Supporting Information

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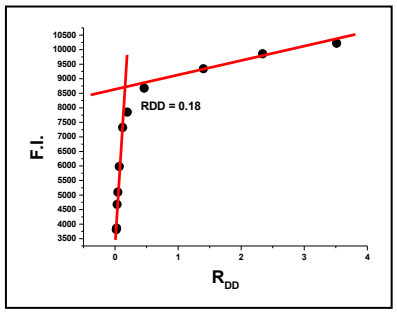
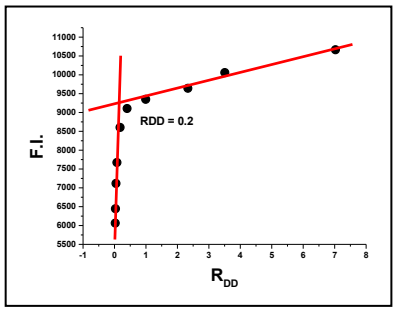
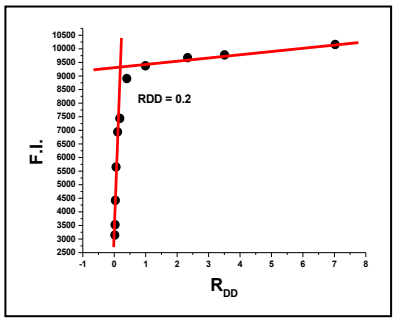
Supplementary Figures.







	(–)-lomaiviticin aglycon (**9**)	(2S,2′S)-lomaiviticin aglycon (**10**)	C-3/C-3′-dideoxylomaiviticin aglycon (**12**)
r_{dd} [a]	0.18	0.20	0.20
r_{bd} [b]	1.8	2	2

[a] r_{dd} denotes the ratio of Dickerson duplex to diazofluorene.
[b] r_{bd} denotes the ratio of base pairs of the Dickerson duplex to diazofluorene.

Figure S1. Binding site size determination by FID titration of diazofluorenes **9**, **10**, and **12** with the Dickerson duplex (0.88 μM/duplex) and ethidium bromide (1.76 μM) in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.5 mM ethylenediaminetetraacetic acid, pH 6.8) at 24 °C.

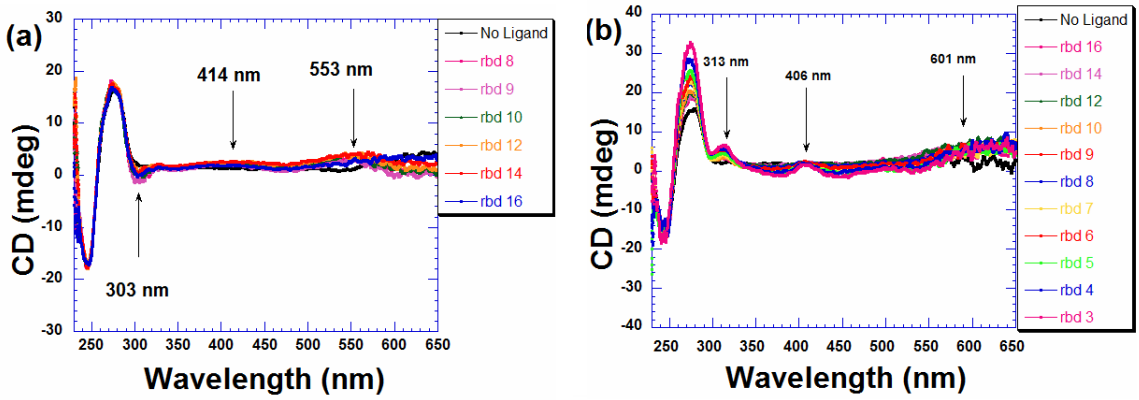


Figure S2. CD titration of (A) (–)-lomaiviticin aglycon (**9**), and (B) (–)-kinamycin C (**6**) with calf thymus DNA (179 μM). Buffer conditions: 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl at pH 6.8 (T = 20 °C).

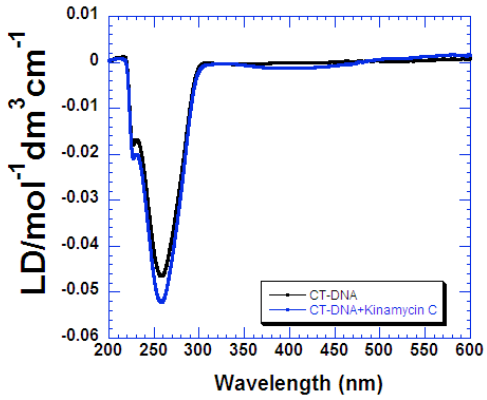


Figure S3. Linear dichroism spectrum of (–)-kinamycin C (**6**) at r_{bd} 8 with calf thymus DNA (1.00 mM). Buffer conditions: 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl at pH 6.8 (T = 22–23 °C).

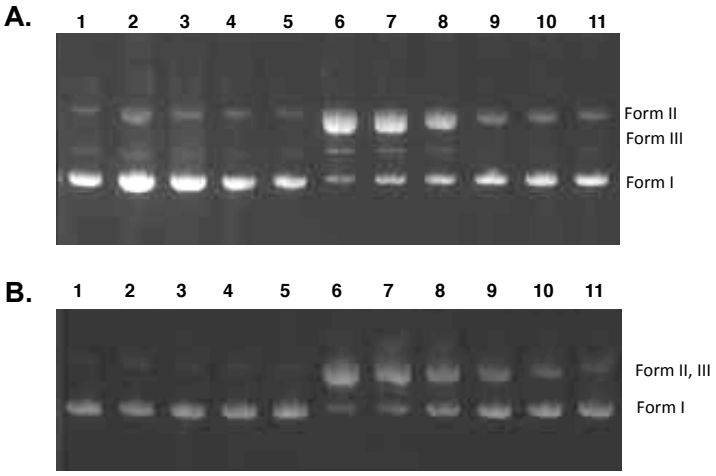
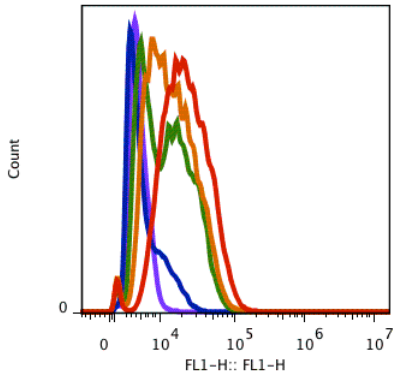


Figure S4. Agarose gel electrophoresis of the DNA cleavage of pBr322 (800 ng) treated with diazofluorenes and (A) glutathione (5 mM) or (B) NADPH (5 mM) cofactor (24 h, 37 °C). Lane 1: pBr322 DNA. Lane 2: [**6**] = 500 μM. Lane 3: [**9**] = 500 μM. Lane 4: [**9**] = 250 μM. Lane 5: [**9**] = 125 μM. Lane 6: [**10**] = 500 μM. Lane 7: [**10**] = 250 μM. Lane 8: [**10**] = 125 μM. Lane 9: [**11**] = 500 μM. Lane 10: [**11**] = 250 μM. Lane 11: [**11**] = 125 μM. Form I = supercoiled DNA; Form II = nicked DNA; Form III = linearized DNA.



Key	Concentration	Compound	Geometric Mean	% Increase
	1 μM	(–)-monomeric lomaiviticin aglycon (**11**)	3.10E4	586%
	500 nM	(–)-monomeric lomaiviticin aglycon (**11**)	1.39E4	208%
	200 nM	(–)-monomeric lomaiviticin aglycon (**11**)	1.20E4	165%
	50 nM	(–)-monomeric lomaiviticin aglycon (**11**)	5556	23%
	–	control	4520	–

Figure S5. H2A.X phosphorylation assay of human leukemia cells (K562) treated with the (–)-monomeric lomaiviticin aglycon (**11**, 50 nM–1 μM) for 4 h at 37 °C. Cells were stained for γ-H2AX. Immunological detection was performed by labeling with anti-γH2AX (Ser139) AB–fluorescein isothiocyanate conjugate. Sample analysis was performed on an Accuri flow cytometer using a 488 nm excitation laser. Emission detected with the filter/bandpass: 530/30 for FITC.

Table S1. Geometric mean H2A.X phosphorylation of PEO1 and PEO4 cell lines treated with the (–)-monomeric lomaiviticin aglycon (**11**) or cisplatin. Cells were stained for γ-H2AX and analyzed by flow cytometry using a 488 nm excitation laser. Emission detected with the filter/bandpass: 530/30 for FITC.

Compound	PEO1 Geometric Mean (% increase)	PEO4 Geometric Mean (% increase)
(–)-monomeric lomaiviticin aglycon (**11**)	2.79E4 (48%)	2.34E4 (193%)
cisplatin	2.27E4 (21%)	7.17E3 (–10%)
control	1.88E4	7.98E3

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Organic solutions were concentrated by rotary evaporation at 30–33 °C. Reversed phase flash-column chromatography was performed as described by Still et al.[1] Reversed phase purifications employ C_{18}-labeled silica gel (125 Å, 55–105 μm particle size) purchased from Waters Corporation (Milford, MA). TLC plates were visualized by exposure to ultraviolet light (UV) and/or submersion in aqueous ceric ammonium molybdate solution (CAM) followed by brief heating on a hot plate (120 °C, 10–15 s).

