Structural basis for DNA cleavage by the potent antiproliferative agent (–)-lomaiviticin A

Christina M. Woo,[1] Zhenwu Li,[1] Eric K. Paulson,[1] Seth B. Herzon[1,2*]

[1]Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States
[2]Department of Pharmacology, Yale School of Medicine, New Haven, Connecticut 06520, United States

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Supporting Information

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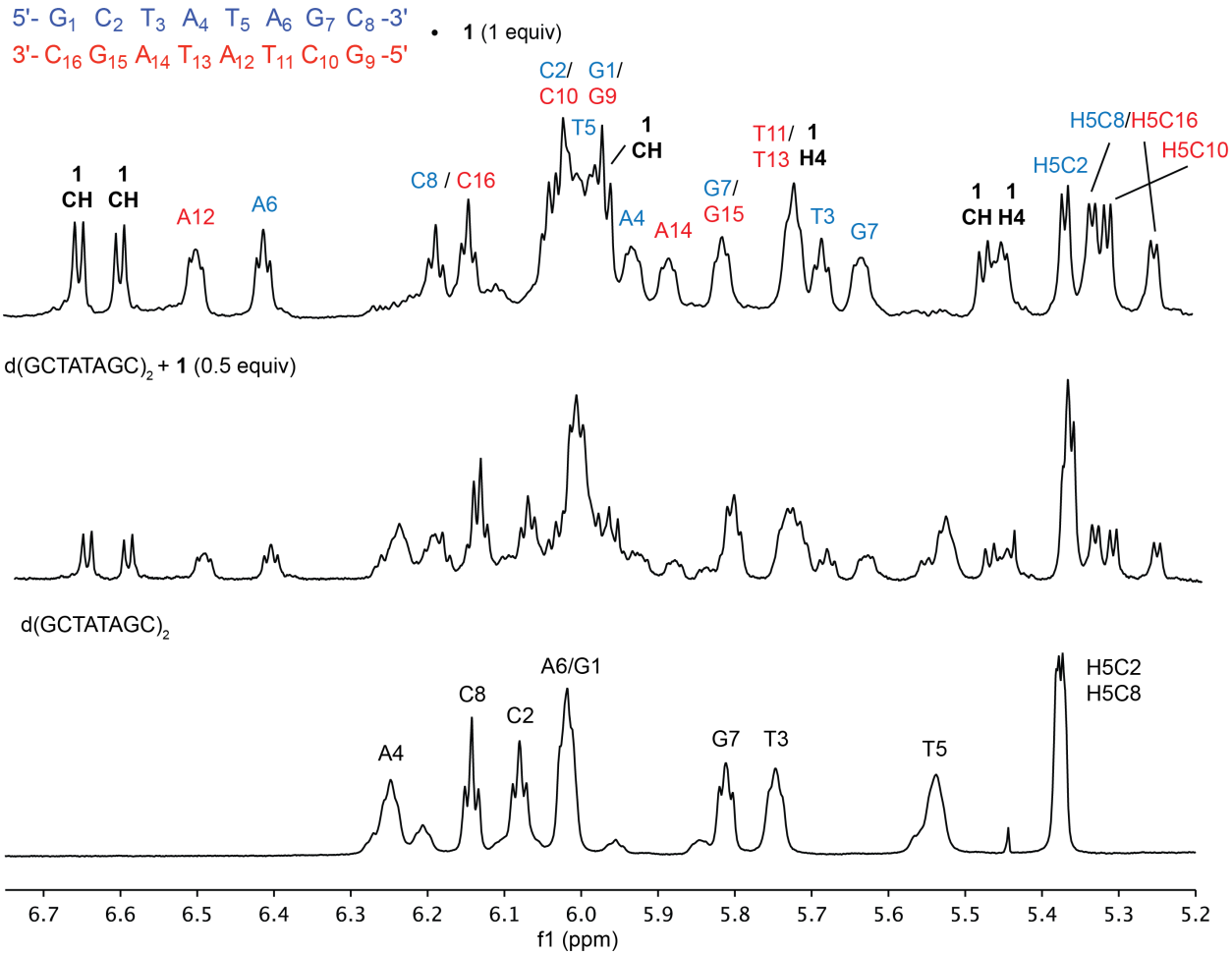


Figure S1. ^{1}H NMR of the nucleotide H1′ region during the sequential addition of (–)-lomaiviticin A (**1**; 0, 0.5, or 1.0 equiv) to d(GCTATAGC)$_2$. Conditions: 10 mM phosphate buffer (pH 7.5), 25 mM sodium chloride, 800 MHz, 24 °C.

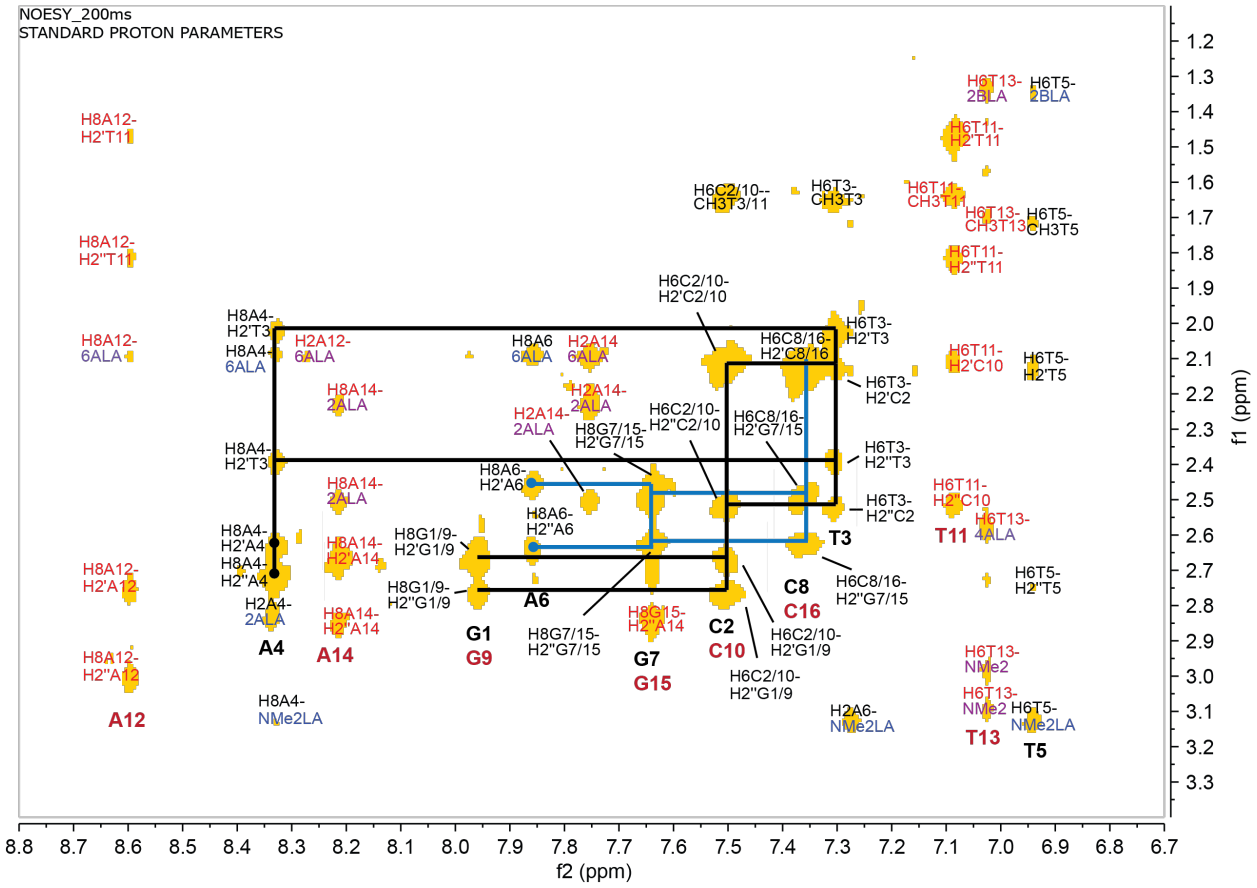


Figure S2. A/G H8→H2′/H2′′(n–1) or T/C H6→H2′/H2′′(n–1) region of the NOESY walk for G1–C8 of the complex between (–)-lomaiviticin A (**1**) and d(GCTATAGC)$_2$. Breaks in the walk are indicated by circular nodes. LA = **1**. For positional numbering of **1**, see Table 2. Conditions: 10 mM phosphate buffer (pH 7.5), 25 mM sodium chloride, 800 MHz, mixing time = 200 ms, 24 °C.

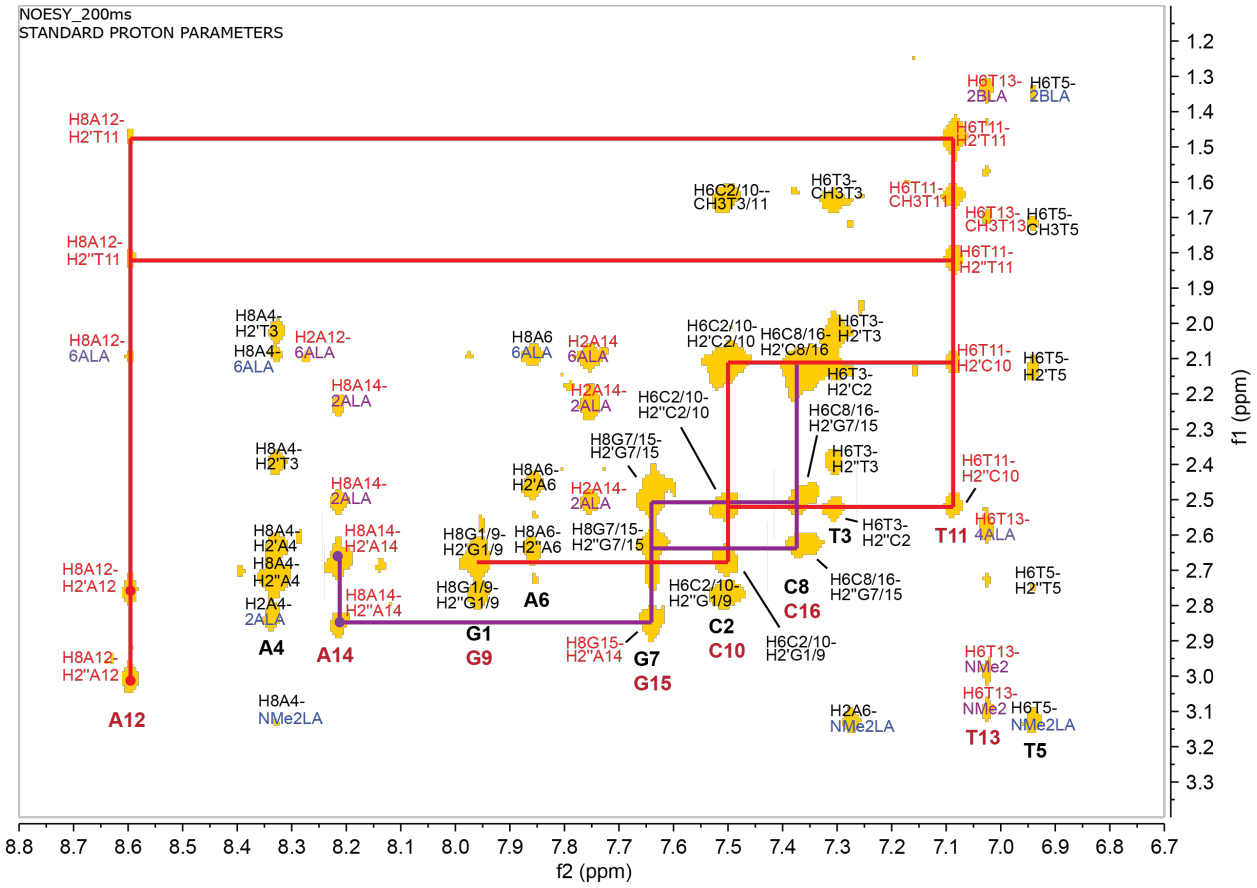


Figure S3. A/G H8→H2′/H2′′(n–1) or T/C H6→H2′/H2′′(n–1) region of the NOESY walk for G9–C16 of the complex between (–)-lomaiviticin A (**1**) and d(GCTATAGC)$_2$. Breaks in the walk are indicated by circular nodes. LA = **1**. For positional numbering of **1**, see Table 2. Conditions: 10 mM phosphate buffer (pH 7.5), 25 mM sodium chloride, 800 MHz, mixing time = 200 ms, 24 °C.

