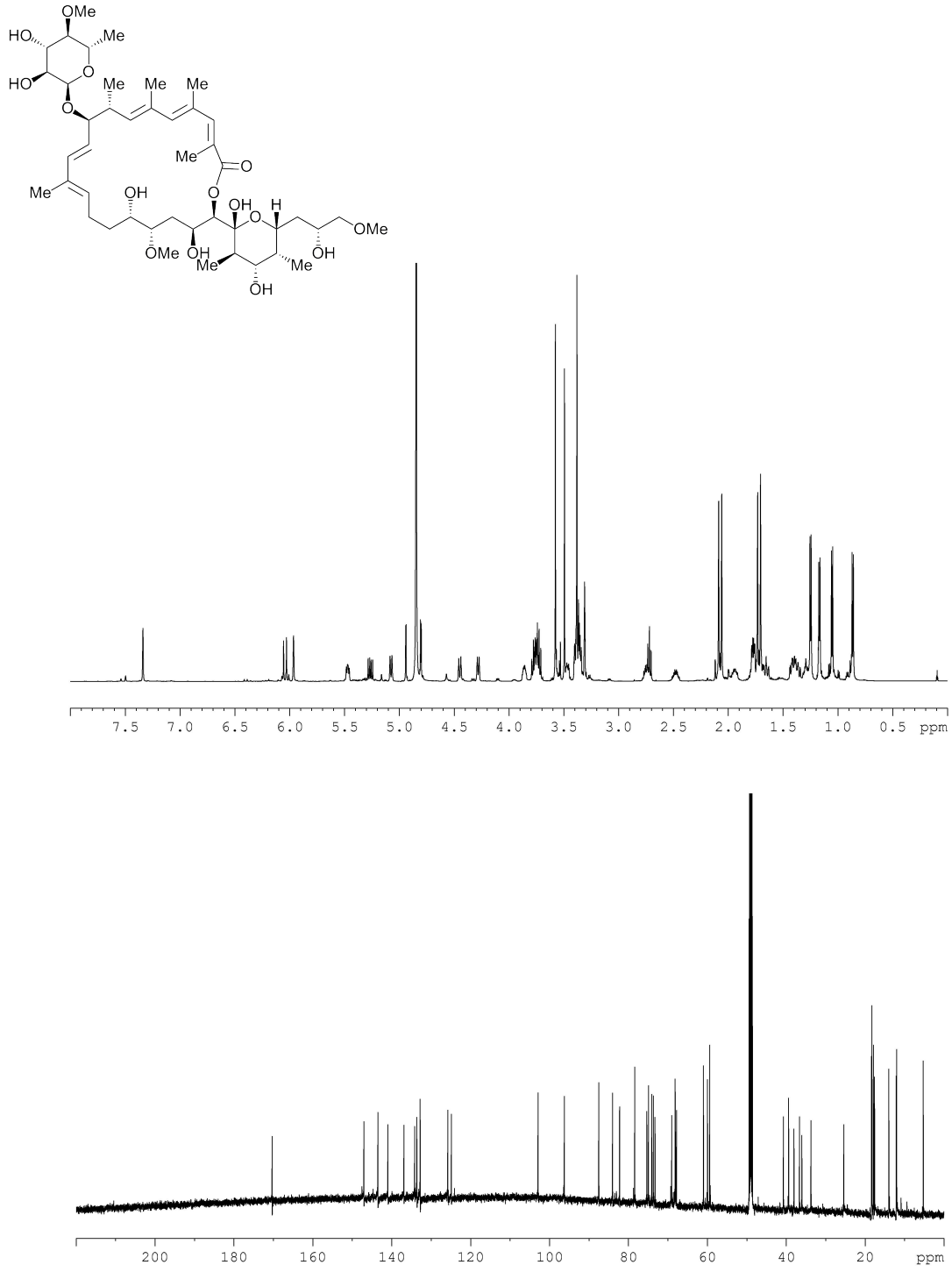

The 400 MHz HSQC NMR spectra of apoptolidin H (**3**) in $CD_3OD$.