Chemical Materials. Commercial solvents and reagents were used as received, with the following exceptions. Benzene and dichloromethane were purified according to the method of Pangborn and co-workers.[2] (–)-Lomaiviticin aglycon (**9**), the (–)-monomeric lomaiviticin aglycon (**11**), and (2*S*,2′*S*)-lomaiviticin aglycon (**10**), was prepared according to the method of Herzon and co-workers.[3] C-3/C-3′-dideoxylomaiviticin aglycon (**12**) and the diazofluorene **S1** was prepared according to the method of Herzon and co-workers.[4] (–)-Kinamycin F (**8**) was prepared according to the method of Herzon and co-workers.[5] (–)-Kinamycin C (**6**) was obtained from the NCI/DTP Open Chemical Repository (http://dtp.cancer.gov).

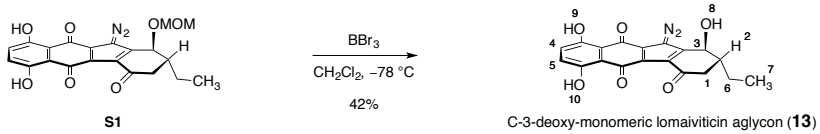
Nucleic Acids. Genomic DNA calf thymus, *M. lysodeiktius*, and *C. perfringens* were purchased from Invitrogen, Sigma Aldrich, and ATCC, respectively, and used as received. The concentrations of all genomic DNA were determined by UV using the following extinction coefficients (in units of base pairs/$L^{-1}cm^{-1}$): ε_{260} = 6,600 for calf thymus, ε_{260} = 6,900 for *M. lysodeiktius*, ε_{260} = 6,300 for *C. perfringens*. Oligomer "Dickerson duplex" (CGCAAATTTGCG)[6] was purchased salt free from Operon. The oligomer was heated for 30 min at 95 °C, cooled over 2 h to 24 °C, and incubated for 24 h at 4 °C to form the duplex. The concentrations of all oligonucleotides were determined by UV using the following extinction coefficients (in units of mol of nucleotide/$L^{-1}cm^{-1}$): ε_{260} = 115,958 for Dickerson duplex. The concentrations of ethidium bromide (EtBr) and thiazole orange (TO) were determined by UV using the following extinction coefficients (in units of mole of fluorophore/$L^{-1}cm^{-1}$): ε_{480} = 5,600 for EtBr, ε_{501} = 63,000 for TO. Plasmid DNA (pBr322) was obtained from Invitrogen and purified using a Qiagen Gel Purification kit. DNA cleavage gels were prepared and run in TAE buffer (pH 8.0, 40 mM tris–acetate).

Cell Culture. K562 cells were obtained from Professor David Spiegel (Yale University). PEO1 and PEO4 cells were obtained from Professor Peter Glazer (Yale University). All cell culture reagents were obtained from Invitrogen. PEO1 and PEO4 cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. K562 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. All cell lines were maintained at 37 ºC under an atmosphere of 5% CO_2 and constant humidity. H2A.X assay was purchased as a kit from Millipore and used as per the manufacturer's instructions.

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 500 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent [$(CD_3)_2NCHO$, δ 8.03]. Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 125 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent [$(CD_3)_2N\mathbf{C}DO$, δ 163.2]. Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR) were obtained using a Thermo Electron Corporation Nicolet 6700 FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows:

frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Analytical ultra high-performance liquid chromatography/mass spectrometry (UPLC/MS) was performed on a Waters UPLC/MS instrument equipped with a reversed-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm), dual atmospheric pressure chemical ionization (API)/electrospray (ESI) mass spectrometry detector, and photodiode array detector. Samples were eluted with a linear gradient of 20% acetonitrile–water containing 0.1% formic acid→100% acetonitrile containing 0.1% formic acid over 3 min, followed by 100% acetonitrile containing 0.1% formic acid for 1 min, at a flow rate of 800 μL/min. Analytical UPLC/MS data are represented as follows: retention time (t_R) in minutes. High-resolution mass spectrometry (HRMS) was obtained at the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. Single-point fluorescence measurements of 96-well plates were carried out on a Genios Multi-Detection Microplate Reader, TECAN with Magellan 5.0 software. Full-spectrum fluorescence titration was obtained on a Photon Technology International (PTI) instrument. Data was analyzed using Kaleidoscope and Origins Suite 5.0 package. Circular dichroism experiments were conducted at 20 °C on a JASCO J-810 spectropolarimeter equipped with a thermoelectrically controlled cell holder. A quartz cell with a 1 cm path length was used in the CD studies. Linear dichroism was obtained at 22–23 °C on a JASCO J-810 spectropolarimeter equipped with Dioptica Scientific linear dichroism accessory. DNA cleavage gels were imaged using UV transillumination on a Biorad Molecular Image ChemiDoc XRS+. Flow cytometry was performed on an Accuri C6 flow cytometer using a 488 nm excitation laser. Emission was detected with the 530/30 filter/bandpass for fluorescein isothiocyanate (FITC).

Synthetic Procedure.[7]



*Deprotection of the Diazofluorene **S1**:*

A solution of boron tribromide in dichloromethane (1.00 M, 201 μL, 201 μmol, 10.0 equiv) was added to a stirred solution of the diazofluorene **S1** (10.0 mg, 20.1 μmol, 1 equiv) in dichloromethane (400 μL) at −78 °C. After 3 h, dichloromethane (10 mL) and 0.1 M aqueous sodium phosphate buffer solution (pH 7, 15 mL) were added in sequence via syringe pump, at a rate of 1 mL/min. Upon completion of the addition, the cooling bath was removed and the biphasic mixture was allowed to warm over 30 min to 21 °C. The reaction vessel was then placed in an ambient temperature water bath and was stirred for 15 min, until the aqueous phase melted completely. The resulting biphasic solution was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (4 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by reversed-phase flash-column chromatography (eluting with 10% acetonitrile–water initially, grading to 30% acetonitrile–water, one step) to afford C-3-deoxymonomeric lomaiviticin aglycon (**13**) as a purple solid (3.0 mg, 42%).

R_f = 0.39 (4% methanol–dichloromethane; UV, CAM). ^{1}H NMR (500 MHz, DMF-d_7): δ 13.38 (s, 1H, H_9/H_{10}), 12.51 (s, 1H, H_9/H_{10}), 7.36 (d, 1H, J = 9.5 Hz, H_4/H_5), 7.32 (d, 1H, J = 9.5 Hz, H_4/H_5), 6.75 (d, 1H, J = 7.5 Hz, H_8), 4.97 (dd, 1H, J = 10.0, 7.0 Hz, H_3), 2.60 (dd, 1H, J = 16.0, 3.5 Hz, H_1), 2.43 (dd, J = 16.0, 13.0 Hz, H_1), 2.18–2.11 (m, 1H, H_2), 1.97–1.93 (m, 1H, H_6), 1.47–1.40 (m, 1H, H_6), 0.98 (t, 3H, J = 7.5 Hz, H_7). ^{13}C NMR (125 MHz, DMF-d_7): δ 190.6 (C), 183.7 (2 × C), 159.1 (C), 158.5 (C), 157.7 (C), 132.1 (C), 131.1 (CH), 129.2 (CH), 128.9 (C), 125.4 (C), 114.4 (C), 113.6 (C), 80.3 (C), 70.9 (CH), 47.6 (CH), 44.4 (CH_2), 24.8 (CH_2), 11.1 (CH_3). IR (ATR-FTIR), cm^{-1}: 3651 (m), 2936 (s), 2162 (m), 1442 (s). HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{15}N_2O_6$, 367.0930; found, 367.0928.

Fluorescence Intercalator Displacement (FID) Procedures.

One to One FID Procedure:

The FID assay was carried out in a 96-well plate. A solution containing calf-thymus polynucleotide (0.88 μM/base pair) and thiazole orange (1.25 μM) was prepared by incubating the two for 30 min in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8) prior to the measurements. Each well of the 96-well plate (flat bottom, black) was loaded with polynucleotide solution (200 μL). The fluorescence of this solution was measured (Ex: 485 nm, Em: 535 nm). A small aliquot of the stock solution of diazofluorene in dimethyl sulfoxide (3.52 μL, 50.0 μM) or dimethyl sulfoxide (negative control, 3.52 μL) was added into each well and the fluorescence was measured in triplicate after incubation for 5 min. Fluorescence readings are reported as a percentage fluorescence relative to the control wells. The reference fluorescence is defined as such: [TO+DNA] gives 100% fluorescence and [TO] gives 0% fluorescence. The change in the fluorescence was plotted as –

$$\% \text{ fluorescence change} = (\Delta F/I_F) \times 100 \text{ where,}$$

ΔF = Change in fluorescence upon ligand addition and I_F = Initial fluorescence of the DNA–TO complex.

