Furan and lactam jadomycin biosynthetic congeners isolated from *Streptomyces venezuelae* ISP5230 cultured with N_{ε}-trifluoroacetyl-L-lysine

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Media:

All media was sterilized by autoclaving at 120°C for 20 minutes. Agar (1.5g/L) and/or apramycin (50 ug/mL) were added when required.

MYM: (1 L aqueous solution, pH 7.0) Maltose (4 g), yeast extract (4 g), malt extract (1 g).

MSM: (1 L aqueous solution, pH 7.5) $MgSO_4$ (0.4 g), MOPS (1.9 g) salt solution (9 mL of 1% *w*/*v* NaCl and 1% *w*/*v* $CaCl_2$), $FeSO_4 \cdot 7H_2O$ (4.5 mL of 0.2% *w*/*v*), trace mineral solution (4.5 mL).

Trace mineral solution: (1 L aqueous solution) $ZnSO_4 \cdot 7H_2O$ (880 mg), $CuSO_4 \cdot 5H_2O$ (39 mg), $MnSO_4 \cdot 4H_2O$ (6.1 mg), H_3BO_3 (5.7 mg), $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ (3.7 mg).

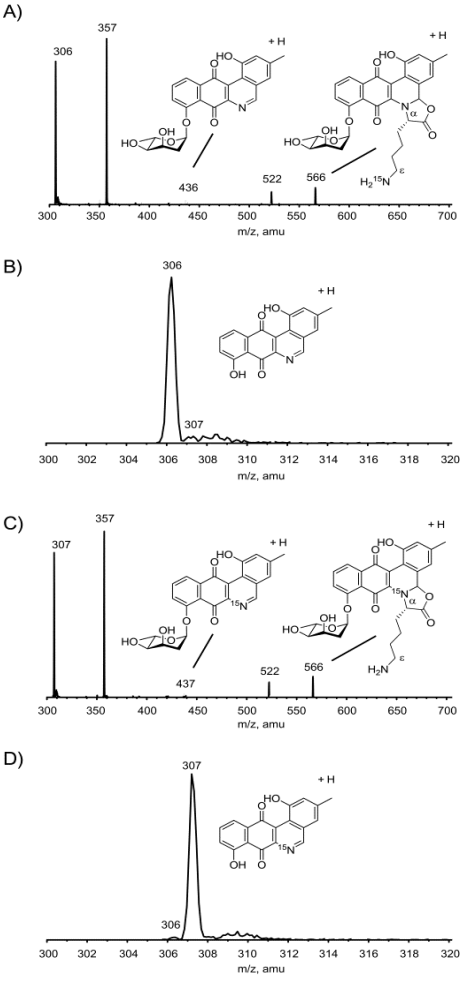
^{15}N labeled Jadomycin K productions and sample preparation for LC-MS2 experiments:

Jadomycin K production was performed as already described, with modification in the amino acid content of the MSM media, to contain 100% ^{15}*N*-α–L-lysine (60 mM) or ^{15}*N*-ε-L-lysine (45 mM). Control fermentations with natural abundance L-lysine (60 and 45 mM) were simultaneously conducted. These productions were performed on a small scale (triplicate 25 mL cultures) using the same culture methods described in the experimental section. After a fermentation period of 2 days, cells were filtered from the solution and samples of the clarified growth media were used without further purification for all qualitative LC-MS/MS analyses. Samples prepared for MS analysis were dissolved and diluted in water.

LC-MS^2 Analysis of ^{15}N labeled lysine for the determination of E-ring size:

Incorporation of the isotopic label was probed using LC-MS^2 experiments of crudely purified organic extracts from these productions. Incorporation of the isotopic label was probed using LC-MS^2 experiments of crudely purified organic extracts from these productions. The mass of singly labelled ^{15}N-α- or ^{15}N-ε- labelled **JdK** ([M(^{15}N) +H]$^+$ 566 m/z) was selected as the parent ion, and the fragmentation pattern was analyzed to pinpoint the incorporation of the isotopic label (**Figure 2**). Fragmentation of the parent ion to m/z 306 in the ^{15}N-ε production, and to 307 in the ^{15}N-α production, demonstrated that in both cases N-α of L-lysine is incorporated into the B-ring to form a five-membered oxazolone ring containing jadomycin (**JdK**).