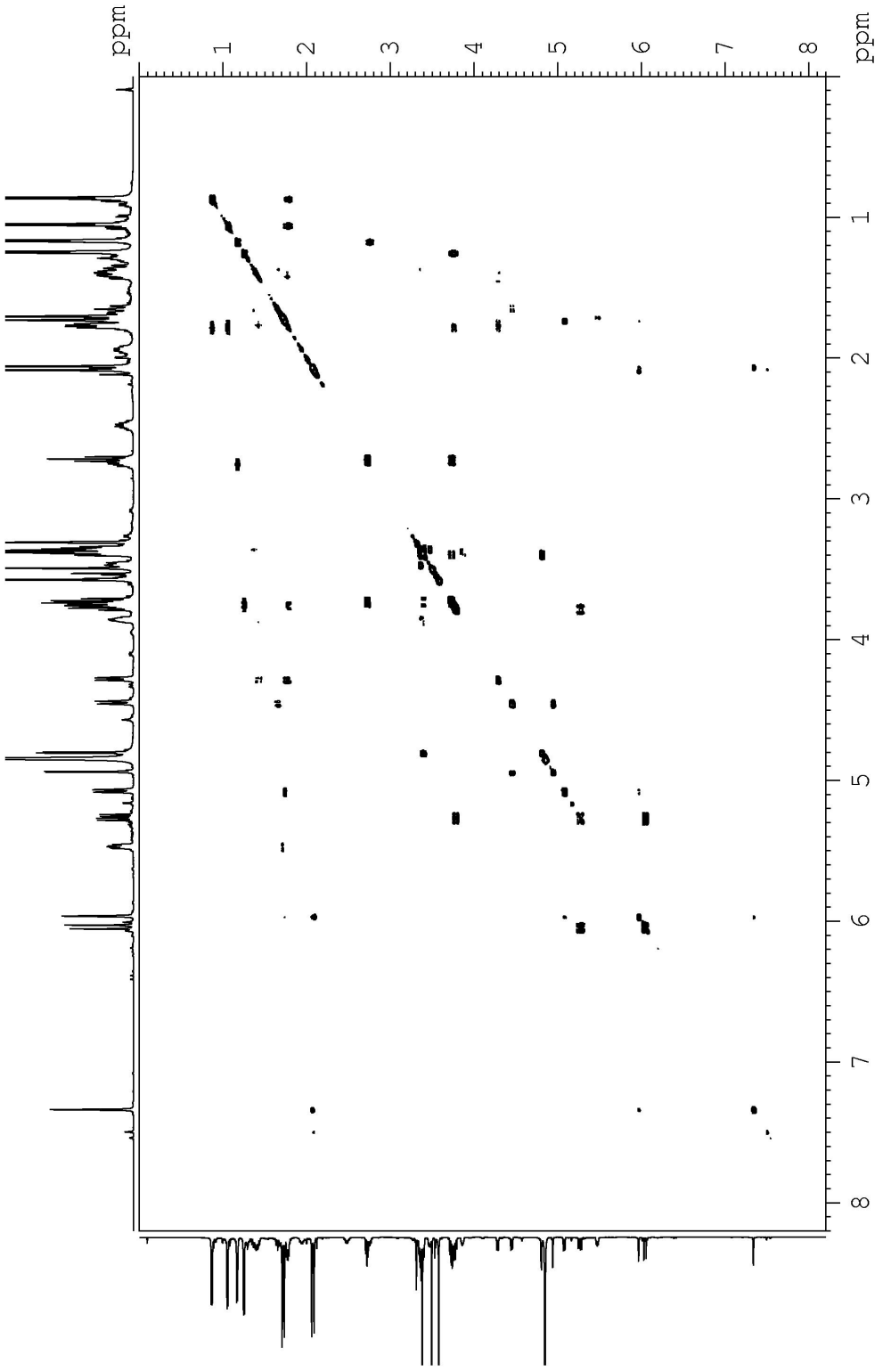

The 400 MHz HMBC NMR spectra of apoptolidin H (**3**) in $CD_3OD$.



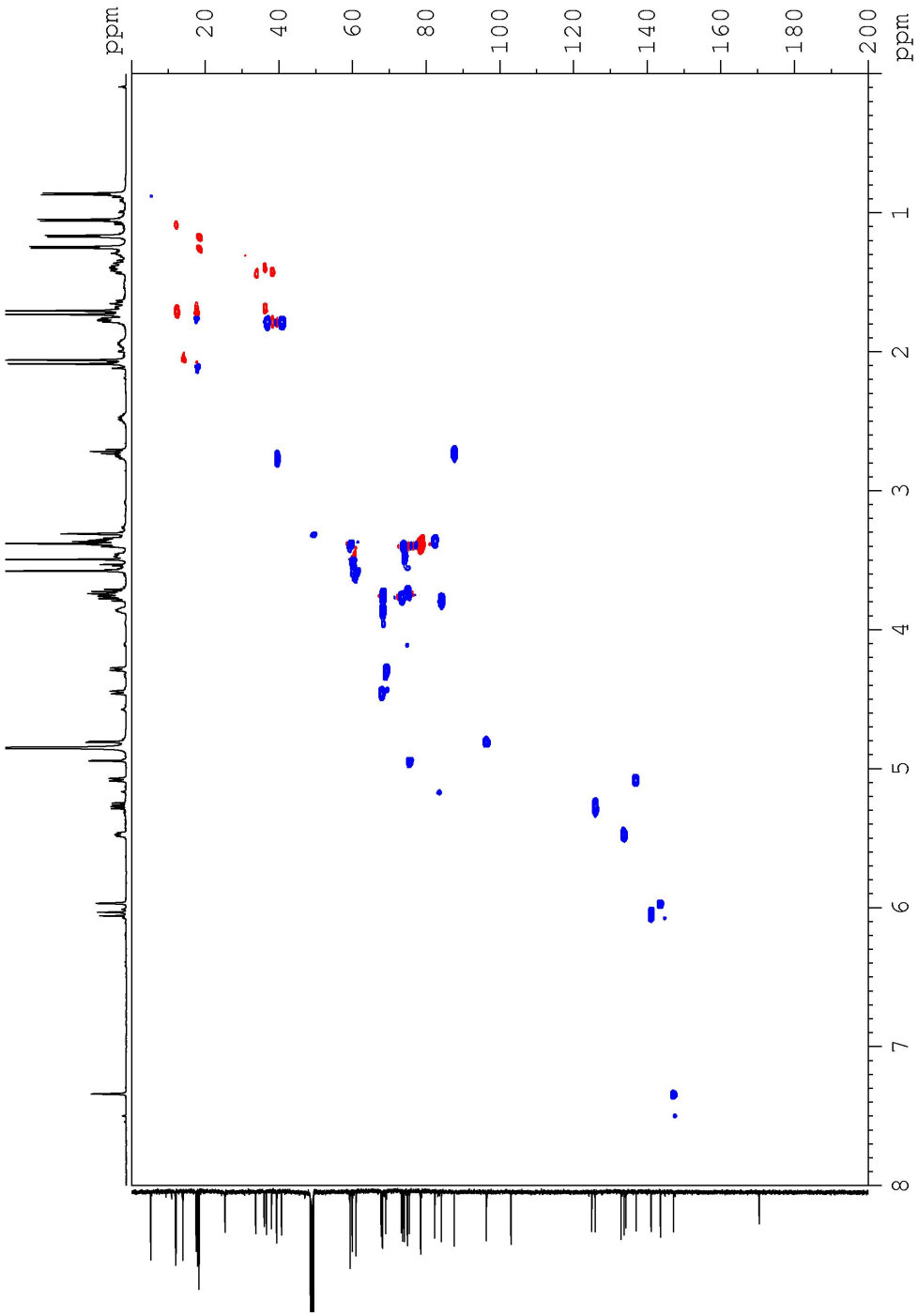

The 400 MHz NOESY NMR spectra of apoptolidin H (**3**) in $CD_3OD$.



