Fluorescent Probes of the Apoptolidins and their Utility in Cellular Localization Studies

Sean M. DeGuire, David Earl, Yu Du, Brenda Crews, Aaron Jacobs, Alessandro Ustione, Cristina Daniel, Katherine Chong, Lawrence J. Marnett, David Piston, Brian O. Bachmann and Gary A. Sulikowski*

*Departments of Chemistry, Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN, 37235, U.S.A**

Supporting Information

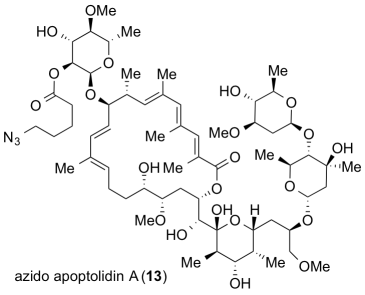
1. General Procedure: All non-aqueous reactions were performed in flame-dried or oven dried round-bottomed flasks under an atmosphere of argon. Stainless steel syringes or cannula were used to transfer air- and moisture-sensitive liquids. Reaction temperatures were controlled using a thermocouple thermometer 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.

Gary Sulikowski 10/8/2015 10:08 AM
Comment [1]: General reaction description

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.

5. Compound preparation: chemical synthesis


azido apoptolidin A (**13**)

Azido Apoptolidin A (13) 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 μL, 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 μL) 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 μm 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 μL 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 **13** 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),

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Comment [2]: Parentheses is used following name otherwise no parentheses

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Comment [3]: Minute abbreviate min (no period)

Gary Sulikowski 10/8/2015 9:54 AM
Comment [4]: In this sentence order of reagents not specified-if important needs to be specified.

Gary Sulikowski 10/8/2015 9:53 AM
Comment [5]: Weights are typically reported to three significant figures (never 4). If weight is accurate to only 2-as in this case or NaH-then only use 2. Mmol reported with same number of SF.

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Comment [6R5]: Weights of less then 0.1 gram use mg. less then 0.5 mol use mmol

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Comment [7]: Just need to define room temperature in general procedure as ca. 23°

Gary Sulikowski 10/8/2015 9:44 AM
Comment [8]: Hour abbreviate h (no period)

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Comment [9]: Indicate number of extracts and approximate folumen

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Comment [10]: Indicate in parenthesis drying agent

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Comment [11]: Should say "filtered" here

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Comment [12]: Yield reported to two significant figures and include weight of product

Gary Sulikowski 10/8/2015 9:56 AM
Comment [13]: Physical state of product needs to be indicated

Gary Sulikowski 10/8/2015 9:57 AM
Comment [14]: Characterization data follows a colon and data separated by semicolon

Gary Sulikowski 10/8/2015 9:46 AM
Comment [15]: Should provide only key absorbance such as carbonyl

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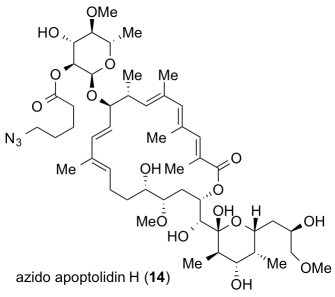
Comment [16]: cm-1 at end of absorptions. Also indicated (film/neat), KBr for sample prep

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 (**14**)

Azido Apoptolidin H (14) 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 μL, 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 μL) 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 μm 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 μL 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 **14** as a white solid: IR (neat) 3425, 2928, 2098, 1669, 1385, 1257 cm^{-1}; ^{1}H 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* =

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Comment [17]: 13C data to a tenth of ppm unless two peaks are too close to resolve

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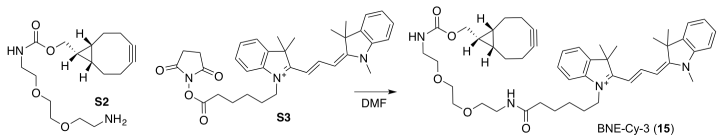
Comment [18]: probably more appropriate to say "allowed to warm" not warmed

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 (15) 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 **15** 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* = 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,