Figure S1. (**A**) LC-MS^2 fragmentation pattern of ^{15}N-ε labeled jadomycin K showing parent ion 566 *m/z* [^{15}NM + H]$^+$, and fragmentation resulting from the cleavage of the amino acid side chain [M + H – $^{15}NC_6H_{11}O_2$]$^+$; (**B**) expanded region (300-320 *m/z*) illustrating the unlabeled 306 *m/z* fragment resulting from the cleavage of both the amino acid and L-digitoxose [M + H – $^{15}NC_6H_{11}O_2$ – digitoxose]$^+$; (**C**) LC-MS^2 fragmentation pattern of ^{15}N-α labeled jadomycin K showing parent ion 566 *m/z* [^{15}NM + H]$^+$, and fragmentation resulting from the cleavage of the amino acid side chain [^{15}NM + H – $C_6H_{11}NO_2$]$^+$; (D) expanded region (300-320 *m/z*) illustrating the labeled 307 *m/z* fragment resulting from the cleavage of both the amino acid and L-digitoxose [^{15}NM + H – $C_6H_{11}NO_2$ – digitoxose]$^+$



Growth curves (OD_{600} and A_{526}) for jadomycin productions with *N*-ε-trifluoroacetyl-L-lysine (TFAL)

Figure S2. OD_{600} curve for *S. venezuelae* VS1099 production with *TFAL.*

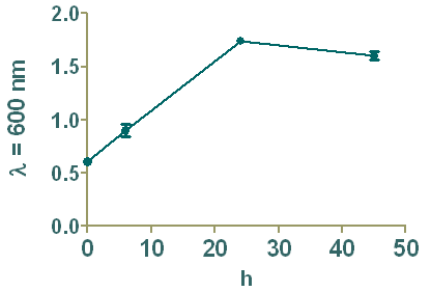
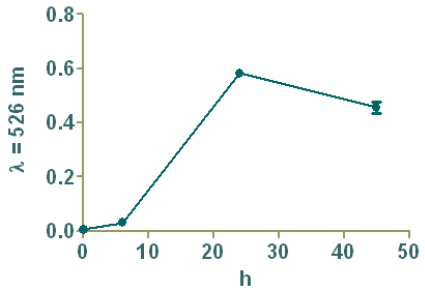


Figure S3. A_{526} curve for *S. venezuelae* VS1099 production with *N*-ε-trifluoroacetyl-L-lysine.



LCMS^2 fragmentation data

Table S1. Low resolution MS^2 (ESI) data for jadomycins examined in this study.

Compound	$[M+H]^+$ parent ion	ESI^+ fragmentation	$[M-H]^-$ parent ion	ESI^- fragmentation
1	661	531, 306		
2			675	631, 527
JdK	565	521, 436, 356, 306		
JdK	566 (isotope peak)	522, 437, 357 and 356[a], 307 and 306[a]		
Jdε^{15}NK	566	522, 436,357, 306		
Jdα^{15}NK	566	522,437, 357,307		

[a] Indicated ions were approximately of equal intensity.