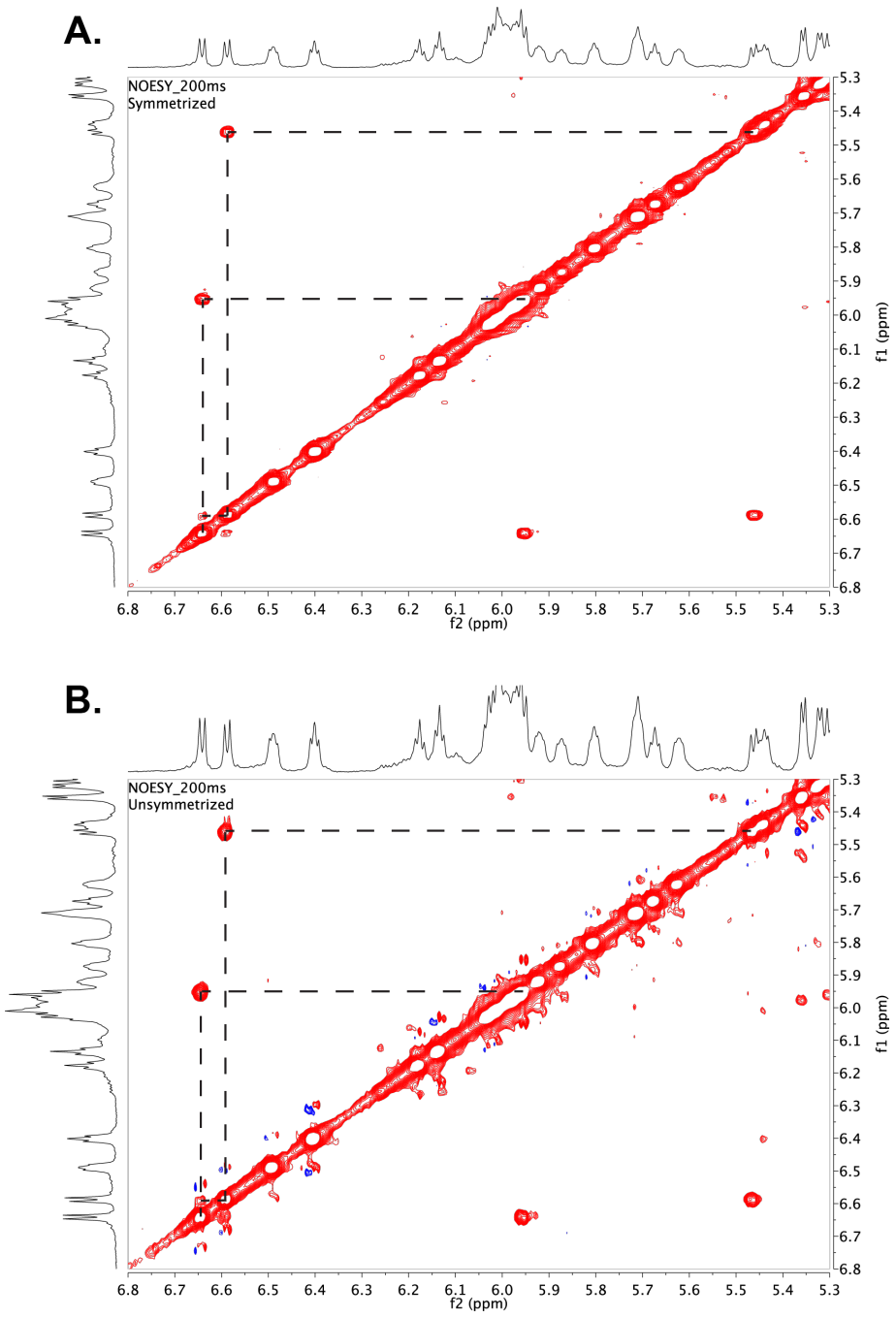


Figure S4. NOESY cross-peaks between the quinone protons (positions 8, 8′, 9, or 9′) of (–)-lomaiviticin A (**1**) after complexation with d(GCTATAGC)$_2$. **A.** Symmetrized NOESY spectrum (for clarity). **B.** Unsymmetrized NOESY spectrum. For positional numbering, see Fig. 1A. Conditions: 10 mM phosphate buffer (pH 7.5), 25 mM sodium chloride, 800 MHz, mixing time = 200 ms, 24 °C.

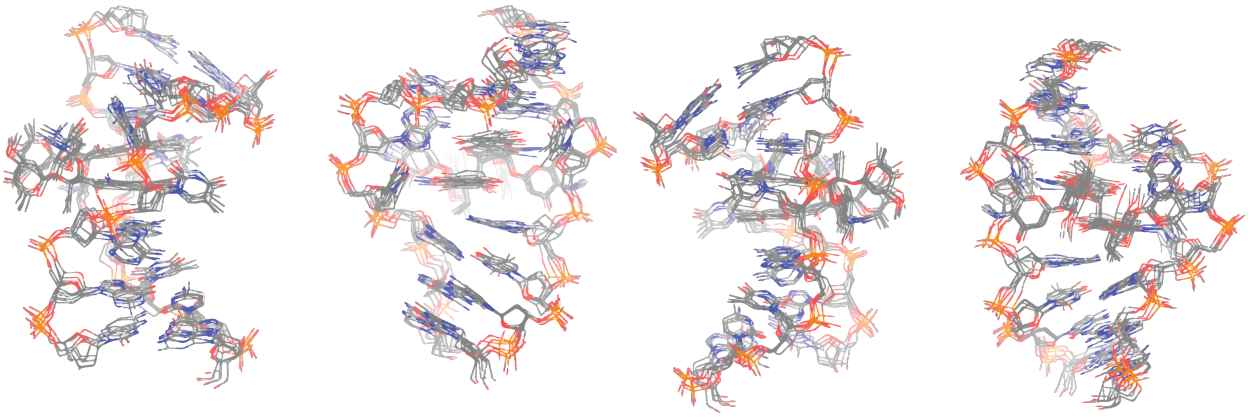


Figure S5. Rotational view of the six representative structures of (–)-lomaiviticin A (**1**) complexed with d(GCTATAGC)$_2$. Hydrogens are omitted for clarity.

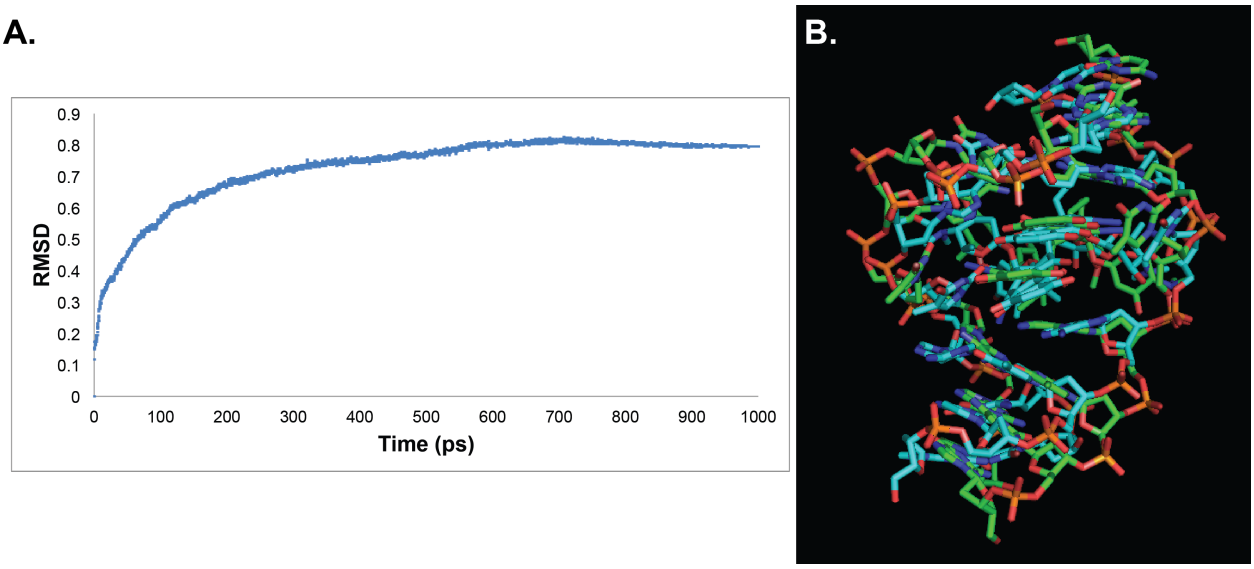


Figure S6. A. Pictorial statistics of RMSD over time (ps) during molecular dynamics simulation of the annealed structure of (–)-lomaiviticin A (**1**) associated with d(GCTATAGC)$_2$. **B.** Overlay of annealed structure (turquoise) and final structure after unrestrained molecular dynamics (green).

