Mechanism of nucleophilic activation of (–)-lomaiviticin A

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J. Am. Chem. Soc.

Supporting Information

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Supplementary Figures.

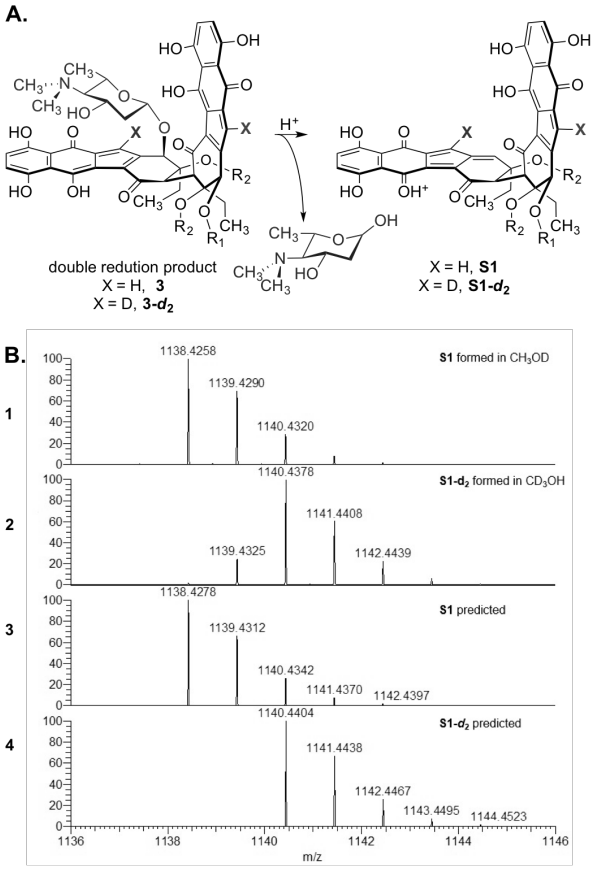


Figure S1. A. Ionization of **3** or **3-*d*$_2$** leads to ejection of the aminosugar residue and observation of the elimination product **S1** or **S1-*d*$_2$** by HRMS analysis. **B.** 1, 2: Selected region of the HRMS spectrum of **S1** or **S1-*d*$_2$**, generated by hydrodediazotization of **1** in CH_3OD or CD_3OH, respectively. 3, 4: Predicted isotope distribution of **S1** and **S1-*d*$_2$**, respectively. **S1**: $[M]^+ = C_{60}H_{68}NO_{21}^+$, calculated = 1138.4278, observed = 1138.4258, error = 1.76 ppm; **S1-*d*$_2$**: $[M]^+ = C_{60}H_{66}D_2NO_{21}^+$, calculated = 1140.4404, observed = 1140.4378, error = 2.28 ppm.

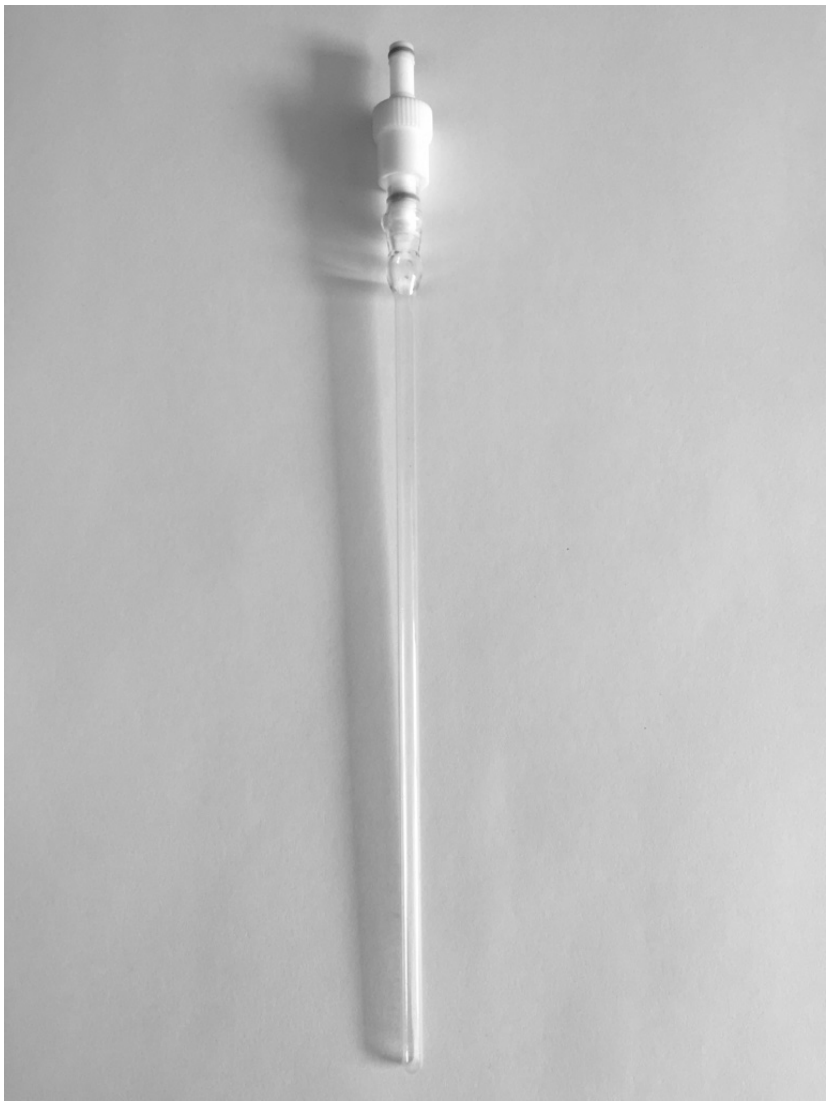


Figure S2. Modified 535PP 5 mm 600 MHz NMR tubes from Wilmad (Vineland, NJ) fused to CG-513 European style NMR tube valves purchased from Chemglass (Vineland, NJ) by Daryl Smith (Glassblower, Yale Chemistry Department).

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. All air-free NMR experiments were conducted in 535PP 5 mm 600 MHz NMR tubes (Wilmad, Vineland, NJ) fitted with a rubber septum under argon. All NMR experiments under air were conducted in modified 535PP 5 mm 600 MHz NMR tubes (Wilmad, Vineland, NJ) fused to CG-513 European style NMR tube valves purchased from Chemglass (Vineland, NJ) by Daryl Smith (Glassblower, Yale Chemistry Department, see Figure S2). Organic solutions were concentrated by rotary evaporation (<25 °C). Liquid reagents and solvents were transferred via gastight syringe (air-free experiments) or positive-displacement pipette (experiments under air). Normal and reverse phased flash-column chromatography was performed as described by Still et al.[1] Normal phase purifications employed silica gel (60 Å, 40–63 μm particle size) purchased from Sorbent Technologies (Atlanta, GA). Reverse phase purifications employed C_{18}-labeled silica gel (125 Å, 55–105 μm particle size) purchased from Waters Corporation (Milford, MA). Analytical thin-layered chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV) and/or submersion in aqueous potassium permanganate solution ($KMnO_4$), followed by brief heating on a hot plate (120 °C, 10–15 s).

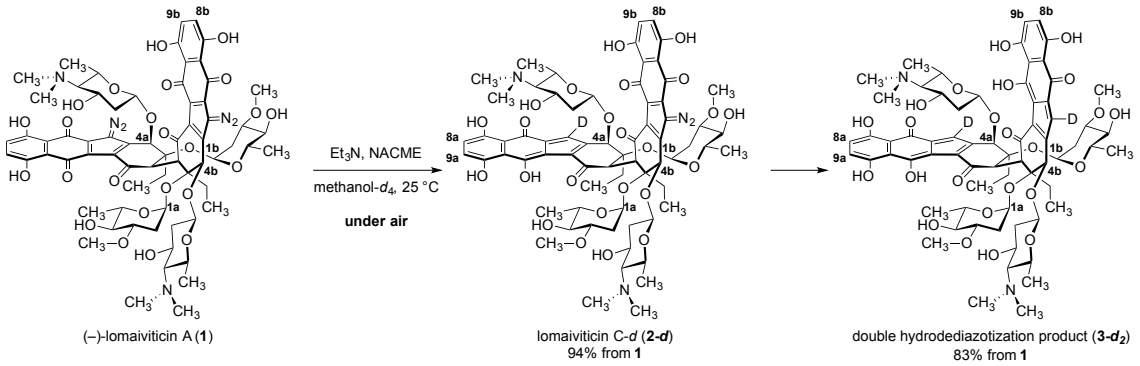
Chemical Materials. Commercial solvents and reagents were used as received with the following exceptions. Triethylamine was distilled from calcium hydride under an atmosphere of nitrogen immediately before use. (–)-Lomaiviticins A (**1**) and C (**2**) were prepared according to the procedures of Herzon and co-workers.[2] Methanol-d_4 and acetone-d_6 employed in air-free experiments were degassed by three freeze–pump–thaw cycles, vacuum transferred, and stored under argon.

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were collected in the Yale Chemical and Biophysical Instrumentation Center (CBIC) on a 500 MHz two-channel Agilent ProPulse NMR spectrometer equipped with a gradient OneNMR probe, at 25 °C, unless otherwise noted. Data was processed using the VnmrJ 4.2A software. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the residual protons in the NMR solvent (CHD_2OD, 3.31; $(CHD_2)(CD_3)CO$, 2.05). 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. Heteronuclear single quantum coherence spectra (HSQC) and heteronuclear multiple bond correlation spectra (HMBC) were recorded on the same instrument at –50 °C. Chemical shifts for carbon are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonance of the solvent ($(CHD_2)_2CO$, δ 206.7 and δ 29.9). Variable temperature (VT) NMR experiments employed nitrogen gas as the coolant (chilled by passing through an external sample of liquid nitrogen).

Samples were precooled to –80 °C in a dry ice bath before introduction into the precooled instruments. NMR data were acquired immediately after the temperature stabilized. High-resolution mass spectrometry (HRMS) was obtained at the Mass Spectrometry and Proteomics Resource of the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University (New Haven, CT). All HRMS samples were prepared in 2 mL Cert High Rec vials purchased from Thermo Scientific (Rockwood, TN). UPLC/HRMS analysis was performed on a Waters nanoAcquity UPLC system equipped with a Thermo Scientific Orbitrap Elite detector, a Waters Symmetry® C_{18} 180 µm × 20 mm trap column, and an ACQUITY UPLC PST (BEH) C_{18} nanoACQUITY column (1.7 µm, 75 µm × 250 mm), at 37 ºC. Trapping was performed at 5 µL/min, 97% water for 0.2 min. Samples were eluted at 300 nL/min with a linear gradient of 3%–90% acetonitrile–water over 10 min followed by 90% acetonitrile–water for an additional 4.5 min. Water and acetonitrile employed in UPLC/MS separations contained 0.1% formic acid. Mass spectra were acquired in the Orbitrap in profile mode scanning the 300–2,000 m/z range using 1 microscan, 120,000 resolution, AGC target of 1E7, and a full max ion time of 50 ms. Data was analyzed using the Thermo Xcalibur Qual Browser software (version 3.0.63). Alternatively, HRMS was obtained at the Yale West Campus (West Haven, CT) on an Agilent TOF/Q-TOF mass spectrometer equipped with an Agilent Eclipse Plus C_{18} column (4.6 µm × 50 mm, 1.8 µm partial size) and an Agilent 1290 LC Model pump (Santa Clara). Samples were eluted at 500 µL/min with a linear gradient of 0%–95% acetonitrile–water over 5 min at 25 ˚C. Water and acetonitrile employed in UPLC/MS separations contained 0.1% formic acid. Data was analyzed using the Agilent Mass Hunter Qualitative Analysis software (Version B.07.00).

Experiment Procedures.

*Hydrodediazotization of (–)-lomaiviticin A (**1**) in methanol-d_4 under air (Table 1, entries 1 and 3):*

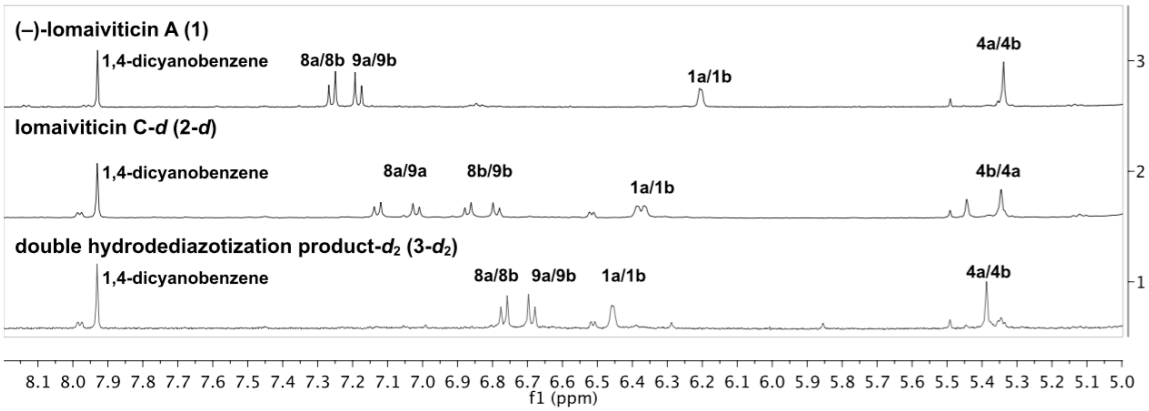


A solution of 1,4-dicyanobenzene in methanol-d_4 (1.56 mM, 50.0 µL, 78.0 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in methanol-d_4 (500 µL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. A solution of NACME in methanol-d_4 (586 mM, 50.0 µL, 29.3 µmol, 40.0 equiv) was then added. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition, and then subsequently every 20 min. After 22 h at 25 °C, triethylamine (4.1 µL, 29.3 µmol, 40.0 equiv) was added. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition, and then subsequently every 20 min. The yields of **1**, **2-*d***, and **3-*d*$_2$** were determined by integration of resolved peaks against 1,4-dicyanobenzene. Data were analyzed using Microsoft Excel and Prism 9.0.

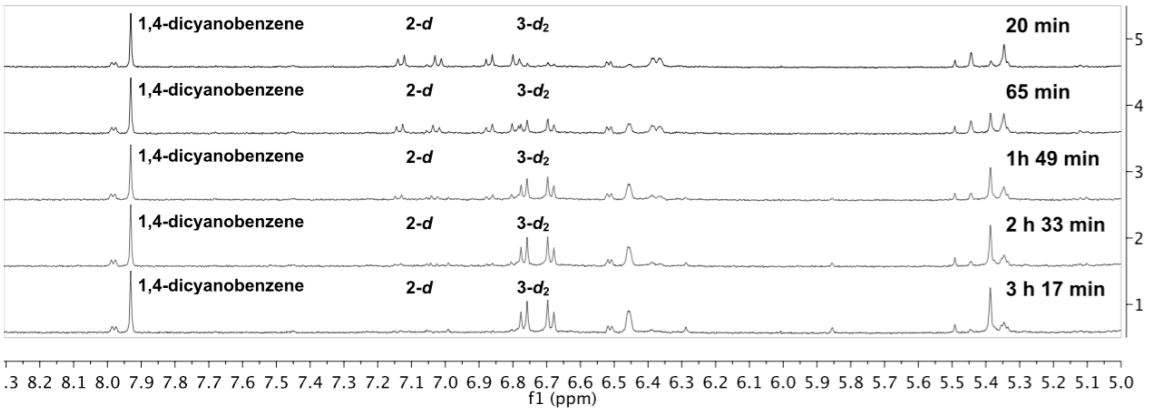
Table S1. Yields of **2-*d*** and **3-*d*$_2$** in the hydrodediazotization of **1** (methanol-d_4, air).

product	yield based on **1**	time
2-*d*	94%	<1 min
3-*d*$_2$	83%	$t_{1/2}$ = 49 min

Selected regions of the ^{1}H NMR spectra of **1**, **2**-***d***, and **3**-d_2.



Representative NMR spectra showing the conversion of **2**-***d*** to **3**-d_2.



The half-life ($t_{1/2}$) and pseudo first-order rate constant (k_{obs}) for the conversion of **2-*d*** to **3-d_2** were calculated according to the following equations:

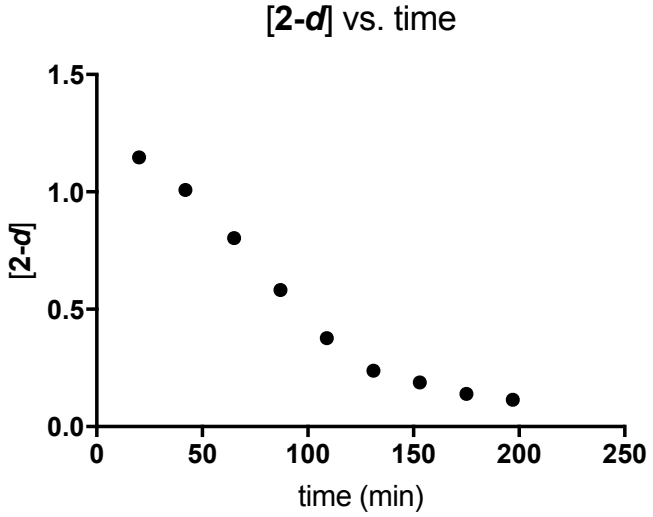
$$\ln[A] = -kt + [A]_0$$

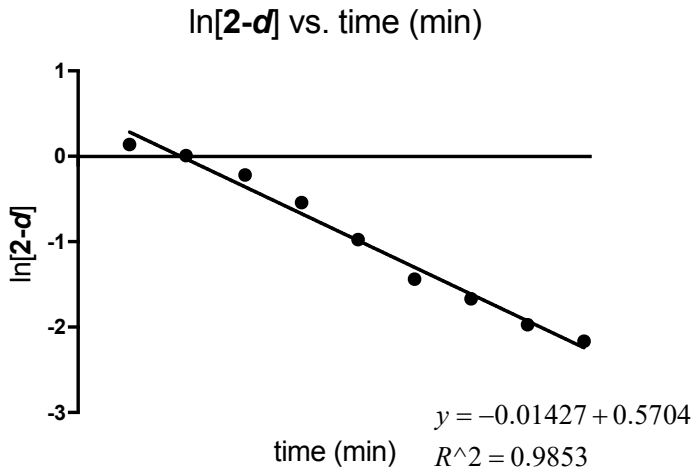
$$t_{1/2} = \frac{\ln(2)}{k}$$

(where $k = k_{obs}$)

Table S2. % of lomaiviticin C-*d* (**2-*d***) in solution as a function of time (methanol-d_4, air).