HPLC traces for purified 1, 2, 3 +4

Figure S4. HPLC trace of **1.**

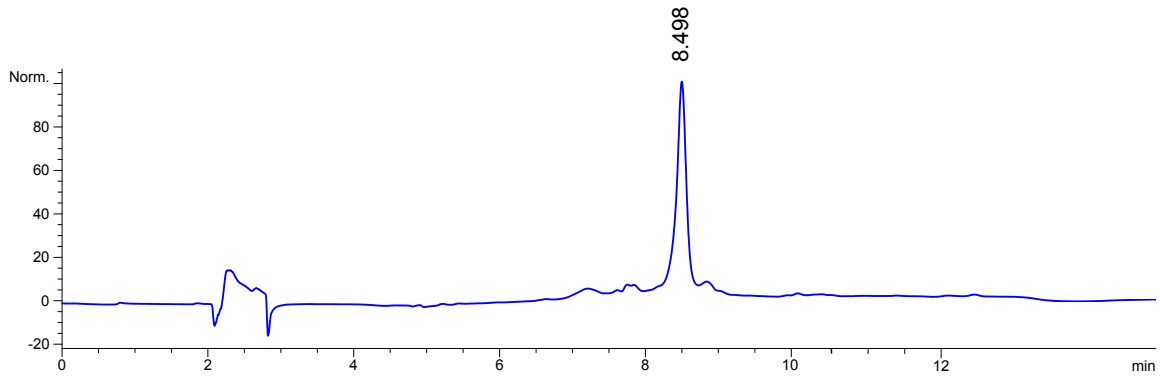


Figure S5. HPLC trace of **2.**

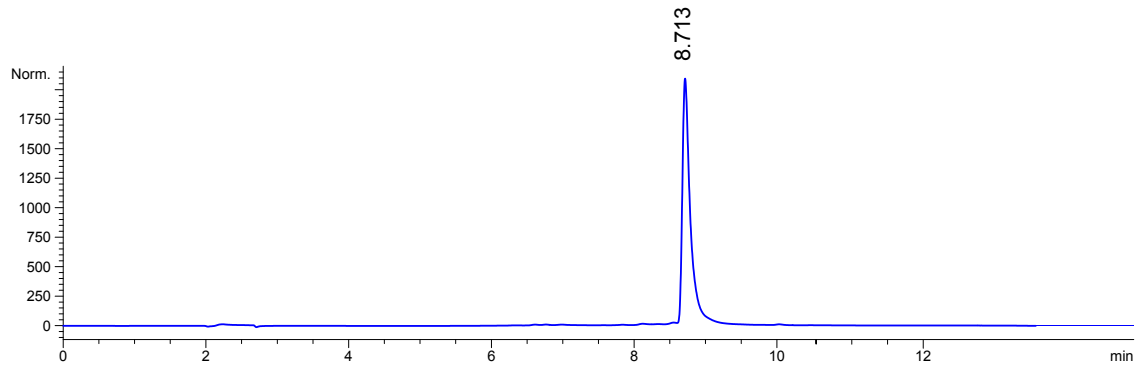
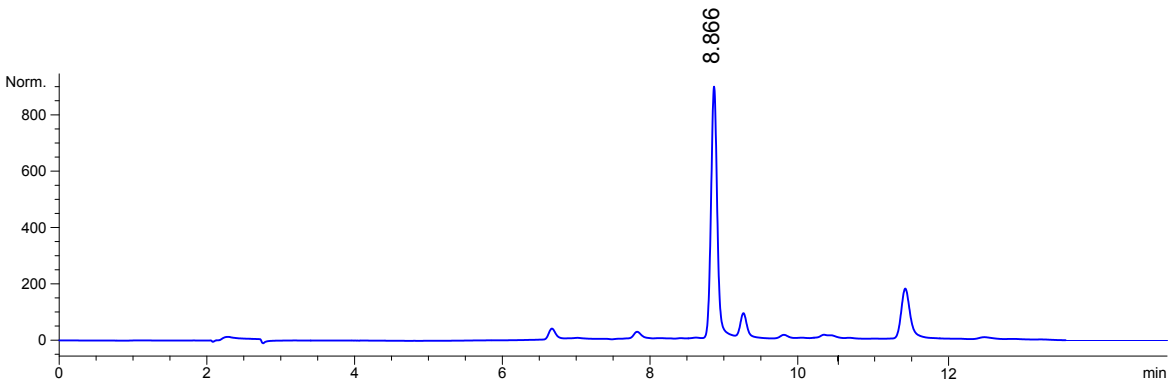
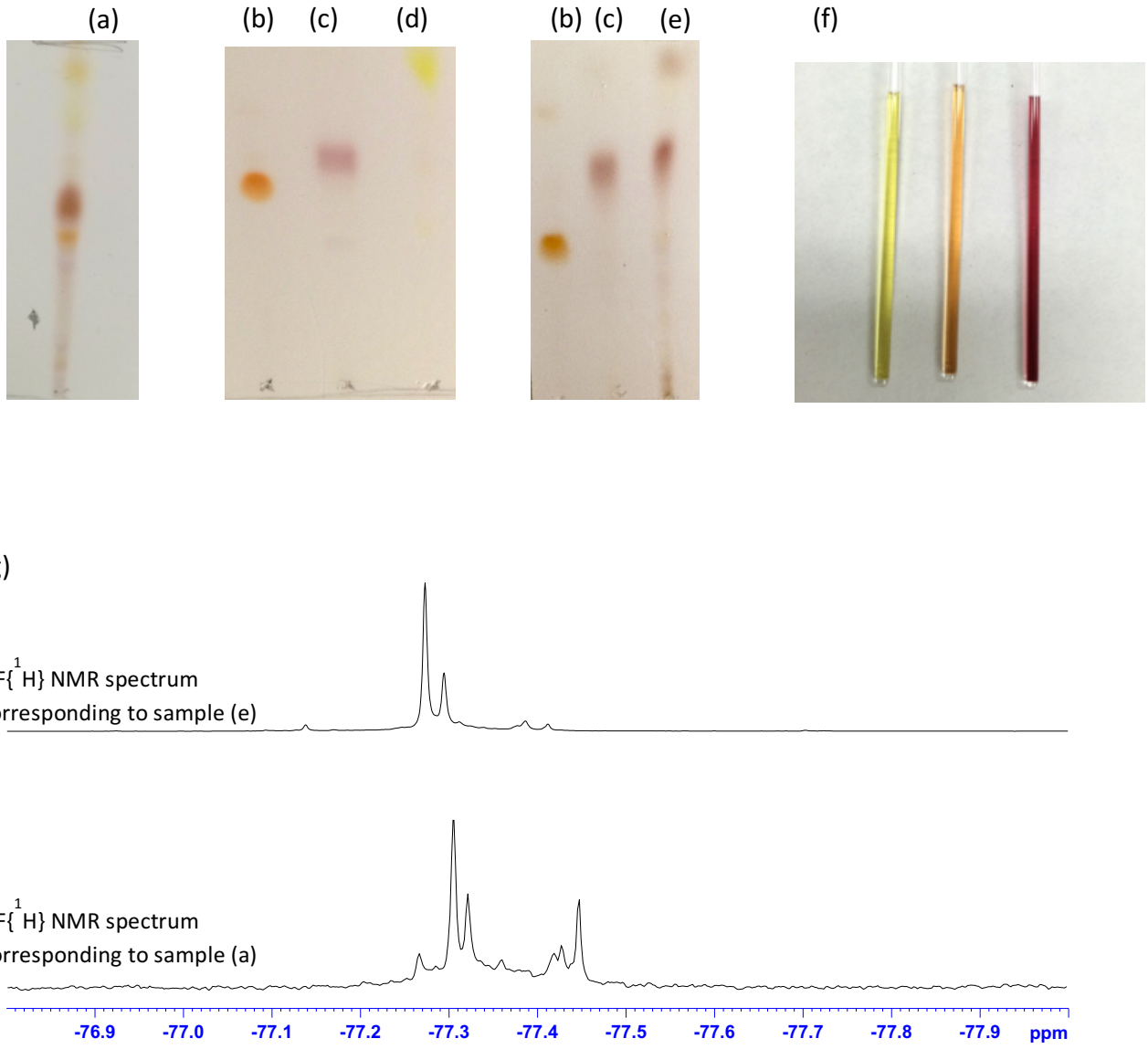