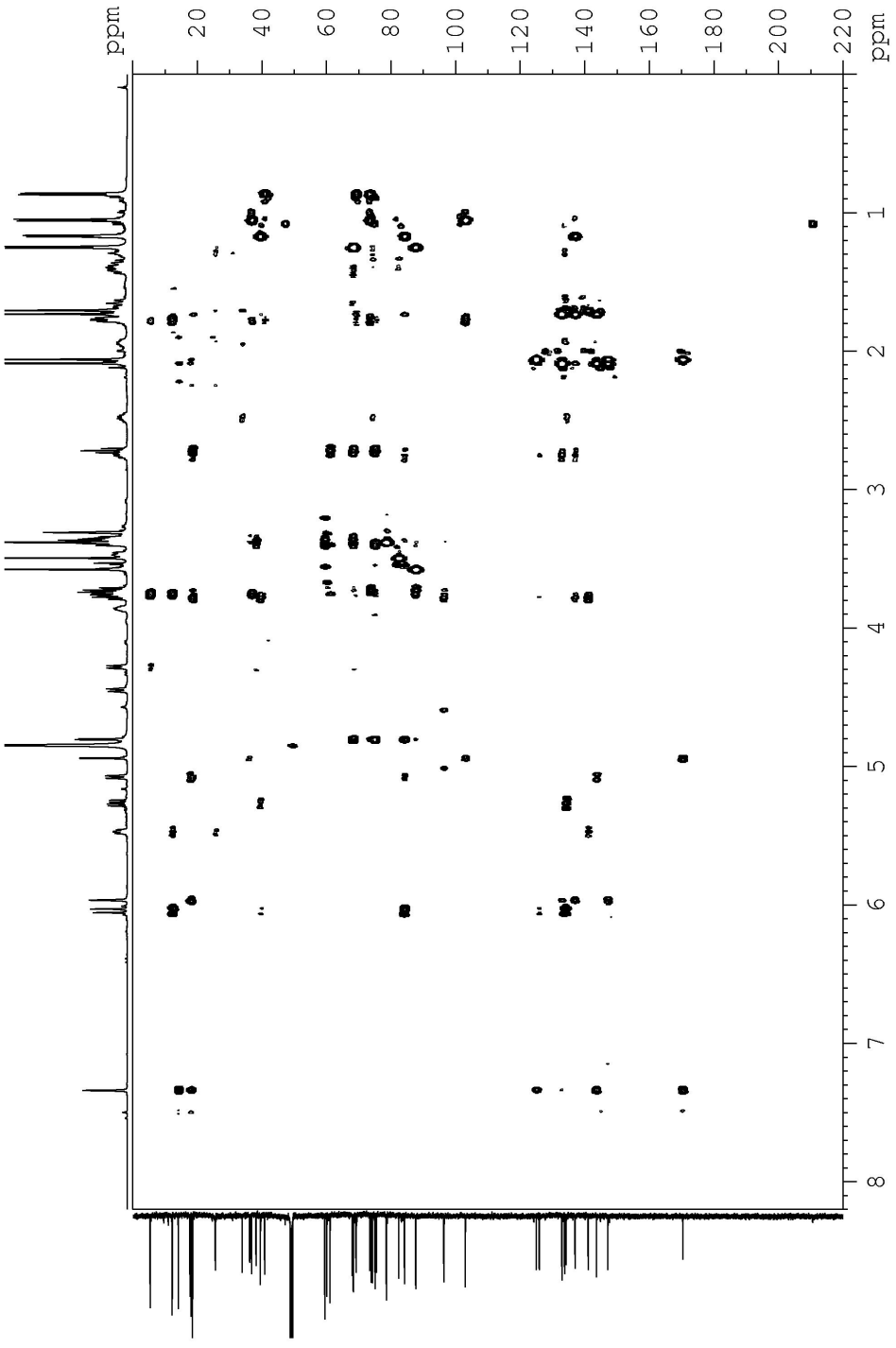

The 600 MHz $^{1}H$ and 150 MHz $^{13}C$ NMR spectra of isoapoptolidin H (**S1**) in $CD_3OD$.