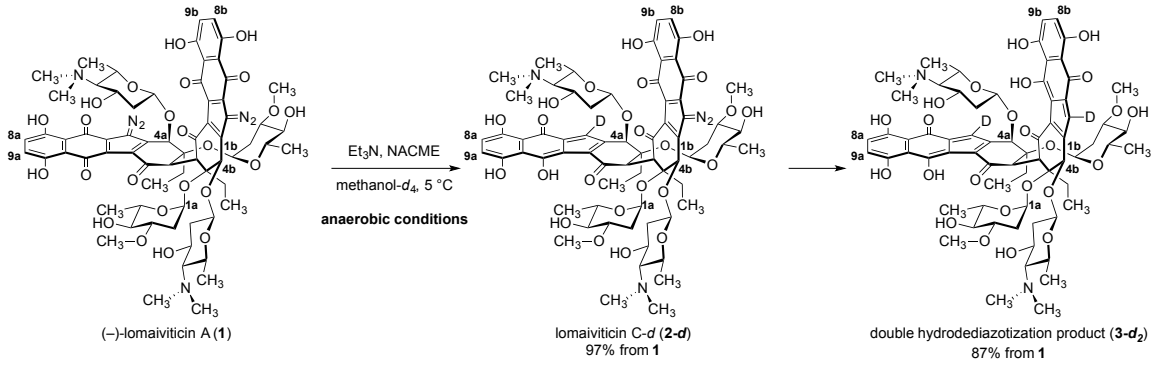
time (min)	% **2-*d*** remaining	[**2-*d***] (mM)	ln[**2-*d***]
20	100	1.1468	0.136975
42	87.86	1.007578	0.00755
65	70	0.80276	-0.219699
87	50.71	0.581542	-0.542072
109	32.86	0.376838	-0.975939
131	20.71	0.237502	-1.437578
153	16.43	0.188419	-1.669086
175	12.14	0.139222	-1.971689
197	10	0.11468	-2.16561





Therefore, k_{obs} = 0.01427 min^{-1}, and $t_{1/2}$ = 48.6 min.

*Hydrodediazotization of (–)-lomaiviticin A (**1**) in methanol-d_4 under anaerobic conditions:*



Note: The rate of hydrodediazotization of **2-*d*** under anaerobic conditions was somewhat faster than that obtained under air. Consequently, this experiment was performed at 5 °C to allow for an accurate determination of the half-life for the transformation of **2-*d*** to **3-d_2**.

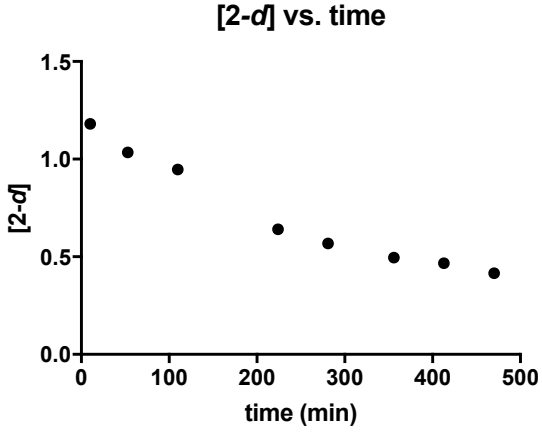
A solution of 1,4-dicyanobenzene in methanol-d_4 (7.79 mM, 20.0 µL, 155.8 nmol, 0.213 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in methanol-d_4 (530 µL) at 25 °C. A ^{1}H NMR spectra was acquired at 5 °C <5 min after mixing. A solution of NACME and triethylamine in methanol-d_4 (586 mM in each, 50.0 µL, 29.3 µmol of each, 40.0 equiv of each) was then added and the sample was returned to the NMR instrument. A ^{1}H NMR spectrum was acquired immediately after the temperature equilibrated to 5 ˚C, and then subsequently every 10 min at 5 ˚C for 8 h until 75% of **2-*d*** was converted to **3-d_2**. The sample was then warmed to 25 °C and a final spectrum was acquired after 5 h at 25 °C to determine the final percent conversion to **3-d_2**. The yields of **1**, **2-*d***, and **3-d_2** were determined by integration of resolved peaks against 1,4-dicyanobenzene. Data were analyzed using Microsoft Excel and Prism 9.0.

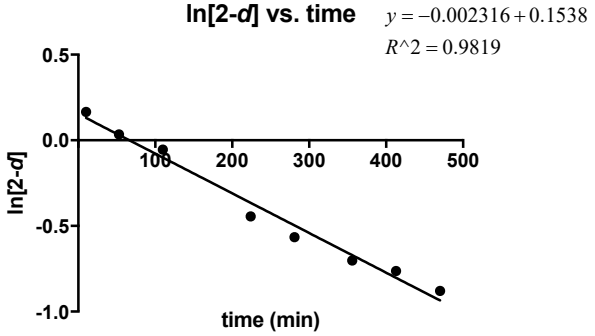
Table S3. Yields of **2-*d*** and **3-d_2** in the hydrodediazotization of **1** (methanol-d_4, anaerobic conditions).

product	final yield based on **1**	time
2-*d*	97%	<1 min
3-d_2	87%	$t_{1/2}$ = 4.99 h (5 ˚C)

Table S4. % of lomaiviticin C-*d* (**2-*d***) in solution as a function of time (methanol-d_4, anaerobic conditions).

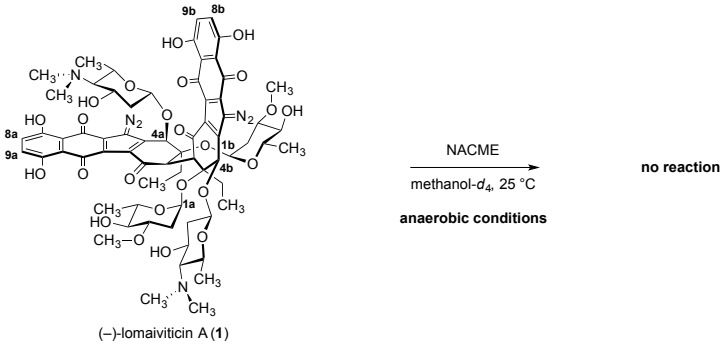
time (min)	% **2-*d*** remaining	[**2-*d***] (mM)	ln[**2-*d***]
10	100	1.1802	0.165684
53	87.6543	1.034496	0.033914
110	80.2469	0.947074	-0.05438
224	54.321	0.641096	-0.44458
281	48.1481	0.568244	-0.5652
355	41.9753	0.495392	-0.7024
413	39.5062	0.466252	-0.76303
470	35.1852	0.415256	-0.87886





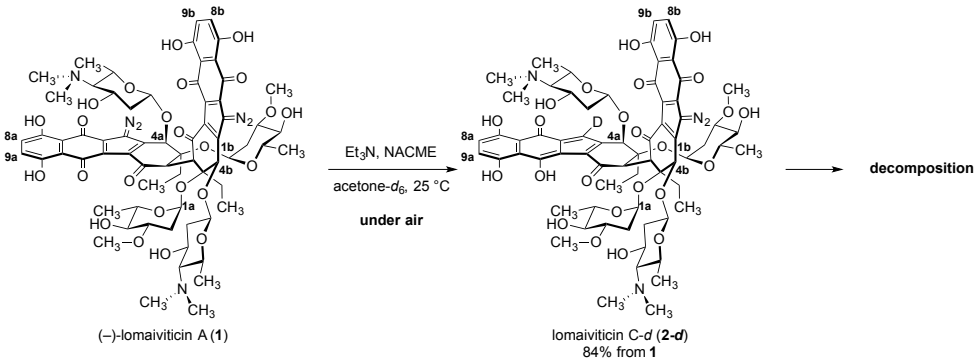
Therefore, k_{obs} = 0.002316 min^{-1}, and $t_{1/2}$ = 299 min (5 h) at 5 ˚C.

*Evaluation of the stability of (–)-lomaiviticin A (**1**) toward N-acetyl-L-cysteine methyl ester in methanol-d_4 under anaerobic conditions:*



A solution of 1,4-dicyanobenzene in methanol-d_4 (7.79 mM, 20.0 μL, 155.8 nmol, 0.213 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in methanol-d_4 (530 μL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. A solution of NACME in methanol-d_4 (586 mM, 50.0 μL, 29.3 μmol, 40.0 equiv) was then added. A ^{1}H NMR spectrum (64 scans) immediately acquired. The mixture was monitored by NMR spectroscopy for 8 h at 25 °C. The yields of **1** and **2-*d*** were determined by integration of resolved peaks against 1,4-dicyanobenzene. After 8 h at 25 °C, 94% of **1** remained; **2-*d*** was not detected.

*Hydrodediazotization of (–)-lomaiviticin A (**1**) in acetone-d_6 (Table 1, entries 2 and 4):*

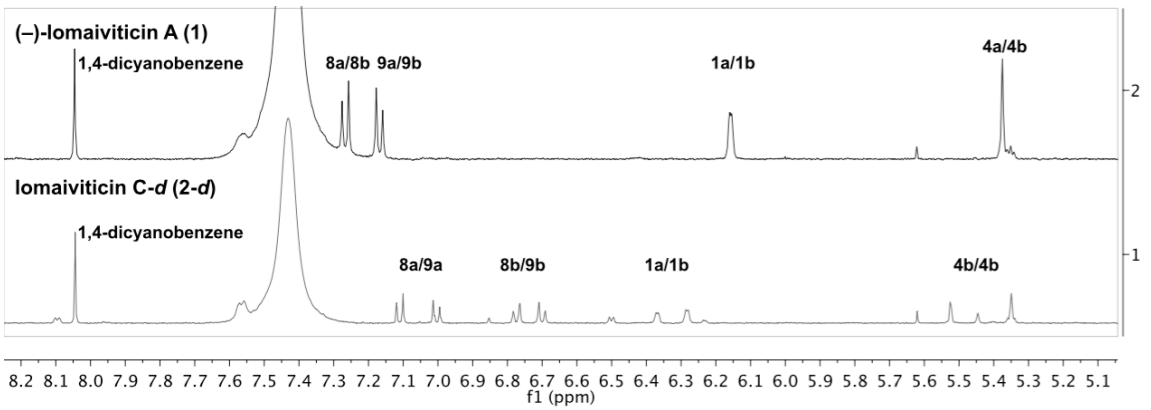


A solution of 1,4-dicyanobenzene in methanol-d_4 (1.56 mM, 50.0 μL, 78.0 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in acetone-d_6 (500 μL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. A solution of NACME in acetone-d_6 (586 mM, 50.0 μL, 29.3 μmol, 40 equiv) was then added. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition, and then subsequently every 20 min. After 22 h at 25 °C, triethylamine (4.1 μL, 29.3 μmol, 40 equiv) was added. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition, and then subsequently every 20 min for an additional 10 h. Additional ^{1}H NMR spectra were acquired at 36 h and 80 h. The yields of **1** and **2-*d*** were determined by integration of resolved peaks against 1,4-dicyanobenzene.

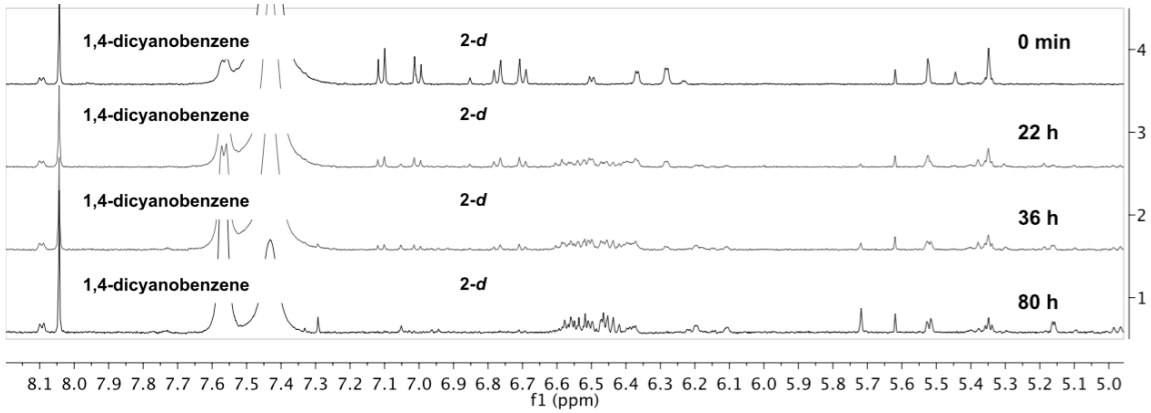
Table S5. Yields of **2-*d*** and **3-d_2** in the hydrodediazotization of **1** (acetone-d_6, air).

product	yield based on **1**	time
2-*d*	84%	<1 min
3-d_2	not observed	–

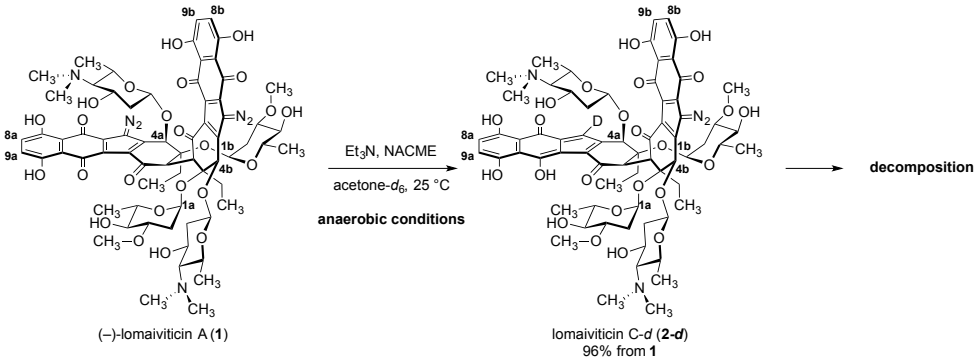
Selected regions of the ^{1}H NMR spectra of **1** and **2-*d***.



Representative NMR spectra showing the decomposition of **2-*d***.



*Hydrodediazotization of (–)-lomaiviticin A (**1**) in acetone-d_6 under anaerobic conditions:*

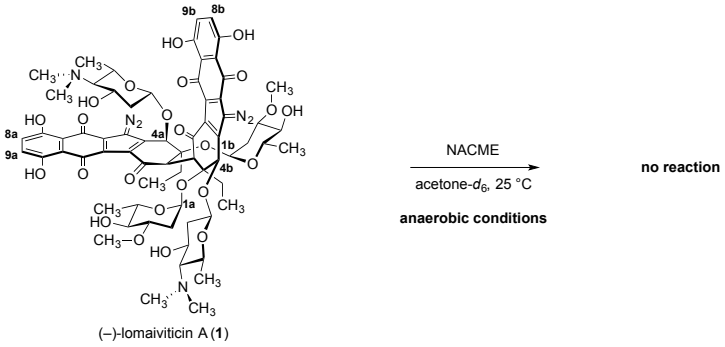


A solution of 1,4-dicyanobenzene in acetone-d_6 (7.79 mM, 20.0 μL, 155.8 nmol, 0.213 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in acetone-d_6 (530 μL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. A solution of NACME and triethylamine in acetone-d_6 (586 mM in each, 50.0 μL, 29.3 μmol of each, 40.0 equiv of each) was then added. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition at 25 ˚C, and then subsequently every 20 min for 12 h. Additional ^{1}H NMR spectra were acquired after 22, 31, and 46 h at 25 °C. The yields of **1** and **2-*d*** were determined by integration of resolved peaks against 1,4-dicyanobenzene.

Table S6. Yields of **2-*d*** and **3-d_2** in the hydrodediazotization of **1** (acetone-d_6, anaerobic conditions).

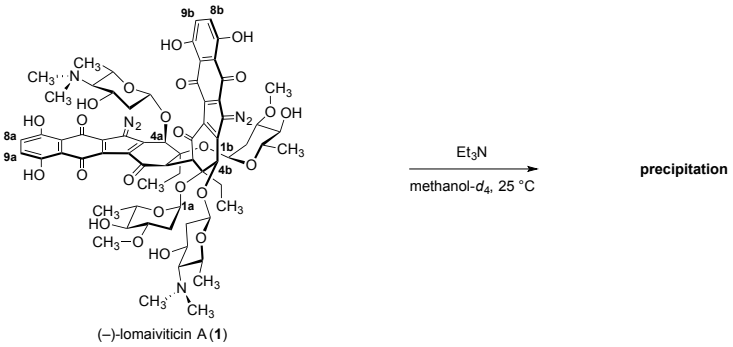
product	yield based on **1**	time
2-*d*	96%	<1 min
3-d_2	not observed	–

*Evaluation of the stability of (–)-lomaiviticin A (**1**) toward N-acetyl-L-cysteine methyl ester in acetone-d_6 under anaerobic conditions:*



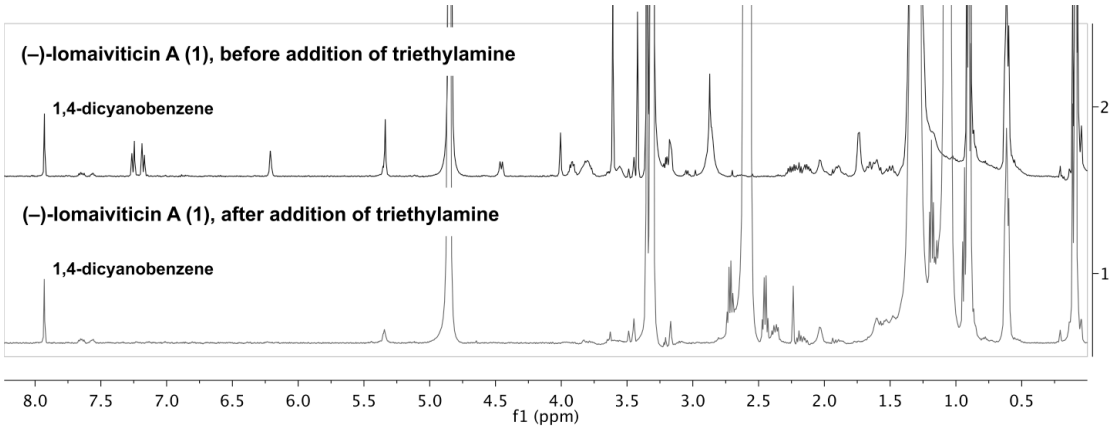
A solution of 1,4-dicyanobenzene in acetone-d_6 (7.79 mM, 20.0 µL, 155.8 nmol, 0.213 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in acetone-d_6 (530 µL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. A solution of NACME in acetone-d_6 (586 mM, 50.0 µL, 29.3 µmol, 40.0 equiv) was then added. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition at 25 ˚C, and then subsequently every 10 min for 8 h. A final spectrum was acquired after 12 h at 25 °C. The yields of **1** and **2-*d*** were determined by integration of resolved peaks against 1,4-dicyanobenzene. After 12 h at 25 °C, 92% of **1** remained; **2-*d*** was not detected.

*Evaluation of the stability of (–)-lomaiviticin A (**1**) toward triethylamine in methanol-d_4:*

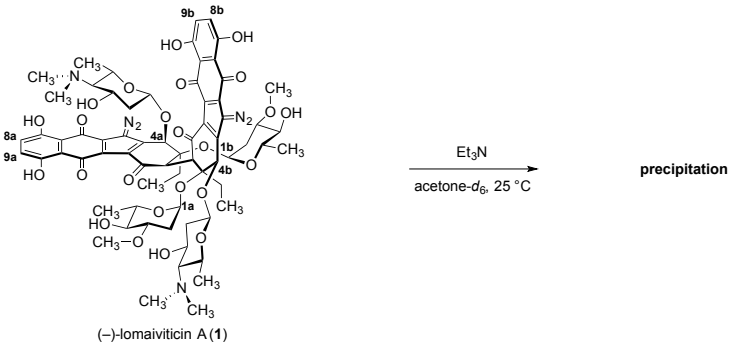


A solution of 1,4-dicyanobenzene in methanol-d_4 (1.56 mM, 50.0 µL, 78.0 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in methanol-d_4 (550 µL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. Triethylamine (4.1 µL, 29.3 µmol, 40.0 equiv) was then added. The red solution immediately turned deep blue, and formation of a precipitate was observed. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition of base, but **1** was not detected.