Figure S6. HPLC trace of **3** and **4**.



TLC of crude and purified culture extracts, and $^{19}F\{^{1}H\}$ NMR data for crude culture extracts

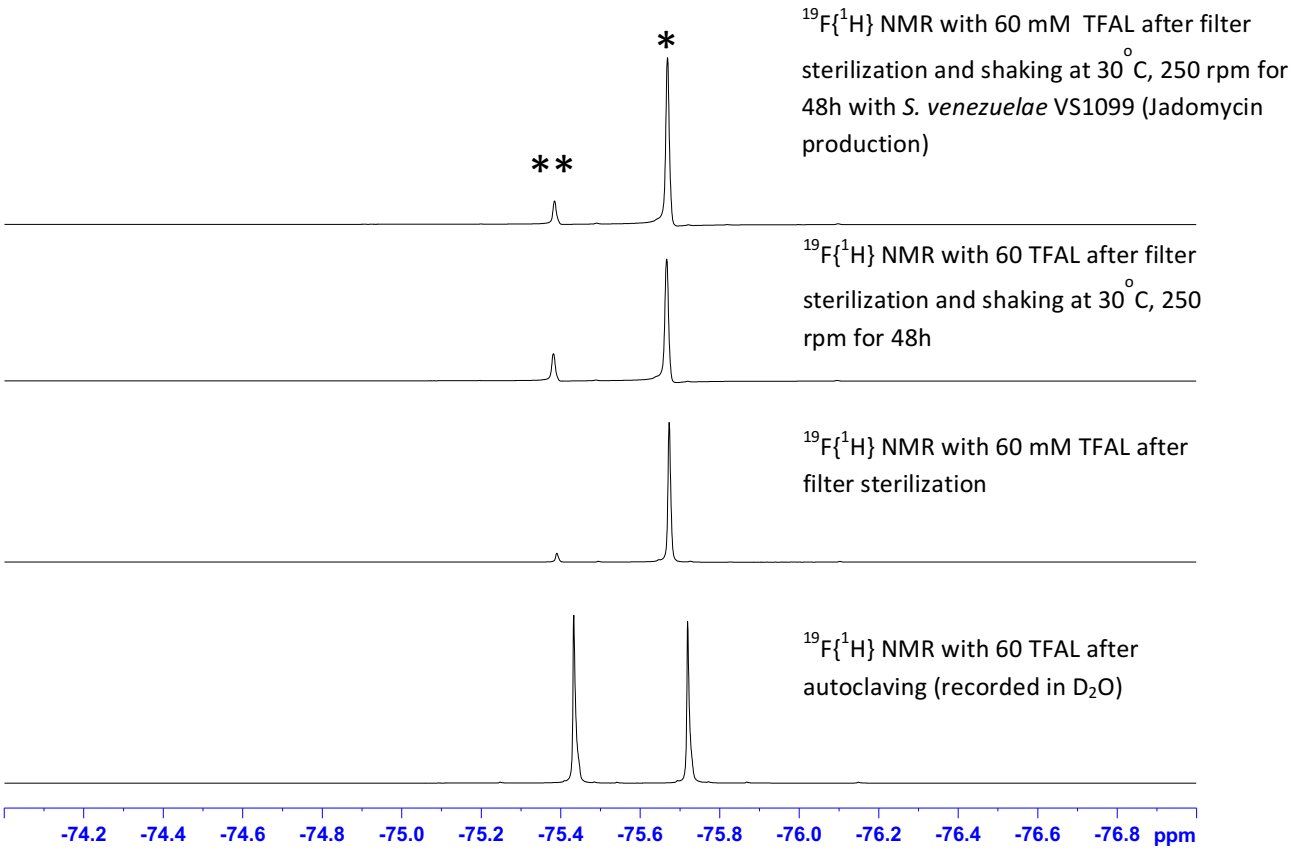
Figure S7. TLC plates developed with 5:5:1 EtOAc:CH_3CN:H_2O as the eluent. (a) Methanol extract from silica phenyl column from a production with *S. venezuelae* VS1099 and TFAL; (b) purified compound **2**; (c) purified compound **1**; (d) purified mixture of **3** and **4;** (e) Methanol extract from silica phenyl column for *S. venezuelae VS1099* production with TFAL using media that was filter sterilized to reduce the hydrolysis of the trifluoroacetyl group prior to bacterial culture. The filter sterilized media does not produce significant amounts of the orange compound **2** in comparison to autoclaved media (visual comparison of (a) and (e); (f) NMR samples of **1** (purple) in methanol-*d4;* **2** (orange) in methanol-*d4*; **3** and **4** (yellow) in chloroform-*d1*; (g) $^{19}F\{^{1}H\}$ NMR spectra 470 MHz, methanol-*d4* of the silica phenyl extracts from the indicated productions.