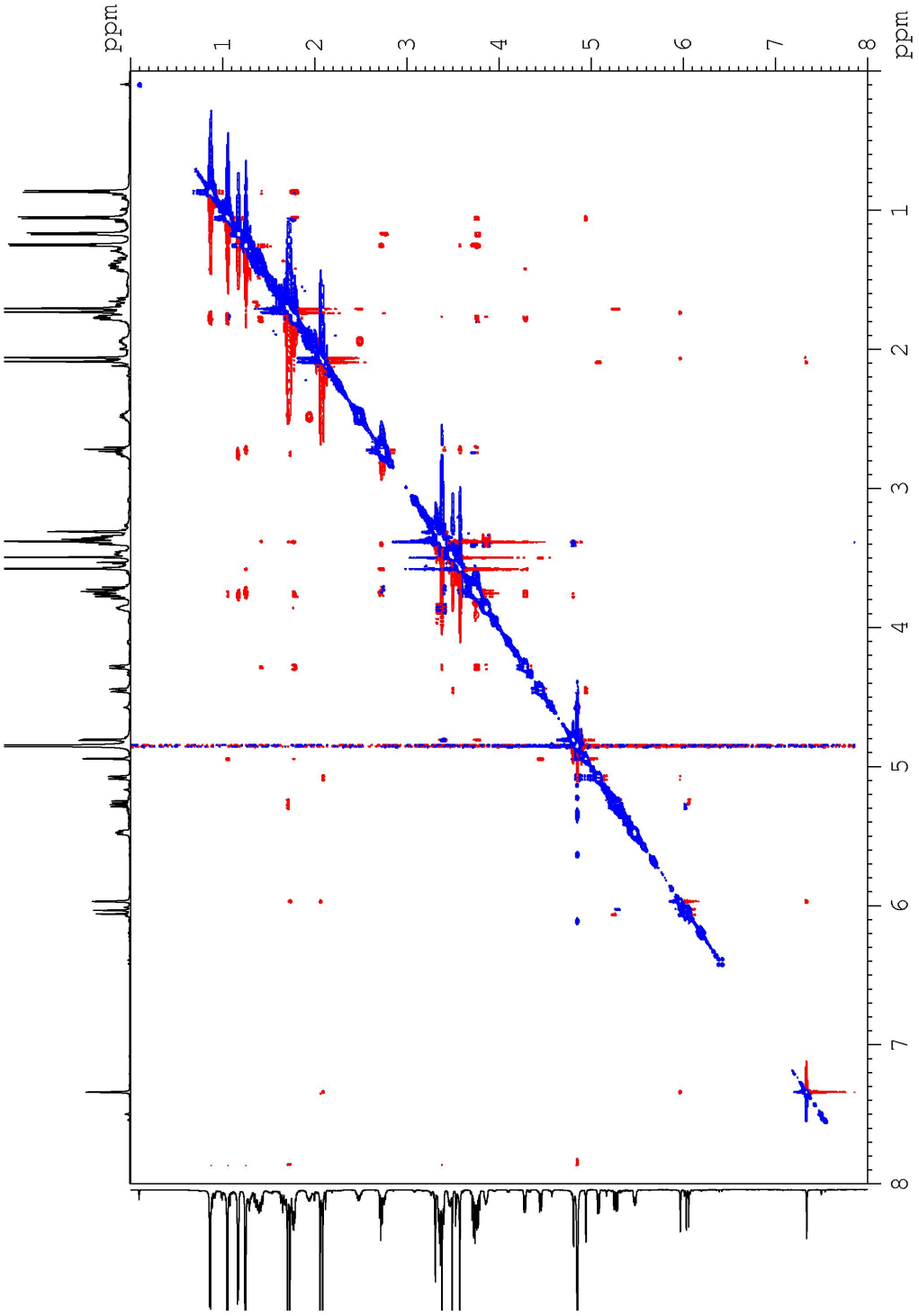

The 400 MHz COSY NMR spectra of isoapoptolidin H (**S1**) in $CD_3OD$.



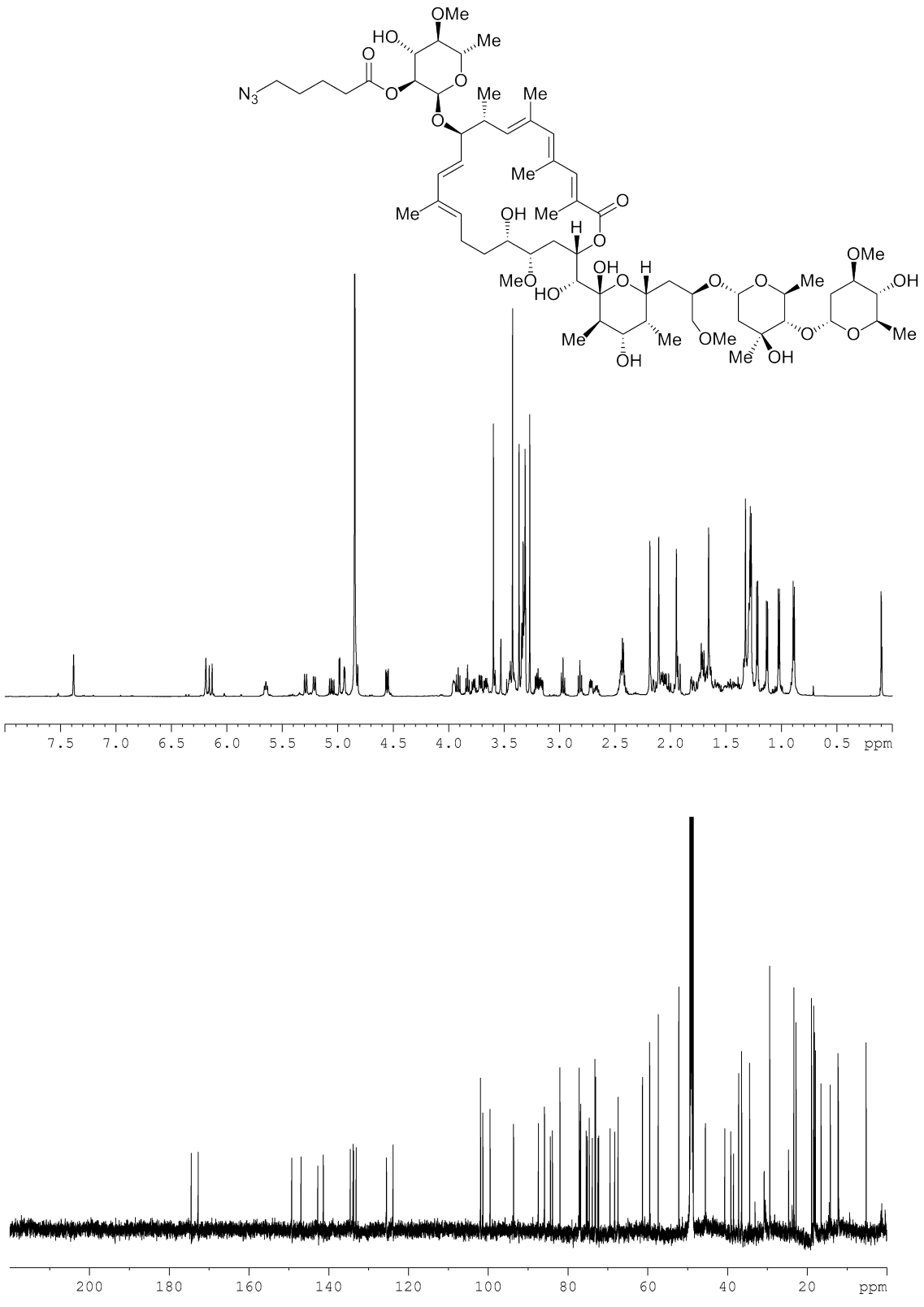

The 400 MHz HSQC NMR spectra of isoapoptolidin H (**S1**) in $CD_3OD$.