Selected region of the NMR spectra before and after the addition of triethylamine:

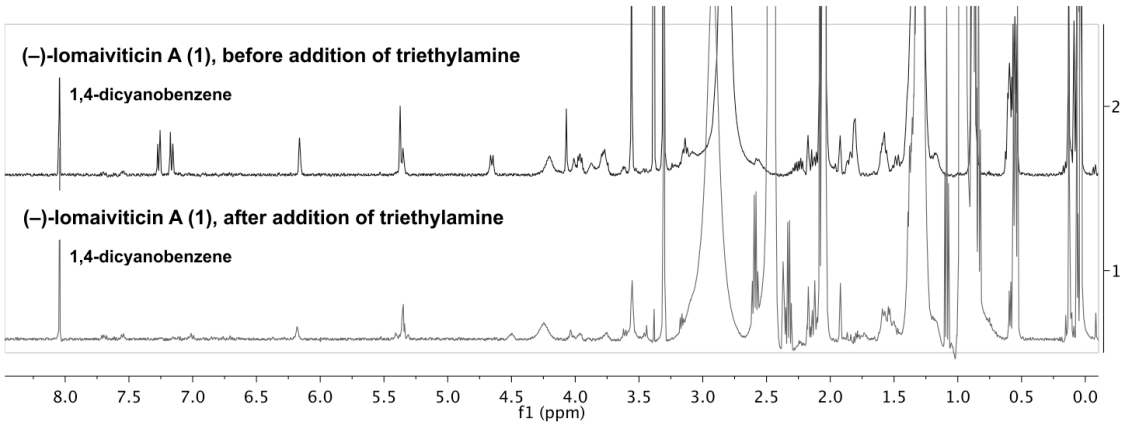


*Evaluation of the stability of (–)-lomaiviticin A (**1**) toward triethylamine in acetone-d_6:*

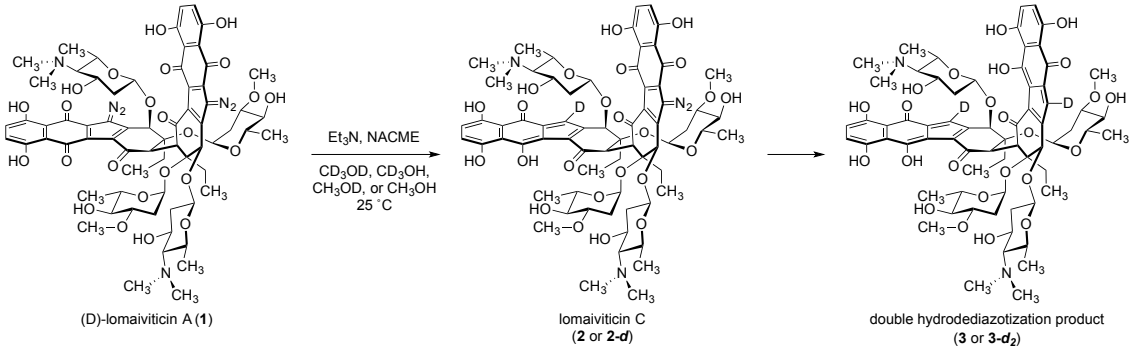


A solution of 1,4-dicyanobenzene in acetone-d_6 (1.56 mM, 50.0 µL, 78.0 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in acetone-d_6 (550 µL) at 25 °C. A ^{1}H NMR spectra was acquired at 25 °C <5 min after mixing. Triethylamine (4.1 µL, 29.3 µmol, 40.0 equiv) was then added. The red solution immediately turned deep blue, and formation of a precipitate was observed. A ^{1}H NMR spectrum (64 scans) was acquired immediately after the addition of base, but **1** was not detected.

Selected region of the NMR spectra before and after the addition of triethylamine:



*Hydrodediazotization of (–)-lomaiviticin A (**1**) in methanol (CH_3OH), methanol-d_1 (CH_3OD), methanol-d_3 (CD_3OH), or methanol-d_4 (CD_3OD).*



General experimental procedure:

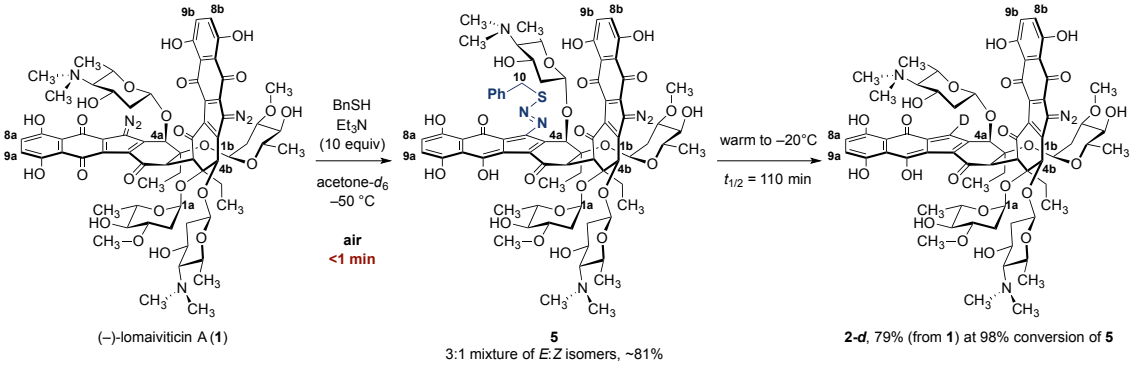
A solution of NACME and triethylamine (81.8 μM in each component, 50.0 μL, 4.10 μmol of each, 40.0 equiv of each) in methanol (CH_3OH), methanol-d_1 (CH_3OD), methanol-d_3 (CD_3OH), or methanol-d_4 (CD_3OD) was added to a solution of (–)-lomaiviticin A (**1**, 0.14 mg, 102 nmol, 1 equiv) in the same solvent (500 μL) at 25 °C. The final volume of the reaction mixture was 550 μL, and the mixture was allowed to stand at 25 °C. After 4 d at 25 °C, an aliquot (2 μL) of the remaining reaction mixture was removed and diluted with a solution of 0.1% formic acid in water (v/v, 36 μL). The diluted solution was then analyzed by UPLC–HRMS.

The retention times of **2**/**2-*d*** and **3**/**3-d_2** are 13.93 and 16.98 min, respectively.

Table S7. UPLC/HRMS analysis of the hydrodediazotization studies.

compound	solvent	observed product	observed mass	predicted mass	error in ppm
2	CH_3OH	**4**	1164.4169	1164.4183	1.20
2	CH_3OD	**4**	1164.4162	1164.4183	1.80
2-*d*	CD_3OH	**4-*d***	1165.4222	1165.4246	2.06
2-*d*	CD_3OD	**4-*d***	1165.4220	1165.4246	2.23
3	CH_3OD	**S1**	1138.4258	1138.4278	1.76
3-d_2	CD_3OH	**S1-d_2**	1140.4378	1140.4404	2.28

*Addition of benzylthiol to (–)-lomaiviticin A (**1**) under air:*



A solution of 1,4-dicyanobenzene in acetone-d_6 (1.56 mM, 50.0 µL, 78 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 1.0 mg, 730 nmol, 1 equiv) in acetone-d_6 (500 µmol) at 25 °C. The resulting mixture was cooled to –50 ˚C and then transferred to an NMR spectrometer that had been precooled to –50 °C. A ^{1}H NMR spectrum was then acquired. The sample was then removed from the spectrometer and cooled to –78 °C. A solution of benzylthiol and triethylamine in acetone-d_6 (146 mM in each, 50.0 µL, 7.3 µmol of each, 10.0 equiv of each) was then added to the cold sample. The sample was transferred to an NMR spectrometer that had been precooled to –50 °C, and the sample was allowed to equilibrate to –50 °C in the NMR spectrometer (<5 min). ^{1}H spectra were taken every 10 min for 1 h to monitor the isomerization of **5** (initially, 1:1 *E:Z*, equilibrating to 3:1 *E:Z*). Once a 3:1 ratio of *E:Z* isomers was achieved, no further isomerization was observed, and COSY (256 t1 increments, 8 scans per increment), and HMBC (200 t1 increments, 50 scans per t1 increment) spectra were obtained. Upon completion of the acquisition, the sample was warmed up to –20 °C, and a ^{1}H NMR spectrum was acquired every 10 min. The conversion to **2-*d*** was >80% complete in 4 h 40 min. After this time, the sample was warmed to 25 °C and a final ^{1}H NMR spectrum was acquired at 25 °C. The final % conversion of **5** to **2-*d*** was 98% after warming to 25 °C.

Table S8. Yields of **5** and **2-*d*** (acetone-d_6, air).

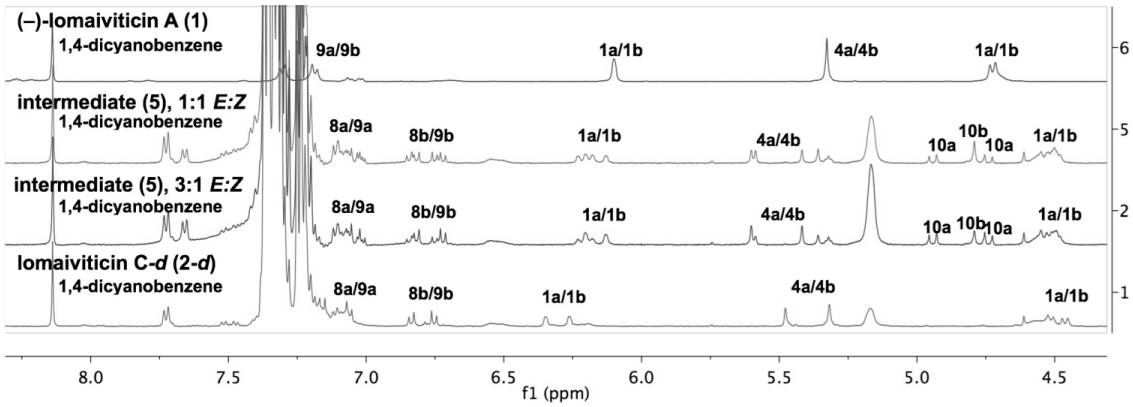
product	yield based on **1**	time
5	81%	immediate
2-*d*	79% (at 98% conversion of **5**)	$t_{1/2}$ = 110 min

An HSQC spectrum of **5** was acquired under slightly modified conditions:

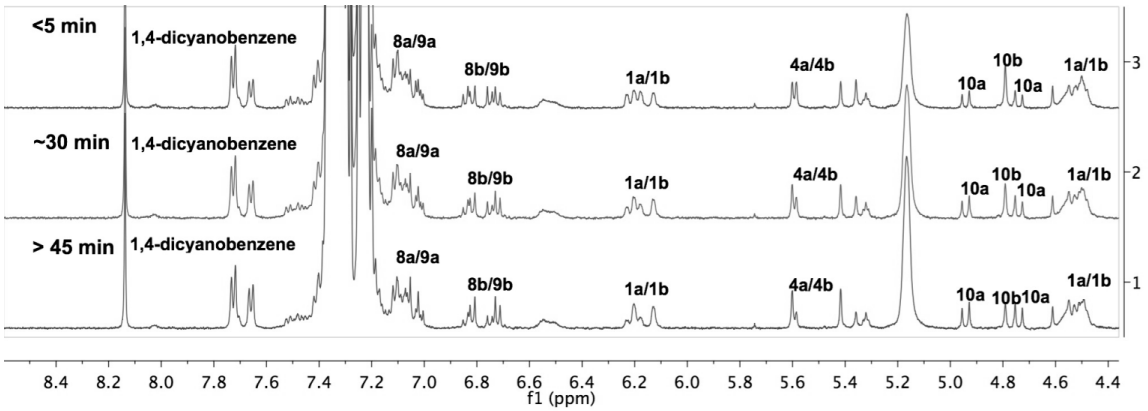
A solution of 1,4-dicyanobenzene in acetone-d_6 (15.6 mM, 5.0 µL, 78 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 2.0 mg, 1.46 µmol, 1 equiv) in acetone-d_6 (500 µmol)

at 25 °C. The resulting mixture was cooled to –50 ˚C and transferred to an NMR spectrometer that had been precooled to –50 °C. A ^{1}H NMR spectrum was then acquired. The sample was then removed from the spectrometer and cooled to –78 °C. A solution of benzylthiol and triethylamine in acetone-d_6 (146 mM in each, 20.0 μL, 2.92 μmol of each, 2.00 equiv of each) was then added to the cold sample. The sample was transferred to an NMR spectrometer that had been precooled to –50 °C, and the sample was allowed to equilibrate to –50 °C in the NMR spectrometer (<5 min). Immediately after the internal temperature reached –50 °C (<5 min), a ^{1}H spectra was taken to verify the formation of **5**, and then the sample was carefully monitored until a 3:1 ratio of *E* and *Z* isomers was formed (~ 1 h). A HSQC (200 t1 increments, 32 scans per increment) NMR spectra was then immediately acquired.

Selected regions of the ^{1}H NMR spectra of **1**, **5**, and **2-*d***.



Selected regions of the ^{1}H NMR spectra in the isomerization of **5**.



Representative NMR spectra showing the conversion of **5** to **2-*d***.

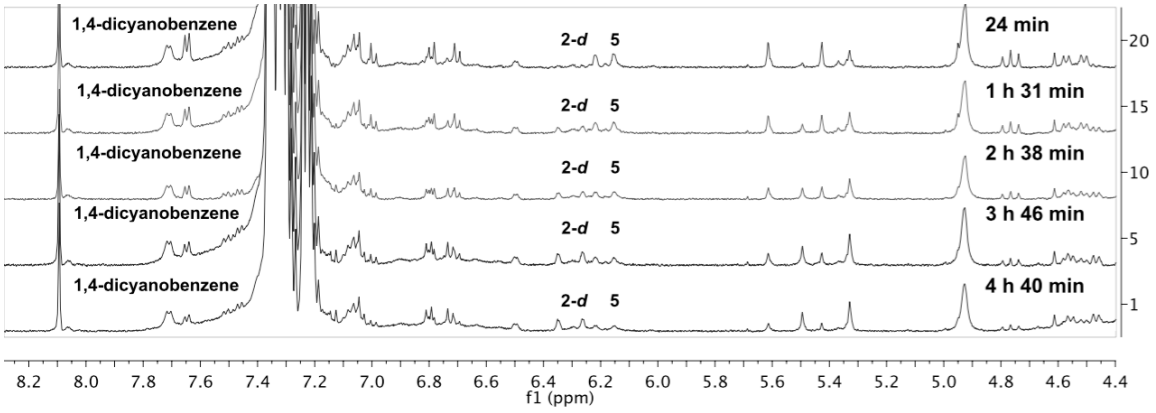
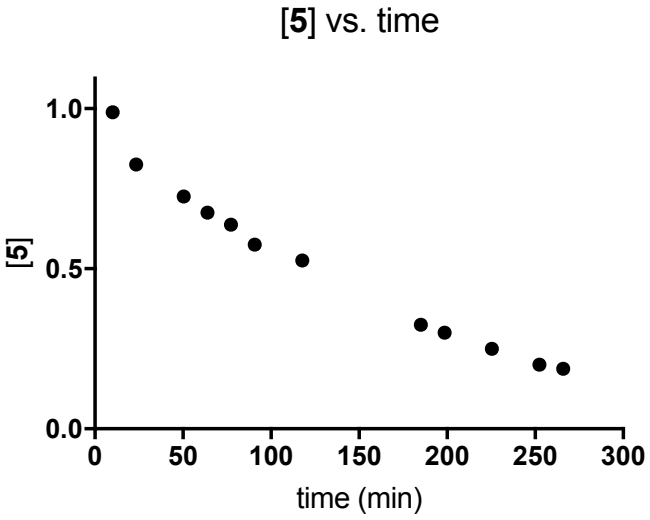
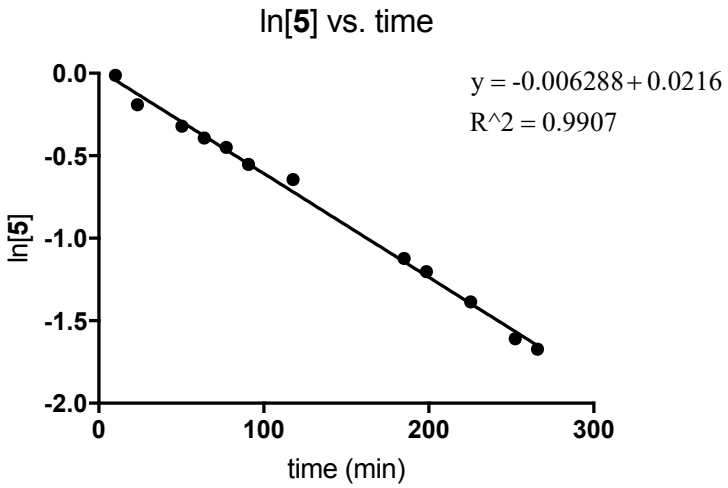


Table S9. % of **5** remaining in solution as a function of time (air).

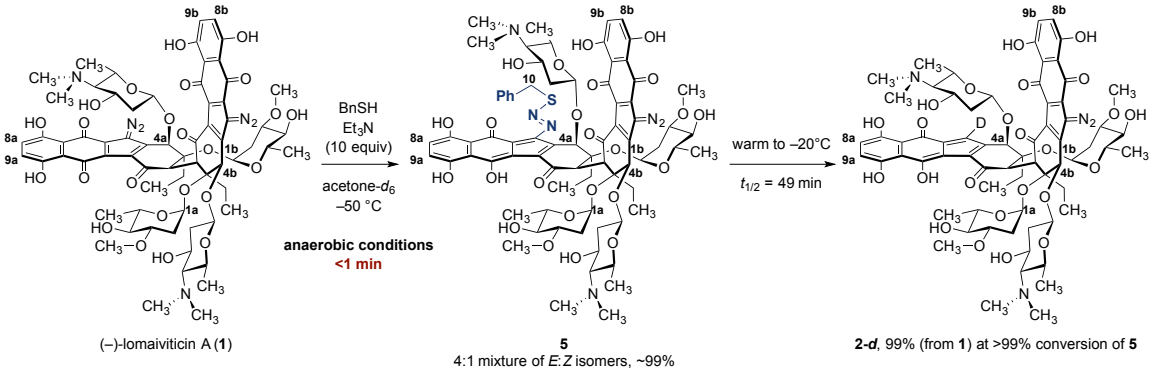
time (min)	% 5 remaining	[5] (mM)	ln[5]
10	100	0.9882	-0.011870173
24	83.544	0.82558481	-0.191663283
51	73.418	0.725513924	-0.320875014
64	68.354	0.675478481	-0.392333978
77	64.557	0.637951899	-0.449492392
91	58.228	0.575407595	-0.552676629
118	53.165	0.525372152	-0.643648407
185	32.911	0.32523038	-1.123221487
199	30.38	0.300212658	-1.203264195
226	25.316	0.250177215	-1.385585751
252	20.253	0.200141772	-1.608729303
266	18.987	0.187632911	-1.673267824





Therefore, k_{obs} = 0.006288 min^{-1}, and $t_{1/2}$ = 110 min.