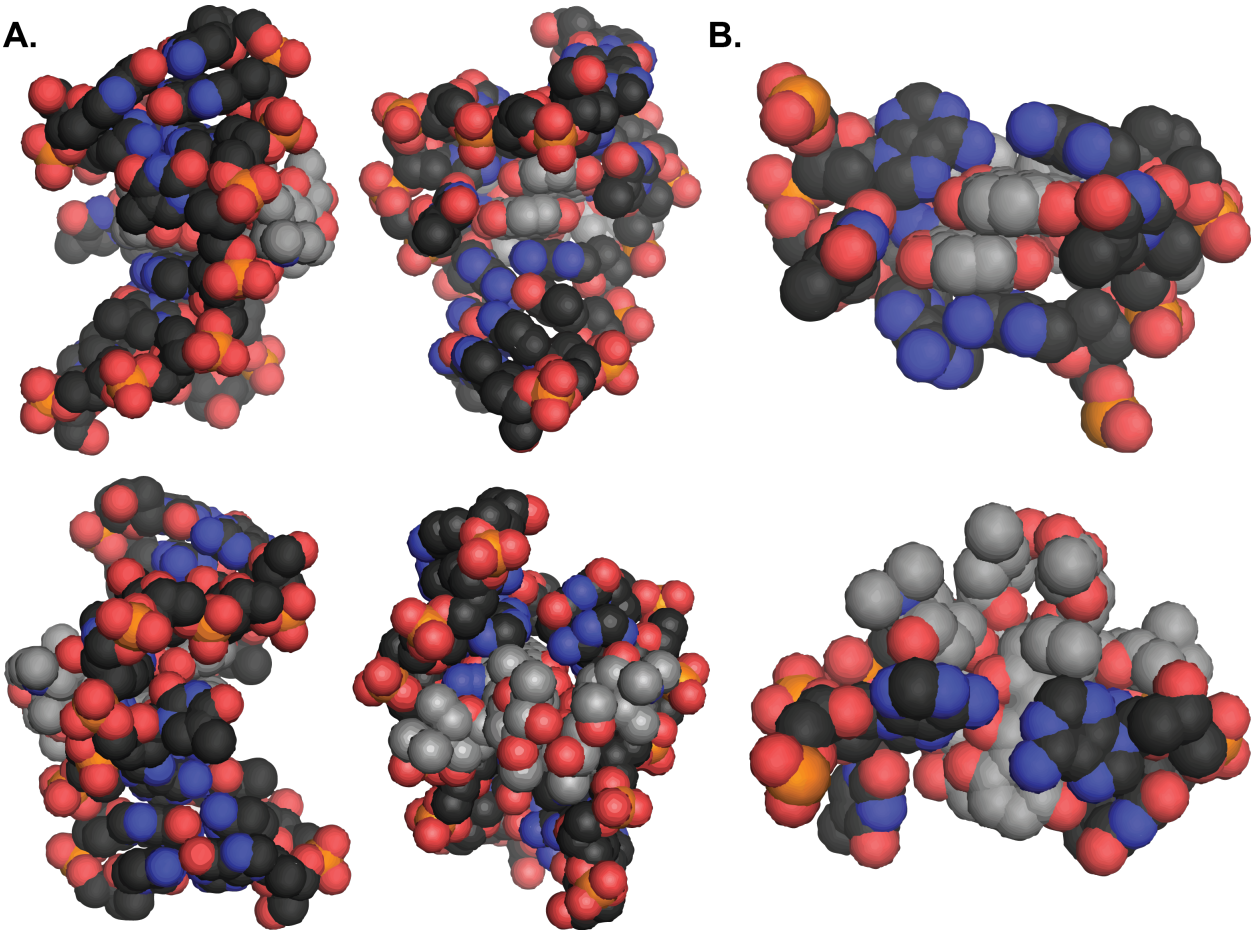


Figure S7. A. Rotational view and **B.** intercalation site of the NMR solution structure of (–)-lomaiviticin A (**1**) complexed with d(GCTATAGC)$_2$ in space filling form. Hydrogens are omitted for clarity.

Table S1. Proton resonance assignments based on NOE contacts of the free oligonucleotide d(GCTATAGC)$_2$ in 10 mM phosphate buffer (pH 7.5), 25 mM sodium chloride.[a]

Residue	H8/H6	H1′	H5/H2 /CH_3	H2′	H2′′	H3′	H4′	H5′/H5′′	H1/H3[b]	H4/H2 (A/B)[b]
G1	7.98	6.01	–	2.67	2.78	4.84	4.25	3.74	12.90	7.74/NA[c]
C2	7.54	5.37	5.37	2.16	2.55	4.81	4.26	4.20/4.11	–	8.11/6.62
T3	7.45	5.74	1.68	2.24	2.56	4.91	4.27	4.15/4.19	13.63	–
A4	8.37	6.24	7.29	2.67	2.92	5.02	4.43	4.14/4.26	–	6.76
T5	7.18	5.53	1.47	2.01	2.35	4.84	4.26	4.06/4.15	13.30	–
A6	8.15	6.01	7.29	2.68	2.84	5.02	4.38	4.06/4.14	–	6.80
G7	7.66	5.80	–	2.45	2.63	4.93	4.34	4.20/4.20	12.90	7.74/NA[c]
C8	7.41	6.13	5.36	2.10	2.16	4.93	4.45	4.03/4.20	–	8.32/6.68

[a]All chemical shifts are referenced to a silyl signal at 0.148 ppm in D_2O(1) and confirmed by the HOD chemical shift at 4.81 ppm (800 MHz, 24 °C). [b]Assigned by Watergate NOESY (800 MHz, 24 °C). [c]NA = not assigned.

Table S2. Proton resonance assignments based on NOE contacts of the oligonucleotide d(GCTATAGC)$_2$ complexed with (–)-lomaiviticin A (**1**) in 10 mM phosphate buffer (pH 7.5), 25 mM sodium chloride.[a]

Strand	H8/H6	H5/H2 /CH3	H1'	H2'	H2''	H3'	H4'	H5'/H5''	H1/H3[b]	H4/H2 (A/B)[b]
G1	7.96	–	5.97	2.66	2.75	4.83	4.23	3.72/3.72	12.93	NA[c]
C2	7.51	5.36	6.03	2.12	2.51	4.78	4.20	4.19	–	6.69/8.33
T3	7.31	1.64	5.67	2.01	2.38	4.87	NA	4.11	13.02	–
A4	8.33	8.34	5.92	2.62	2.71	5.09	4.34	4.15/4.23	–	NA[c]
T5	6.94	1.70	5.97	2.13	2.75	4.84	4.02	3.72/3.78	11.11	–
A6	7.86	7.28	6.41	2.45	2.64	4.93	4.23	4.11/4.11	–	NA[c]
G7	7.64	–	5.81	2.49	2.62	4.94	4.30	4.15/4.18	13.10	NA[c]
C8*	7.37	5.32	6.14	2.11	2.12	4.48	4.18	4.02/NA	–	6.55/8.11
G9	7.96	–	5.97	2.66	2.75	4.83	4.23	3.72	12.93	NA[c]
C10	7.50	5.30	6.01	2.10	2.50	4.78	4.20	4.19	–	6.64/8.44
T11	7.09	1.63	5.71	1.46	1.80	4.86	4.25	4.05	13.60	–
A12	8.60	8.28	6.49	2.75	3.00	5.15	4.24	4.04	–	NA[c]
T13	7.03	1.69	5.71	2.03	2.19	4.89	4.25	3.84/3.64	11.44	–
A14	8.22	7.76	5.87	2.67	2.84	5.00	4.17	3.80/NA	–	NA[c]
G15	7.64	–	5.81	2.66	2.75	4.94	4.30	4.03/4.18	13.10	NA[c]
C16*	7.35	5.24	6.18	2.11	2.12	4.46	4.47	4.02/NA	–	6.52/8.08

[a]All chemical shifts are referenced to a silyl signal at 0.148 ppm in D_2O(1) and confirmed by the HOD chemical shift at 4.81 ppm (800 MHz, 24 °C). [b]Assigned by Watergate NOESY (800 MHz, 24 °C). * = interchangable assignments. [c]NA = not assigned.

Chemical Materials. (–)-Lomaiviticins A–C (**1**–**3**) were prepared according to the procedure of Herzon and co-workers (2). (–)-Kinamycin C (**4**) was a gift from the Developmental Therapeutics Program of the National Cancer Institute (NSC 138425).

Nucleic Acids. Genomic DNA calf thymus and *M. lysodeiktius* were purchased from Invitrogen and Sigma Aldrich, respectively, and were used as received. The concentrations of all genomic DNA were determined by UV spectroscopy 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*. Polynucleotides dAdT, dGdC, and dA•dT were purchased from Invitrogen, and were used as received. The concentrations of all polynucleotides were determined by UV spectroscopy using the following extinction coefficients (in units of base pairs/$L^{-1}cm^{-1}$): ε_{260} = 6,500 for dAdT, ε_{260} = 6,500 for dGdC, and ε_{260} = 6,500 for dA•dT. The oligomers CGCAAATTTGCG (3), CGCATATATCGC, CGCGCGCGCGCG, CGCAAAAAAGCG, GCGTTTTTTCGC, and CGCATATGCG were purchased salt free from Operon. For the NMR solution structure, the oligomer GCTATAGC was purchased HPLC-purified from IDT. Prior to use, the oligomers were solubilized in 10 mM phosphate buffer, pH 7.5, 25 mM sodium chloride, and heated at 100 °C for 30 min, cooled to 24 °C over 2 h, and incubated at 4 °C for 24 h to anneal the duplex. The concentrations of all oligonucleotides were determined by UV spectroscopy using the following extinction coefficients (in units of mol of nucleotide/$L^{-1}cm^{-1}$): ε_{260} = 115,958 for CGCAAATTTGCG, ε_{260} = 115,958 for CGCATATATCGC, ε_{260} = 101,994 for CGCGCGCGCGCG, ε_{260} = 133,660 for CGCAAAAAAGCG, ε_{260} = 98,256 for GCGTTTTTTCGC, ε_{260} = 94,304 for CGCATATGCG, and ε_{260} = 84,400 for CGCTATAGC. The concentration of thiazole orange (TO) was determined by UV spectroscopy using the following extinction coefficient (in units of mol of fluorophore/$L^{-1}cm^{-1}$): ε_{260} = 63,000 for TO.

Instrumentation. Singe-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. All nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 800 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 a silyl signal at 0.148 ppm in D_2O(1) and confirmed by the HOD chemical shift at 4.81 ppm. 800 MHz NMR data was collected on an Agilent DD2 800 MHz NMR spectrometer running VnmrJ version 4.0 with a 5mm ^{13}C-sensitivity enhanced salt-tolerant HCN cold probe. The pulse sequences and other parameters had the default settings as provided by Agilent, except as noted. 1D spectra of samples in D_2O used 64 scans of the 's2pul' pulse sequence with a 5 second relaxation delay, a 45-degree pulse, and 43395 points collected in a 2.569 second acquisition time. 1D spectra of samples in 90% H_2O–10% D_2O used 64 scans of the 'water' pulse sequence with W5 watergate solvent suppression, a 1.5 second relaxation delay, and 8192 points collected in a 0.485 second acquisition time. 2D NOESY spectra of samples in D_2O used 2 scans and 1024 increments of the 'NOESY' pulse sequence with a 1-second relaxation delay, a mixing time of 100 or 200 milliseconds, and 4096 points collected in a 0.2425 second acquisition time. 2D NOESY spectra of samples in 90% H_2O–10% D_2O used 8 scans and 1024 increments of the 'wgNOESY' pulse sequence with 3-9-19 watergate solvent suppression, a 1 second relaxation delay, a mixing time of 100 or 200 milliseconds, and 4096 points collected in a 0.2425 second acquisition time. A 2D H-C HSQC spectrum was collected in D_2O using 32 scans and 256 increments of the 'gHSQCAD' pulse sequence using a 1 second relaxation delay, a 140 Hz JXH coupling, and 2533 points collected in a 0.150 second acquisition time. A 2D DQF-COSY spectrum was collected in D_2O using 4 scans and 1024 increments of the 'gDQCOSY' pulse sequence using a 1 second relaxation delay and 4096 points collected in a 0.5112 second acquisition time. Data were analyzed using

MestReNova version 10. Computational structural modeling was performed on a Linux x86_64 interface, 8 CPU system running AMBER 14.0.

Fluorescence Intercalator Displacement (FID) Procedures.

1:1 FID Procedure:

The FID assay was carried out in a 96-well plate in triplicate. A solution containing genomic or polymeric DNA (1.76 μM/base pair) and thiazole orange (TO, 3.52 μM, 2.00 equiv/base pair) was prepared by incubating the two components for 30 min in buffer (100 mM potassium chloride, 10 mM sodium cacodylate, 0.5 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 ligand in dimethylsulfoxide (1.14 μL, 310 μM stock solution) or dimethylsulfoxide alone (negative control, 1.14 μL) was added into each well in triplicate and the fluorescence was measured after incubation for 1 h at 24 °C. Fluorescence readings are reported as percentage fluorescence relative to the negative control wells. The reference fluorescence is defined as such: [TO+DNA] gives 100% fluorescence and [TO] only gives 0% fluorescence. The change in the fluorescence was plotted as:

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

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

FID Titration Experiments with Polynucleotides:

The FID titrations of DNA were carried out in a quartz cuvette in duplicate. A solution of polymeric DNA (2.50 μM/base pair) saturated with thiazole orange (1.25 μM, 0.50 equiv/base pair) in buffer (100 mM potassium chloride, 10.0 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8, 200 μL) was prepared, and the fluorescence of this solution was measured (Ex: 504 nm, Em: 505–650 nm). An aliquot of the stock solution of ligand (2.50 μM–62.5 μM) was added to the mixture, and the fluorescence was measured after incubation for 5 min at 24 °C. The addition of ligand was continued until changes in the fluorescence intensity were no longer observed. The fluorescence maxima were then plotted against the ratio of base pairs to ligand (r_{bl}) to obtain binding site size, 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.