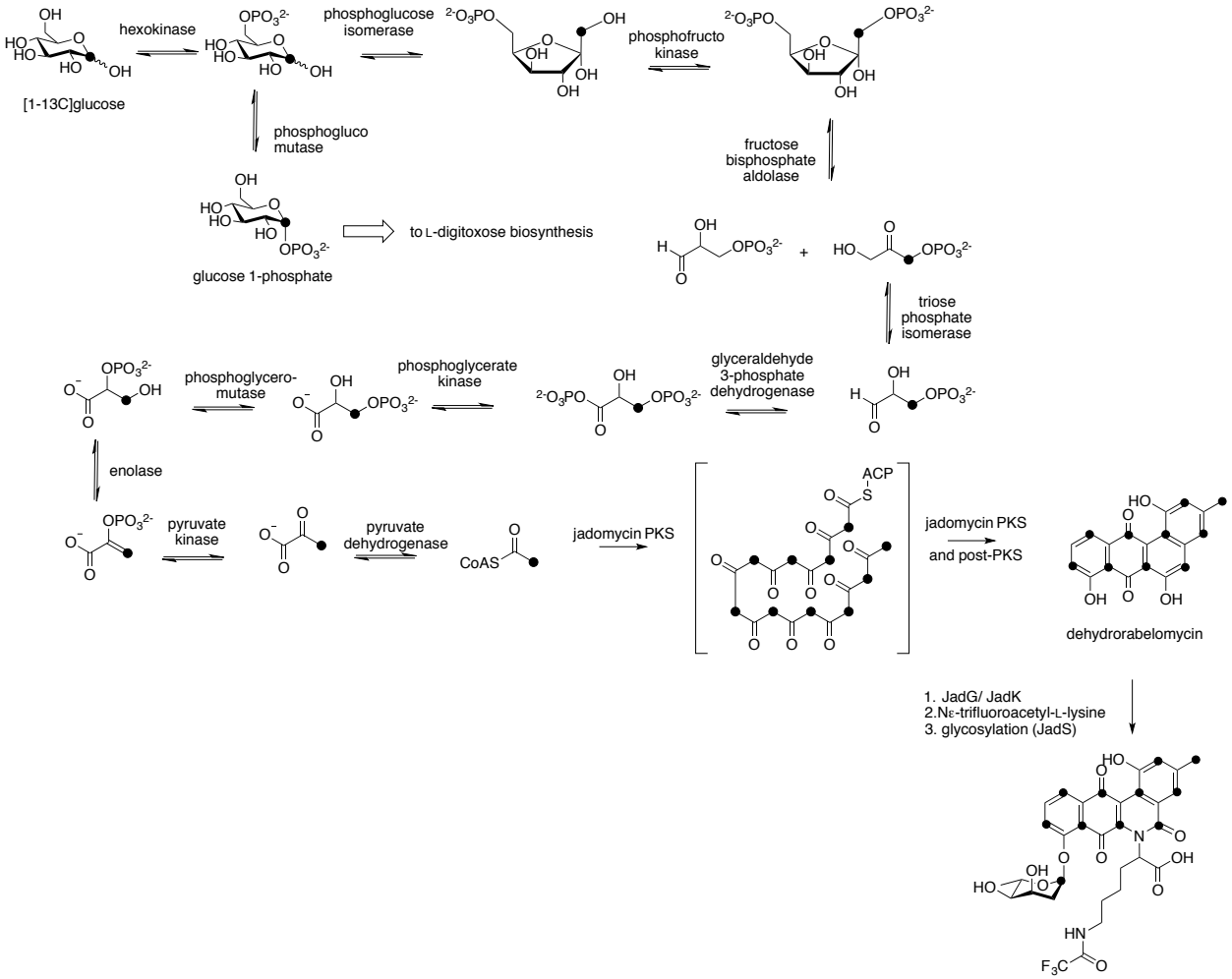
^{19}F NMR spectral analysis of the stability of TFAL in MS culture media and effect of media preparation on bacterial culture