*Addition of benzylthiol to (–)-lomaiviticin A (**1**) under anaerobic conditions:*



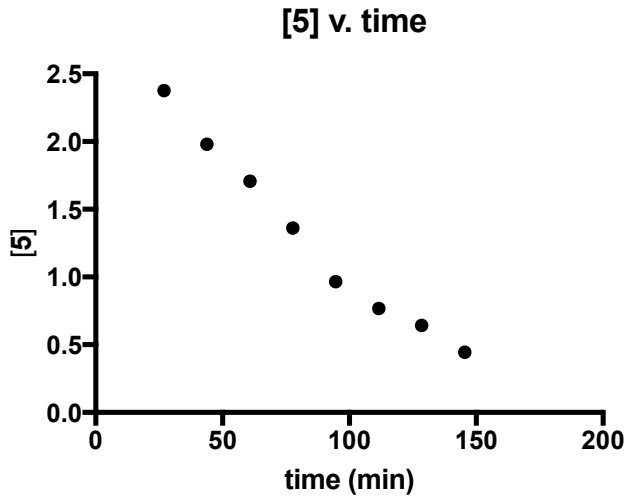
A solution of 1,4-dicyanobenzene in acetone-d_6 (7.79 mM, 20.0 µL, 155.8 nmol, 0.11 equiv) was added to a solution of (–)-lomaiviticin A (**1**, 2.0 mg, 1.46 µmol, 1 equiv) in acetone-d_6 (530 µL) at 25 °C, and the resulting mixture was cooled to –50 °C in an NMR spectrometer. A ^{1}H NMR spectra was acquired at –50 ˚C. A solution of benzylthiol and triethylamine in acetone-d_6 (1.17 M in each, 12.5 µL, 14.6 µmol of each, 10.0 equiv of each) was then added, and the mixture was immediately cooled to –50 °C. Once the temperature was equilibrated at –50 °C(<3 min), a ^{1}H NMR spectrum (64 scans) was acquired. Additional ^{1}H NMR spectra were acquired every 10 min for 5 h to monitor the isomerization and stability of **5** at –50 °C (initially, 1:1 *E:Z*, equilibrating to 4:1 *E:Z*). Once a 4:1 ratio of *E:Z* isomers was achieved, and no further reaction was observed, the sample was warmed to –20 °C, and a ^{1}H NMR spectrum was acquired every 10 min for 8 h. The conversion to **2-*d*** was >75% complete within 2 h. After this time, the sample was warmed to 25 °C and a final ^{1}H NMR spectrum was acquired at 25 °C. The final % conversion of **5** to **2-*d*** was >99% after warming to 25 °C.

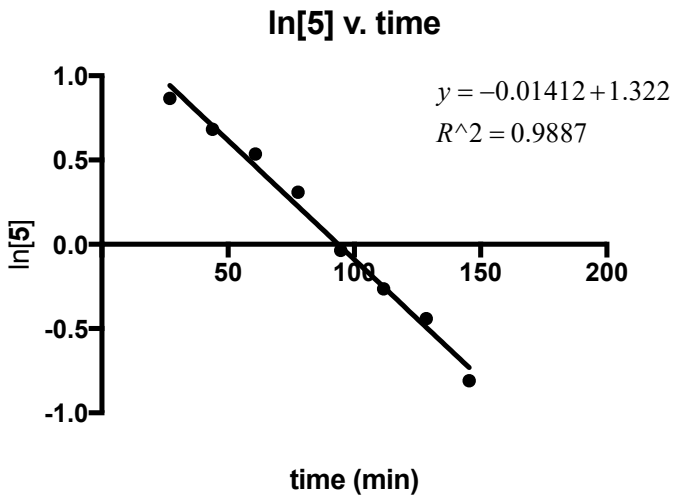
Table S10. Yields of **5** and **2-*d*** (acetone-d_6, anaerobic conditions).

product	yield based on **1**	time
5	>99%	immediate
2-*d*	>99%	$t_{1/2}$ = 49 min

Table S11. % of **5** remaining in solution as a function of time (anaerobic conditions).

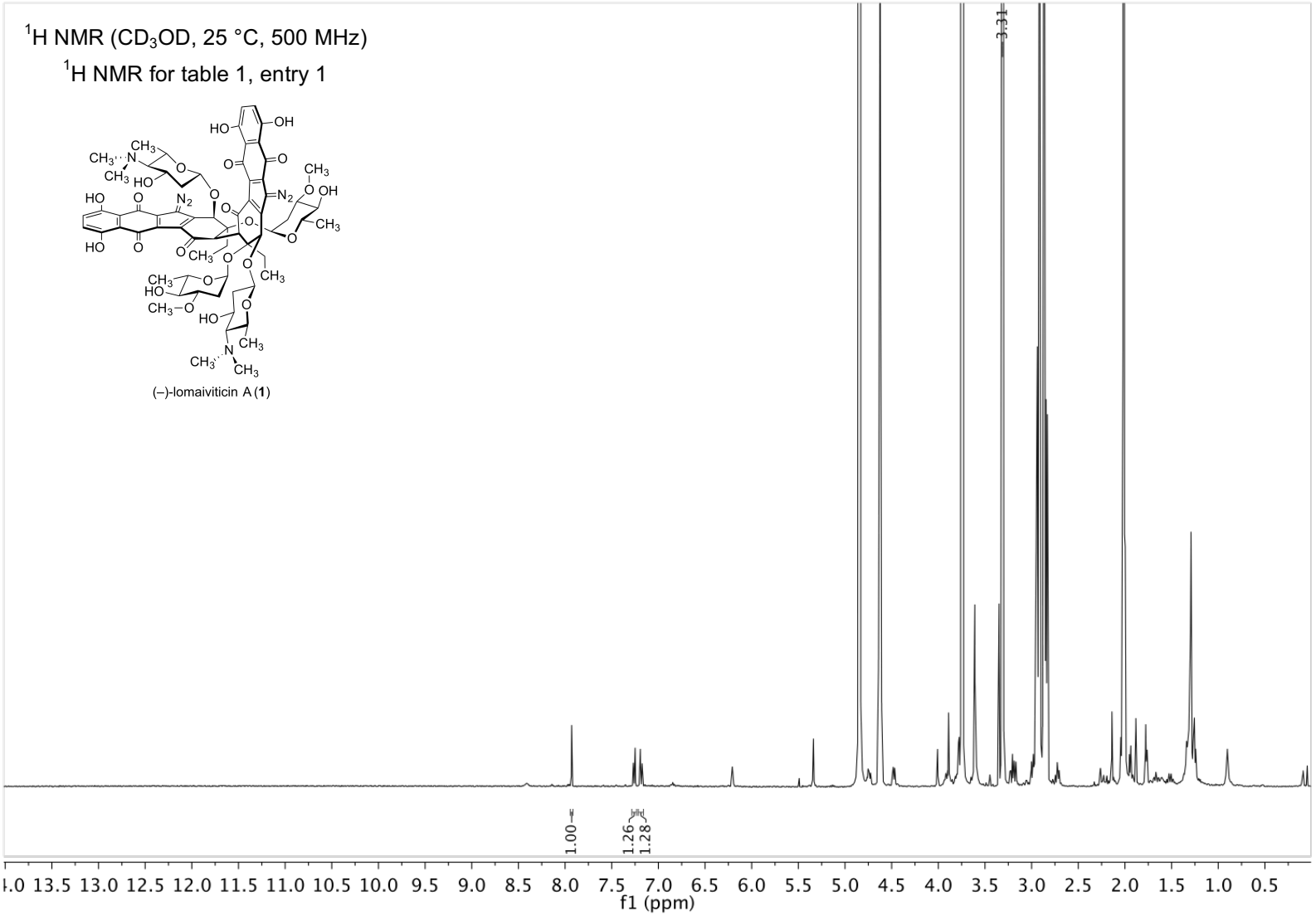
time (min)	% 5 remaining	[5] (mM)	ln[5]
26.93333333	87.27272727	2.376	0.865418402
43.86666667	72.72727273	1.98	0.683096845
60.79983333	62.72727273	1.70775	0.535176715
77.73316667	50	1.36125	0.308403395
94.6665	35.45454545	0.96525	-0.035368144
111.5998333	28.18181818	0.76725	-0.264942585
128.5331667	23.63636364	0.6435	-0.440833252
145.4665	16.36363636	0.4455	-0.808558032

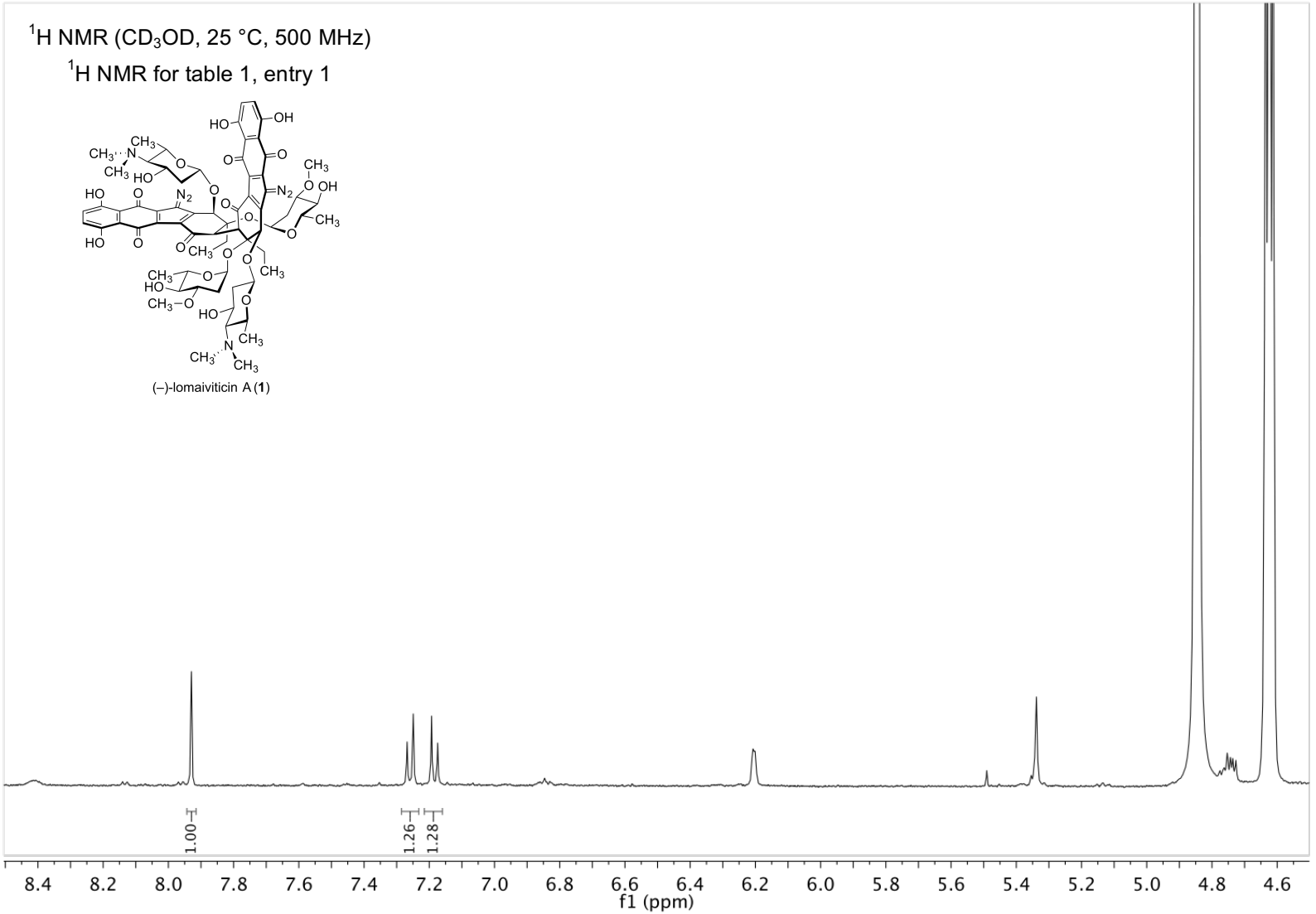


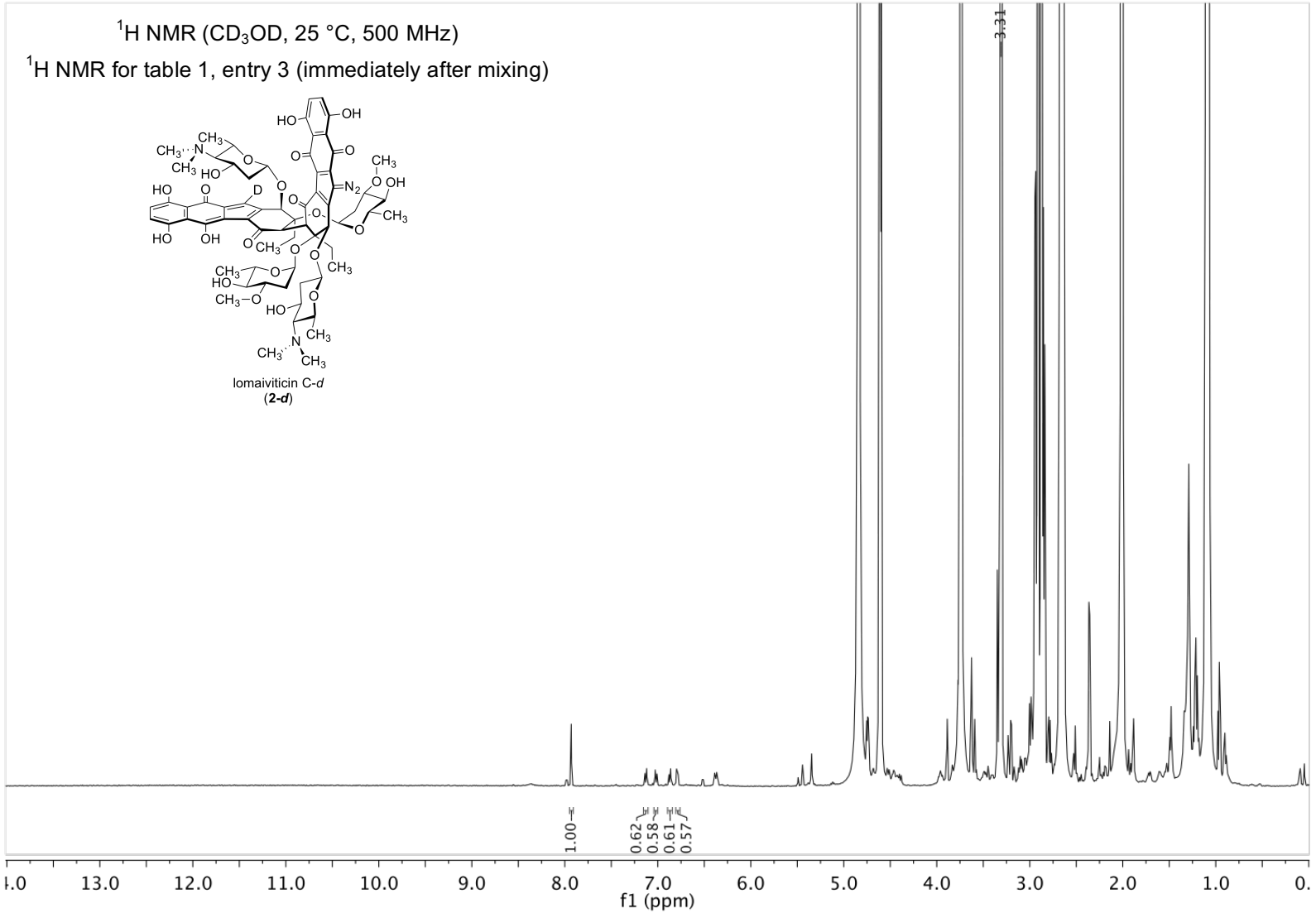


Therefore, k_{obs} = 0.01412 min^{-1}, and $t_{1/2}$ = 49 min.

Catalog of Nuclear Magnetic Resonance Spectra.

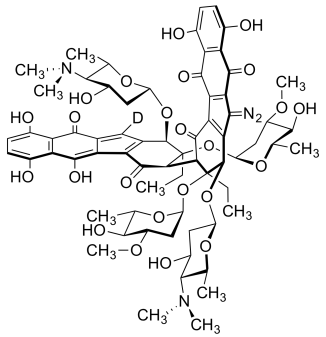



1H NMR (CD3OD, 25 °C, 500 MHz)
1H NMR for table 1, entry 1
(–)-lomaiviticin A (1)
1.00
1.26
1.28
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
f1 (ppm)


^{1}H NMR (CD_3OD, 25 °C, 500 MHz)
^{1}H NMR for table 1, entry 3 (immediately after mixing)
lomaiviticin C-*d*
(**2-*d***)
3.31
1.00
0.62
0.58
0.61
0.57
f1 (ppm)

^{1}H NMR (CD_3OD, 25 °C, 500 MHz)

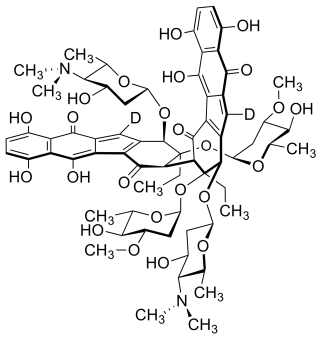
^{1}H NMR for table 1, entry 3 (immediately after mixing)



lomaiviticin C-*d*
(**2-*d***)

^{1}H NMR (CD_3OD, 25 °C, 500 MHz)

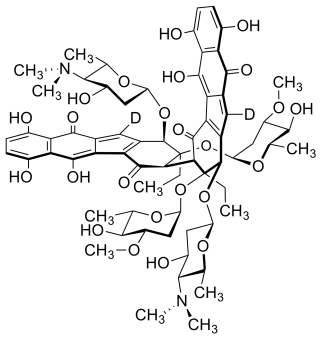
^{1}H NMR for table 1, entry 3 (after full conversion of **2-*d***, end of the experiment)



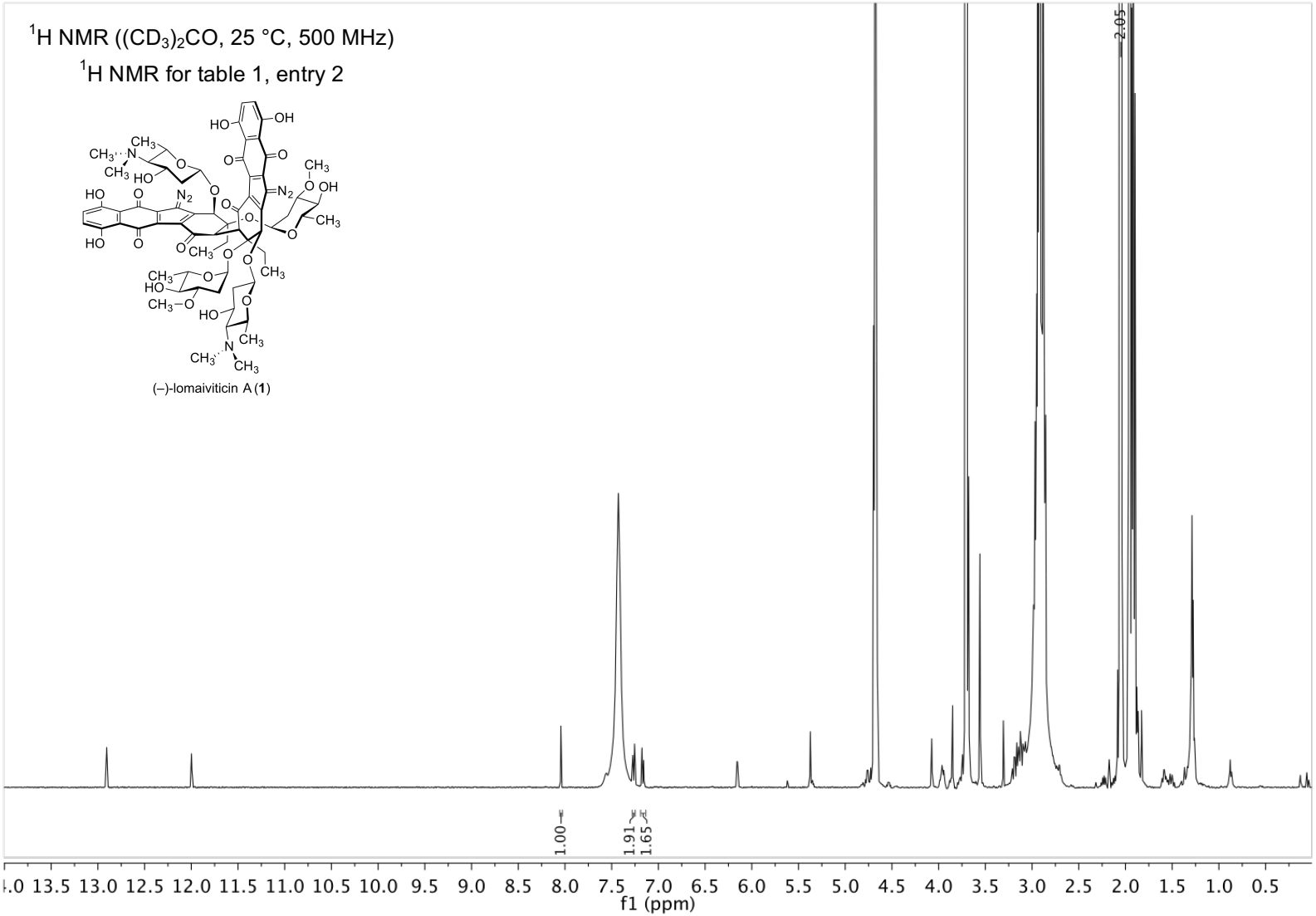
double hydrodediazotization product (**3-d_2**)