FID Titration Experiments with Oligonucleotides:

The FID titrations of 12mer and 10mer oligonucleotides were carried out in a quartz cuvette in duplicate. A solution of the duplex (1.25 μM, 1 equiv) saturated with thiazole orange (2.50 μM, 2.00 equiv/duplex) in buffer (100 mM potassium chloride, 10.0 mM sodium cacodylate, 0.50 mM ethylenediaminetetraacetic acid, pH 6.8, 200 μL) was prepared, and the fluorescence of this solution was measured (Ex: 504 nm, Em: 505–650 nm). An aliquot of the stock solution of ligand (2.50 μM–2.50 mM) was added to the mixture, and the fluorescence was measured after incubation for 5 min at 24 °C. The addition of ligand was continued until changes in the fluorescence were no longer observed. To determine the binding stoichiometry, the fluorescence maxima were plotted against the ratio of the duplex to ligand (r_{dl}). To determine the dissociation constant, the percentage change in fluorescence was plotted against the log(concentration of drug).

NMR Solution Structure.

Solution Structure of 5′-$G_1C_2T_3A_4T_5A_6G_7C_8$-3′:

The chemical shifts of 5′-$G_1C_2T_3A_4T_5A_6G_7C_8$-3′ have been previously determined by Sakurai and co-workers(4) and these shifts were confirmed by reassignment (Table S1).

*Titration of (–)-Lomaiviticin A (**1**) into 5′-$G_1C_2T_3A_4T_5A_6G_7C_8$-3′:*

Aliquots of (–)-lomaiviticin A (**1**) in D_2O (6 × 10.0 μL, 1.10 μmol, 1 equiv) were titrated into 5′-$G_1C_2T_3A_4T_5A_6G_7C_8$-3′ in phosphate buffered D_2O (350 μL, 1.10 μM/duplex, 1.00 equiv) and the ^{1}H NMR spectrum was immediately recorded at 24 °C.

*Solution Structure of (–)-Lomaiviticin A (**1**) Complexed to 5′-$G_1C_2T_3A_4T_5A_6G_7C_8$-3′:*

Structure calculations were performed using Leap, a module of AMBER 14 (5, 6). The 5′-$G_1C_2T_3A_4T_5A_6G_7C_8$-3′ duplex starting model was created using the make-na server (http://structure.usc.edu/make-na/). The starting structure for (–)-lomaiviticin A (**1**) was generated by calculation of the conformational minimum of 100,000 structures on Spartan followed by DFT geometry optimization in Gaussian 09 [B3LYP 6-31G(d,p)$^+$]. The minimized structure of **1** was imported to Leap using the Antechamber tools. Ten starting structures were prepared with (–)-lomaiviticin A (**1**) positioned varying distances from the minor groove of the duplex, and four starting structures were prepared with (–)-lomaiviticin A (**1**) positioned in the center of the separated stands of DNA (20–40 Å apart). The structures were neutralized with Na^+ ions and solvated with explicit water using the LEAP module of AMBER 14. A periodic octagonal box of TIP3P water was created around the structure. Initially, 500 steps of steepest descent minimization followed by 500 steps of conjugate gradient minimization were conducted for the waters and sodium ions with 500 kcal/(mol•Å^2) restraints placed on the DNA duplex. Then, 1500 steps of steepest descent minimization followed by 1000 steps of conjugate gradient minimization were carried out for the entire system without restraints.

Distance restraints were derived from the observed cross-peak intensities in the NOESY spectrum obtained in phosphate buffered D_2O with a mixing time of 100 ms. Distance restraints were binned into strong, medium, and weak NOEs with upper bound restraints of 3.0 Å, 5.0 Å, and 6.0 Å, respectively. For NOEs involving methyl protons, distances were measured from the center of the methyl group, and 1 Å was added to the upper bound distance restraint. Distance restraints for exchangeable protons were obtained from cross-peaks observed in a 200 ms NOESY spectrum in a 90% H_2O–10% D_2O solvent. Hydrogen bonds from clearly defined base pairs were restrained within 0.2 Å of Watson–Crick base pairing. Base pairs with no defined pairing (A4–T13, T5–A12) were not restrained by Watson–Crick base pairing. Sugar pucker torsion angles were measured from the DQF-COSY and were bounded for an S-type conformation if a typical coupling constant was found. Sugar torsion bounds were given pseudorotation angles of 100–165° for guanine and cytosine; 125–165° for adenine; 90–130° for thymine, and the component angles generated using libraries within AMBER 14. Dihedral angles along the phosphate backbone were included for values typical for B-form DNA (α = –39 ± 75°, β = –151 ± 75°, γ = 31 ± 75°, δ = 156 ± 75°, ε = 159 ± 75°, ζ = –99 ± 75°, χ = –99 ± 75°), excluding base pairs where interruptions in the NOE walk was observed (T5, T13).

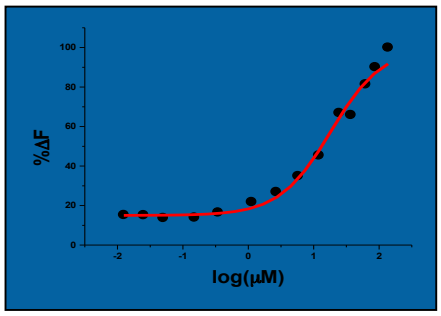
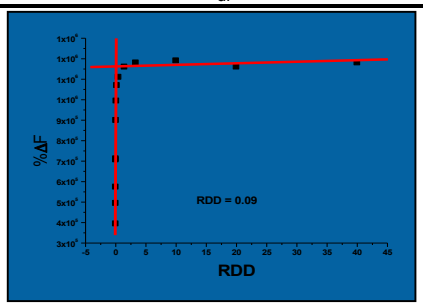
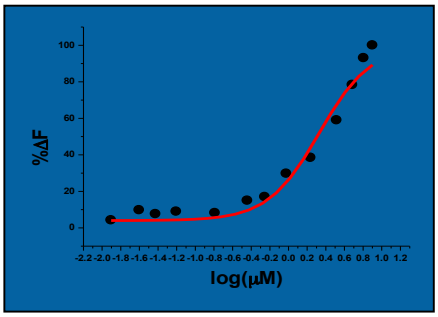
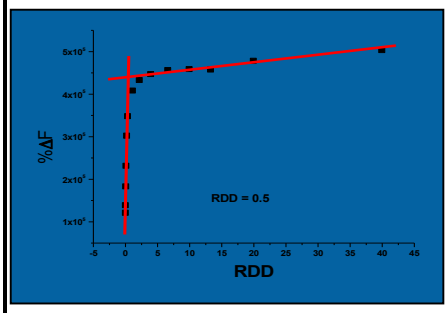
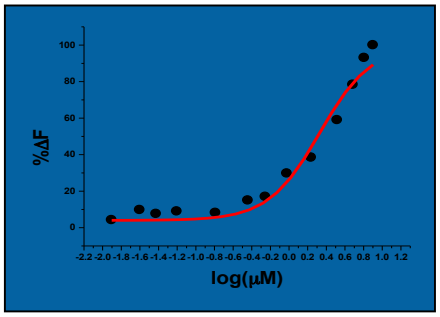
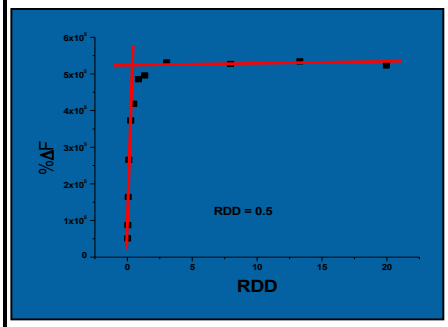
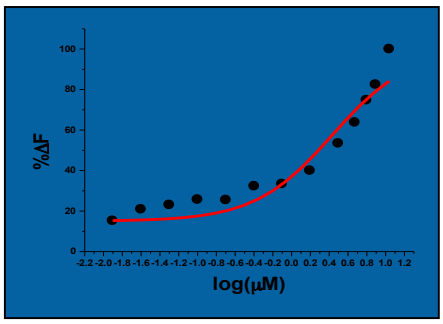
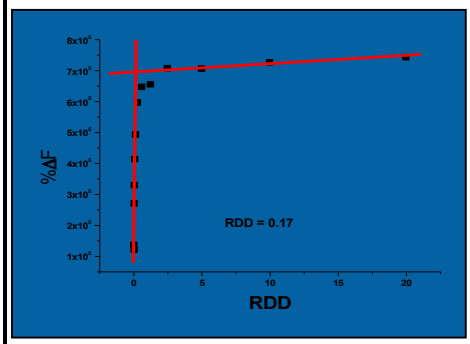
Annealing simulations were performed using Sander, a module of AMBER 14. Initial annealing was performed with two protocols. In the first protocol, a 30 ps annealing was performed with a 1 fs time step using Langevin dynamics and a collision frequency of 1 ps^{-1}. In the first 7.5 ps, the system was heated from 0 K to 300 K with a short temperature coupling of 0.4 ps and an increase in the weights of the restraints (0.1→1.0). Until 27 ps, the system temperature was maintained at 300 K, the temperature coupling maintained at 4 ps. In the final 3 ps, the system temperature was cooled from 300 K to 0 K, and the temperature coupling

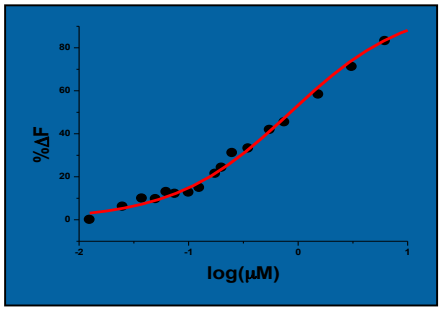
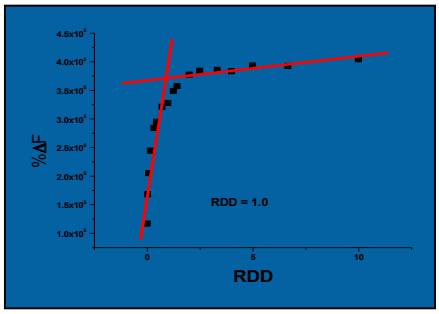
shortened to 1 ps (for 2000 steps), and then to 0.1 ps (for 1000 steps). NMR restraints weights were maintained throughout (1.0). In the second protocol, a 100 ps annealing was performed with a 1 fs time step using Langevin dynamics and a collision frequency of 1 ps^{-1}. In the first 7.5 ps, the system was heated from 0 K to 300 K with a short temperature coupling of 0.4 ps and an increase in the weights of the restraints (0.1→1.0). Until 70 ps, the system temperature was maintained at 300 K, the temperature coupling maintained at 4 ps. In the final 30 ps, the system temperature was cooled from 300 K to 0 K, and temperature coupling shortened to 1 ps (for 16665 steps), and then to 0.1 ps (for 13333 steps). NMR restraints weights were maintained throughout (1.0).