The aqueous extract from the TFAL cultures were also deeply colored after jadomycin production, and was analysis by LC-MS^2 was performed in order to identify produced natural products. The mass and fragmentation pattern corresponding to **JdK** was observed, undoubtedly a product arising from hydrolysis to lysine during autoclave sterilization (Figure S11). Isolation of **JdK** using standard chromatographic techniques was unsuccessful, consistent with the previous reports in which apparent challenges associated with isolation and stability resulted in incomplete characterization of the natural product. (K. Fan, X. Zhang, H. Liu, H. Han, Y. Luo, Q. Wang, M. Geng, K. Yang, *J. Antibiot.,* 2012, **65**, 449). Remarkably, productions with media sterilized by filtration did not produce significant amounts of compound **2,** but did reduce the production of **JdK**.

Figure S8. Stability of TFAL(*) evaluated by ^{19}F{^{1}H} NMR spectroscopy. (**) indicates the hydrolysis product, trifluoroacetate. Approximately half of the protecting group is hydrolyzed after autoclaving, while some hydrolysis is observed after shaking in the culture media for 2 days. The presence of streptomyces does not affect the rate of hydrolysis over two days. All samples recorded in 10% D_2O, 90% H_2O at 470 MHz unless specified otherwise.



Metabolism of 1-^{13}C D-glucose



Scheme S1. Detailed scheme demonstrating 1-^{13}C glucose metabolism and incorporation in jadomycins.

Antibiotic and cytotoxicity screening

Microbroth Antimicrobial Assay:

All microbroth antibiotic susceptibility testing was carried out in 96 well plates in accordance with Clinical Laboratory Standards Institute testing standards (2003) using the following pathogens: methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *S. warneri* ATCC 17917, vancomycin-resistant *Enterococcus faecium* EF 379 (VRE), *Pseudomonas aeruginosa* ATCC 14210, *Proteus vulgaris* ATCC 12454, and *Candida albicans* ATCC 14035. Compounds were tested in three replicates against each organism. Compounds were serially diluted to generate a range of concentrations in a final well volume concentration of 2% DMSO. Each plate contained eight uninoculated positive controls (media with 2% DMSO), eight untreated negative controls (Media with 2% DMSO + organism), and one column containing a concentration range of a control antibiotic (vancomycin for MRSA and *S. warneri*, rifampicin for VRE, gentamycin for *P. aeruginosa*, ciprofloxacin for *P. vulgaris*, or nystatin for *C. albicans*). The optical density of the plate was recorded on a BioTek Synergy HT plate reader at 600nm at time zero and then again after incubation of the plates for 22 h at 37 °C. After subtracting the time zero OD_{600} from the final reading the percentages of microorganism survival relative to vehicle control wells were calculated and the IC_{50} was determined.

Table S2. Antibiotic screening results for jadomycins **1** and **2**.

	MRSA		*S. warneri*		VRE		*P. aeruginosa*		*P. vulgaris*		*C. albicans*	
	MIC_{90} (µg/ml)	IC_{50} (µg/ml)	MIC_{90} (µg/ml)	IC_{50} (µg/ml)	MIC_{90} (µg/ml)	IC_{50} (µg/ml)	MIC_{90} (µg/ml)	IC_{50} (µg/ml)	MIC_{90} (µg/ml)	IC_{50} (µg/ml)	MIC_{90} (µg/ml)	IC_{50} (µg/ml)
1	3.13	2.09 ± 0.2	3.13	2.84 ± 0.14	12.5	1.5 ± 0.3	>100	>100	>100	>100	>100	>100
2	100	47.4 ± 4.5	100	84.1 ± 0.01	>100	24.5 ± 0.3	>100	>100	>100	>100	>100	>100
Vancomycin	0.78	0.51 ± 0.08	0.39	0.24 ± 0.05								
Rifampicin					3.13	1.40 ± 0.02						
Gentamicin							1.56	1.04 ± 0.31				
Ciprofloxacin									0.004	0.0037 ± 0.0001		

Cell Cytotoxicity Assay:

Human foreskin BJ fibroblast cells (ATCC CRL-2522) and *Cercopithecus aethiops* kidney epithelial cells (Vero, ATCC CCL-81) were grown and maintained in 15 mL of Eagle's minimal essential medium (Sigma M5650) supplemented with 10% fetal bovine serum (VWR#CA95043-976) and 100 µU penicillin and 0.1 mg/mL streptomycin (VWR#CA12001-692) in T75cm^2 cell culture flasks (VWR# CABD353136) at 37^{o}C in a humidified atmosphere of 5% CO_2. Culture medium was refreshed every two to three days and cells were not allowed to exceed 80% confluency.