FID Titration Experiments with Polynucleotides:

FID titrations of genomic DNA were carried out in a quartz cuvette. A solution of genomic DNA (10.0 μM/base pair) saturated with ethidium bromide (5.00 μM) in buffer (100 mM potassium chloride, 10.0 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8, 1.00 mL) was prepared, and the fluorescence of this solution was measured (Ex: 481 nm, Em: 490–750 nm). An aliquot of the stock solution of the diazofluorene (500 μM–10.0 mM) was added to the mixture, and the fluorescence was measured in triplicate after incubation for 5 min at 24 °C. The addition of diazofluorene was continued until no more change in the fluorescence was observed. The fluorescence maxima was then plotted against the ratio of base pair to drug to obtain binding site size wherever applicable, and the percentage change in fluorescence against the log(concentration of drug) was plotted to obtain DC_{50} values using a sigmoidal fitting of the graph in Origin 7.0.

Table S2. FID-based determination of DC_{50} values of diazofluorenes with different polynucleotides.

Polynucleotide	(−)-lomaiviticin aglycon (**9**)	(2*S*,2′*S*)-lomaiviticin aglycon (**10**)	C-3/C-3′-dideoxylomaiviticin aglycon (**12**)	monomeric diazofluorenes **6**, **8**, **11**, **13**
Calf thymus	7.5 ± 1.1 μM	40.7 ± 1.1 μM	19.8 ± 1.1 μM	DC_{50} > 100 μM
M. lysodeiktius	10.0 ± 1.1 μM	63.2 ± 1.2 μM	59.7 ± 1.1 μM	DC_{50} > 100 μM
C. perfringens	14.4 ± 1.7 μM	56.2 ± 1.1 μM	18.6 ± 1.1 μM	DC_{50} > 100 μM

FID Titration Experiments with Dickerson Duplex:

FID titrations of Dickerson duplex were carried out in a quartz cuvette. A solution of the duplex (0.88 μM/duplex, 1 equiv) saturated with ethidium bromide (1.76 μM, 2.00 equiv/duplex) in buffer (100 mM potassium chloride, 10.0 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8, 1.00 mL) was prepared, and the fluorescence of this solution was measured (Ex: 480 nm, Em: 490–700 nm). An aliquot of the stock solution of diazofluorene (25.0 μM–5.00 mM) was added to the mixture, and the fluorescence was measured in triplicate after incubation for 5 min at 24 °C. The addition of diazofluorene was continued until no more change in the fluorescence was observed. The fluorescence maxima were then plotted against the ratio of base pair to drug, and the percentage change in fluorescence against the log(concentration of drug).

Circular Dichroism and Linear Dichroism.

*Circular Dichroism of (–)-Lomaiviticin Aglycon (**9**) with Calf Thymus DNA:*

Small aliquots (0.63–4.48 μL) of a concentrated solution of (–)-lomaiviticin aglycon (**9**, 5.00 mM) were added to a solution of calf thymus DNA (179 μM, 100 mM KCl, 10.0 mM sodium cacodylate, 0.50 mM EDTA, pH 6.8, 3% dimethylsulfoxide, 2.00 mL). The solution was inverted twice and incubated for 4 min at 20 °C. The CD spectra were then recorded as an average of 3 scans from 200–650 nm and data recorded in 0.1 nm increments with an averaging time of 2 s.

*Circular Dichroism of (–)-Kinamycin C (**6**) with Calf Thymus DNA:*

Small aliquots (0.64–4.45 μL) of a concentrated solution of (–)-kinamycin C (**6**, 5.00 mM) were added to a solution of calf thymus DNA (179 μM, 100 mM KCl, 10.0 mM sodium cacodylate, 0.50 mM EDTA, pH 6.8, 2.00 mL). The solution was inverted twice and incubated for 4 min at 20 °C. The CD spectra were then recorded as an average of 3 scans from 200–650 nm and data recorded in 0.1 nm increments with an averaging time of 2 s.

Linear Dichroism (LD) Experiments:

LD spectra were measured on a Jasco J-810 spectropolarimeter equipped with the microcuvette flow linear dichroism accessory (Dioptica Scientific Ltd). Orientation of the ligand/nucleic acid samples was achieved in a microcuvette flow cell with an outer rotating cylinder. The experimental path length was 0.3 cm. The total volume used in the LD experiments was 70.0 μL. The calf thymus DNA solution (1.00 mM/base pair) was rotated at a speed of 3000 rpm for five minutes before the scan was taken. The LD signal thus obtained was subtracted with a control experiment in which only buffer was used to give the corrected LD signal. Experiments in which a dimethylsulfoxide soluble ligand was needed, a comparative LD of DNA alone was also obtained in the same dimethylsulfoxide dilution (typically 1–4%, v/v). Dimethylsulfoxide has an overlapping signal with the base absorption at ~220 nm. The amount of dimethylsulfoxide present in buffer significantly impacts the magnitude of DNA base signal at ~220 nm. Each scan represents an average of three readings. To obtain the LD spectrum in the presence of ligands, the DNA solution (1.00 mM/base pair) was mixed with the ligands at nucleic acid/ligand ratio of 8.

Cleavage Assays.

Cleavage Assay with Dithiothreitol (DTT) Cofactor:

An aliquot of the stock solution of diazofluorene in dimethylsulfoxide (1.25–5.00 mM, 1.00 μL, 1 equiv) or dimethylsulfoxide (negative control, 1.00 μL) was added to a solution of pBr322 DNA (800 ng) in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 8.00 μL) in a microcentrifuge tube. Dithiothreitol in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 50.0 mM, 1.00 μL, 5.00 equiv) was added, and the microcentrifuge tube was sealed. The sealed tube was briefly vortexed and centrifuged, and was then incubated for 24 h at 37 °C. Gel loading dye blue (6×, 2.00 μL) was added to the microcentrifuge tube, and the contents (11.0 μL) were loaded on a 1% agarose gel stained with 0.1% ethidium bromide. The gel was placed in an electrophoresis tank containing TAE buffer solution (400 mL), and electrophoresis (101 V) was performed for 2 h at 24 °C.

Cleavage Assay with Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Cofactor:

An aliquot of the stock solution of diazofluorene in dimethylsulfoxide (1.25–5.00 mM, 1.00 μL, 1 equiv) or dimethylsulfoxide (negative control, 1.00 μL) was added to a solution of pBr322 DNA (800 ng) in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 8.00 μL) in a microcentrifuge tube. NADPH in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 50.0 mM, 1.00 μL, 5.00 equiv) was added, and the microcentrifuge tube was sealed. The sealed tube was briefly vortexed and centrifuged, and was then incubated for 24 h at 37 °C. Gel loading dye blue (6×, 2.00 μL) was added to the microcentrifuge tube, and the contents (11.0 μL) were loaded on a 1% agarose gel stained with 0.1% ethidium bromide. The gel was placed in an electrophoresis tank containing TAE buffer solution (400 mL), and electrophoresis (101 V) was performed for 2 h at 24 °C.

Cleavage Assay with Glutathione Cofactor:

An aliquot of the stock solution of diazofluorene in dimethylsulfoxide (1.25–5.00 mM, 1.00 μL, 1 equiv) or dimethylsulfoxide (negative control, 1.00 μL) was added to a solution of pBr322 DNA (800 ng) in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 8.00 μL) in a microcentrifuge tube. Glutathione in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 50.0 mM, 1.00 μL, 5.00 equiv) was added, and the microcentrifuge tube was sealed. The sealed tube was briefly vortexed and centrifuged, and then incubated for 24 h at 37 °C. Gel loading dye blue (6×, 2.00 μL) was added to the microcentrifuge tube, and the contents (11.0 μL) were loaded on a 1% agarose gel stained with 0.1% ethidium bromide. The gel was placed in an electrophoresis tank containing TAE buffer solution (400 mL), and electrophoresis (101 V) was performed for 2 h at 24 °C.

Cleavage Assay without Reducing Cofactor:

An aliquot of the stock solution of diazofluorene in dimethylsulfoxide (2.50–5.00 mM, 1.00 μL, 1 equiv) or dimethylsulfoxide (negative control, 1.00 μL) was added to a solution of pBr322 DNA (800 ng) in 10.0 mM tris–hydrogen chloride buffer (pH 7.5, 9.00 μL) in a microcentrifuge tube, and the microcentrifuge tube was sealed. The sealed tube was briefly vortexed and centrifuged, and then incubated for 24 h at 37 °C. Gel loading dye blue (6×, 2.00 μL) was added to the microcentrifuge tube, and the contents (11.0 μL) were loaded on a 1% agarose gel stained with 0.1% ethidium bromide. The gel was placed in an electrophoresis tank containing TAE buffer solution (400 mL), and electrophoresis (101 V) was performed for 2 h at 24 °C.

H2A.X Assays.