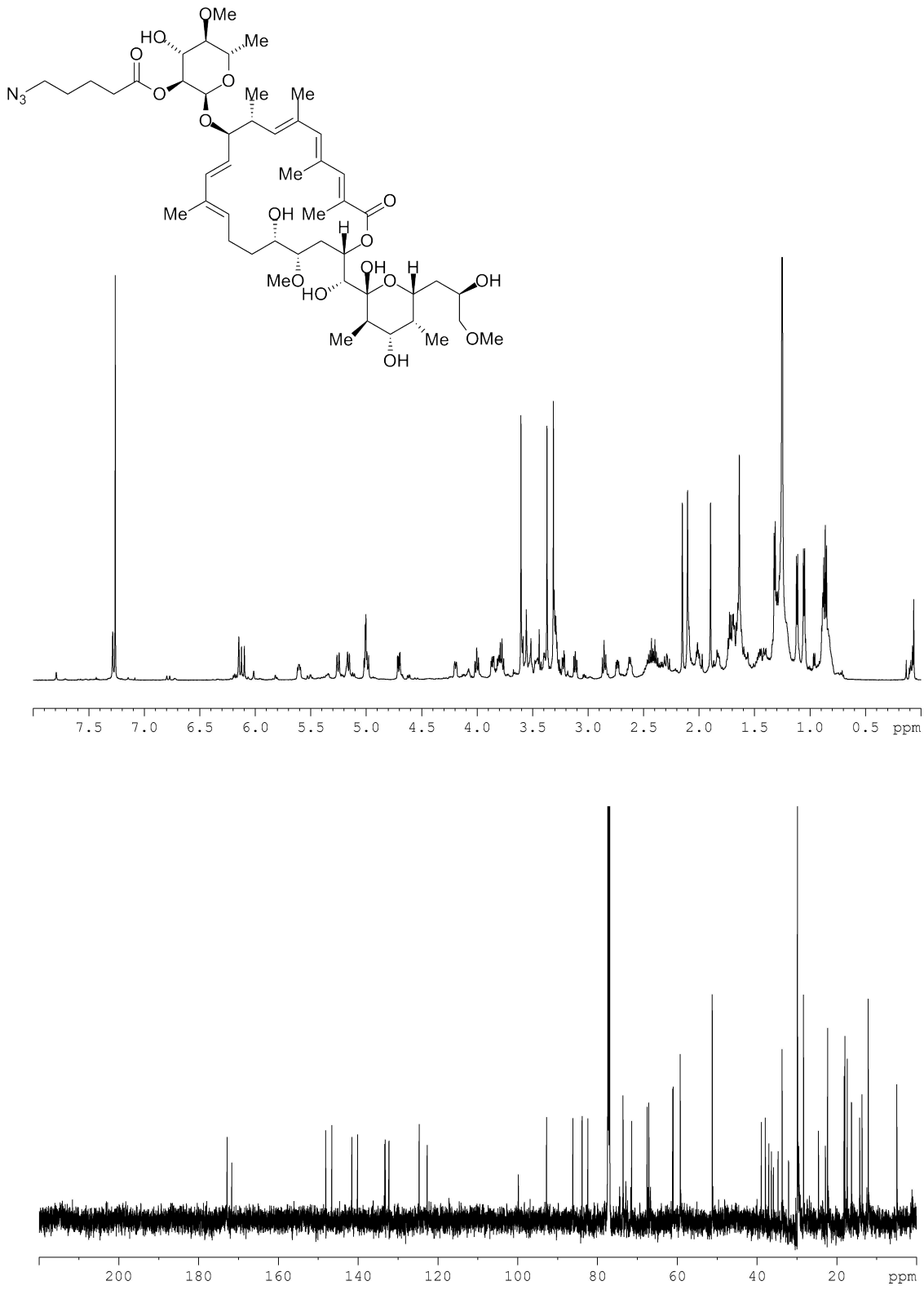

The 400 MHz HMBC NMR spectra of isoapoptolidin H (**S1**) in $CD_3OD$.