At 80% confluency, the cells were counted, diluted and plated into 96 well treated cell culture plates (VWR#29442-054) at a cell density of 10000 cells per well in 90 µL of growth medium (minus the addition of antibiotics). The plates were incubated at 37 ^{o}C in a humidified atmosphere of 5% CO_2 to allow cells to adhere to the plates for 24 h before treatment. DMSO was used as the vehicle at a final concentration of 1% in the wells. All tested compounds were resolublized in sterile DMSO (Sigma#D2438) and a dilution series was prepared for each cell line, added to the respective assay plate, and plates were incubated at 37 ^{o}C in a humidified atmosphere of 5% CO_2 for 24 h. All samples were

tested in triplicate. Each plate contained eight un-inoculated positive controls (media with 1% DMSO), eight untreated negative controls (media with 1% DMSO + cells), and a concentration range of a positive cytotoxin control (zinc pyrithione for fibroblast cells and phenoxyethanol for kidney cells). Alamar blue (Invitrogen#Dal1100) was added 24 h after treatment, to each well at 10% of the culture volume (11µL in 100 µL). Fluorescence was monitored using a BioTek Synergy HT plate reader at 530/25 excitation, 590/35 emission at both time zero and 4 h after Alamar blue was added. After subtracting the time zero emission 590 nm measurement from the final reading, the inferred percentage of microorganism survival relative to vehicle control wells were calculated and the IC_{50} was determined.

Table S3. Cytotoxicity screening results for compounds **1** and **2**

	Fibroblast		Kidney	
	MIC_{90} (µg/ml)	IC_{50} (µg/ml)	MIC_{90} (µg/ml)	IC_{50} (µg/ml)
1	>128	>128	>128	>128
2	64	57.5 ± 3.7	>128	>128
zinc pyrithione	16	7.8 ± 0.4		
phenoxyethanol			0.63*	0.32 ± 0.01 *

***units are a % based on total well volume**

References:

National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Cancer cell line screening:

Compounds **1**, **2**, **3**+**4** were submitted to the National Cancer Institute's Developmental Therapeutics NCI-60 Human Tumor cell lines screen. See https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm (accessed May 31 2016) for detailed experimental procedures. **1** was not accepted for screening. Compounds **2** and **3**+**4** were not selected for further screening after the one-dose NC-60 cell panel.