Structures that fit the NMR restraints best were annealed with the NMR restraints for additional 1 ns. Annealing was performed for 1 fs time step using Langevin dynamics and a collision frequency of 1 $ps^{-1.}$ In the first 75 ps, the system was heated from 0 K to 300 K with a tight temperature coupling of 0.4 ps and an increase in the weights of the restraints (0.1→1.0). Until 700 ps, the system temperature was maintained at 300 K, the temperature coupling maintained at 4 ps. In the final 300 ps, the system temperature was cooled from 300 K to 0 K, and temperature coupling shortened to 1 ps (for 166665 steps), and then to 0.1 ps (for 133333 steps). NMR restraints weights were maintained throughout (1.0).

The best structure was subsequently modeled using molecular dynamics (MD) simulations. MD simulations were performed using Sander, a module of AMBER 14. A 2 ps time step was applied in the MD simulations. Production was carried out for 1 ns at 300 K with a temperature coupling of 2.0 ps, and a distant-dependent dielectric constant. The structure was validated with cpptraj in AMBER 14, and the RMSD converged over time. Six structures from the final 100 ps were selected to represent the final structure with a final RMSD = 0.740 ± 0.141.

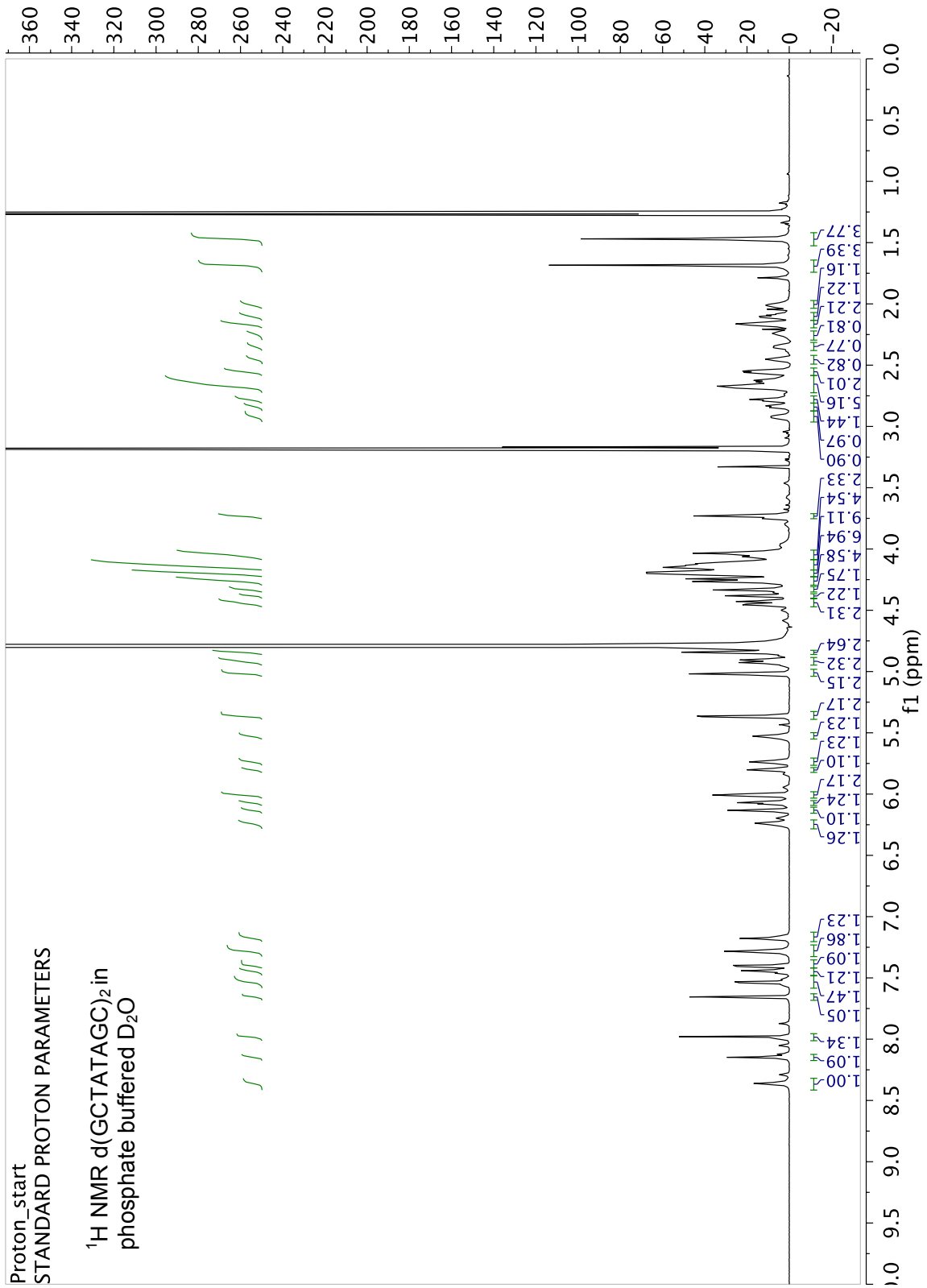
Catalog of Fluorescent Intercalator Displacement Spectra.

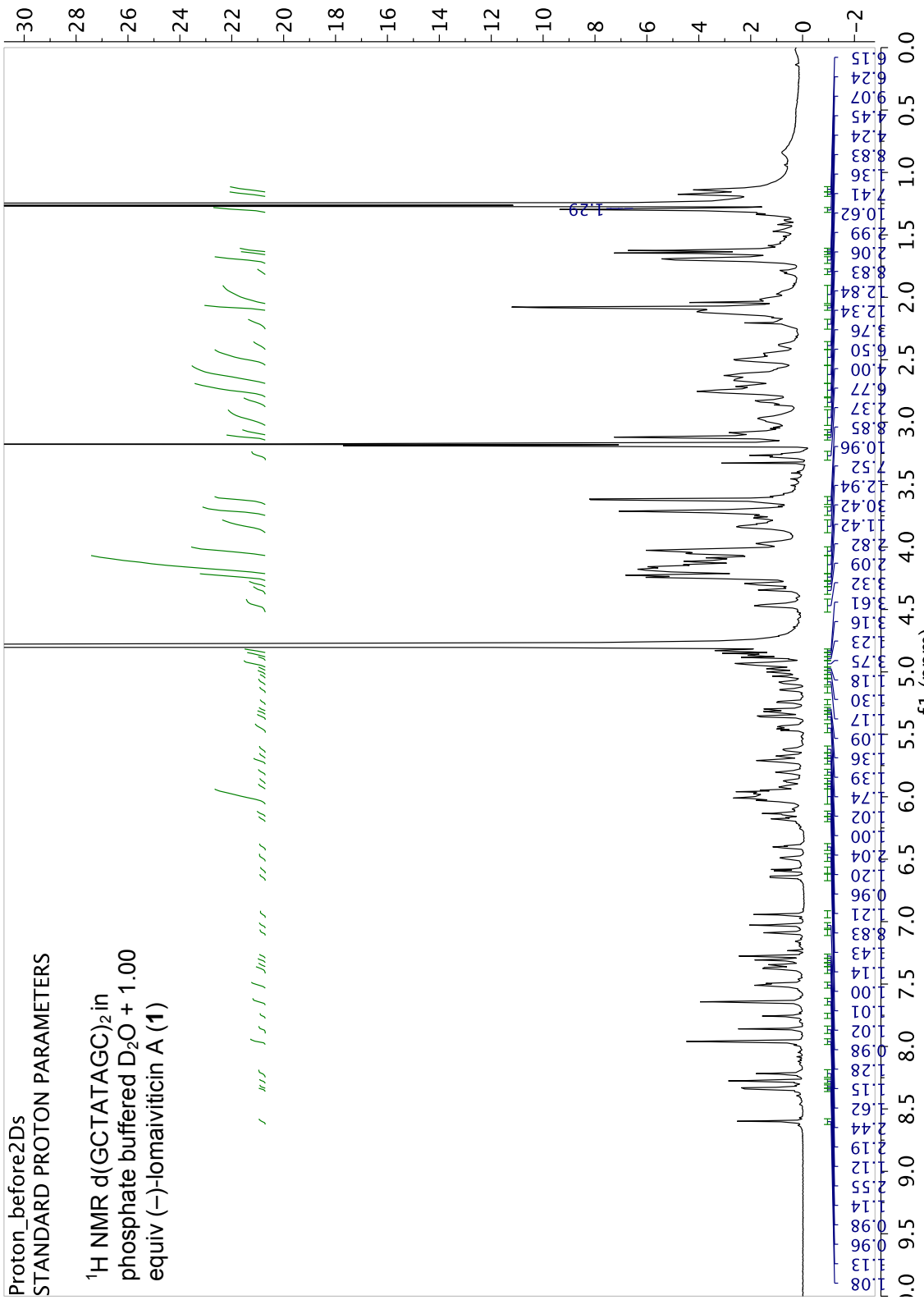
Oligonucleotide duplexes	DC_{50} (per duplex)	r_{dl}[a]
d(CGCGCGCGCGCG)$_2$	 18.3 ± 1.4 μM	
d(CGCATATATGCG)$_2$	 1.87 ± 0.14 μM	
d(CGCAAATTTGCG)$_2$	 2.14 ± 0.18 μM	
d(CGCAAAAAAGCG)· d(GCGTTTTTTCGC)	 2.70 ± 0.38 μM	