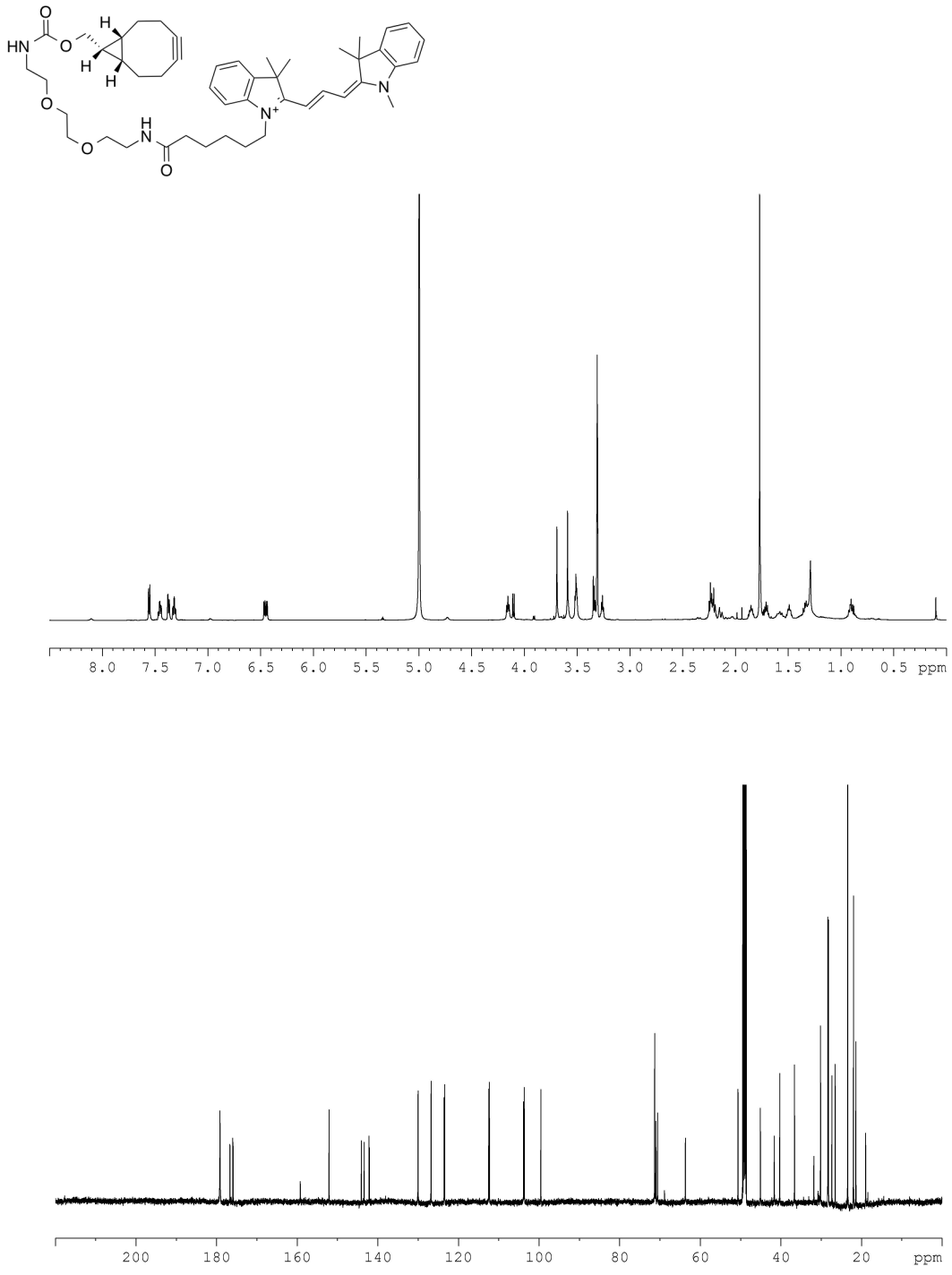

The 400 MHz NOESY NMR spectra of isoapoptolidin H (**S1**) in $CD_3OD$.



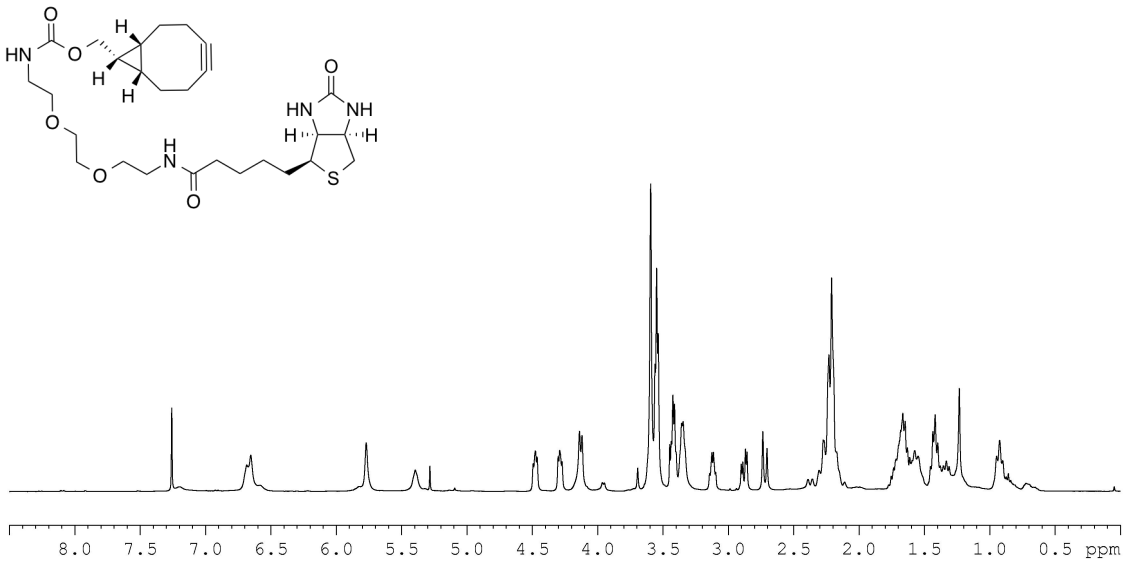

The 600 MHz $^{1}H$ and 150 MHz $^{13}C$ NMR spectra of azido apoptolidin A (**7**) in $CD_3OD$.

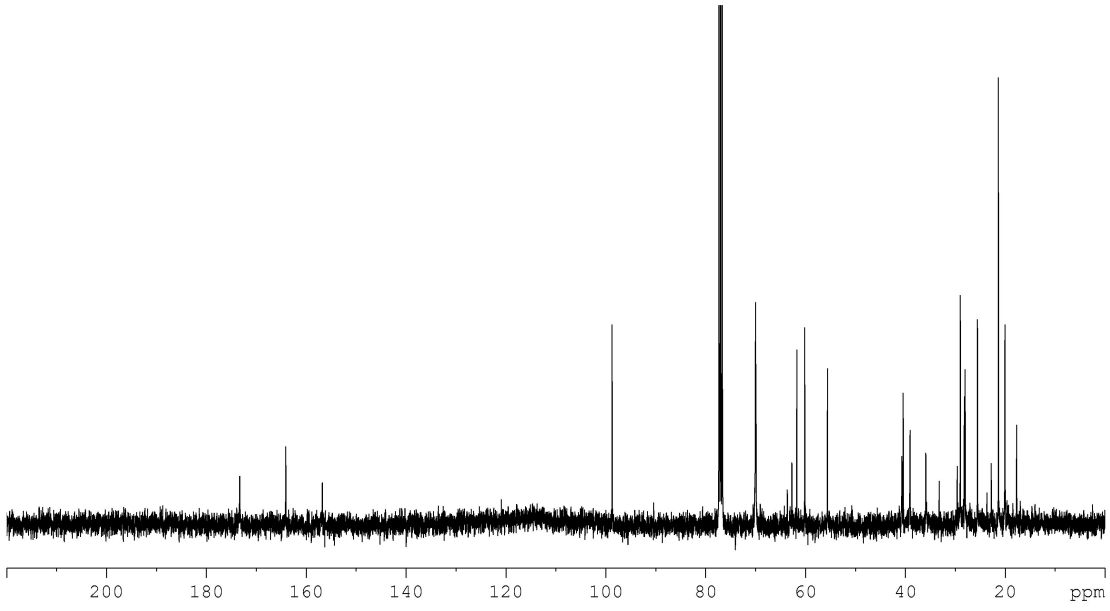



The 600 MHz $^{1}H$ and 150 MHz $^{13}C$ NMR spectra of azido apoptolidin H (**8**) in $CD_3OD$.