H2A.X Assay with Synthetic Diazofluorenes:

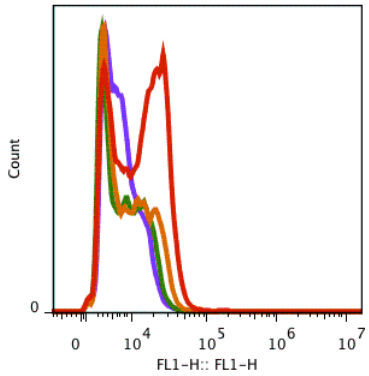
K562 cells (7.05×10^5 cells/mL) in growth medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin, 2.00 mL) were incubated with an aliquot of the stock solution of diazofluorene (1.00 mM, 2.00 μL) or dimethylsulfoxide (negative control, 2.00 μL) for 4 h at 37 °C. The cells were transferred into eppendorf tubes in 2 × 1.0 mL aliquots. The eppendorf tubes were centrifuged (1000 rpm, 6 min), washed with PBS (2 × 0.5 mL), and centrifuged. The cells were treated with 1× fixation solution (16× = 37% formaldehyde, 15% methanol, 350 μL) for 20 min at 0 °C. The fixed cells were washed with PBS (2 × 0.5 mL) and centrifuged. The cells were treated sequentially with 1× ice-cold permeabilization solution (10× = 5% saponin, 1.4 M sodium chloride, 25 mM calcium chloride, 100 mM HEPES, pH 7.4, 100 μL) and anti-phospho-Histone H2A.X (Ser139)–FITC conjugate (60 μg/mL, 4.00 μL) for 20 min at 0 °C. 1× Wash solution (10× = 1% saponin in PBS, 200 μL) was added to each tube, and the cells were centrifuged. The cells were washed with PBS (2 × 1.0 mL), and the washed cells were centrifuged. The centrifuged cells were suspended in PBS (300 μL), and the cells were analyzed by flow cytometry. A minimum of 10,000 cells were analyzed within the gated population.

*Titration of the (–)-Monomeric Lomaiviticin Aglycon (**11**) with the H2A.X Assay:*

K562 cells (7.05×10^5 cells/mL) in growth medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin, 2.00 mL) were incubated with an aliquot of the stock solution of diazofluorene (50 μM–1.00 mM, 2.00 μL) or dimethylsulfoxide (negative control, 2.00 μL) for 4 h at 37 °C. The cells were transferred into eppendorf tubes in 2 × 1.0 mL aliquots. The eppendorf tubes were centrifuged (1000 rpm, 6 min), washed with PBS (2 × 0.5 mL), and centrifuged. The cells were treated with 1× fixation solution (16× = 37% formaldehyde, 15% methanol, 350 μL) for 20 min at 0 °C. The fixed cells were washed with PBS (2 × 0.5 mL) and centrifuged. The cells were treated sequentially with 1× ice-cold permeabilization solution (10× = 5% saponin, 1.4 M sodium chloride, 25 mM calcium chloride, 100 mM HEPES, pH 7.4, 100 μL) and anti-phospho-Histone H2A.X (Ser139)–FITC conjugate (60 μg/mL, 4.00 μL) for 20 min at 0 °C. 1× Wash solution (10× = 1% saponin in PBS, 200 μL) was added to each tube, and the cells were centrifuged. The cells were washed with PBS (2 × 1.0 mL), and the washed cells were centrifuged. The centrifuged cells were suspended in PBS (300 μL), and the cells were analyzed by flow cytometry. A minimum of 10,000 cells were analyzed within the gated population.

Titration of Mitomycin C with the H2A.X Assay:

K562 cells (7.05×10^5 cells/mL) in growth medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin, 2.00 mL) were incubated with an aliquot of the stock solution of mitomycin C (50 μM–1.00 mM, 2.00 μL) or dimethylsulfoxide (negative control, 2.00 μL) for 4 h at 37 °C. The cells were transferred into eppendorf tubes in 2 × 1.0 mL aliquots. The eppendorf tubes were centrifuged (1000 rpm, 6 min), washed with PBS (2 × 0.5 mL), and centrifuged. The cells were treated with 1× fixation solution (16× = 37% formaldehyde, 15% methanol, 350 μL) for 20 min at 0 °C. The fixed cells were washed with PBS (2 × 0.5 mL) and centrifuged. The cells were treated sequentially with 1× ice-cold permeabilization solution (10× = 5% saponin, 1.4 M sodium chloride, 25 mM calcium chloride, 100 mM HEPES, pH 7.4, 100 μL) and anti-phospho-Histone H2AX (Ser139)–FITC conjugate (60 μg/mL, 4.00 μL) for 20 min at 0 °C. 1× Wash solution (10× = 1% saponin in PBS, 200 μL) was added to each tube, and the cells were centrifuged. The cells were washed with PBS (2 × 1.0 mL), and the washed cells were centrifuged. The centrifuged cells were dissolved in PBS (300 μL), and the cells were analyzed by flow cytometry. A minimum of 10,000 cells were analyzed within the gated population.



Key	Concentration	Compound	Geometric Mean	% Increase
	1 μM	mitomycin C	1.20E4	71%
	500 nM	mitomycin C	8503	21%
	200 nM	mitomycin C	7321	4%
	–	control	7026	–

Figure S6. H2AX phosphorylation assay of human leukemia cells (K562) treated with mitomycin C (200 nM–1 μM) for 4 h at 37 °C. Cells were stained for γ-H2AX. Sample analysis performed by flow cytometry. Immunological detection was performed by labeling with anti-γH2AX (Ser139) AB–fluorescein isothiocyanate conjugate. Sample analysis was performed on an Accuri flow cytometer using 488 nm excitation laser. Emission detected with the filter/bandpass: 530/30 for FITC.

*H2AX Assay with the (–)-Monomeric Lomaiviticin Aglycon (**11**) and Cisplatin:*

PEO1 and PEO4 cells (7.5×10^4 cells/mL, respectively) in growth medium (DMEM medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin, 2.00 mL) were plated and incubated 2 days prior to treatment. An aliquot of the stock solution of the (−)-monomeric lomaiviticin aglycon (**11**, 1.00 mM, 2.00 μL), cisplatin (1.00 mM, 2.00 μL), or dimethylsulfoxide (negative control, 2.00 μL) was added to each condition. The treated plate was incubated for 24 h at 37 °C. After incubation, the cells were aspirated and trypsinized (0.25% trypsin−EDTA, 500 μL). The trypsinized cells were diluted with PBS (1.5 mL) transferred into eppendorf tubes in 2 × 1.0 mL aliquots. The eppendorf tubes were centrifuged (1100 rpm, 5 min), washed with PBS (2 × 0.75 mL), and centrifuged. The cells were treated with 1× fixation solution (16× = 37% formaldehyde, 15% methanol, 350 μL) for 20 min at 0 °C. The fixed cells were washed with PBS (2 × 0.5 mL) and centrifuged. The cells were treated sequentially with 1× ice-cold permeabilization solution (10× = 5% saponin, 1.4 M sodium chloride, 25 mM calcium chloride, 100 mM HEPES, pH 7.4, 100 μL) and anti-phospho-Histone H2AX (Ser139)−FITC conjugate (60 μg/mL, 4.00 μL) for 20 min at 0 °C. 1× Wash solution (10× = 1% saponin in PBS, 200 μL) was added to each tube, and the cells were centrifuged. The cells were washed with PBS (2 × 1.0 mL), and the washed cells were centrifuged. The centrifuged cells were dissolved in PBS (300 μL), and the cells were analyzed by flow cytometry. A minimum of 10,000 cells were analyzed within the gated population.

Cell Viability Assays.

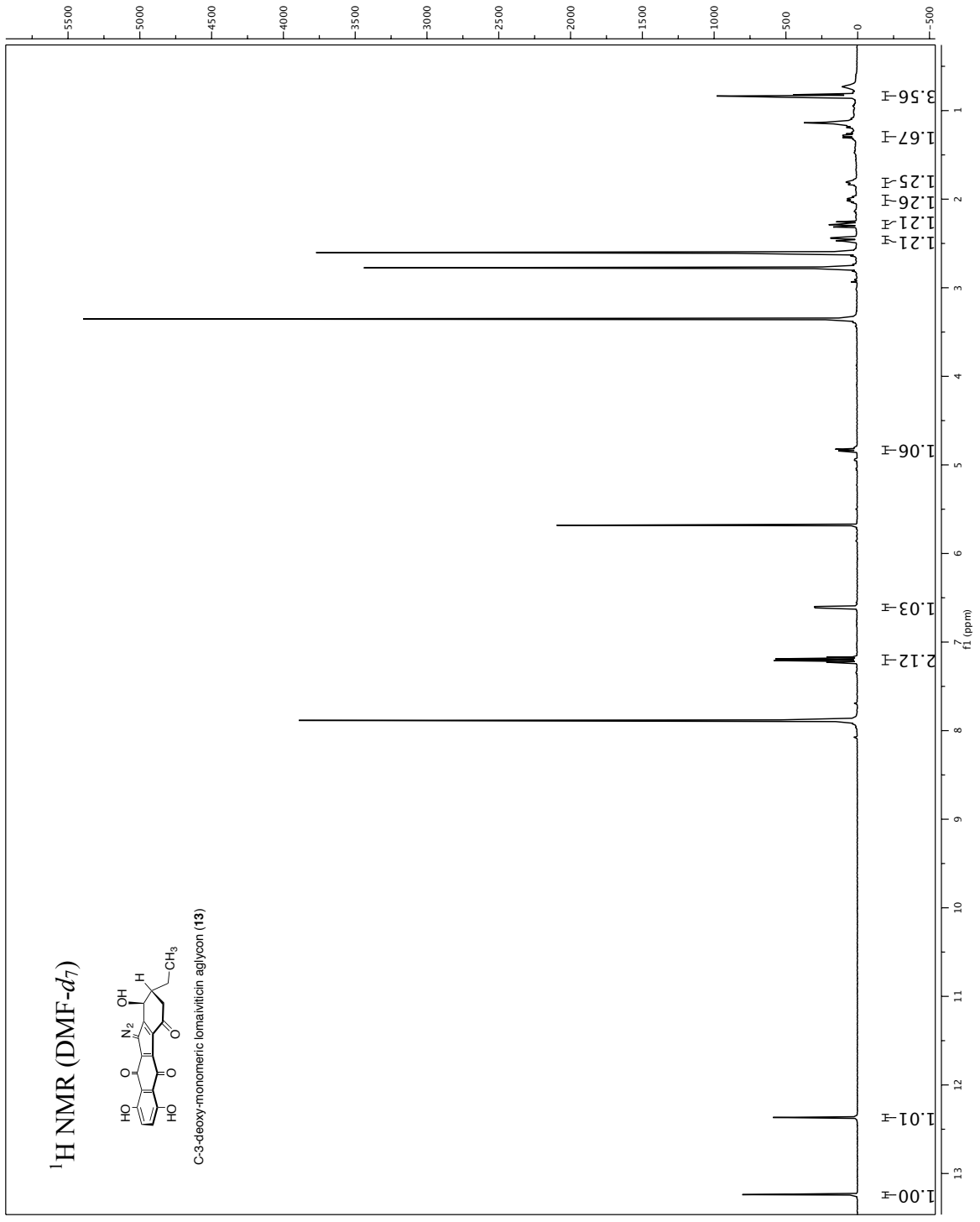
All tissue culture experiments were conducted in a biological safety cabinet in the dark. K562 cells were obtained from Professor David Spiegel (Yale University). All cell culture reagents were obtained from Invitrogen. K562 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. All cell lines were maintained at 37 ºC under an atmosphere of 5% CO_2 and constant humidity.