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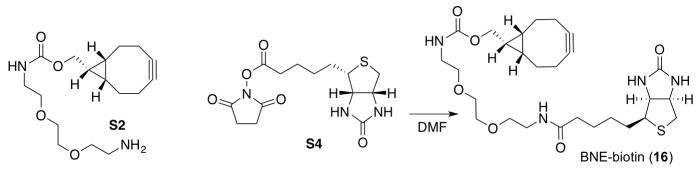
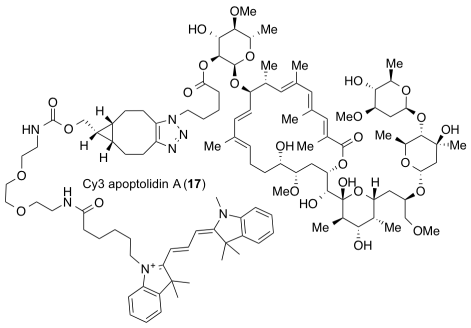
Comment [19]: note residue is purified

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 (16) 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 **16** 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.



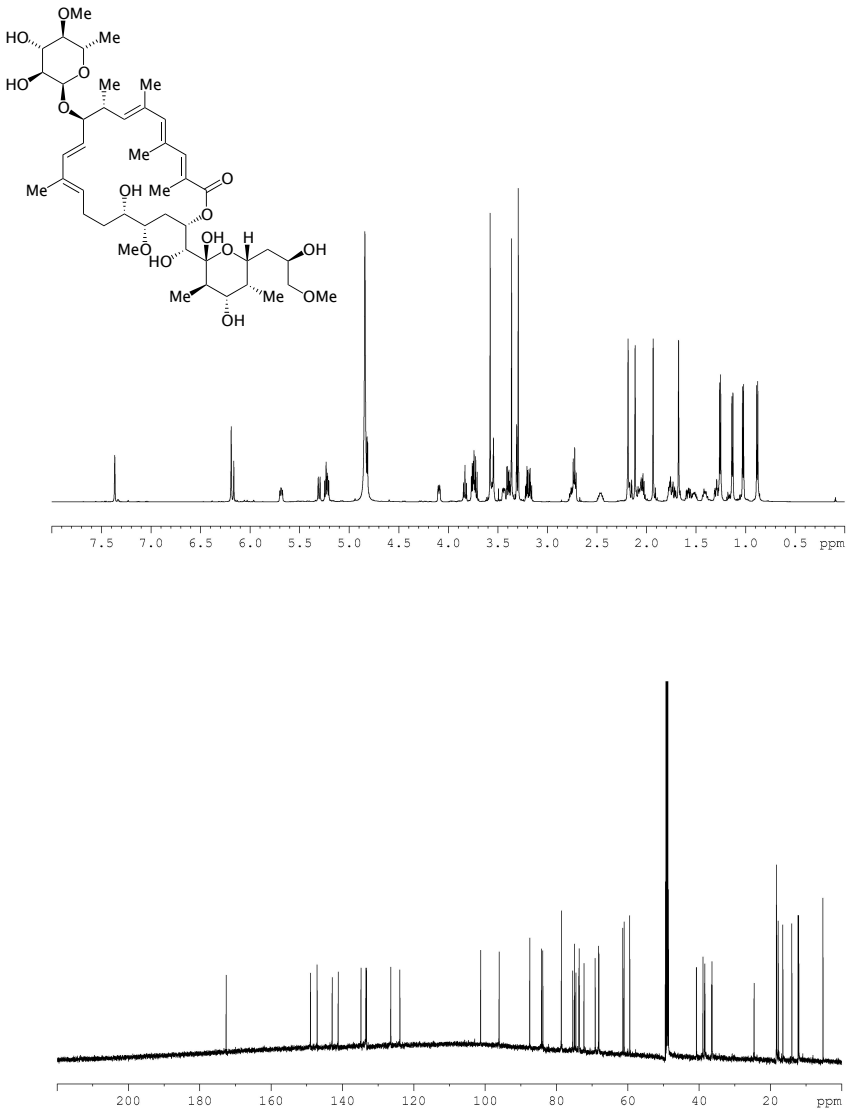
Cy3 Apoptolidin A (17) To a solution of BNE-Cy-3 (**15**) (2.6 mg, 0.0033 mmol) in methanol (0.5 mL) was added azido apoptolidin A (**13**) (2.05 mg, 0.0016 mmol) as a solution in methanol (0.5 mL). The resulting solution was stirred at 37 °C for 4 hours, removed from the bath and concentrated *in vacuo*. 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 (**17**) 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.