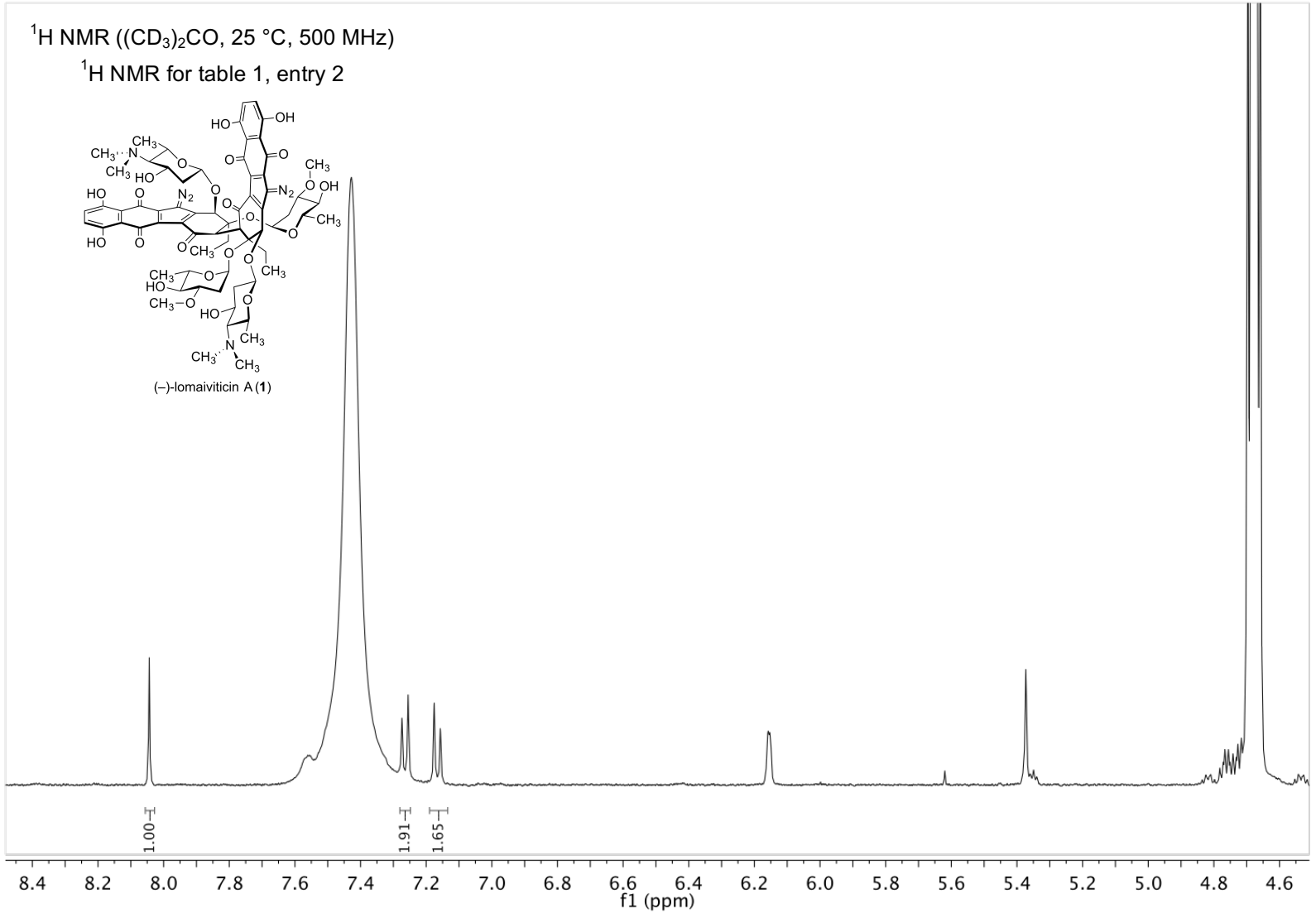
^{1}H NMR (CD_3OD, 25 °C, 500 MHz)

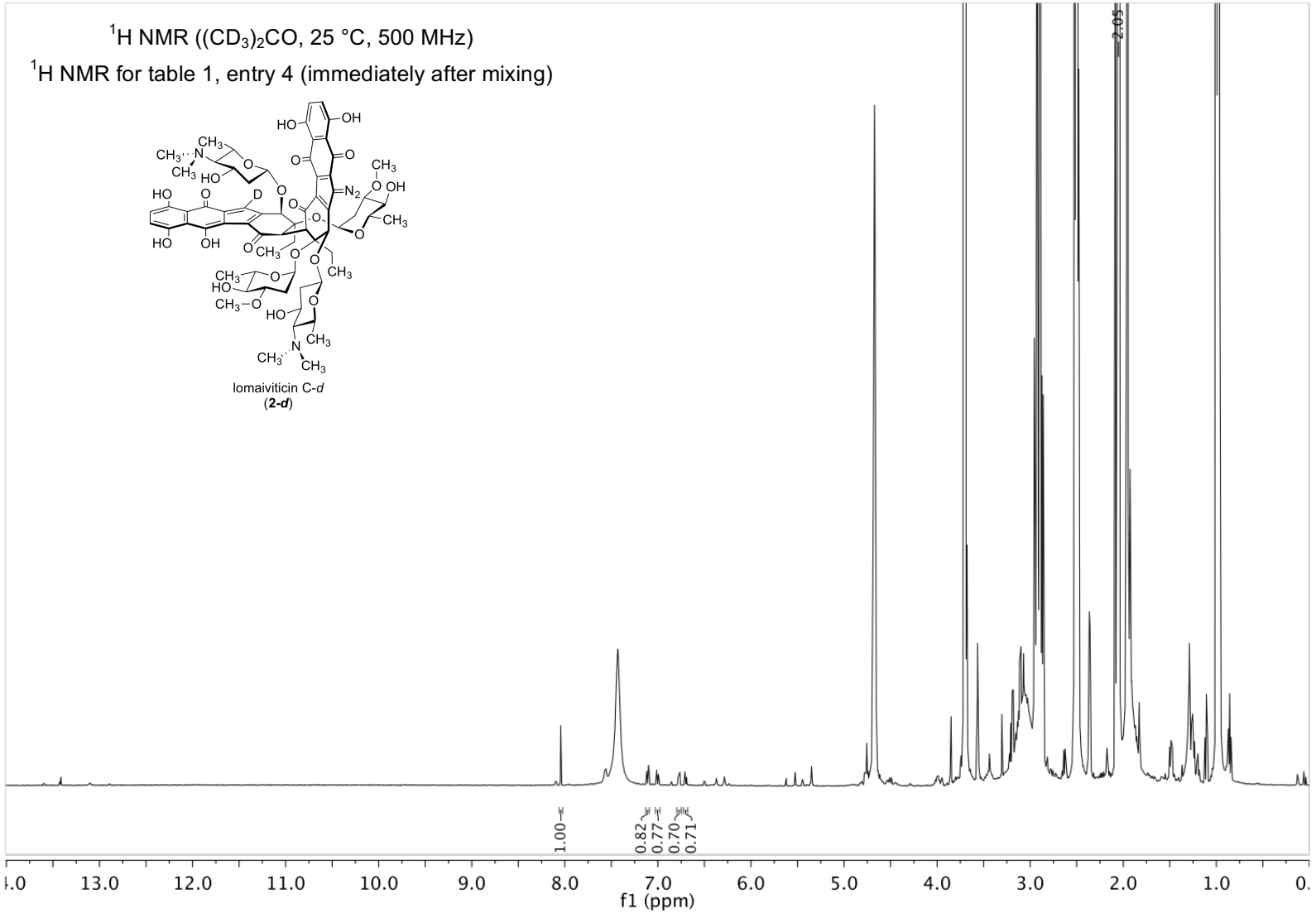
^{1}H NMR for table 1, entry 3 (after full conversion of **2-*d***, end of the experiment)



double hydrodediazotization product (**3-d_2**)

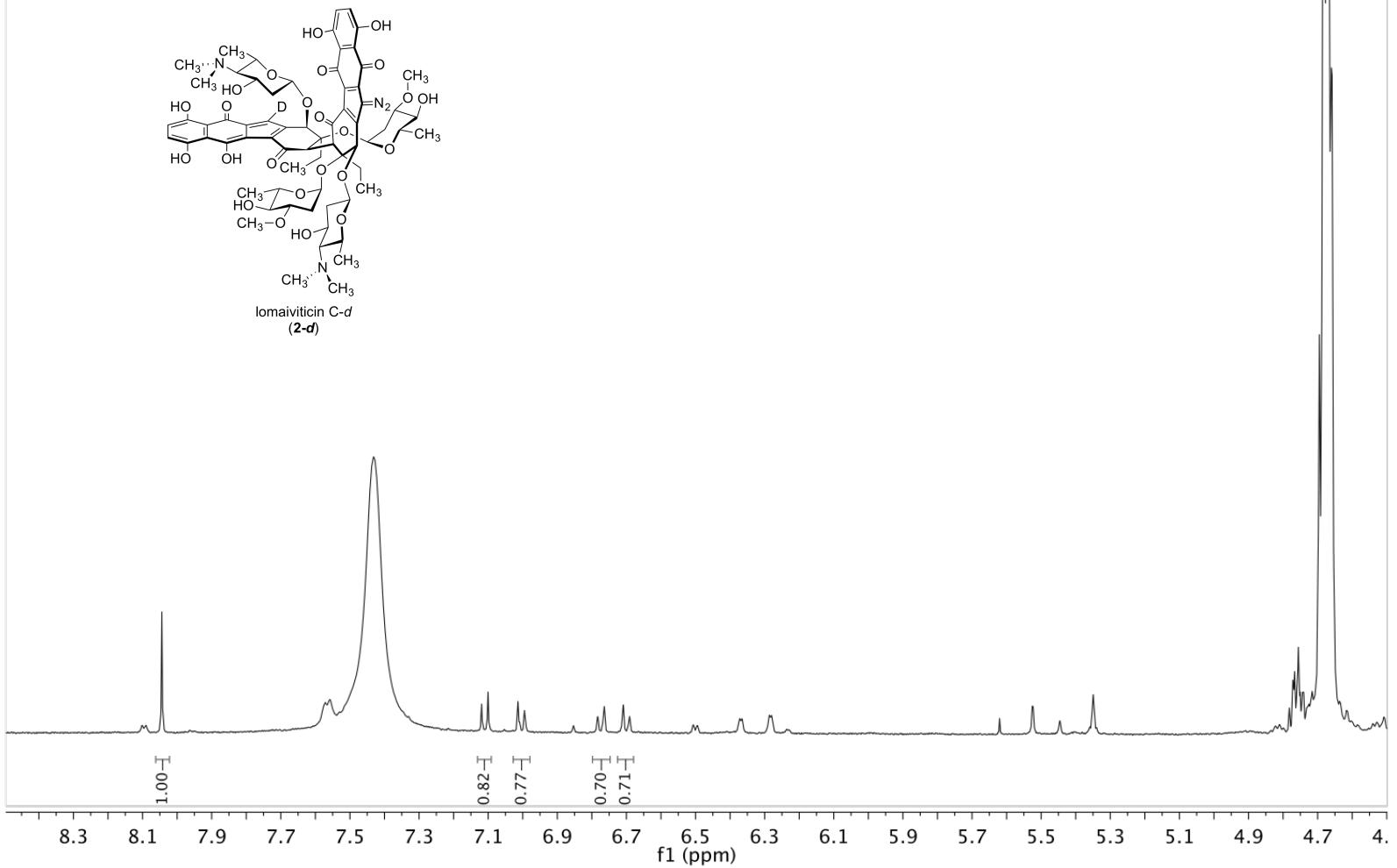

1H NMR ((CD3)2CO, 25 °C, 500 MHz)
1H NMR for table 1, entry 2
(–)-lomaiviticin A (1)
2.05
1.00
1.91
1.65
f1 (ppm)


1H NMR ((CD3)2CO, 25 °C, 500 MHz)
1H NMR for table 1, entry 2
(–)-lomaiviticin A (1)
1.00
1.91
1.65
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
f1 (ppm)

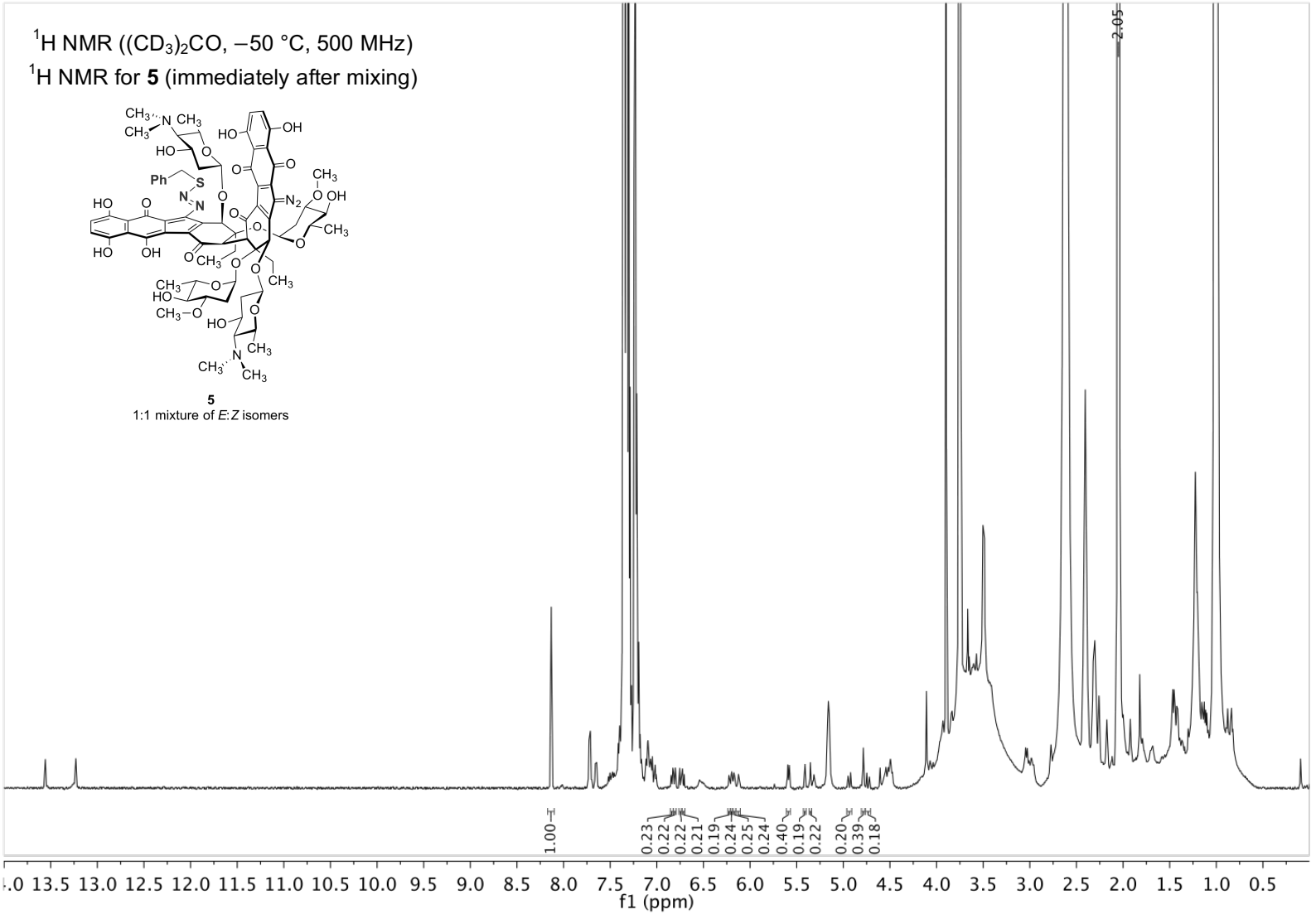

^{1}H NMR ((CD$_3$)$_2$CO, 25 °C, 500 MHz)
^{1}H NMR for table 1, entry 4 (immediately after mixing)
lomaiviticin C-*d*
(**2-*d***)
1.00
0.82
0.77
0.70
0.71
2.05
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
f1 (ppm)

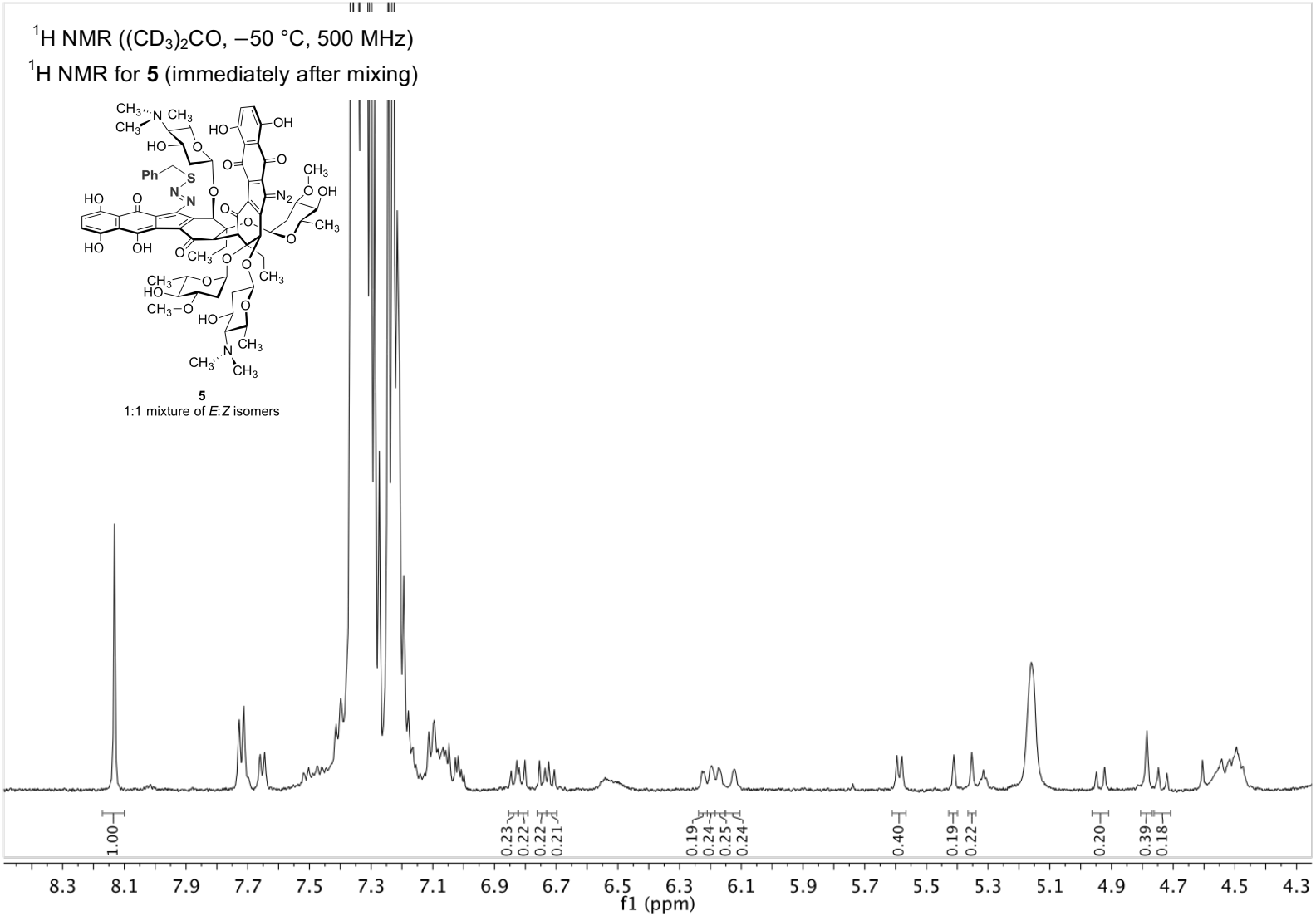
^{1}H NMR ($(CD_3)_2CO$, 25 °C, 500 MHz)