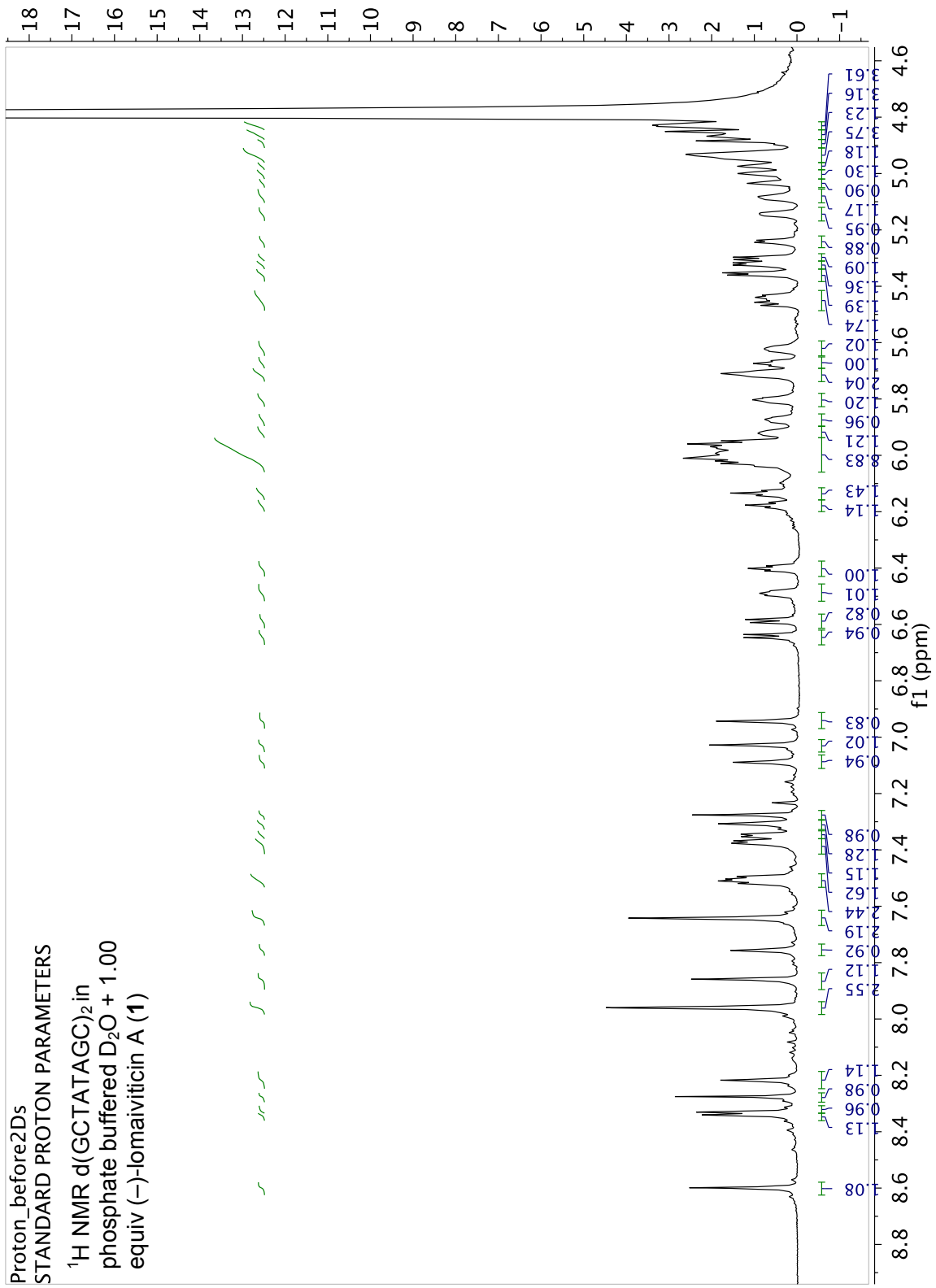
d(CGCATATGCG)$_2$			0.85 ± 0.05 μM

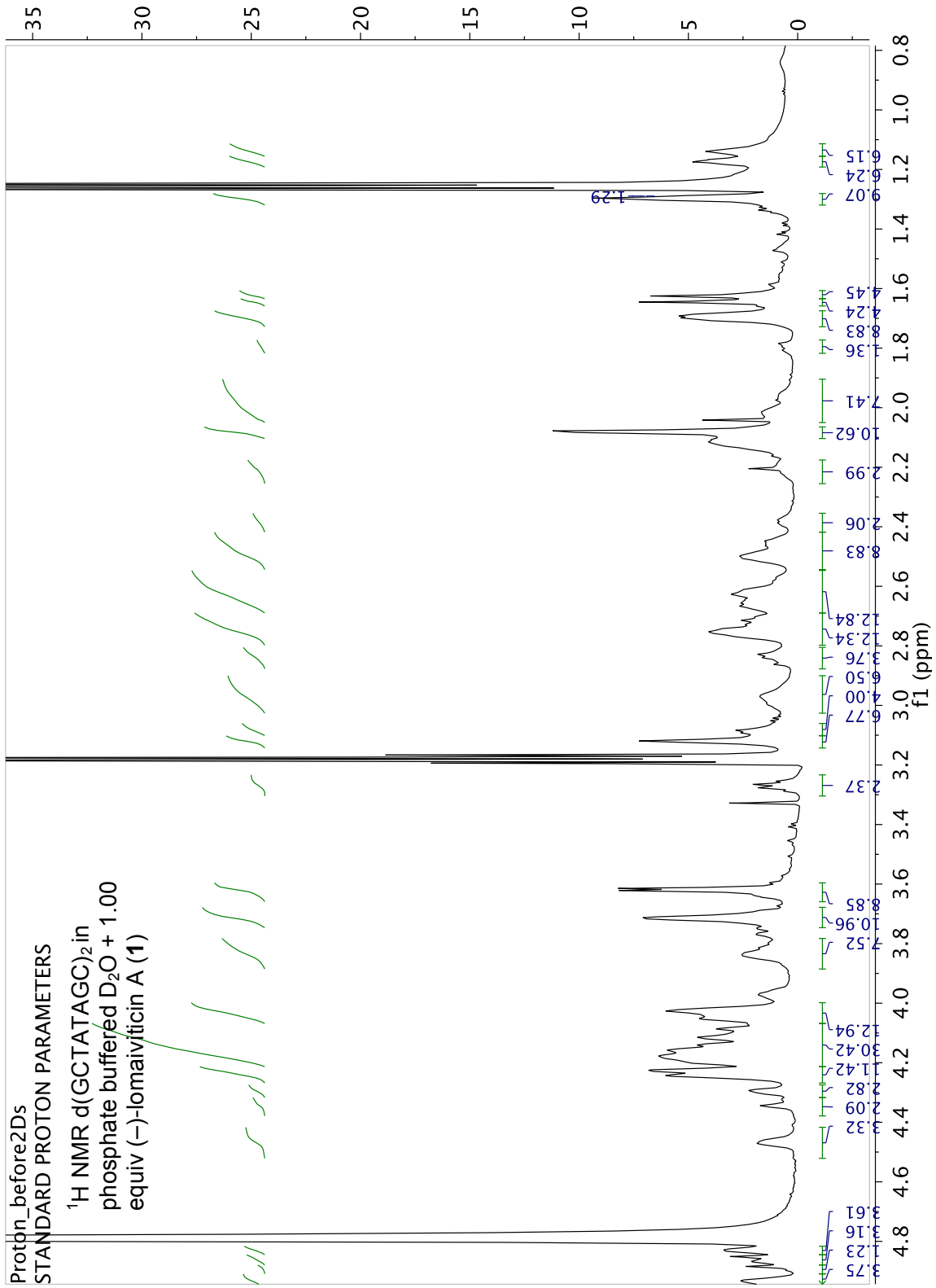
[a] RDD and r_{dl} are equivalent terms

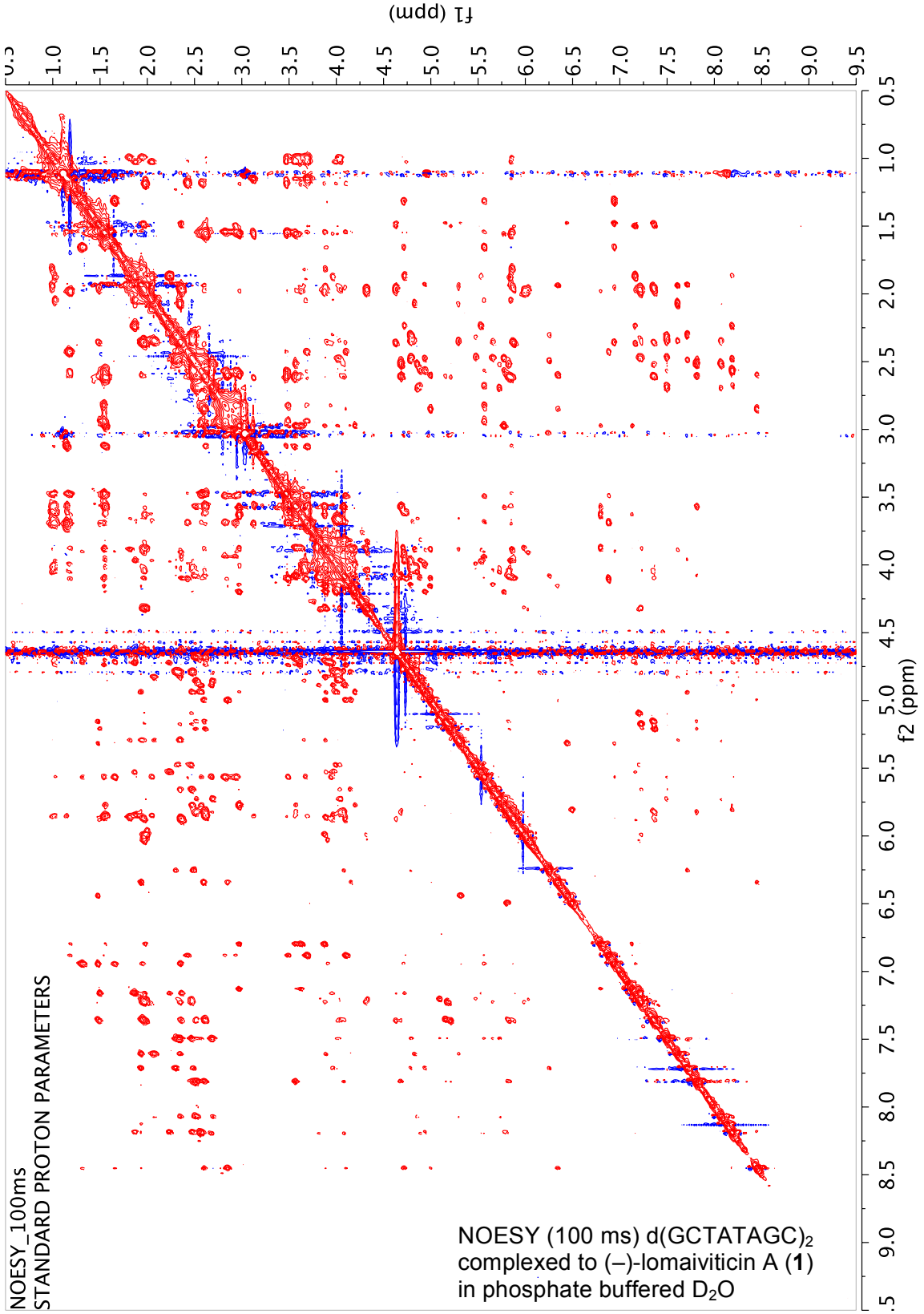
Catalog of Nuclear Magnetic Resonance Spectra.