Common abbreviations-note: periods not typically used
g for grams
mL for millileters
L for lites
mp for melting point
bp for boiling poin
h for hours
min for minutes
mmol for mmoles
mol for moles
ca. (about)
cf. (compare)
e.g. (example)
i.e. (that is)
EtOAc
DCM
DMF
DME
THF
Ether
DCE
rt (room temperature)

Note: most important to be CONSISTENT

9. Copy of ^{1}H, ^{13}C NMR and 2D Spectra



The 600 MHz ^{1}H and 150 MHz ^{13}C NMR spectra of apoptolidin H (**9**) in CD_3OD.

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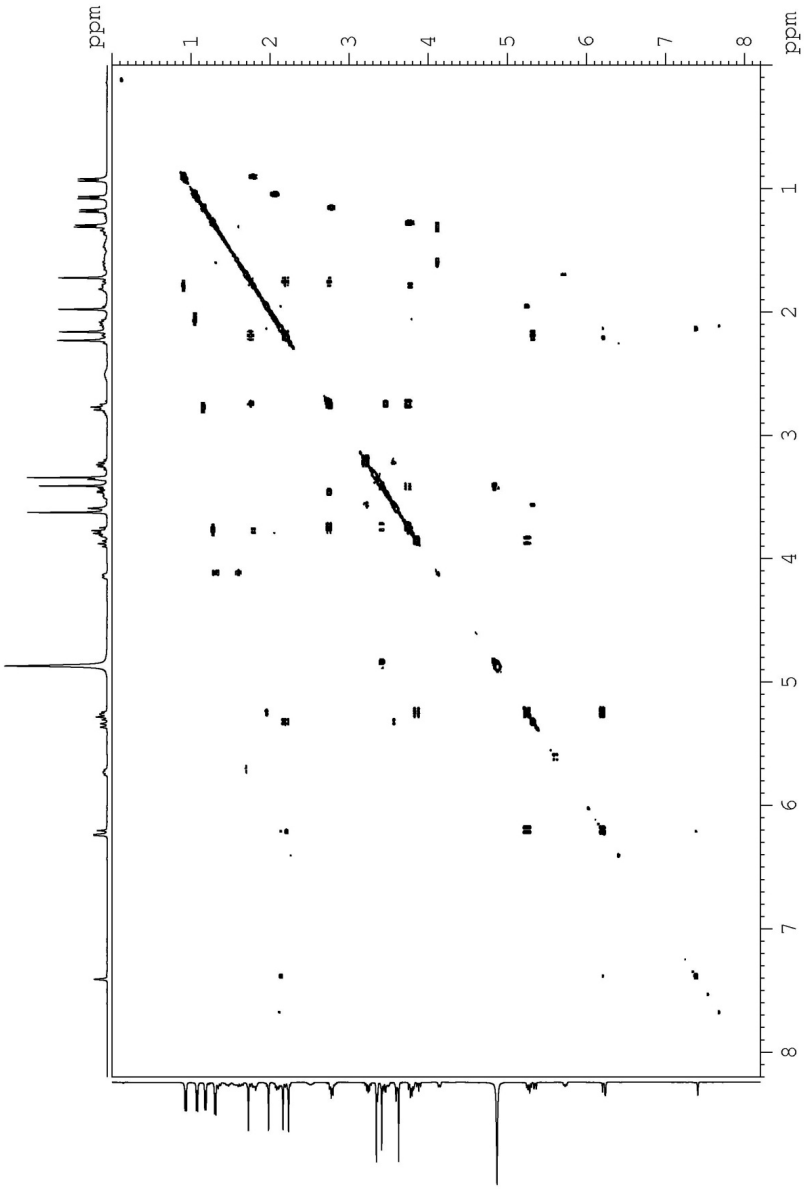
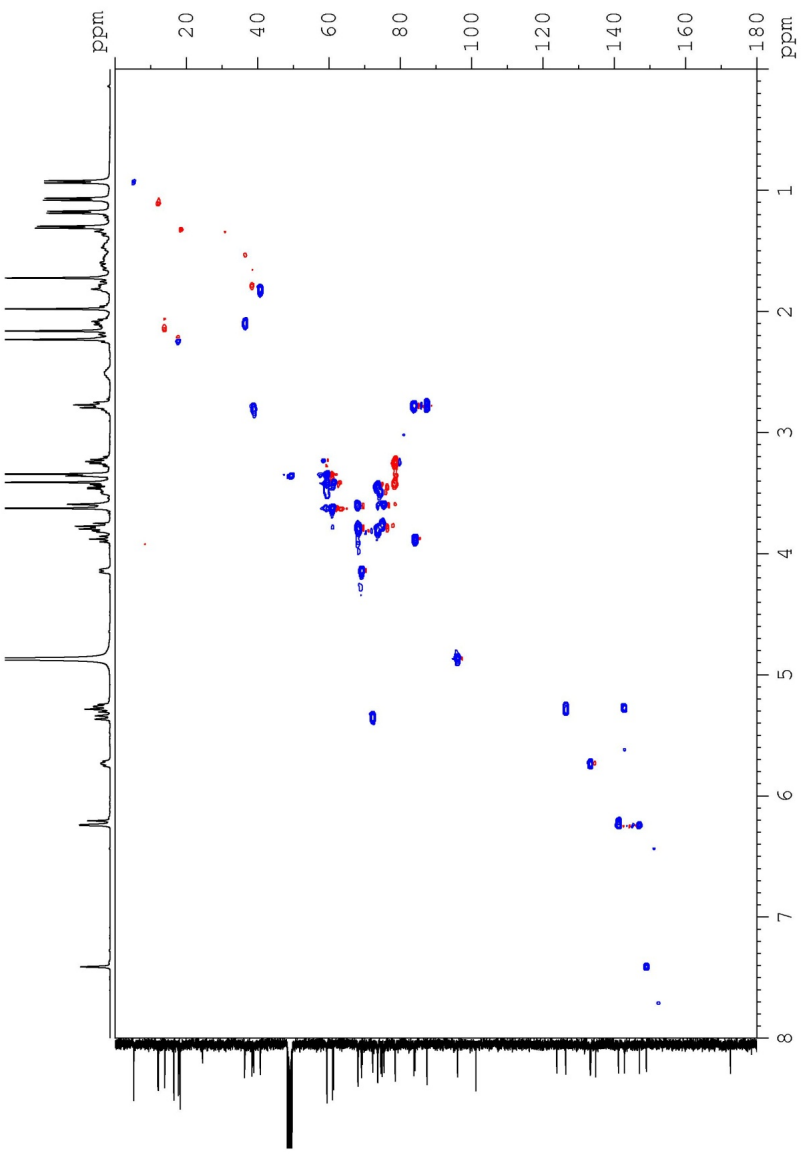
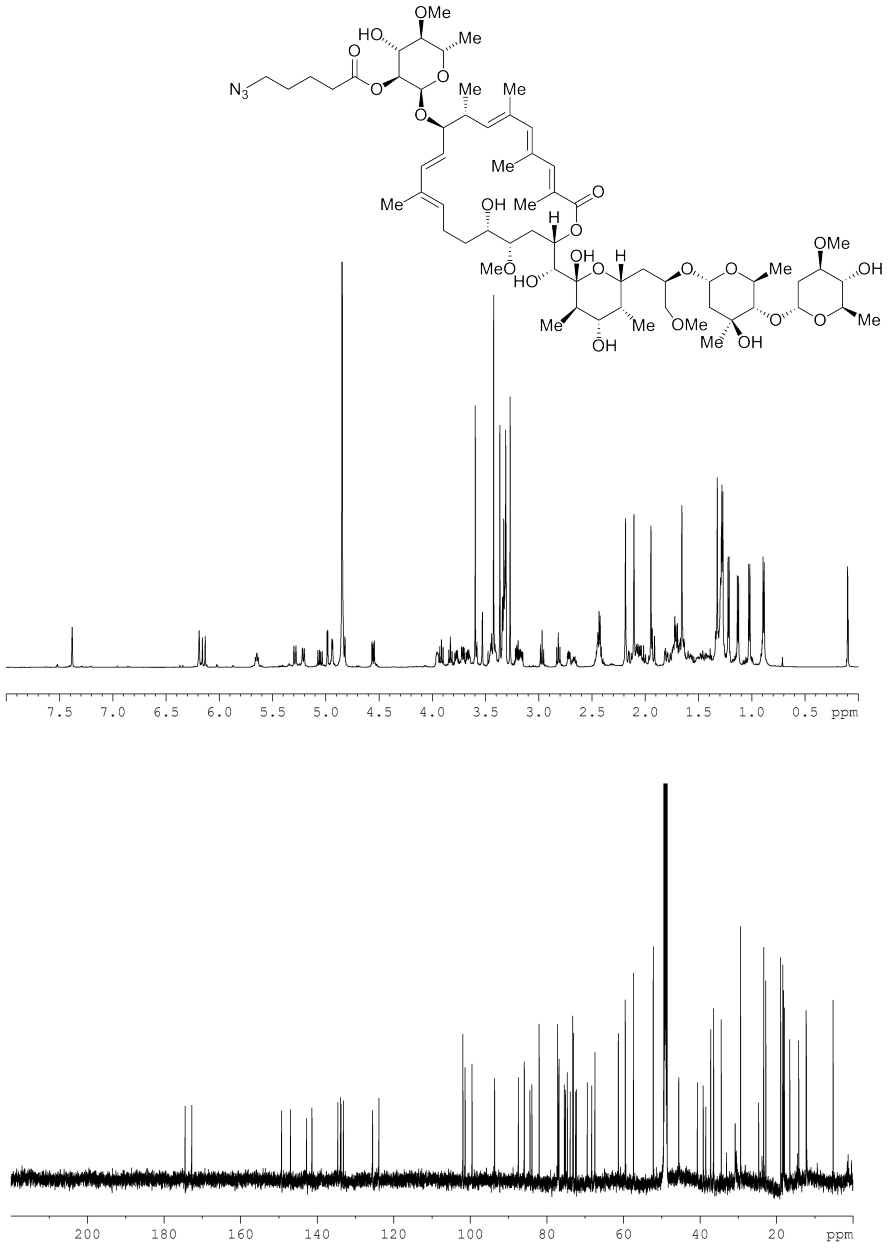
Comment [20]: proton and carbon on same page-insert structure and use captions indicting instrument, solvent