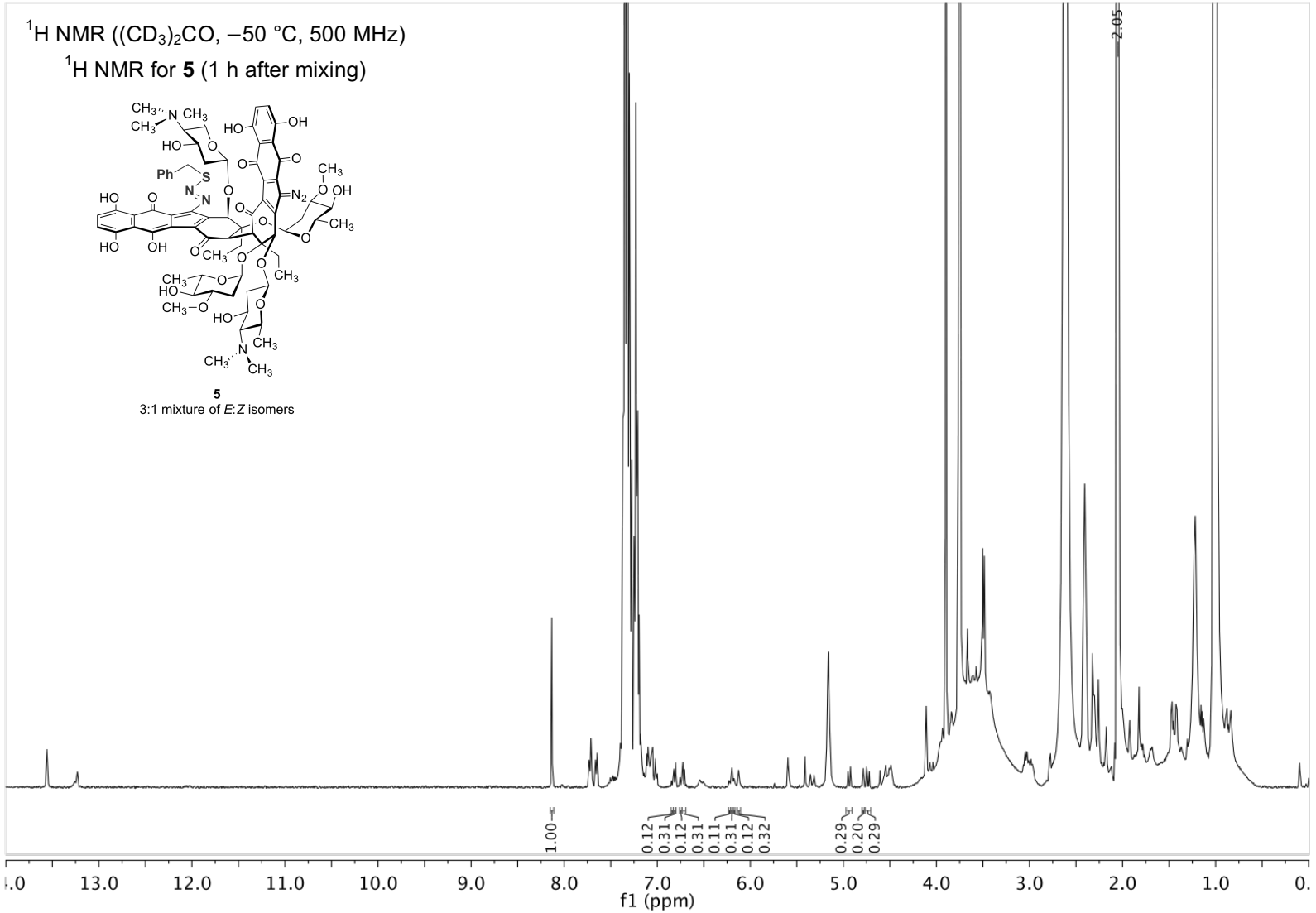
^{1}H NMR for table 1, entry 4 (immediately after mixing)

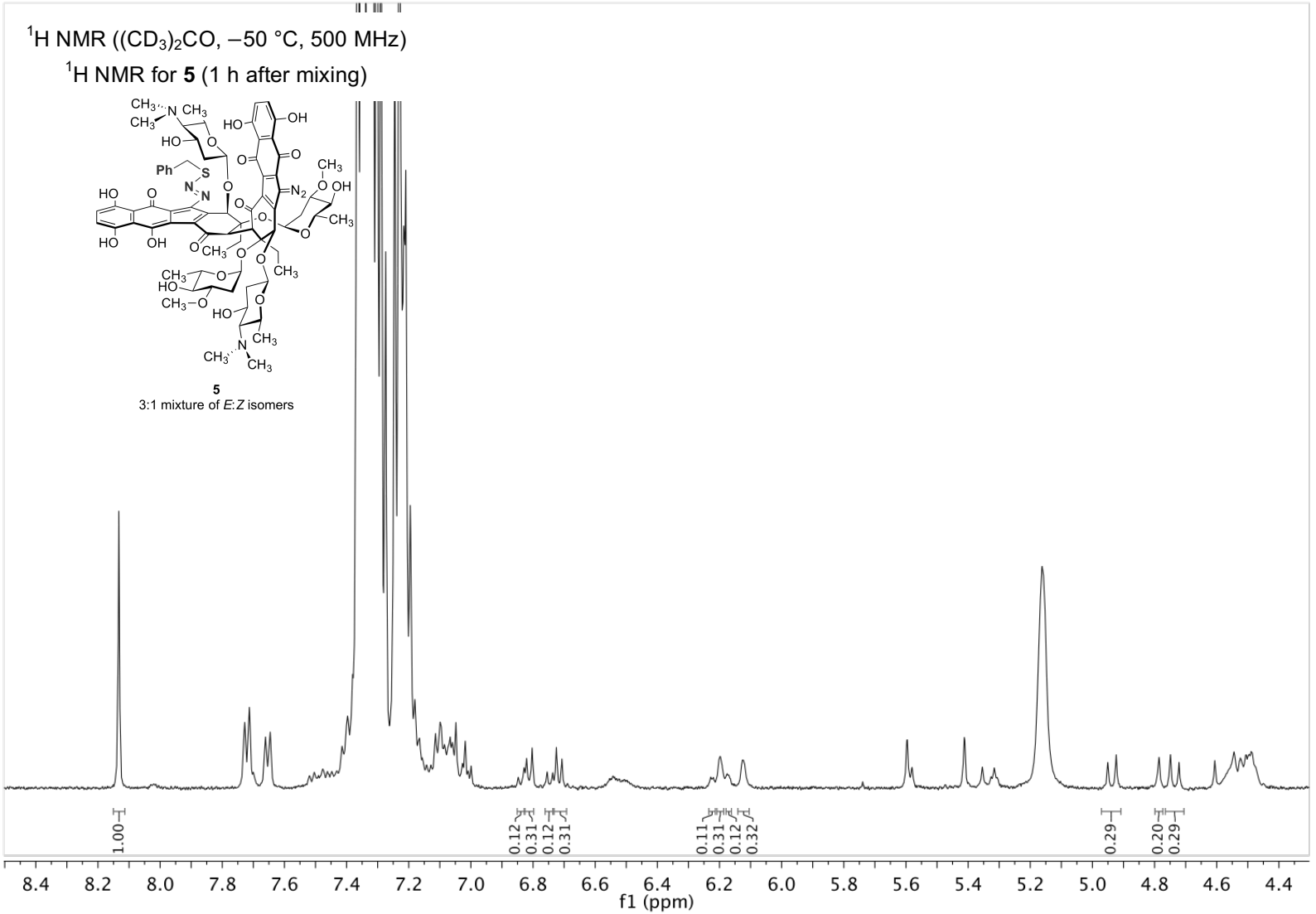


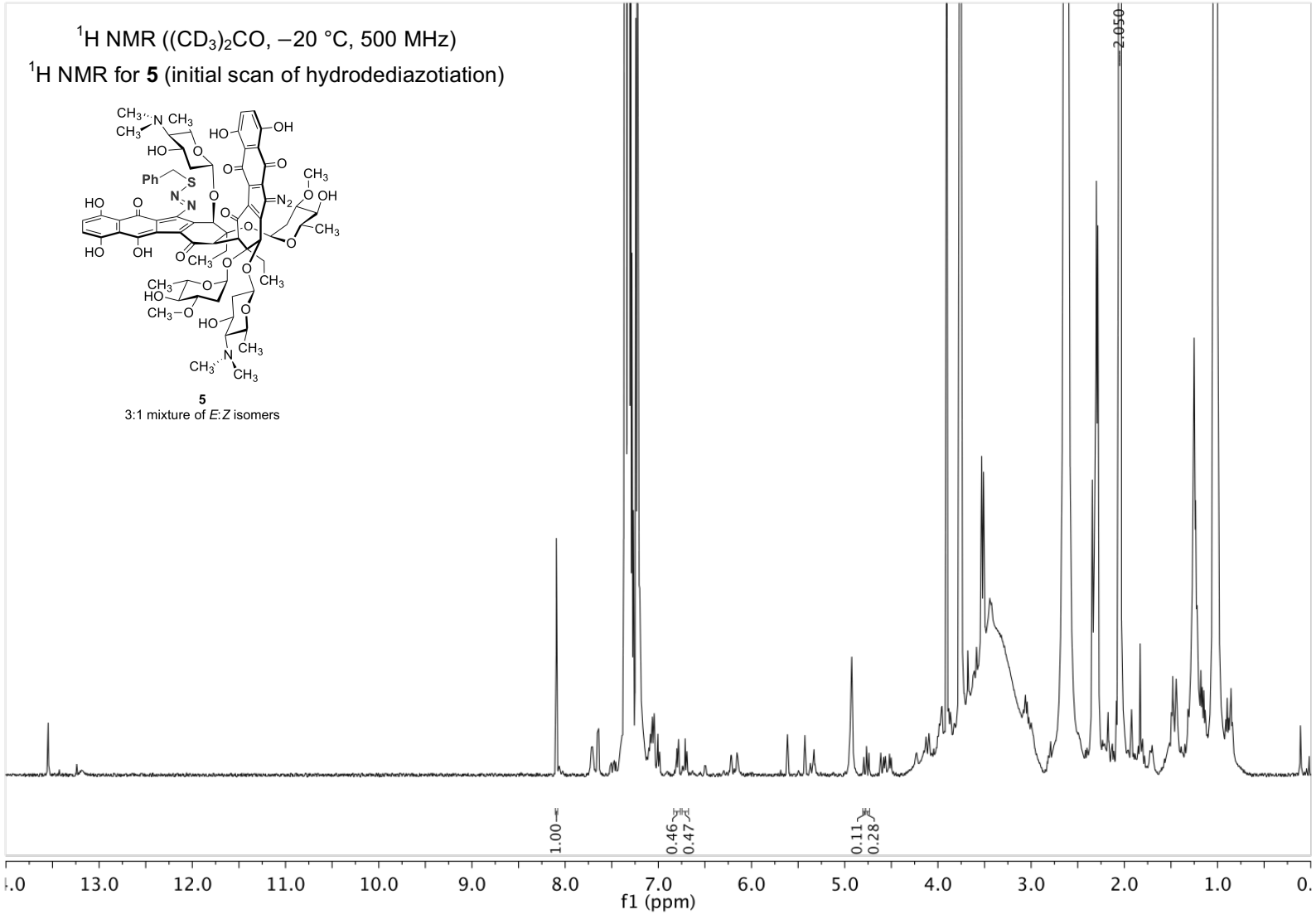
Iomaiviticin C-*d*
(**2-*d***)

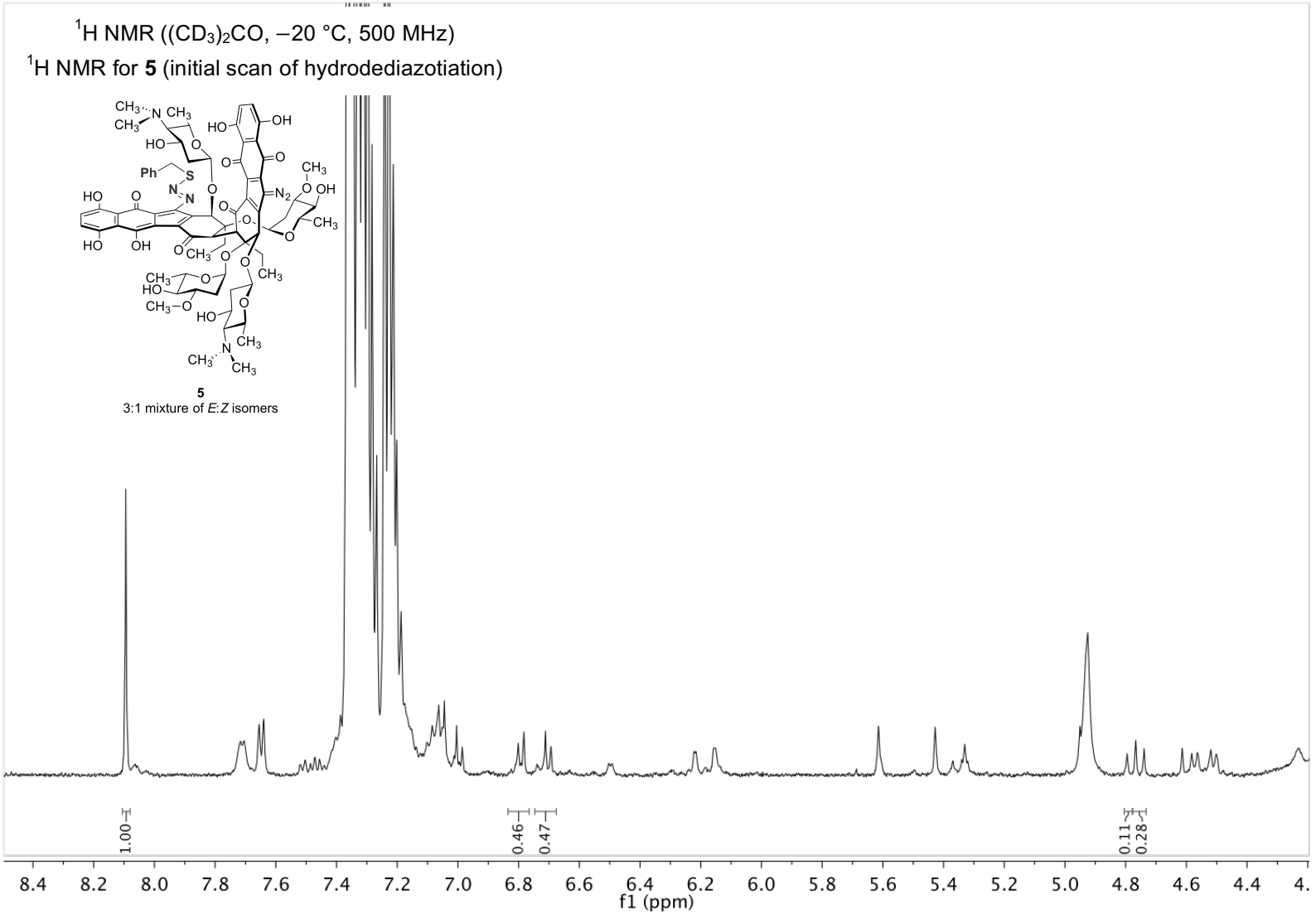

^{1}H NMR ((CD$_3$)$_2$CO, −50 °C, 500 MHz)
^{1}H NMR for **5** (immediately after mixing)
5
1:1 mixture of *E*:*Z* isomers
2.05
1.00
0.23
0.22
0.22
0.21
0.19
0.24
0.25
0.24
0.40
0.19
0.22
0.20
0.39
0.18
f1 (ppm)