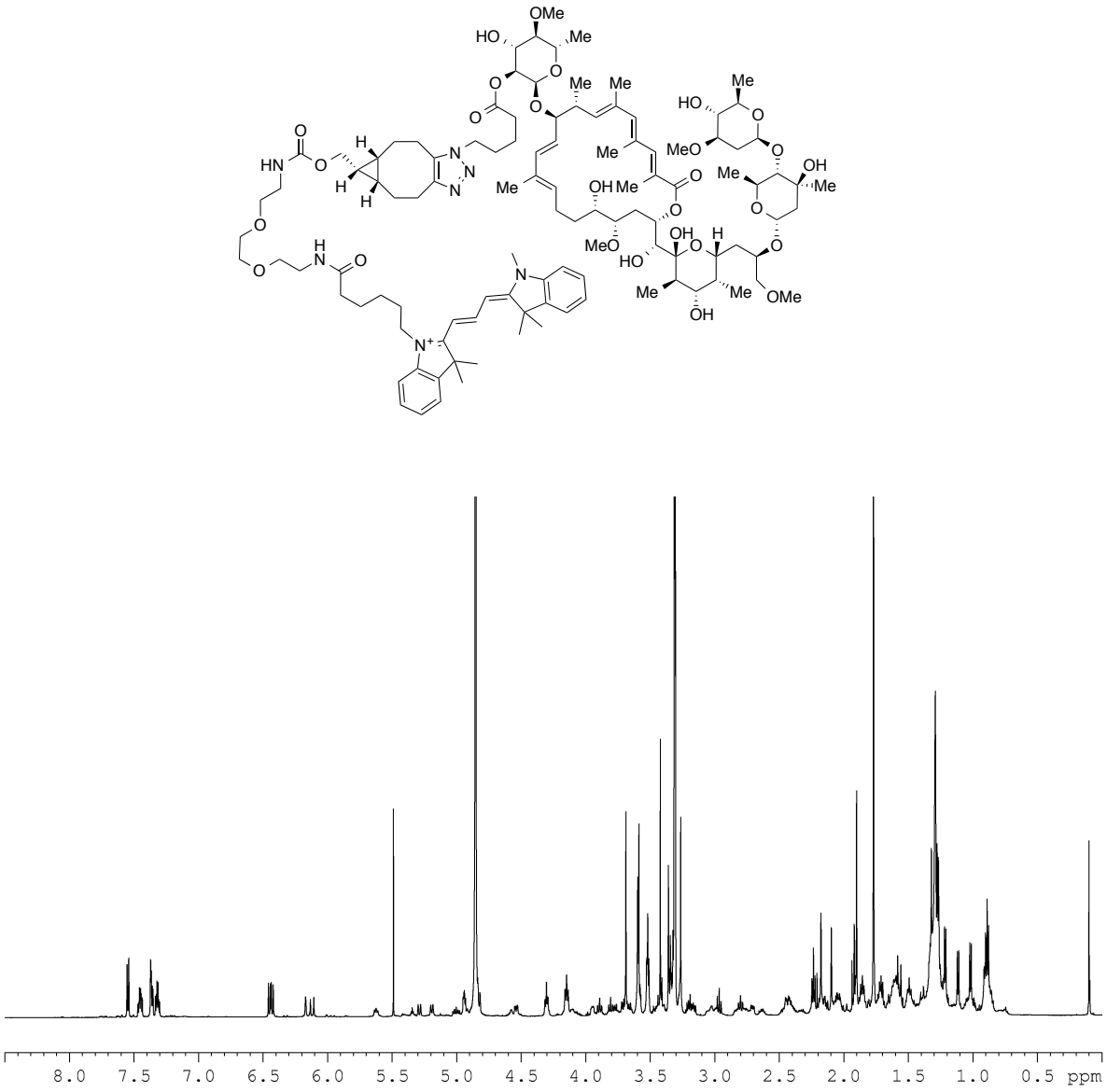



The 600 MHz $^{1}H$ and 150 MHz $^{13}C$ NMR spectra of BNE-Cy-3 (**10**) in $CD_3OD$.