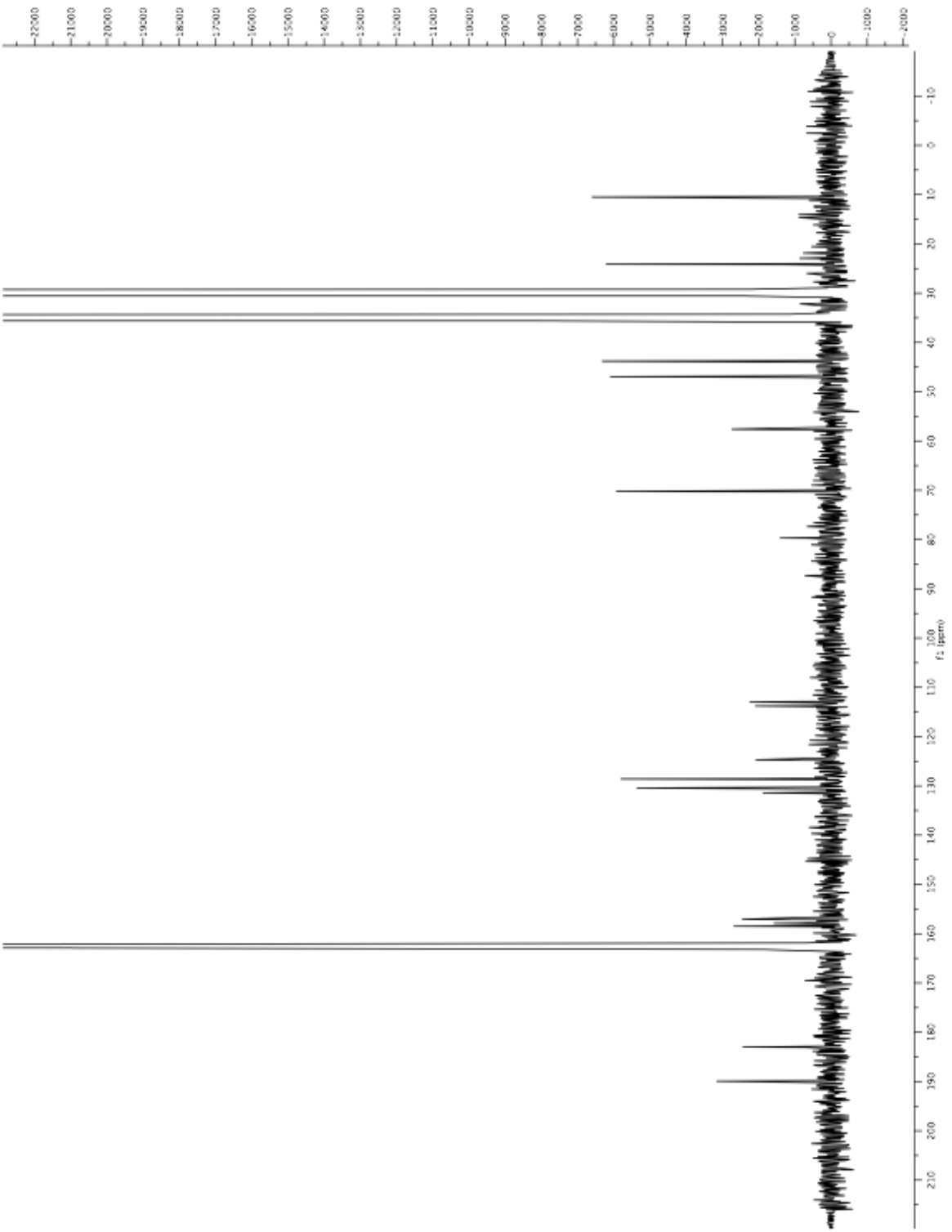
(–)-Kinamycin C (**6**, positive control) and analogs **8**, **9**, **10**, **11**, **12**, and **13** were prepared as 10 mM stock solutions in dimethylsulfoxide (DMSO) and stored with protection from light (aluminum foil) at –20 °C. Compounds were diluted two-fold serially in DMSO from 10 mM to 1.2 mM and arranged in quadruplicate in 2 μL volumes in 384-well low volume plates (Matrical). Vehicle control wells containing DMSO were included on each plate.

K562 cells were pipetted before counting in a hemacytometer. The counted cells were plated into sterile white, clear bottom tissue culture-treated 384-well plates (Corning) at a concentration of 400 cells/well (20 μL total volume) using a MultiDrop (Thermo Fisher). Assay plates were centrifuged at 500 rpm for 2 s and incubated overnight at 37 °C in a humidified 5% CO_2 incubator. After incubation, 20 nL of compound solution was transferred from the compound source plate to the cell assay plate using an Aquarius (Tecan) with a 20 nL 384-well pin tool (V&P Scientific). One microliter (1 μL) of 210 mM (–)-kinamycin C (**6**) diluted in media from a 10 mM stock solution in DMSO was added to the positive control wells on each plate by hand resulting in a final concentration of 10 mM. The final concentration of compounds ranged from 10 mM to 1.2 nM, and the final DMSO concentration was 0.1%. Assay plates were centrifuged at 500 rpm for 2 seconds and incubated for 72 hours at 37 °C in a humidified 5% CO_2 incubator. CellTiter-Glo (Promega) was used to measure cell viability in the assay wells.[63] It was prepared according to the manufacturer's instructions and 20 μL/well was added to the assay plates using a MultiDrop. Luminescence was read on an Envision plate reader (PerkinElmer) with 0.3 second sampling time per well after a 10 min room temperature incubation in the dark. Toxic effects have low luminescence signal relative to the vehicle control. Raw data (luminescence counts per second) was normalized to % effect by the formula [(DMSO vehicle – 10 mM (–)-kinamycin C) /(DMSO vehicle – sample)] * 100. Ten mM (–)-kinamycin C (**6**) was used to show 100% effect (toxicity), and DMSO vehicle was used to show 0% effect (no toxicity). Twenty four (24) wells of each control were run on every plate. Data was plotted in GraphPad Prism using a variable slope 4-parameter fit. The top of the curve was constrained to less than or equal to 101%.

compound	IC_{50} (μM)
(–)-lomaiviticin aglycon (**9**)	0.377
(2*S*,2′*S*)-lomaiviticin aglycon (**10**)	0.945
(–)-monomeric lomaiviticin aglycon (**11**)	0.383
C-3/C-3′-dideoxylomaiviticin aglycon (**12**)	2.60
(–)-kinamycin C (**6**)	1.30