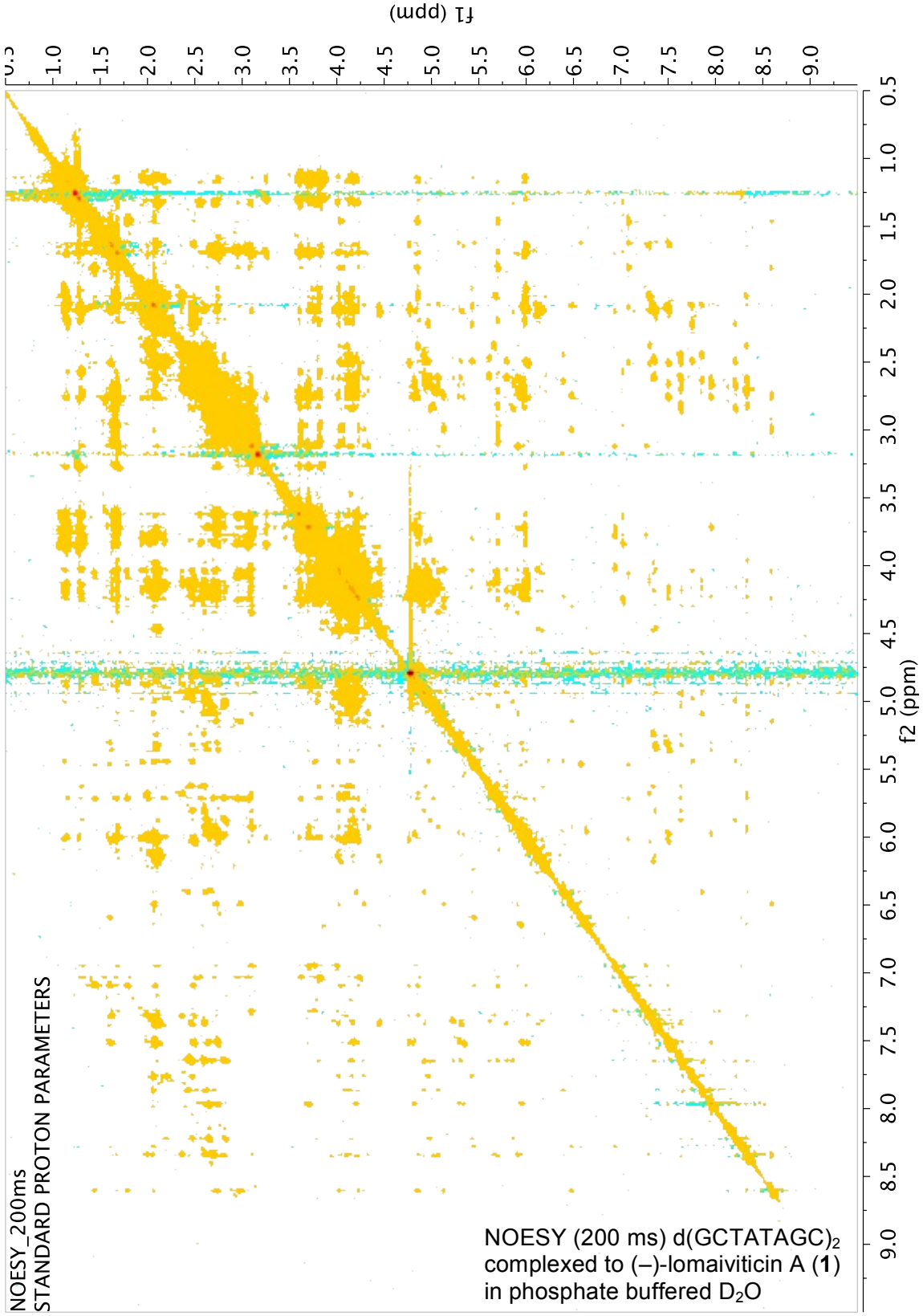


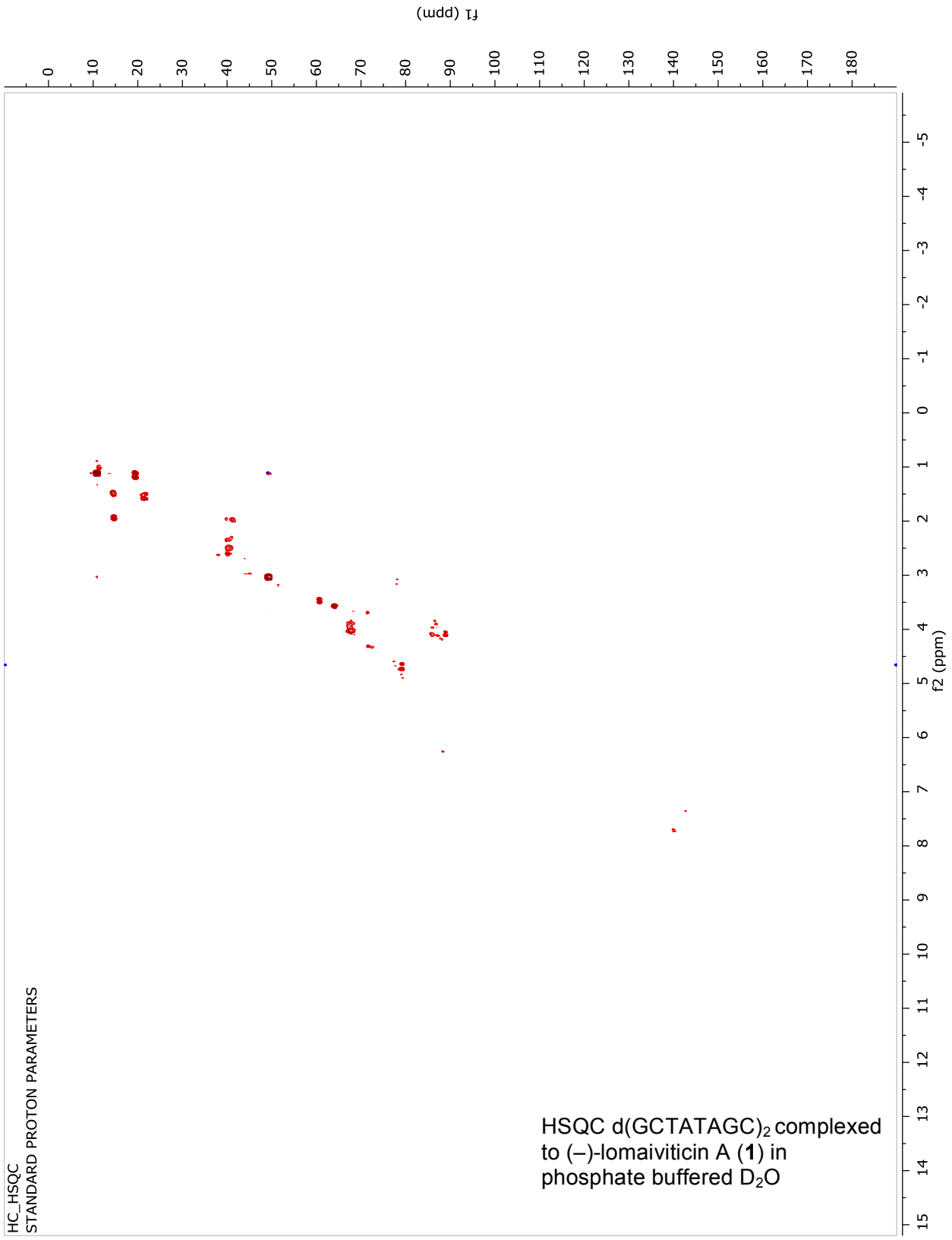

Proton_before2Ds
STANDARD PROTON PARAMETERS
^{1}H NMR d(GCTATAGC)$_2$ in
phosphate buffered D_2O + 1.00
equiv (–)-lomaiviticin A (**1**)
1.29
f1 (ppm)

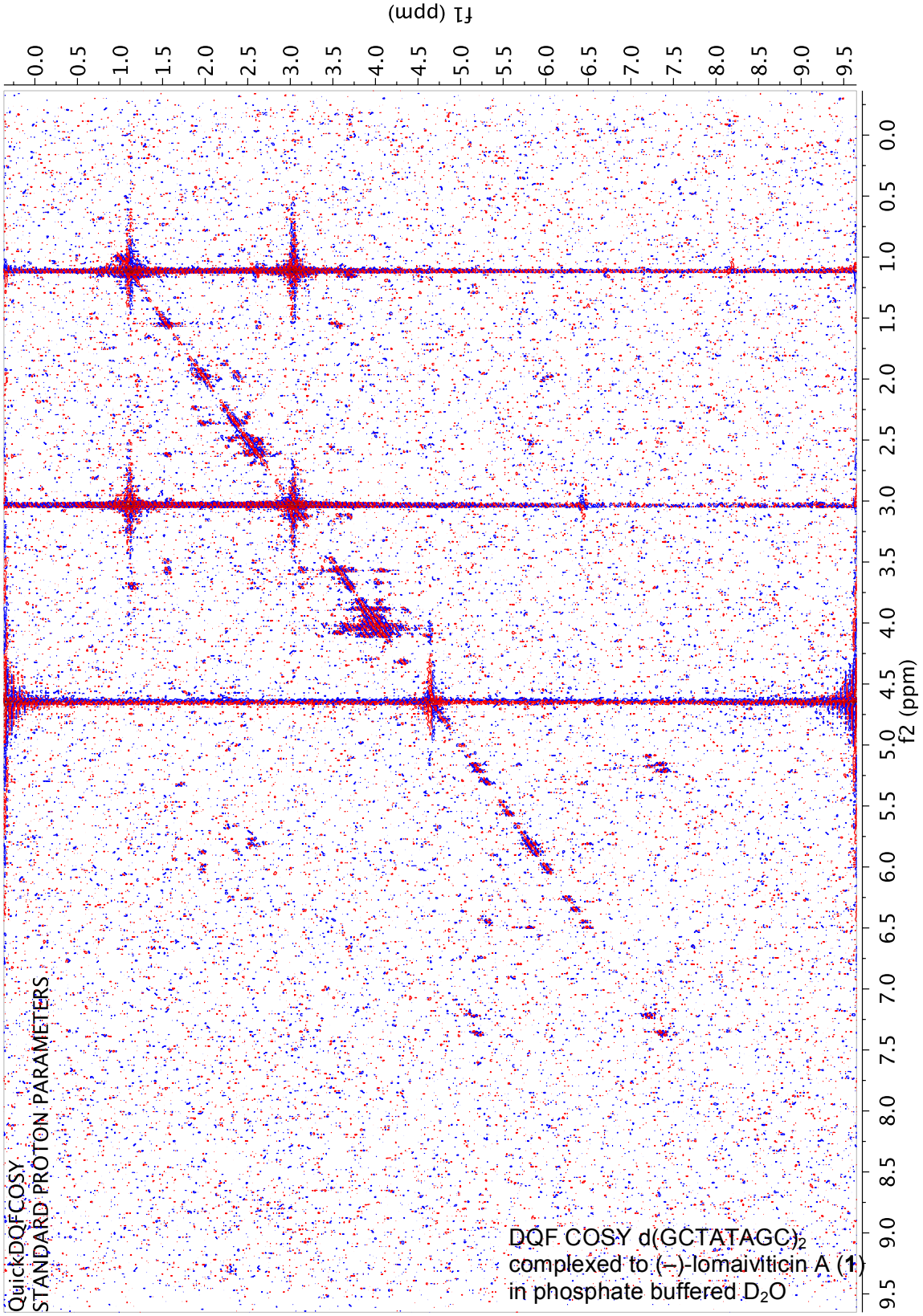

Proton_before2Ds
STANDARD PROTON PARAMETERS
^{1}H NMR d(GCTATAGC)$_2$ in
phosphate buffered D_2O + 1.00
equiv (–)-lomaiviticin A (**1**)
f1 (ppm)