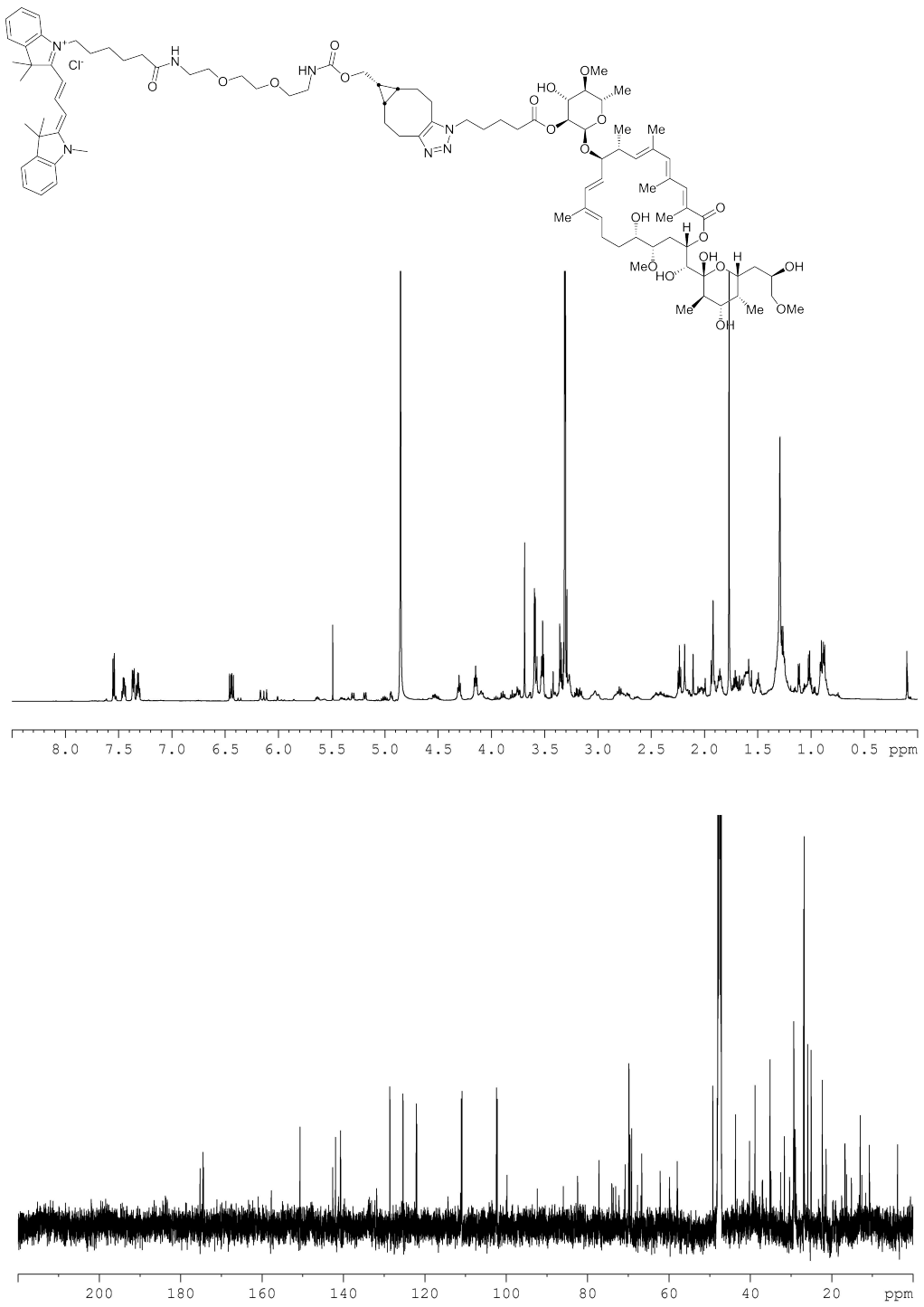


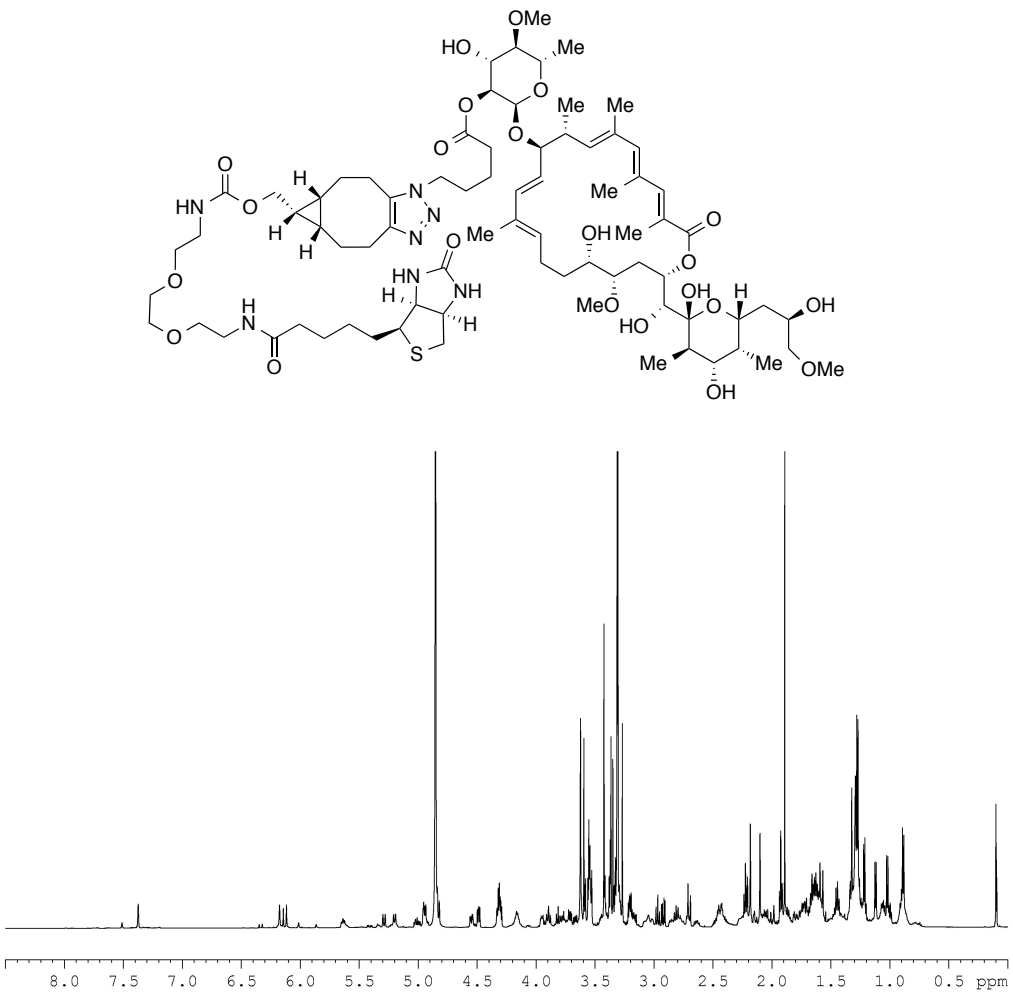

The 400 MHz $^{1}H$ and 100 MHz $^{13}C$ NMR spectra of BNE-biotin (**9**) in $CDCl_3$.




The 600 MHz $^{1}$H NMR spectrum of Cy3 apoptolidin A (**11**) in $CD_3OD$.




The 600 MHz $^{1}H$ and 150 MHz $^{13}C$ NMR spectra of Cy-3 apoptolidin H (**12**) in $CD_3OD$.




The 600 MHz $^{1}$H NMR spectrum of biotin apoptolidin A (**13**) in $CD_3OD$.

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