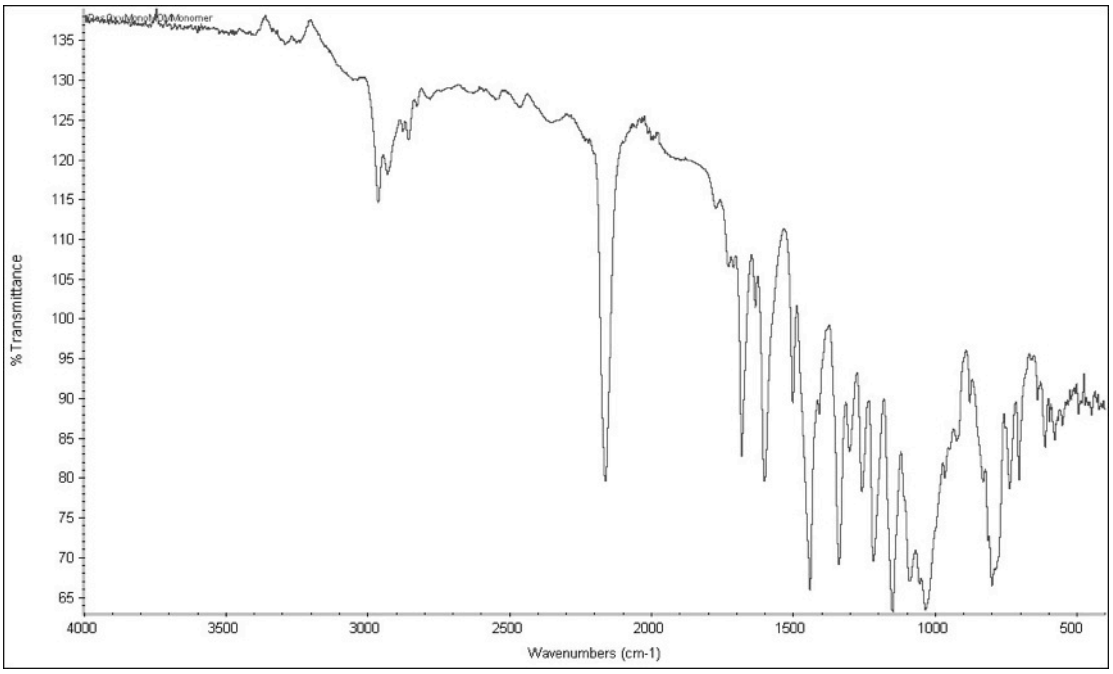
Catalog of NMR and FID Spectra.





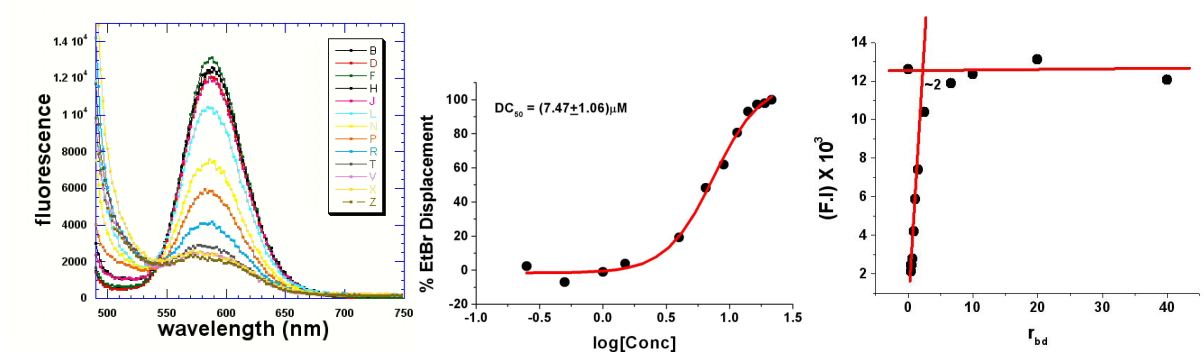
^{13}C NMR (DMF-d_7)

IR

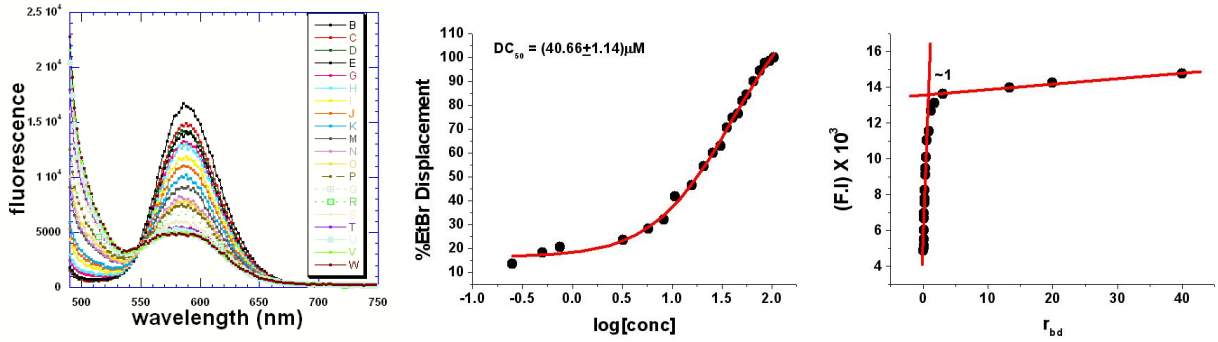

Monomer
% Transmittance
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

FID titration of diazofluorenes with calf thymus DNA (10.0 μM/base pair) and ethidium bromide (5.00 μM) in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8) at 24 °C.

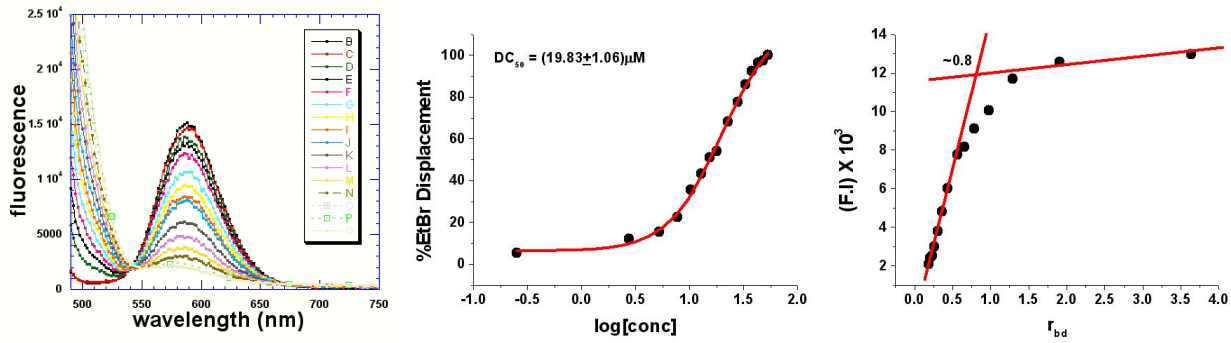
(–)-lomaiviticin aglycon (**9**)–calf thymus DNA

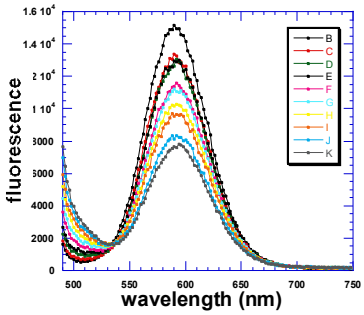


(2*S*,2′*S*)-lomaiviticin aglycon (**10**)–calf thymus DNA



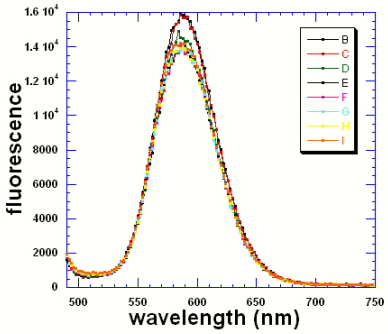
C-3/C-3′-dideoxylomaiviticin aglycon (**12**)–calf thymus DNA





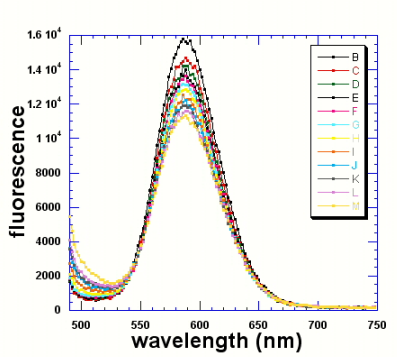
C-3-deoxylomaiviticin monomer (**13**)–calf thymus DNA

DC_{50} > 100 μM



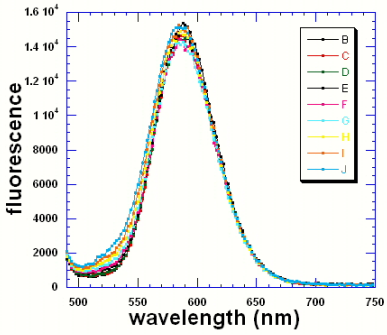
(–)-monomeric lomaiviticin aglycon (**11**)–calf thymus DNA