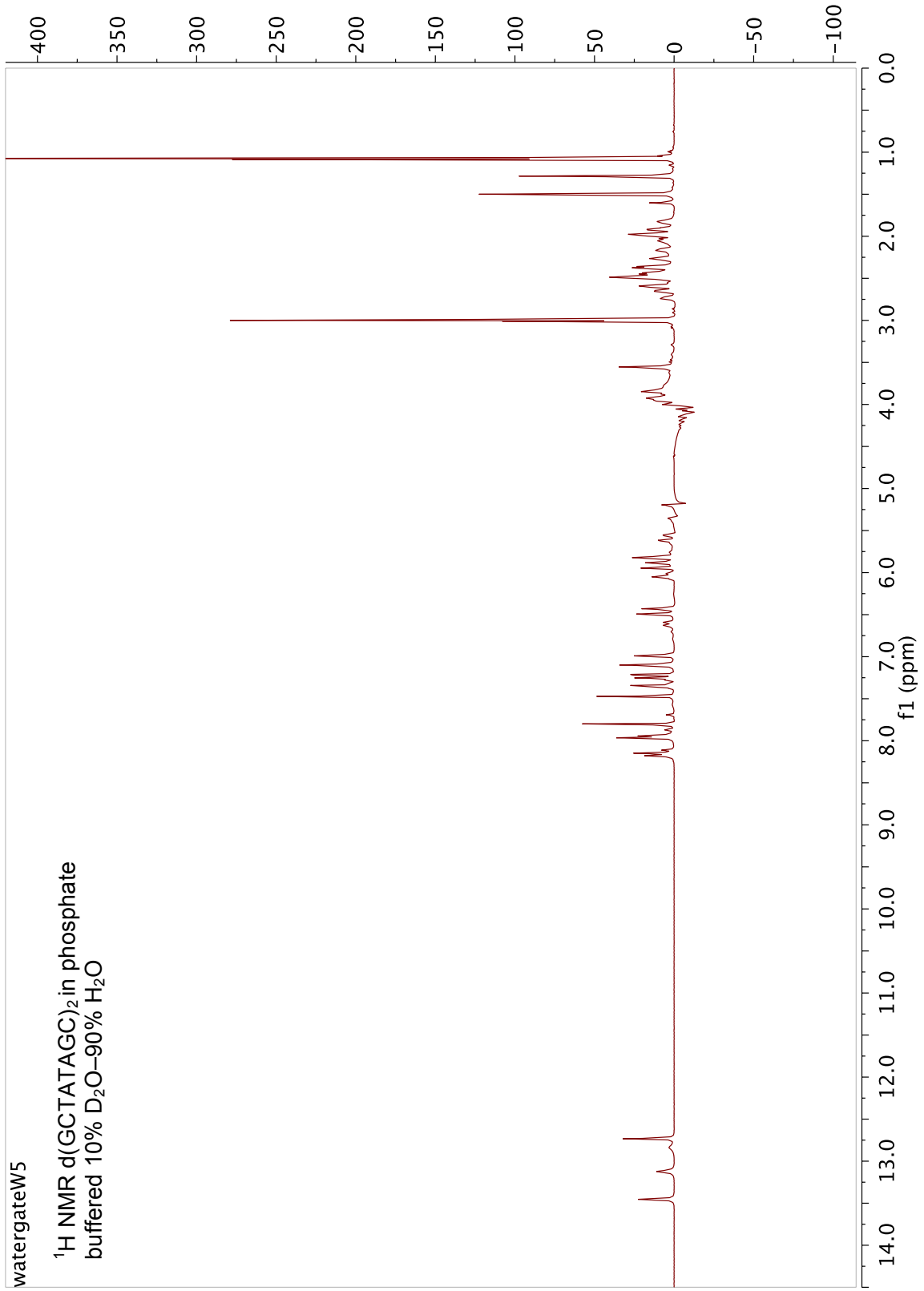

Proton_before2Ds
STANDARD PROTON PARAMETERS
^{1}H NMR d(GCTATAGC)$_2$ in
phosphate buffered D_2O + 1.00
equiv (–)-lomaiviticin A (**1**)
f1 (ppm)

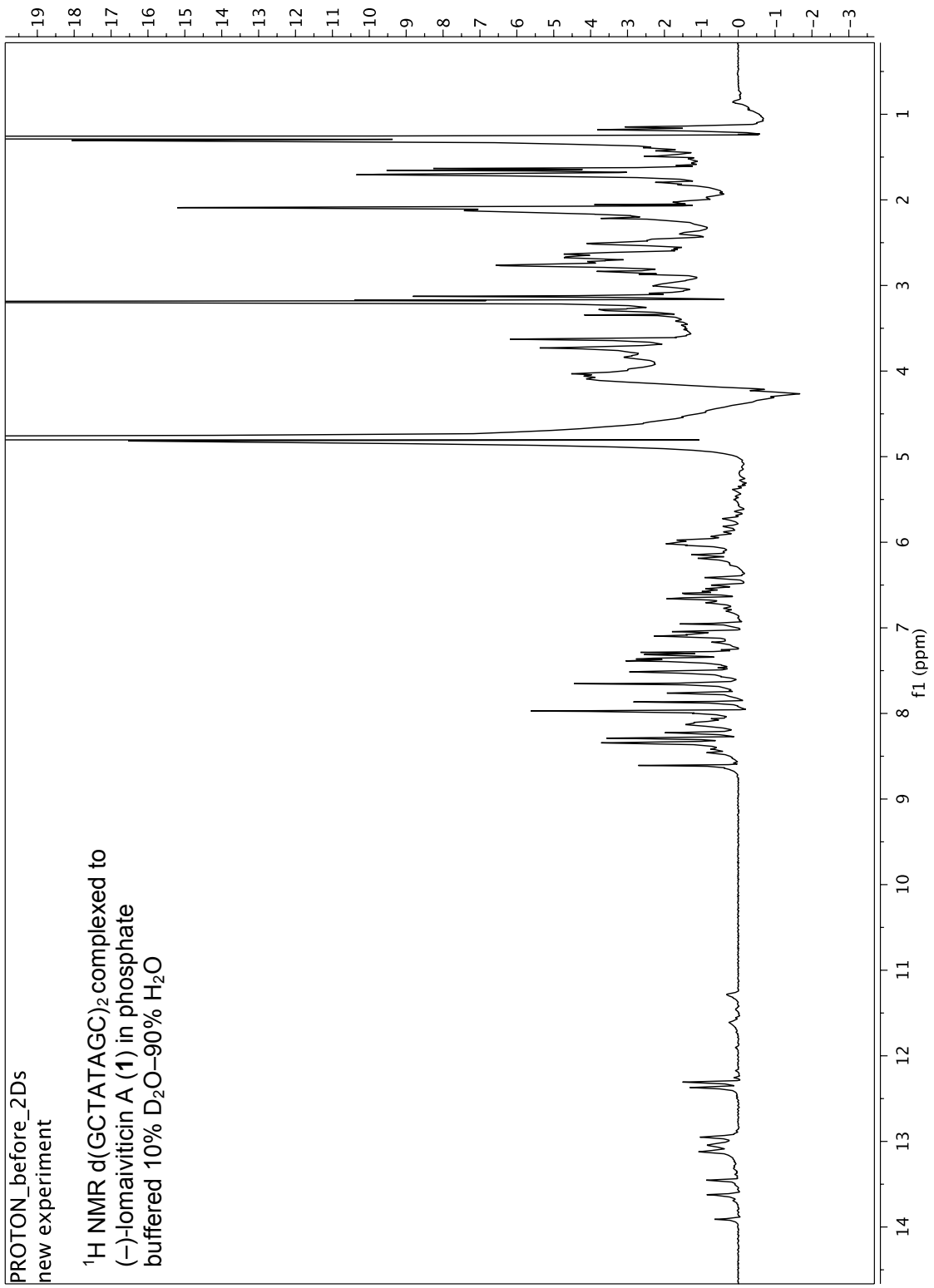

f1 (ppm)
f2 (ppm)
NOESY_100ms
STANDARD PROTON PARAMETERS
NOESY (100 ms) d(GCTATAGC)2
complexed to (–)-lomaiviticin A (1)
in phosphate buffered D2O

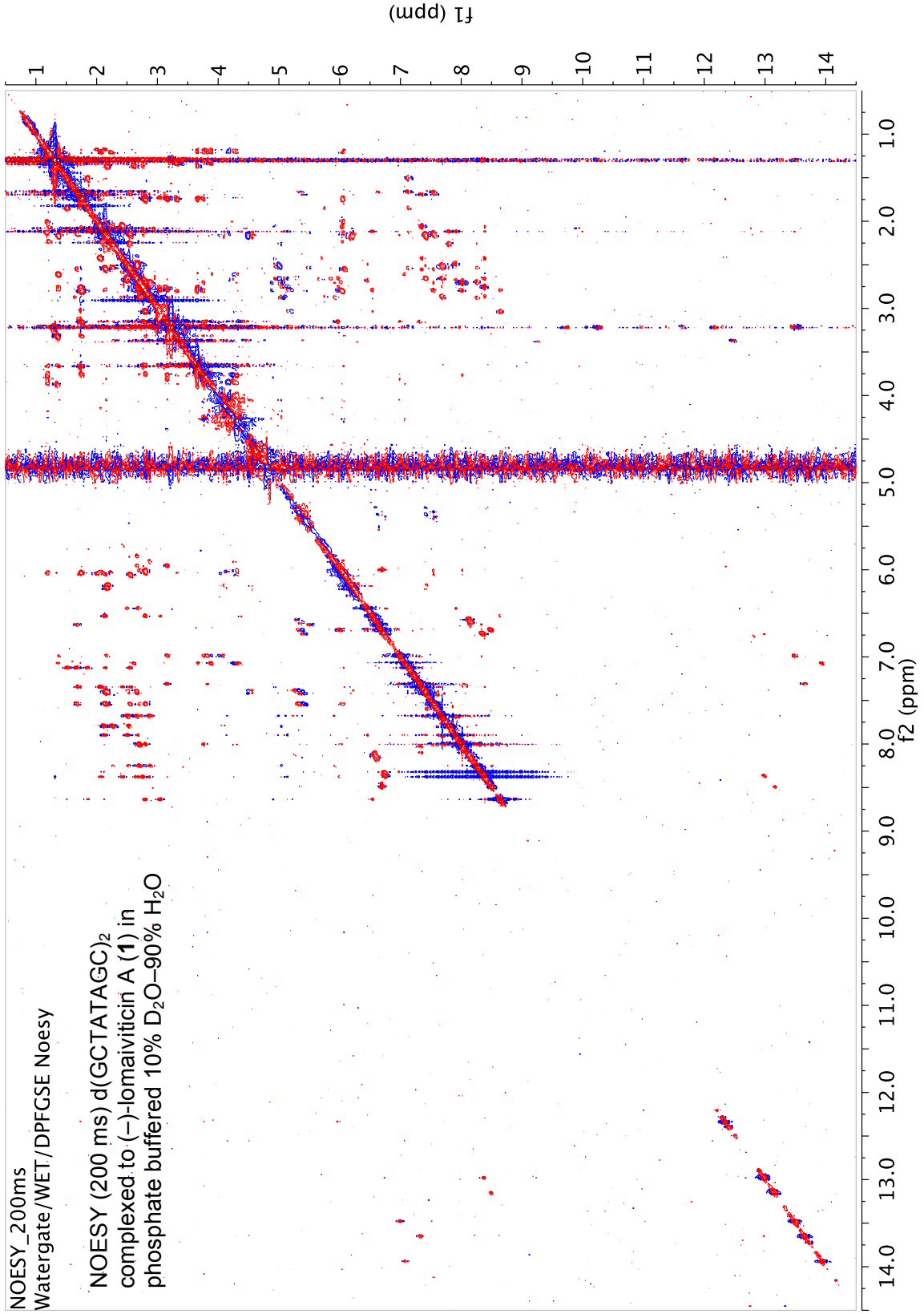

f1 (ppm)
f2 (ppm)
NOESY_200ms
STANDARD PROTON PARAMETERS
NOESY (200 ms) d(GCTATAGC)2
complexed to (–)-lomaiviticin A (1)
in phosphate buffered D2O


HC_HSQC
STANDARD PROTON PARAMETERS
HSQC d(GCTATAGC)2 complexed to (–)-lomaiviticin A (1) in phosphate buffered D2O
f1 (ppm)
f2 (ppm)


f1 (ppm)
f2 (ppm)
QuickDQFCOSY
STANDARD PROTON PARAMETERS
DQF COSY d(GCTATAGC)2
complexed to (–)-lomaiviticin A (1)
in phosphate buffered D2O


watergateW5
^{1}H NMR d(GCTATAGC)$_2$ in phosphate
buffered 10% D_2O–90% H_2O
400
350
300
250
200
150
100
50
0
−50
−100
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
f1 (ppm)


PROTON_before_2Ds
new experiment
^{1}H NMR d(GCTATAGC)$_2$ complexed to
(–)-lomaiviticin A (**1**) in phosphate
buffered 10% D_2O–90% H_2O
f1 (ppm)


NOESY_200ms
Watergate/WET/DPFGSE Noesy
NOESY (200 ms) d(GCTATAGC)2
complexed to (–)-lomaiviticin A (1) in
phosphate buffered 10% D2O–90% H2O
f1 (ppm)
f2 (ppm)

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