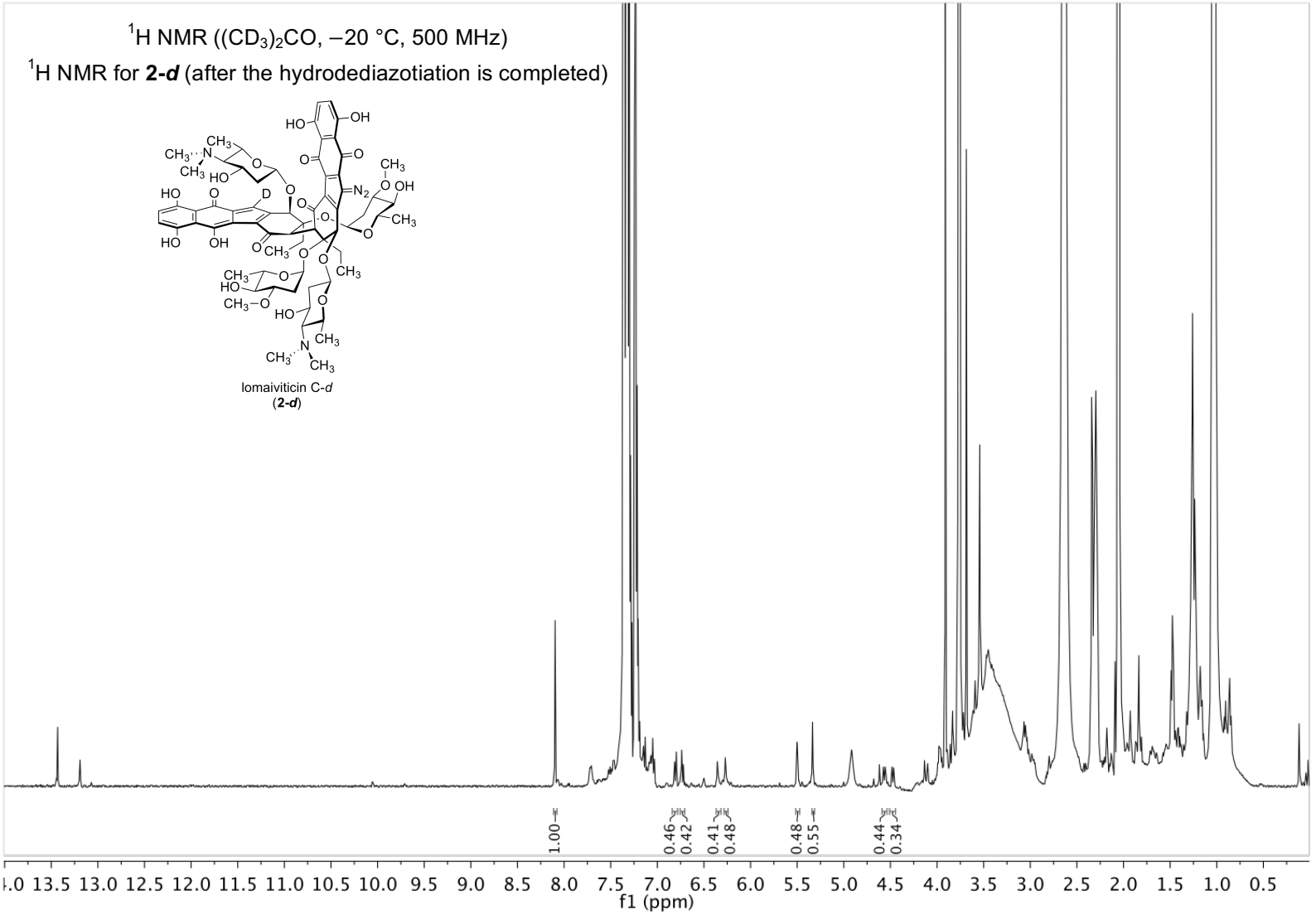

^{1}H NMR ((CD$_3$)$_2$CO, −50 °C, 500 MHz)
^{1}H NMR for **5** (immediately after mixing)
5
1:1 mixture of *E*:*Z* isomers
1.00
0.23
0.22
0.22
0.21
0.19
0.24
0.25
0.24
0.40
0.19
0.22
0.20
0.39
0.18
8.3 8.1 7.9 7.7 7.5 7.3 7.1 6.9 6.7 6.5 6.3 6.1 5.9 5.7 5.5 5.3 5.1 4.9 4.7 4.5 4.3
f1 (ppm)

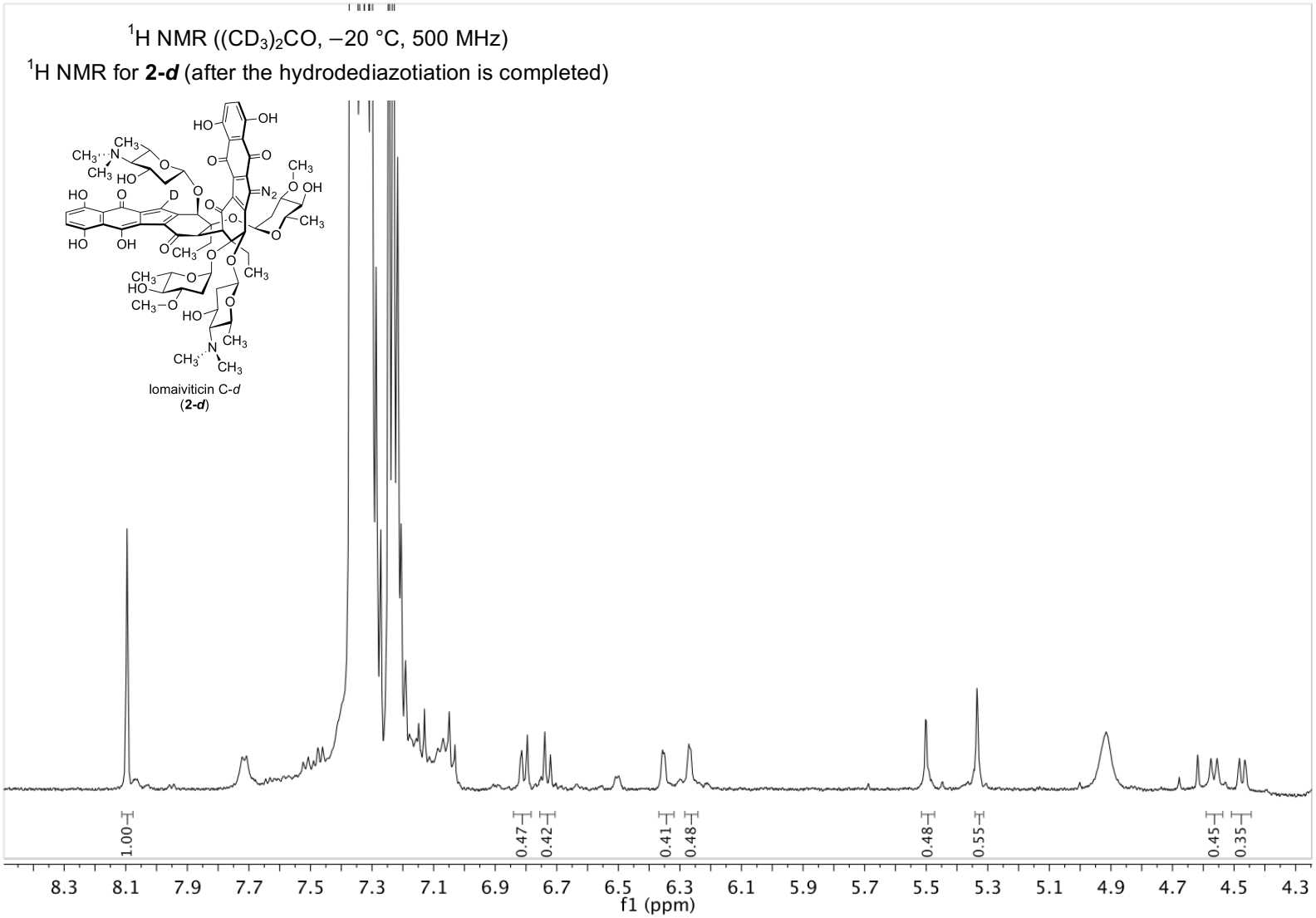

1H NMR ((CD3)2CO, −50 °C, 500 MHz)
1H NMR for 5 (1 h after mixing)
5
3:1 mixture of E:Z isomers
2.05
1.00
0.12
0.31
0.12
0.31
0.11
0.31
0.12
0.32
0.29
0.20
0.29
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
f1 (ppm)

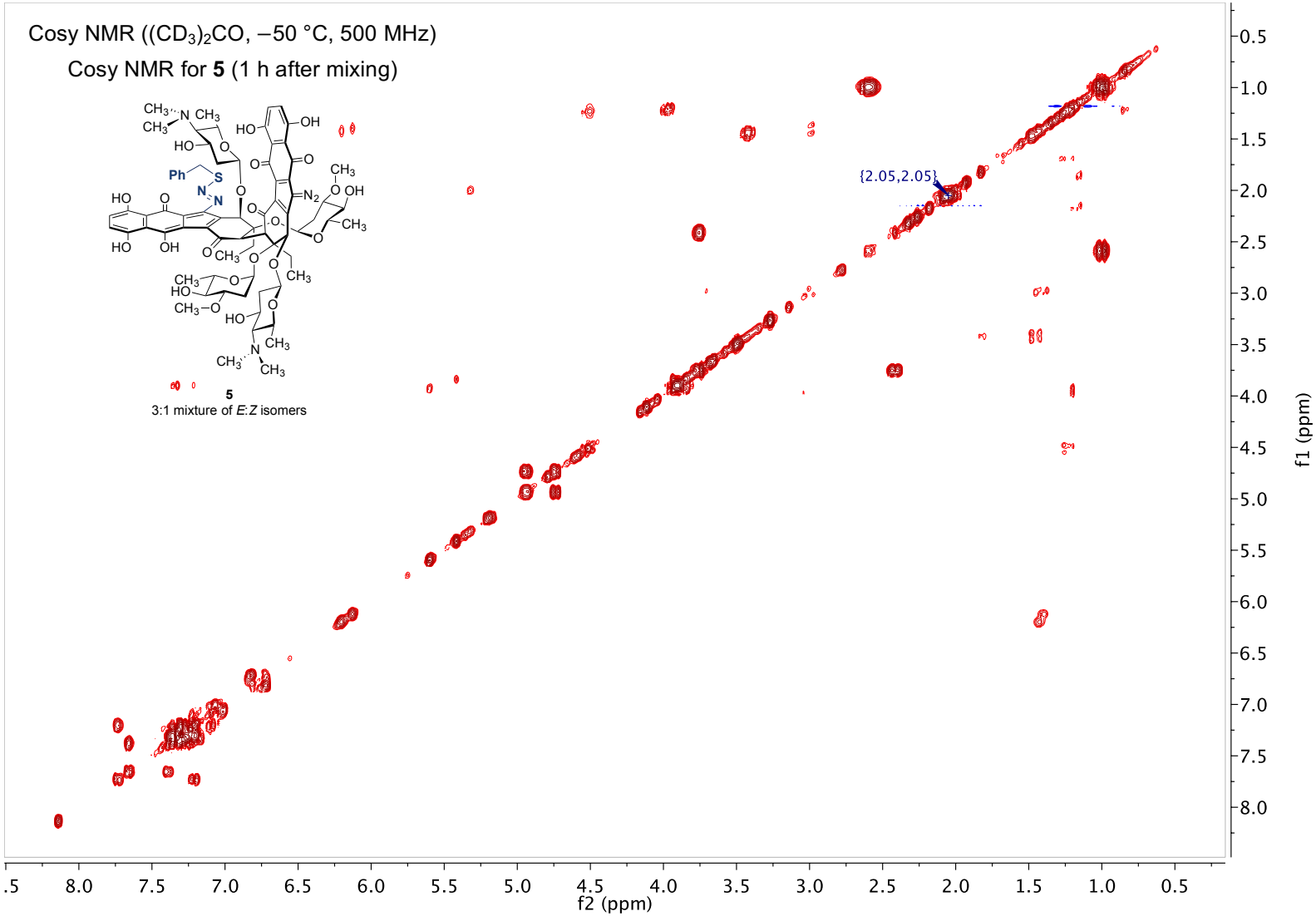

^{1}H NMR ((CD$_3$)$_2$CO, −50 °C, 500 MHz)
^{1}H NMR for **5** (1 h after mixing)
5
3:1 mixture of *E*:*Z* isomers
1.00
0.12
0.31
0.12
0.31
0.11
0.31
0.12
0.32
0.29
0.20
0.29
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4
f1 (ppm)

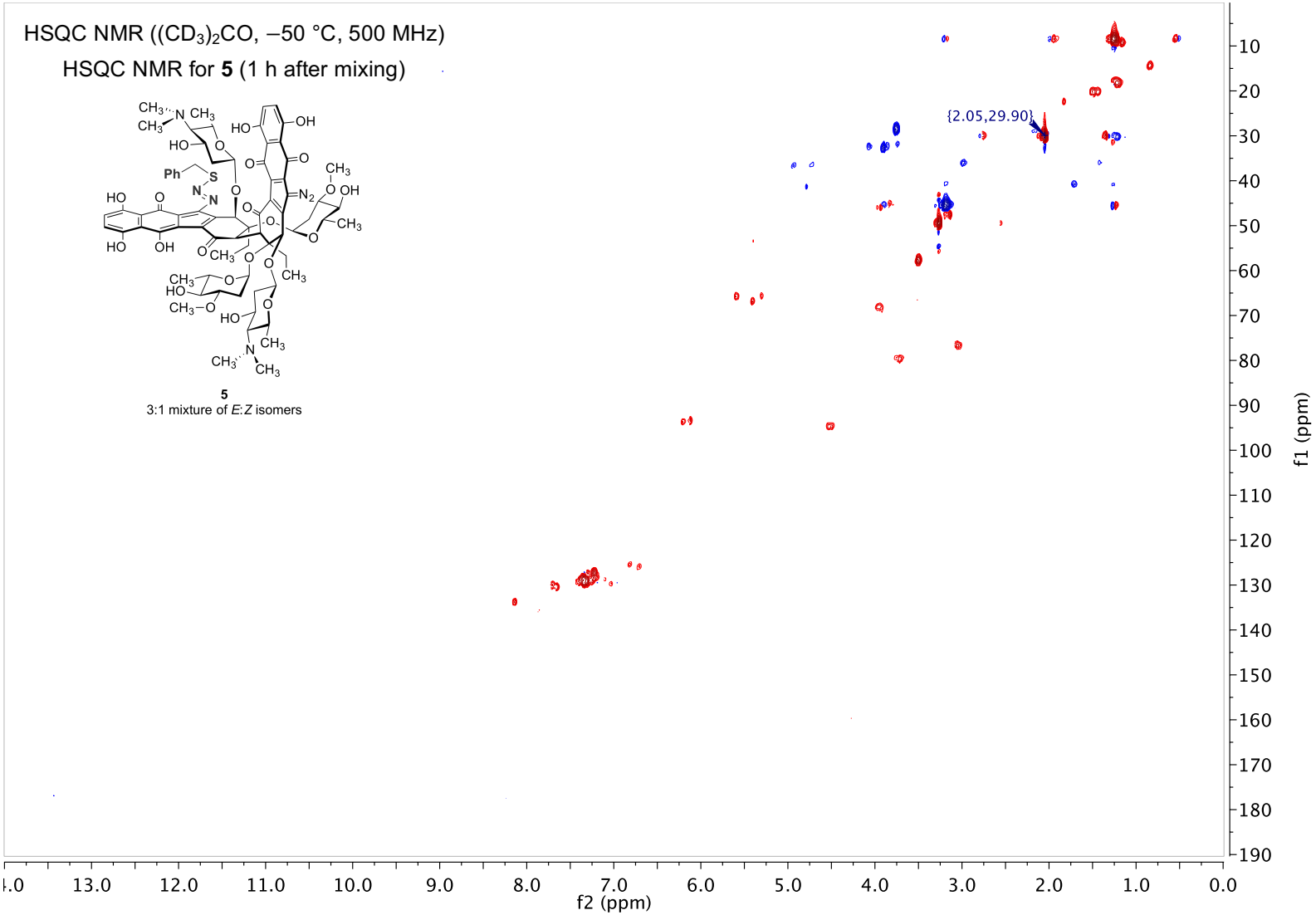

^{1}H NMR ((CD$_3$)$_2$CO, −20 °C, 500 MHz)
^{1}H NMR for **5** (initial scan of hydrodediazotiation)
5
3:1 mixture of *E*:*Z* isomers
2.050
1.00
0.46
0.47
0.11
0.28
f1 (ppm)

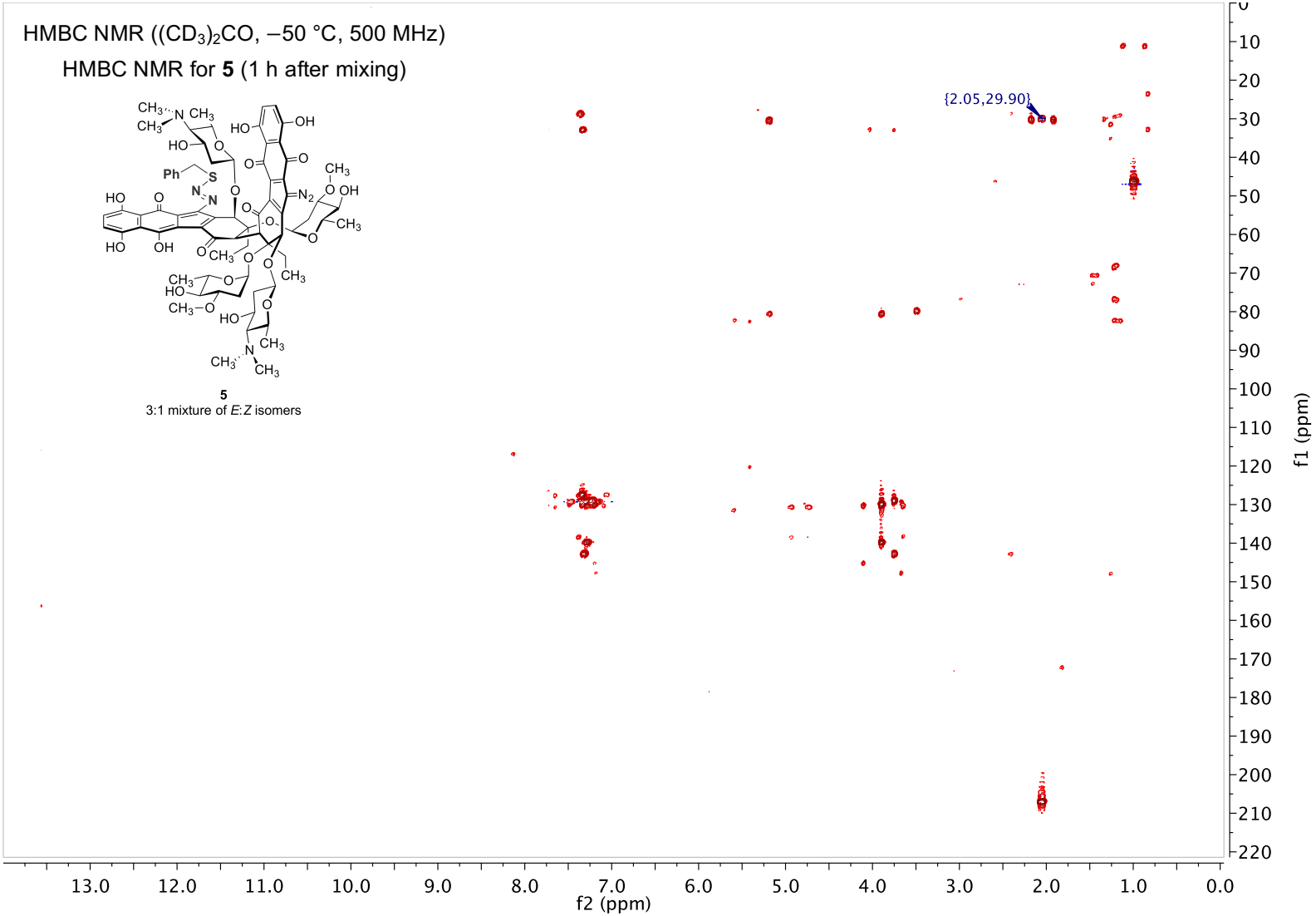

^{1}H NMR ((CD$_3$)$_2$CO, −20 °C, 500 MHz)
^{1}H NMR for **5** (initial scan of hydrodediazotiation)
5
3:1 mixture of *E*:*Z* isomers
1.00
0.46
0.47
0.11
0.28
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.
f1 (ppm)


^{1}H NMR ((CD$_3$)$_2$CO, −20 °C, 500 MHz)
^{1}H NMR for **2-*d*** (after the hydrodediazotiation is completed)
lomaiviticin C-*d*
(**2-*d***)
1.00
0.46
0.42
0.41
0.48
0.48
0.55
0.44
0.34
f1 (ppm)