DC_{50} > 100 μM



(–)-kinamycin C (**6**)–calf thymus DNA

DC_{50} > 100 μM

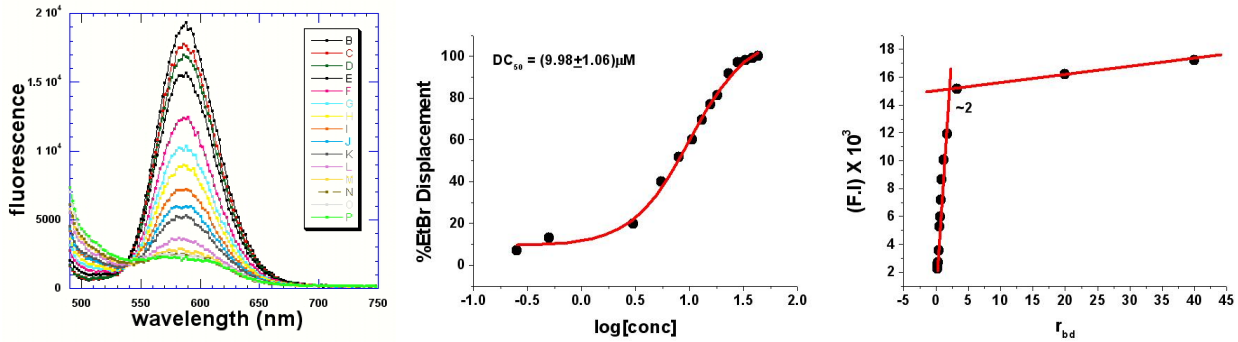


(–)-kinamycin F (**8**)–calf thymus DNA

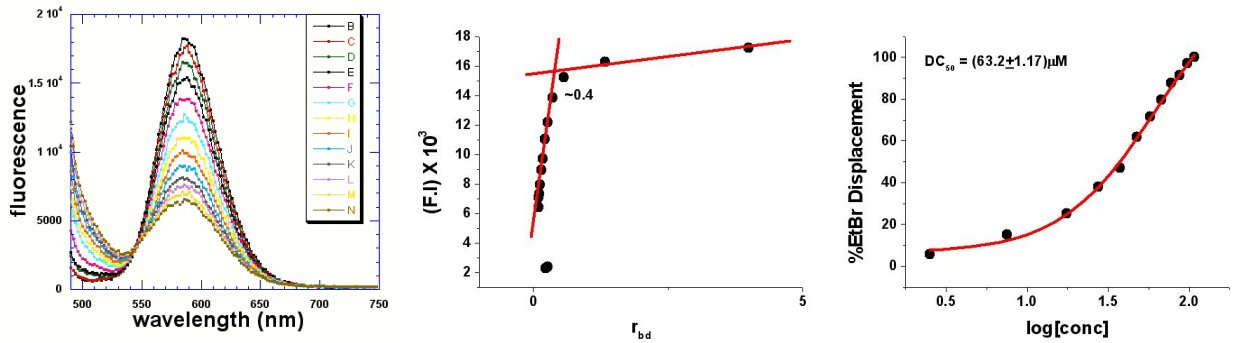
DC_{50} > 100 μM

FID titration of diazofluorenes with *M. lysodeiktius* DNA (10.0 μM/base pair) and ethidium bromide (5.00 μM) in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8) at 24 °C.

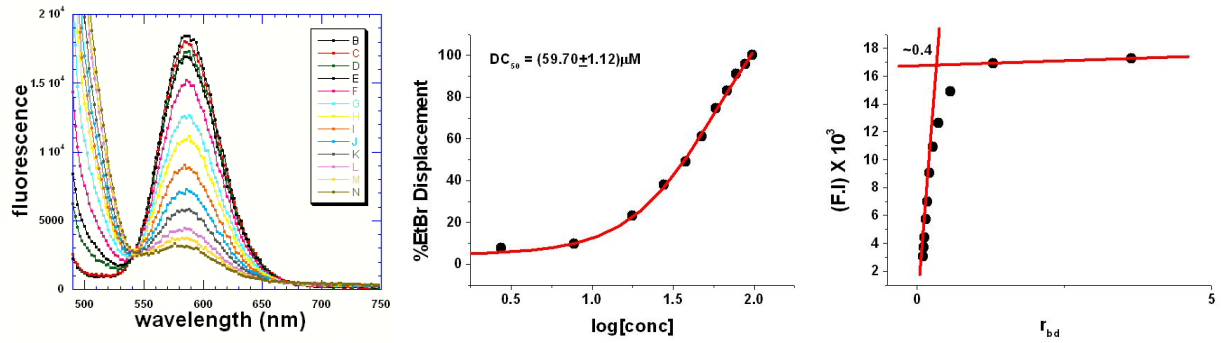
(–)-lomaiviticin aglycon (**9**)–*M. lysodeiktius* DNA

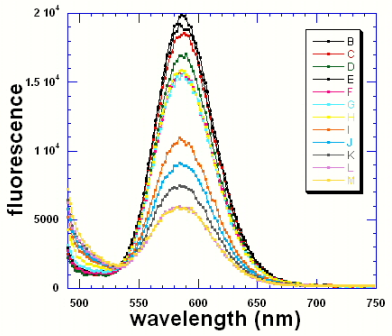


(2*S*,2′*S*)-lomaiviticin aglycon (**10**)–*M. lysodeiktius* DNA

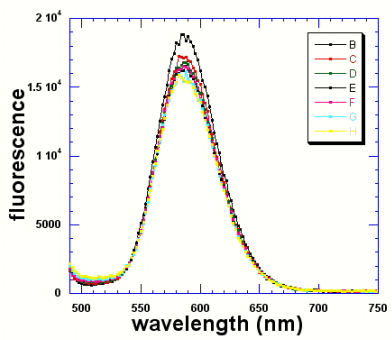


C-3/C-3′-dideoxylomaiviticin aglycon (**12**)–*M. lysodeiktius* DNA

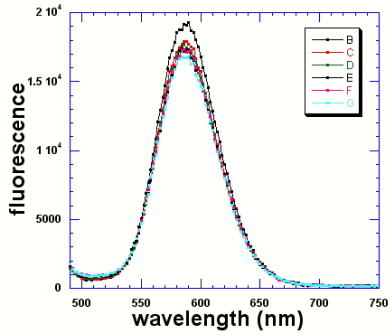




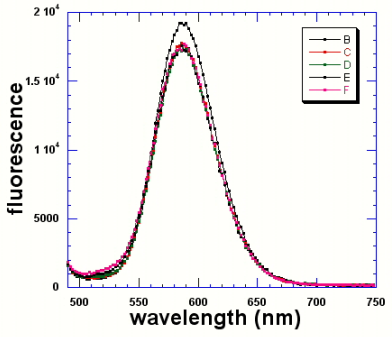
C-3-deoxylomaiviticin monomer (**13**)–*M. lysodeiktius* DNA
DC_{50} > 100 μM



(–)-monomeric lomaiviticin aglycon (**11**)–*M. lysodeiktius* DNA
C_{50} > 100 μM



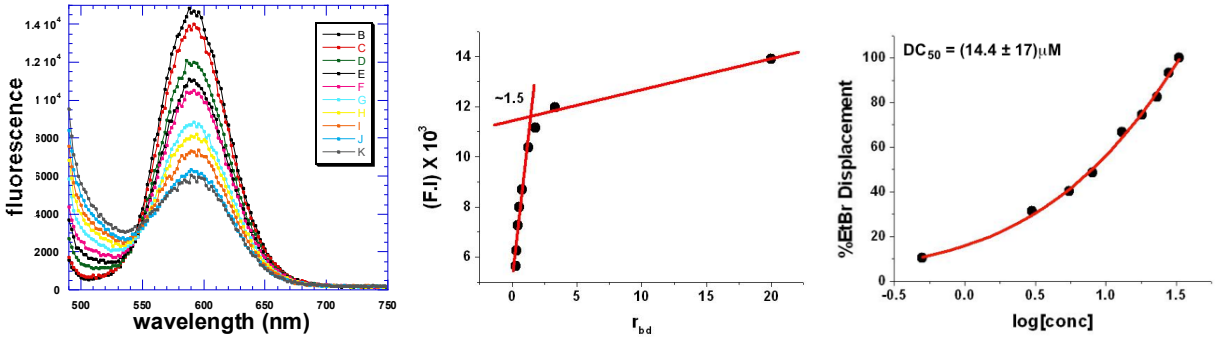
(–)-kinamycin C (**6**)–*M. lysodeiktius* DNA
DC_{50} > 100 μM



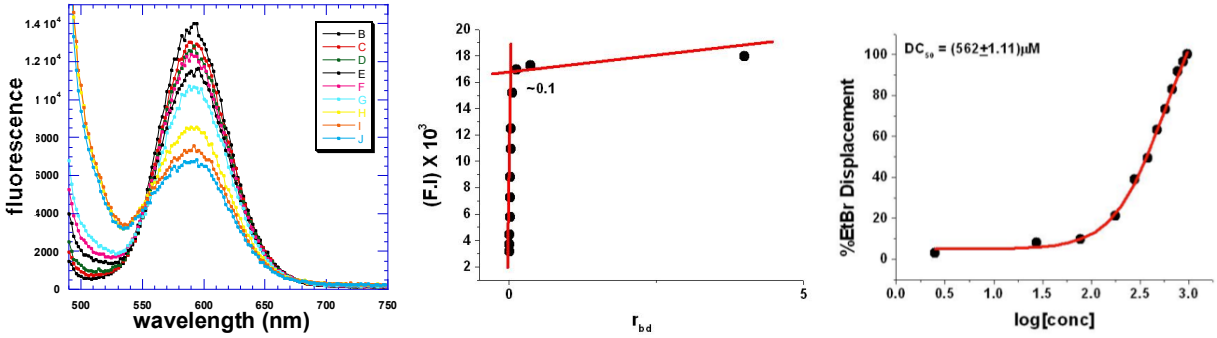
(–)-kinamycin F (**8**)–*M. lysodeiktius* DNA
DC_{50} > 100 μM

FID titration of diazofluorenes with *C. perfringens* DNA (10.0 μM/base pair) and ethidium bromide (5.00 μM) in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8) at 24 °C.