The 400 MHz COSY NMR spectra of apoptolidin H (**9**) in CD_3OD.



The 400 MHz HSQC NMR spectra of apoptolidin H (**9**) in CD_3OD.


OMe
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O
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OMe
OH
Me
Me
OH
OMe
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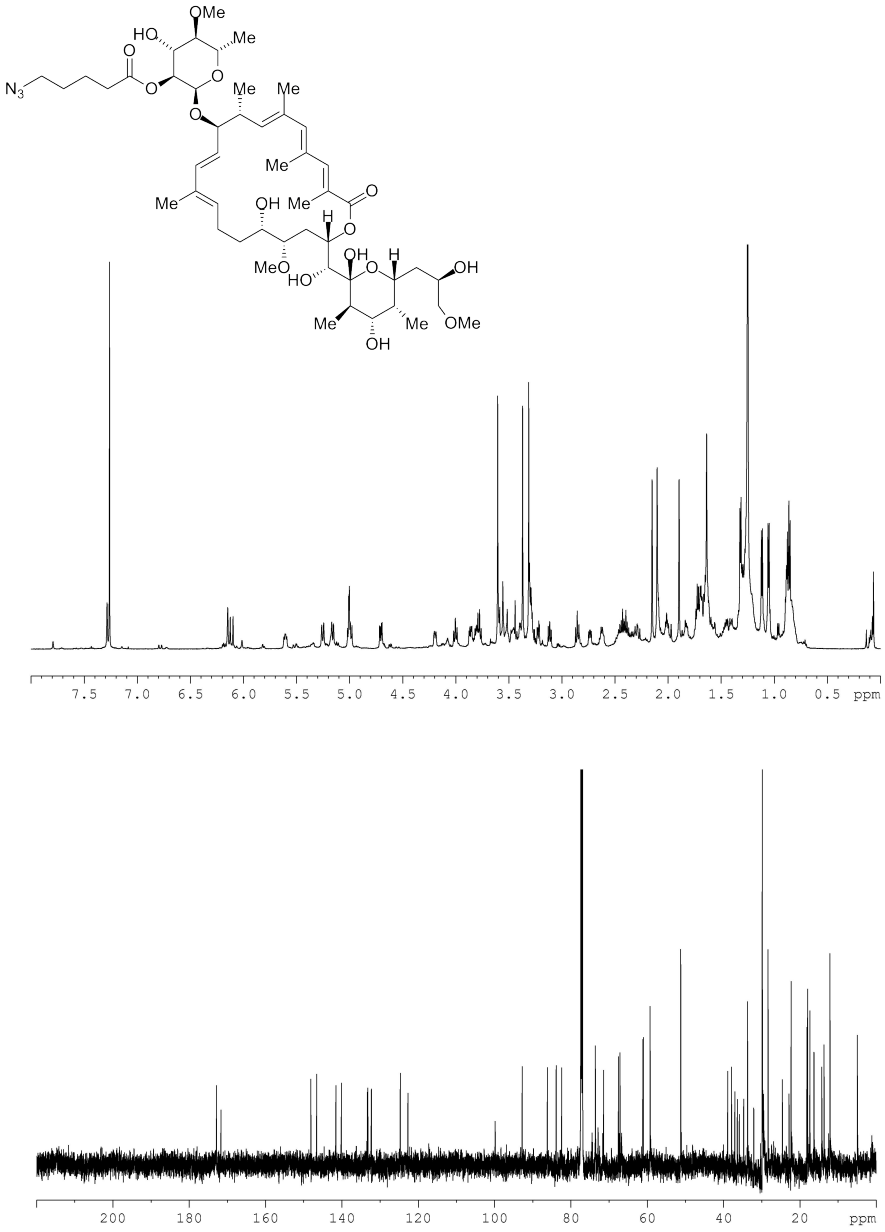