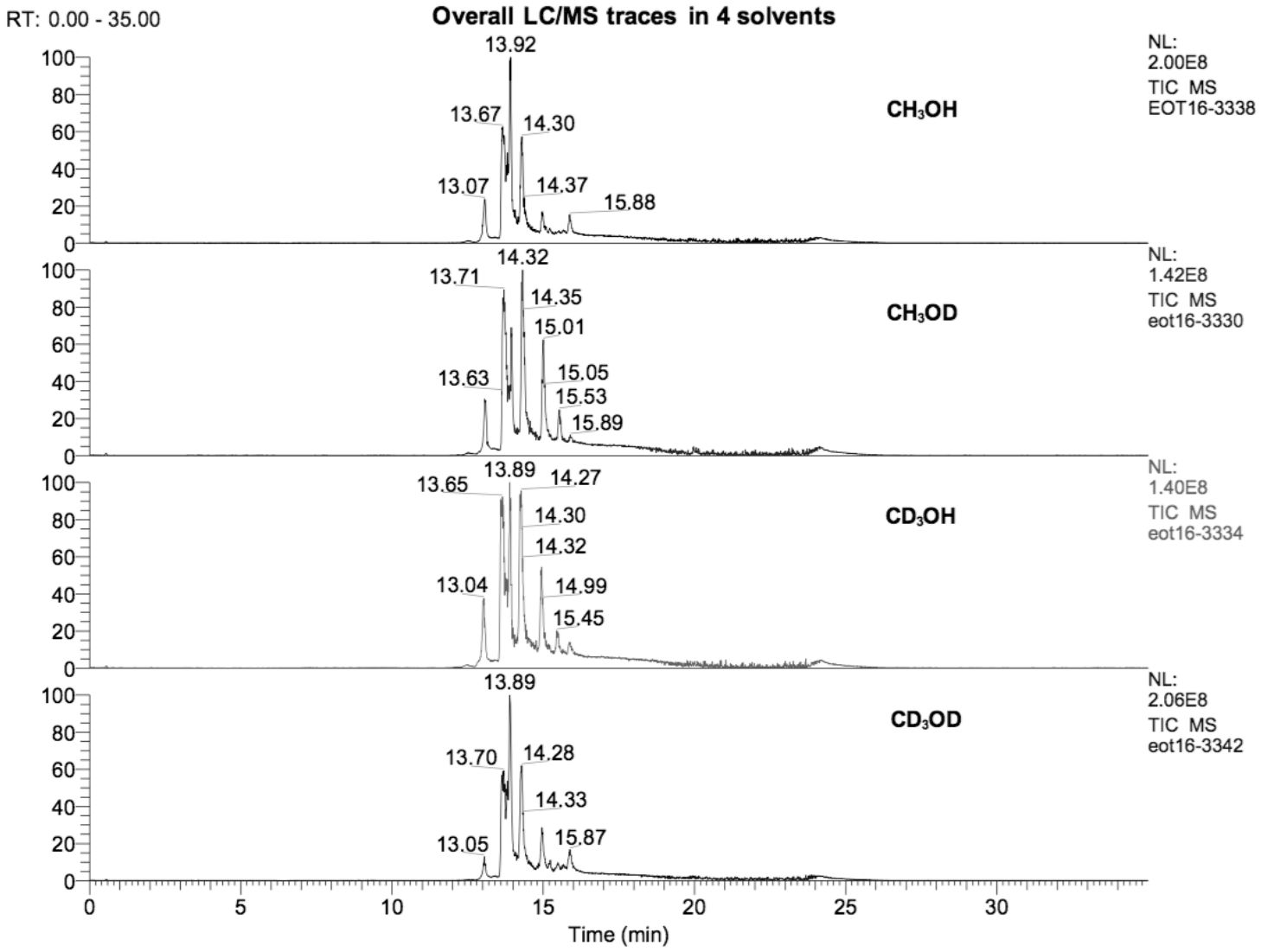

^{1}H NMR ((CD$_3$)$_2$CO, −20 °C, 500 MHz)
^{1}H NMR for **2-*d*** (after the hydrodediazotiation is completed)
lomaiviticin C-*d*
(**2-*d***)
1.00
0.47
0.42
0.41
0.48
0.48
0.55
0.45
0.35
8.3 8.1 7.9 7.7 7.5 7.3 7.1 6.9 6.7 6.5 6.3 6.1 5.9 5.7 5.5 5.3 5.1 4.9 4.7 4.5 4.3
f1 (ppm)

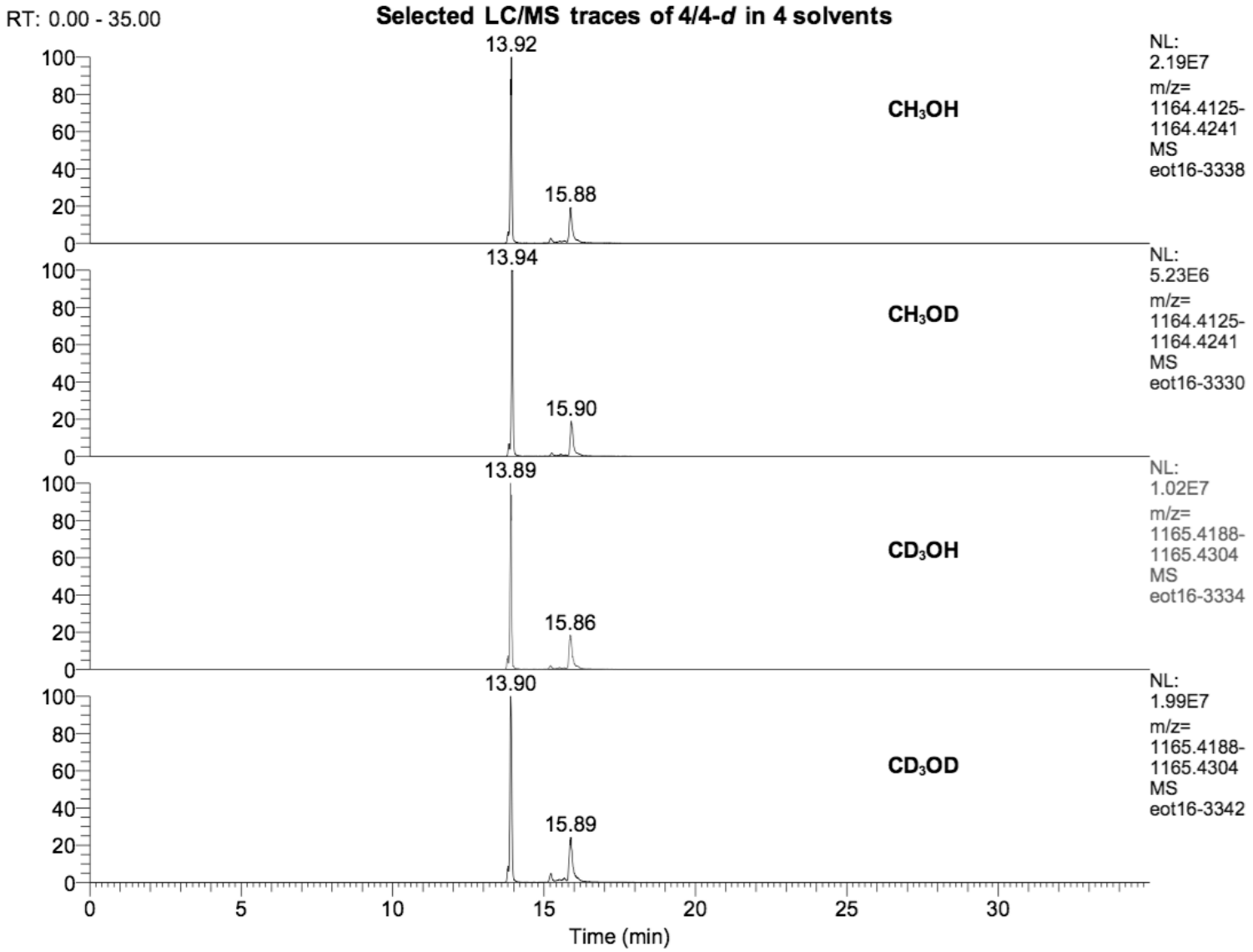

Cosy NMR ((CD3)2CO, −50 °C, 500 MHz)
Cosy NMR for 5 (1 h after mixing)
5
3:1 mixture of E:Z isomers
{2.05,2.05}
f1 (ppm)
f2 (ppm)

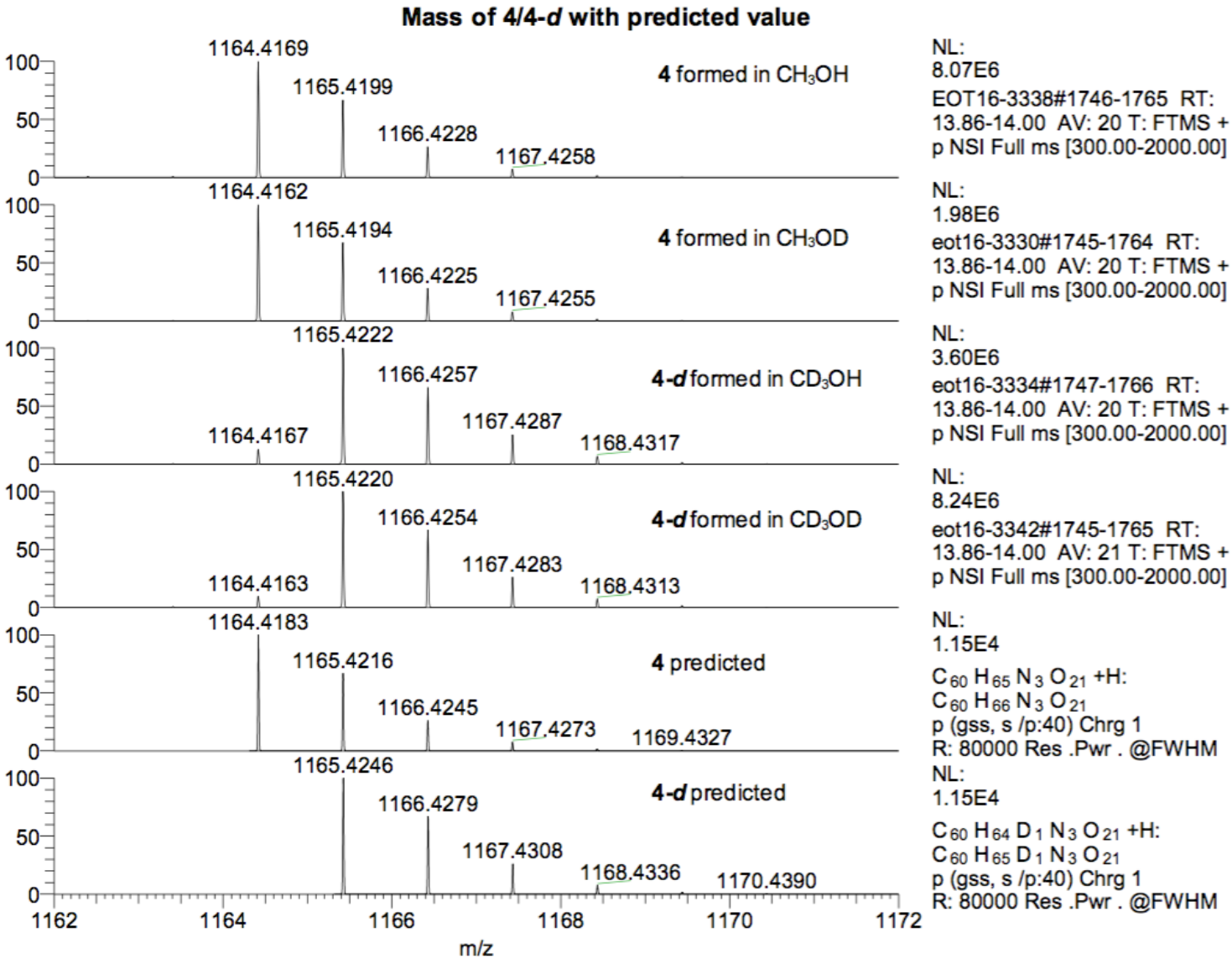

HSQC NMR ((CD3)2CO, −50 °C, 500 MHz)
HSQC NMR for 5 (1 h after mixing)
5
3:1 mixture of E:Z isomers
{2.05,29.90}
f2 (ppm)
f1 (ppm)

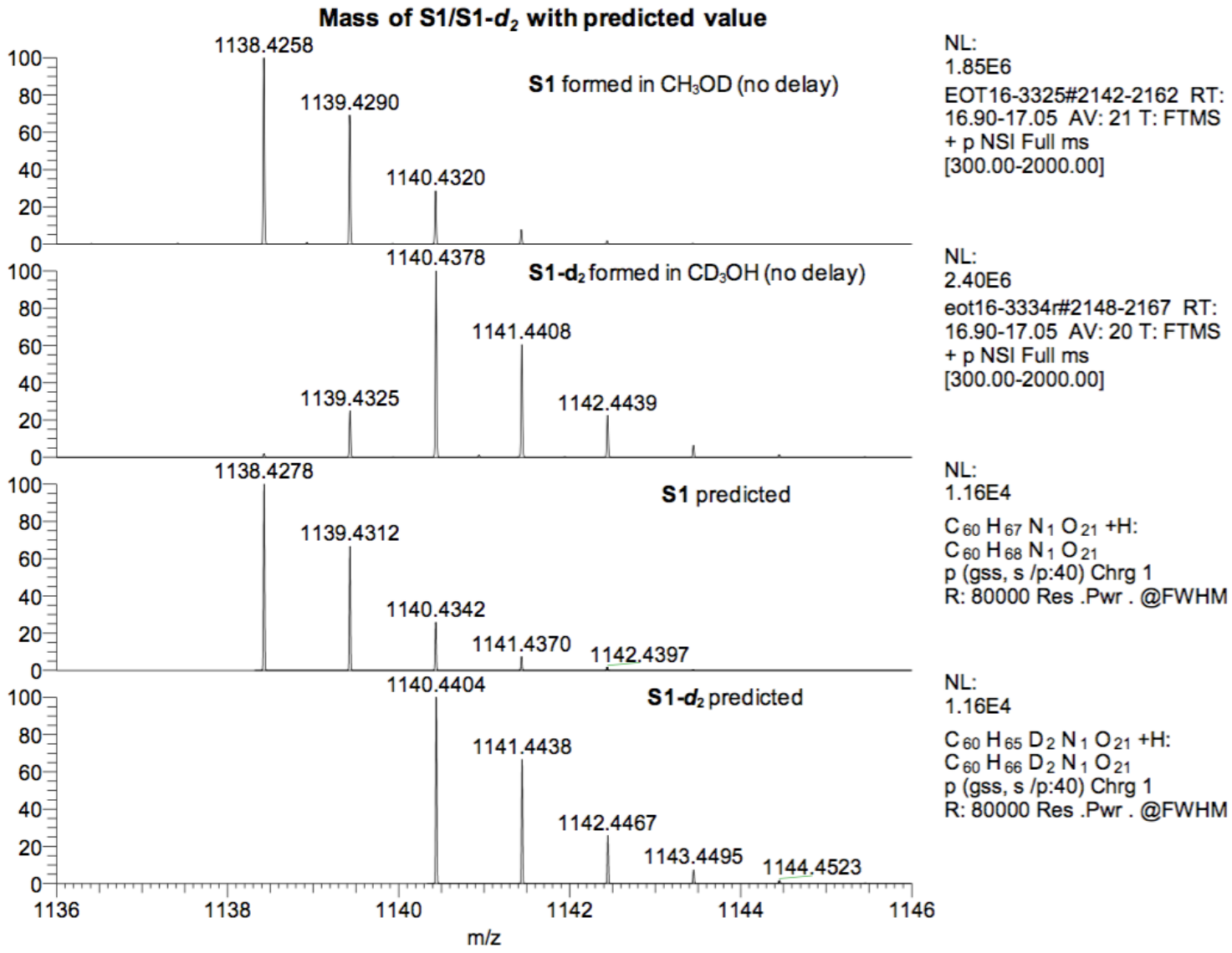

HMBC NMR ((CD3)2CO, −50 °C, 500 MHz)
HMBC NMR for 5 (1 h after mixing)
{2.05,29.90}
5
3:1 mixture of E:Z isomers
f2 (ppm)
f1 (ppm)

Catalog of High Resolution Mass Spectra.




RT: 0.00 - 35.00
Selected LC/MS traces of 4/4-d in 4 solvents
13.92
15.88
CH3OH
NL:
2.19E7
m/z=
1164.4125-
1164.4241
MS
eot16-3338
13.94
15.90
CH3OD
NL:
5.23E6
m/z=
1164.4125-
1164.4241
MS
eot16-3330
13.89
15.86
CD3OH
NL:
1.02E7
m/z=
1165.4188-
1165.4304
MS
eot16-3334
13.90
15.89
CD3OD
NL:
1.99E7
m/z=
1165.4188-
1165.4304
MS
eot16-3342
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (min)


Mass of 4/4-d with predicted value
1164.4169
1165.4199
1166.4228
1167.4258
4 formed in CH3OH
1164.4162
1165.4194
1166.4225
1167.4255
4 formed in CH3OD
1165.4222
1166.4257
1167.4287
1164.4167
1168.4317
4-d formed in CD3OH
1165.4220
1166.4254
1167.4283
1164.4163
1168.4313
4-d formed in CD3OD
1164.4183
1165.4216
1166.4245
1167.4273
1169.4327
4 predicted
1165.4246
1166.4279
1167.4308
1168.4336
1170.4390
4-d predicted
100
50
0
1162
1164
1166
1168
1170
1172
m/z
NL:
8.07E6
EOT16-3338#1746-1765 RT:
13.86-14.00 AV: 20 T: FTMS +
p NSI Full ms [300.00-2000.00]
NL:
1.98E6
eot16-3330#1745-1764 RT:
13.86-14.00 AV: 20 T: FTMS +
p NSI Full ms [300.00-2000.00]
NL:
3.60E6
eot16-3334#1747-1766 RT:
13.86-14.00 AV: 20 T: FTMS +
p NSI Full ms [300.00-2000.00]
NL:
8.24E6
eot16-3342#1745-1765 RT:
13.86-14.00 AV: 21 T: FTMS +
p NSI Full ms [300.00-2000.00]
NL:
1.15E4
C60 H65 N3 O21 +H:
C60 H66 N3 O21
p (gss, s /p:40) Chrg 1
R: 80000 Res .Pwr . @FWHM
NL:
1.15E4
C60 H64 D1 N3 O21 +H:
C60 H65 D1 N3 O21
p (gss, s /p:40) Chrg 1
R: 80000 Res .Pwr . @FWHM


Mass of S1/S1-d_2 with predicted value
1138.4258
1139.4290
1140.4320
S1 formed in CH_3OD (no delay)
NL:
1.85E6
EOT16-3325#2142-2162 RT:
16.90-17.05 AV: 21 T: FTMS
+ p NSI Full ms
[300.00-2000.00]
1140.4378
1141.4408
1139.4325
1142.4439
S1-d_2 formed in CD_3OH (no delay)
NL:
2.40E6
eot16-3334r#2148-2167 RT:
16.90-17.05 AV: 20 T: FTMS
+ p NSI Full ms
[300.00-2000.00]
1138.4278
1139.4312
1140.4342
1141.4370
1142.4397
S1 predicted
NL:
1.16E4
$C_{60}H_{67}N_1O_{21}$ +H:
$C_{60}H_{68}N_1O_{21}$
p (gss, s /p:40) Chrg 1
R: 80000 Res .Pwr . @FWHM
1140.4404
1141.4438
1142.4467
1143.4495
1144.4523
S1-d_2 predicted
NL:
1.16E4
$C_{60}H_{65}D_2N_1O_{21}$ +H:
$C_{60}H_{66}D_2N_1O_{21}$
p (gss, s /p:40) Chrg 1
R: 80000 Res .Pwr . @FWHM
100
80
60
40
20
0
1136
1138
1140
1142
1144
1146
m/z

Bibliography.

1. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
2. Woo, C. M.; Beizer, N. E.; Janso, J. E.; Herzon, S. B. *J. Am. Chem. Soc.* **2012**, *134*, 15285.