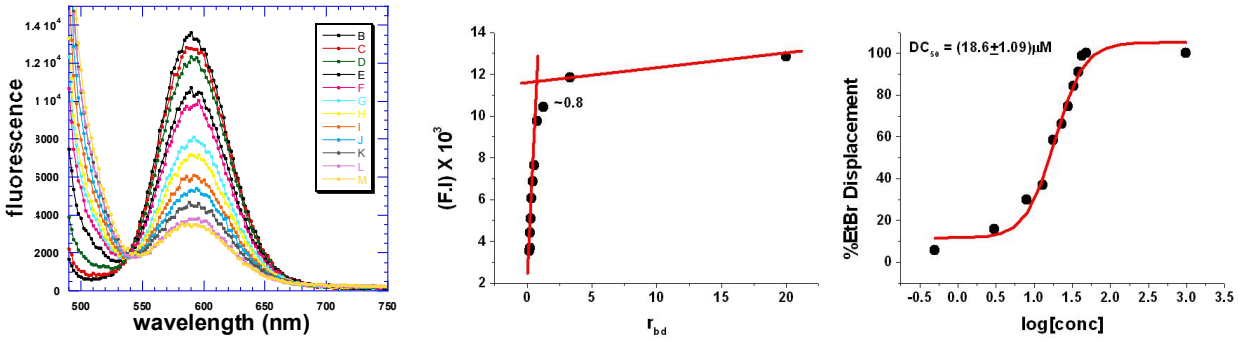
(–)-lomaiviticin aglycon (**9**)–*C. perfringens* DNA

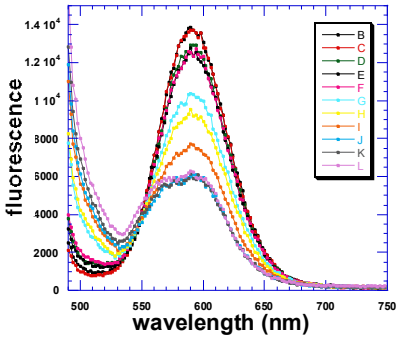


(2*S*,2′*S*)-lomaiviticin aglycon (**10**)–*C. perfringens* DNA

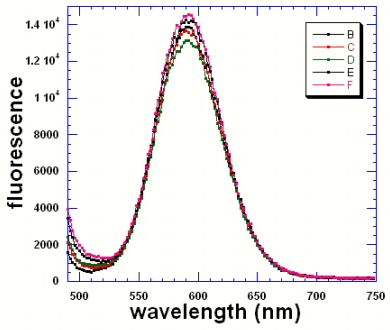


C-3/C-3′-dideoxylomaiviticin aglycon (**12**)–*C. perfringens* DNA

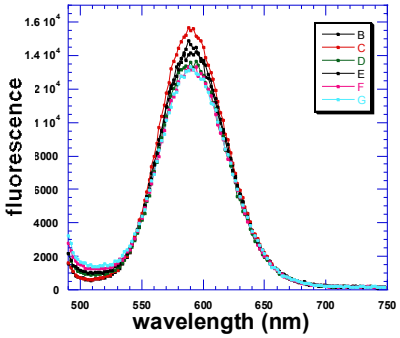




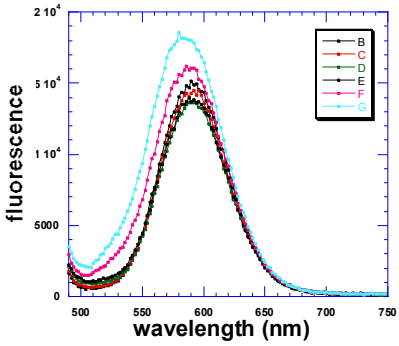
C-3-deoxylomaiviticin monomer (**13**)–*C. perfringens* DNA
DC_{50} > 100 μM



(–)-monomeric lomaiviticin aglycon (**11**)–*C. perfringens* DNA
DC_{50} > 100 μM



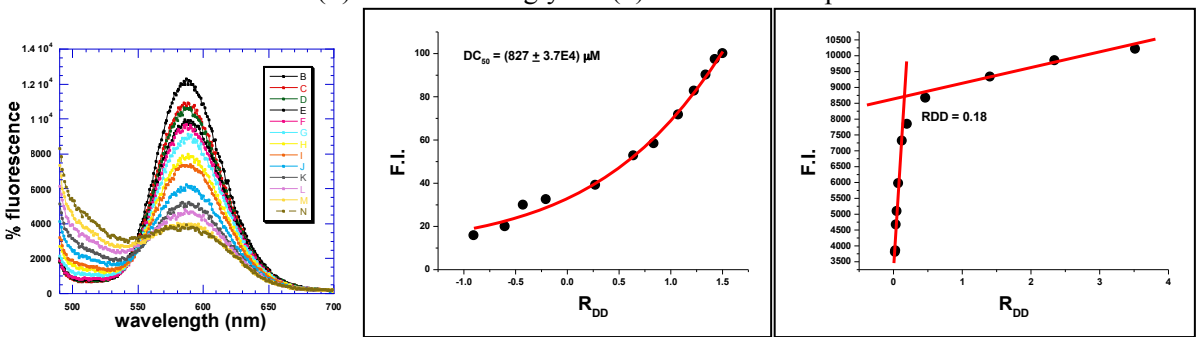
(–)-kinamycin C (**6**)–*C. perfringens* DNA
DC_{50} > 100 μM



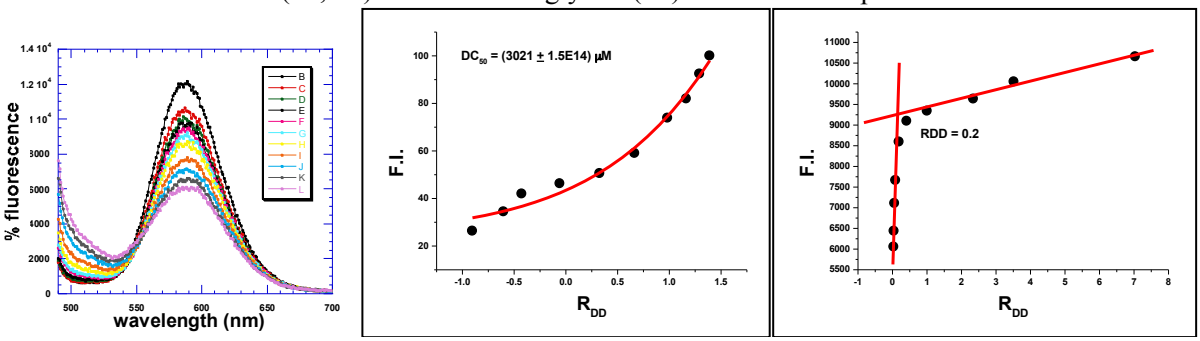
(–)-kinamycin F (**8**)–*C. perfringens* DNA
DC_{50} > 100 μM

FID titration of diazofluorenes **9**, **10**, and **12** with the Dickerson duplex (0.88 μM/duplex) and ethidium bromide (1.76 μM) in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.5 mM ethylenediaminetetraacetic acid, pH 6.8) at 24 °C.

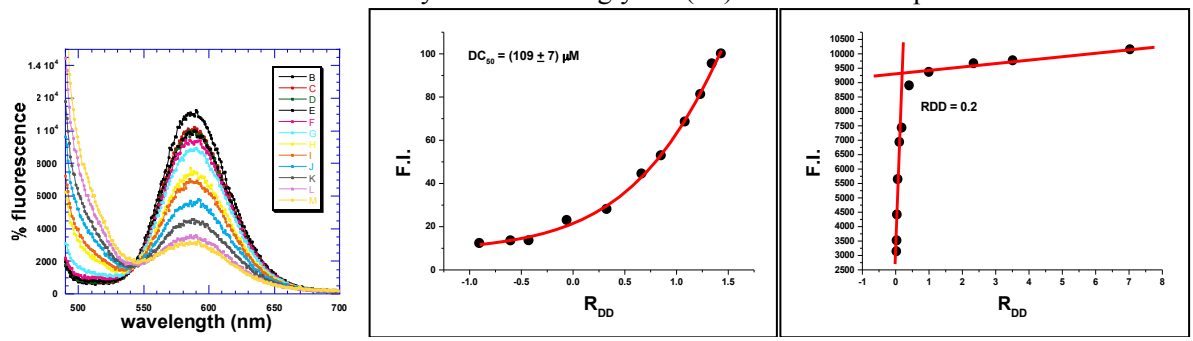
(–)-lomaiviticin aglycon (**9**)–Dickerson Duplex



(2*S*,2′*S*)-lomaiviticin aglycon (**10**)–Dickerson Duplex



C-3/C-3′-dideoxylomaiviticin aglycon (**12**)–Dickerson Duplex



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[1] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923.
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[5] C. M. Woo, L. Lu, S. L. Gholap, D. R. Smith, S. B. Herzon, *J. Am. Chem. Soc.* **2010**, *132*, 2540.
[6] R. E. Dickerson, H. R. Drew, *J. Mol. Biol.* **1981**, *149*, 761.
[7] For clarity, synthetic intermediates not described in the manuscript are numbered in the Supporting Information beginning with **S1**.