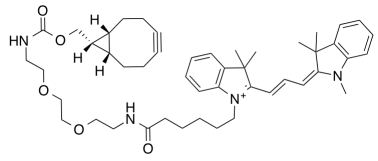
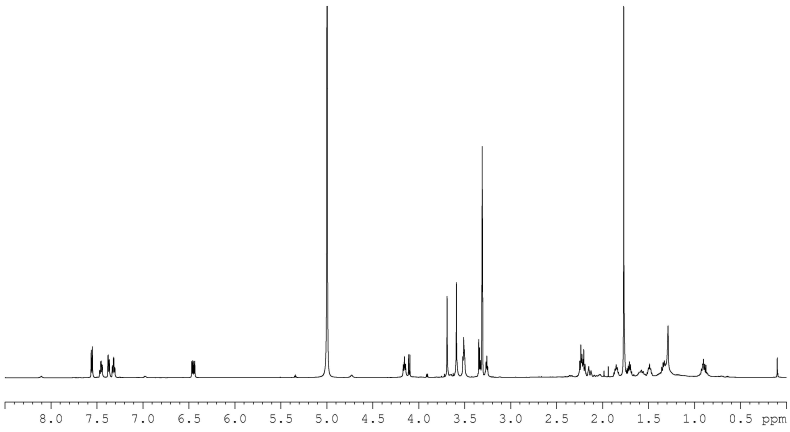
Me
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
200 180 160 140 120 100 80 60 40 20 ppm

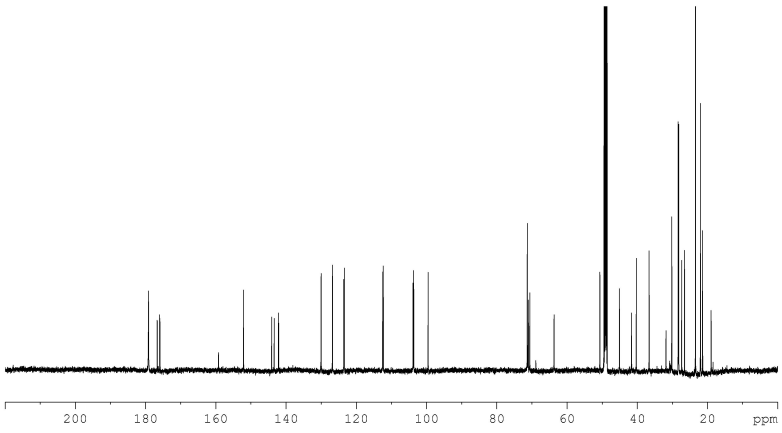
The 600 MHz ^{1}H and 150 MHz ^{13}C NMR spectra of azido apoptolidin A (**13**) in CD_3OD.



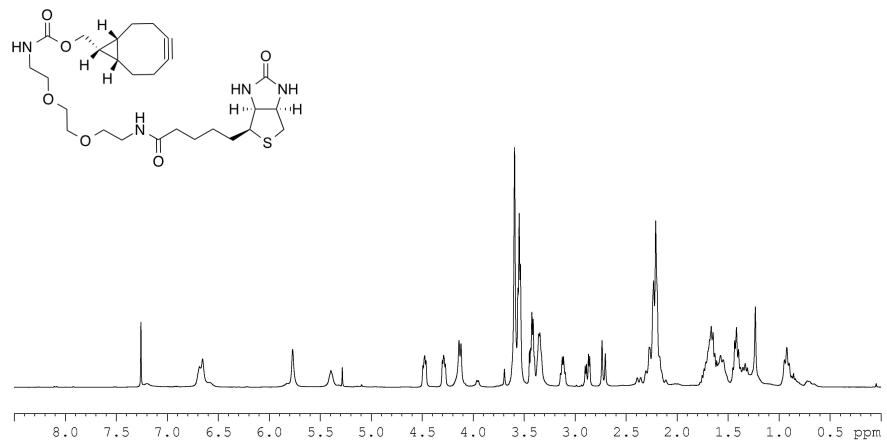
The 600 MHz ^{1}H and 150 MHz ^{13}C NMR spectra of azido apoptolidin H (**14**) in CD_3OD.

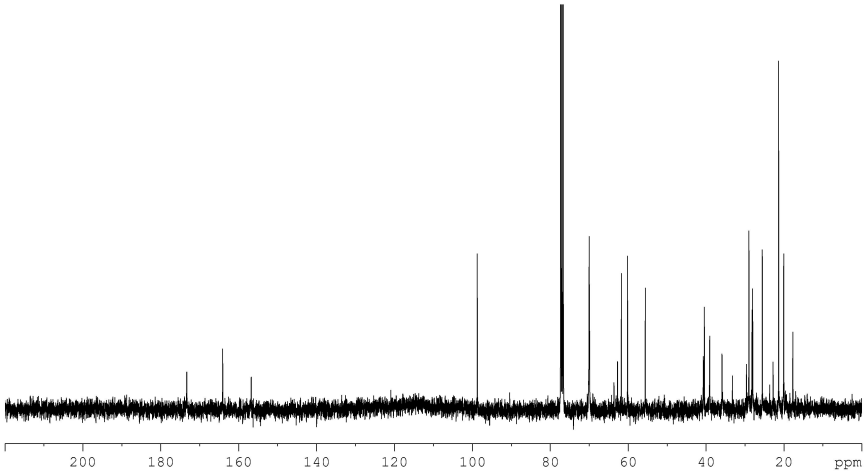






The 600 MHz ^{1}H and 150 MHz ^{13}C NMR spectra of BNE-Cy-3 (**15**) in CD_3OD.





The 400 MHz ^{1}H and 100 MHz ^{13}C NMR spectra of BNE-biotin (**16**) in $CDCl_3$.

10. References

1. Apoptolidin A: Hayakawa, Y.; Kim, J.; Adachi, H.; Shin-ya, K.; Fujita, K.; Seto, H., *J. Am. Chem. Soc.* **1998,** *120*, 3524-3525

2. Isoapoptolidin A: Pennington, J.; Williams, H.; Salomon, A.; Sulikowski, G., *Org. Lett.* **2002**, *4*, 3823-3825.

3. Gust, B.; Chandra, G.; Jakimowicz, D.; Yuqing, T.; Bruton, C. J.; Chater, K. F. *Adv.Appl. Microbiol.* **2004**, *54*, 107-128.

4. Du, Y.; Derewacz, D. K.; DeGuire, S. M.; Teske, J.; Ravel, J.; Sulikowski, G. A.; Bachmann, B. O., *Tetrahedron* **2011**, *67* , 6568-6575.

5. Kieser, T.; Bibb, M. J.; Buttner, M. J.; Chater, K. F.; Hopwood, D. A. Practical Streptomyces Genetics; John Innes Foundation: Norwich, 2000.

6. Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; Van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; Van Delft, F. L., *Angewandte Chemie Int. Ed.* **2010**, *49*, 9422-9425.

7. Bolte, S.; Cordelières, F. P. A guided tour into subcellular colocalization analysis in light microscopy. *J. Microsc.* **2006**, *224*, 213-232.

8. Schneider, C.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **2012**, *9*, 671-675.