Figure S9. One-dose screening results for **2:**

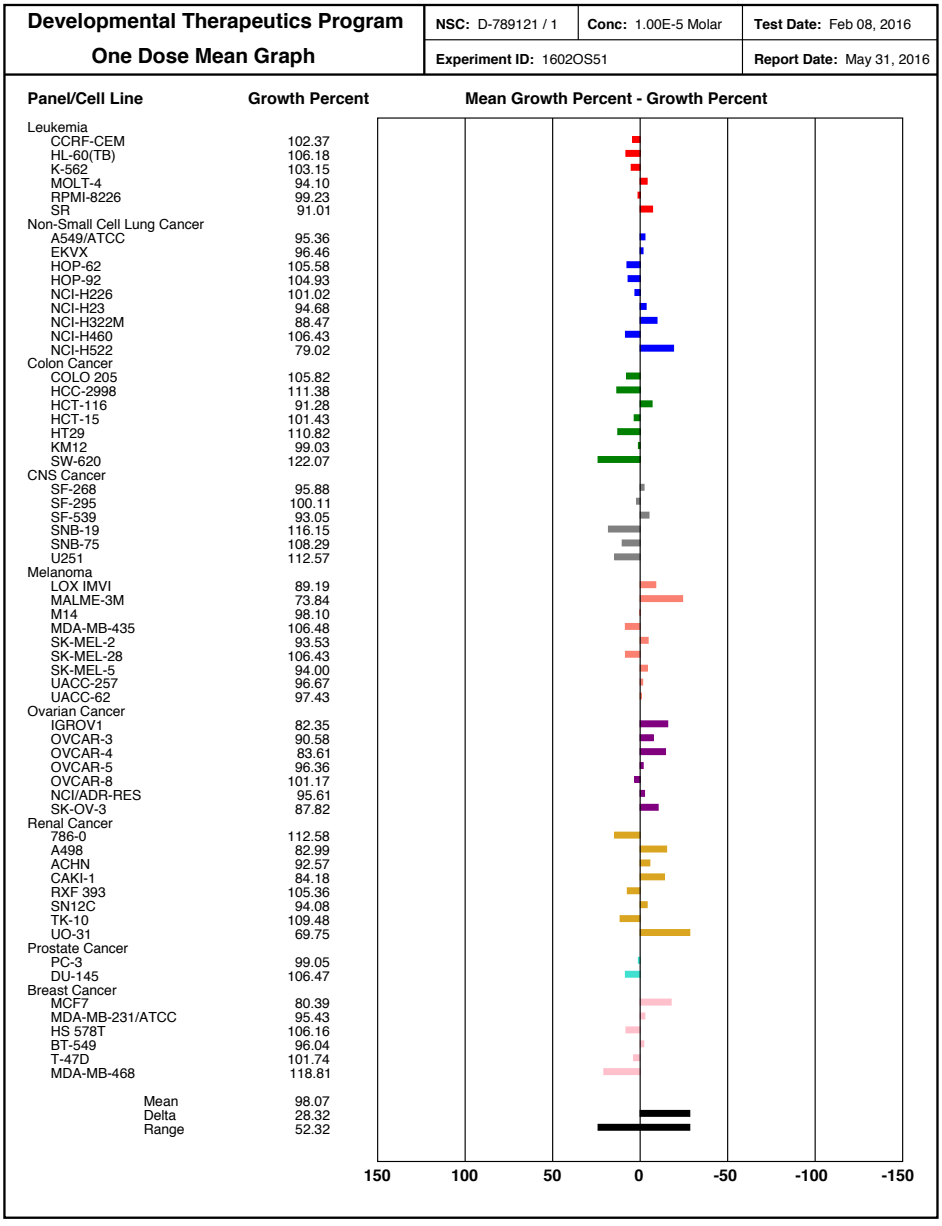
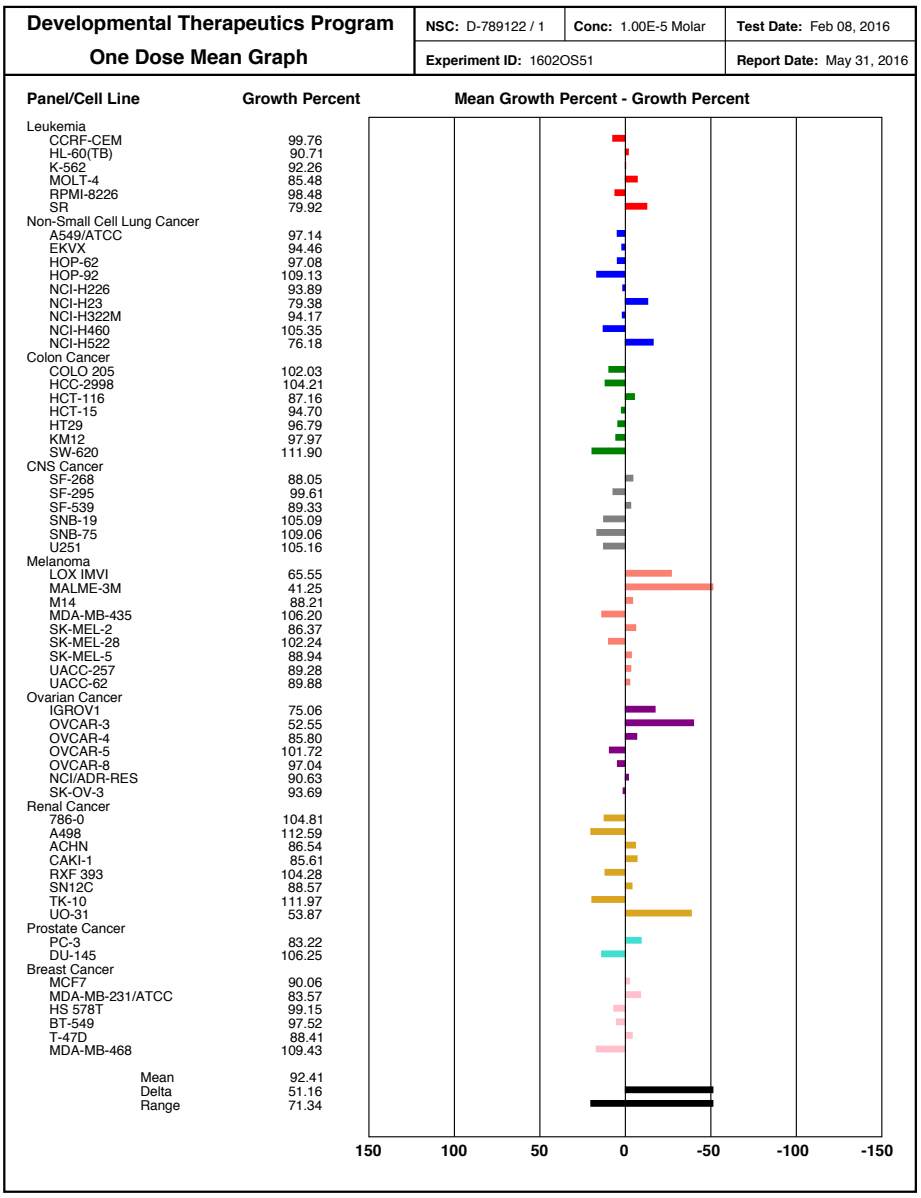


Figure S10. One-dose screening results for **3+4**:



NMR spectra (^{1}H, ^{13}C, ^{19}F{^{1}H}, COSY, HSQC, HMBC) for compounds **1**, **2**, **3**+**4**

Figure S11. ^{1}H NMR spectrum of **1** (700 MHz, methanol-*d4*).

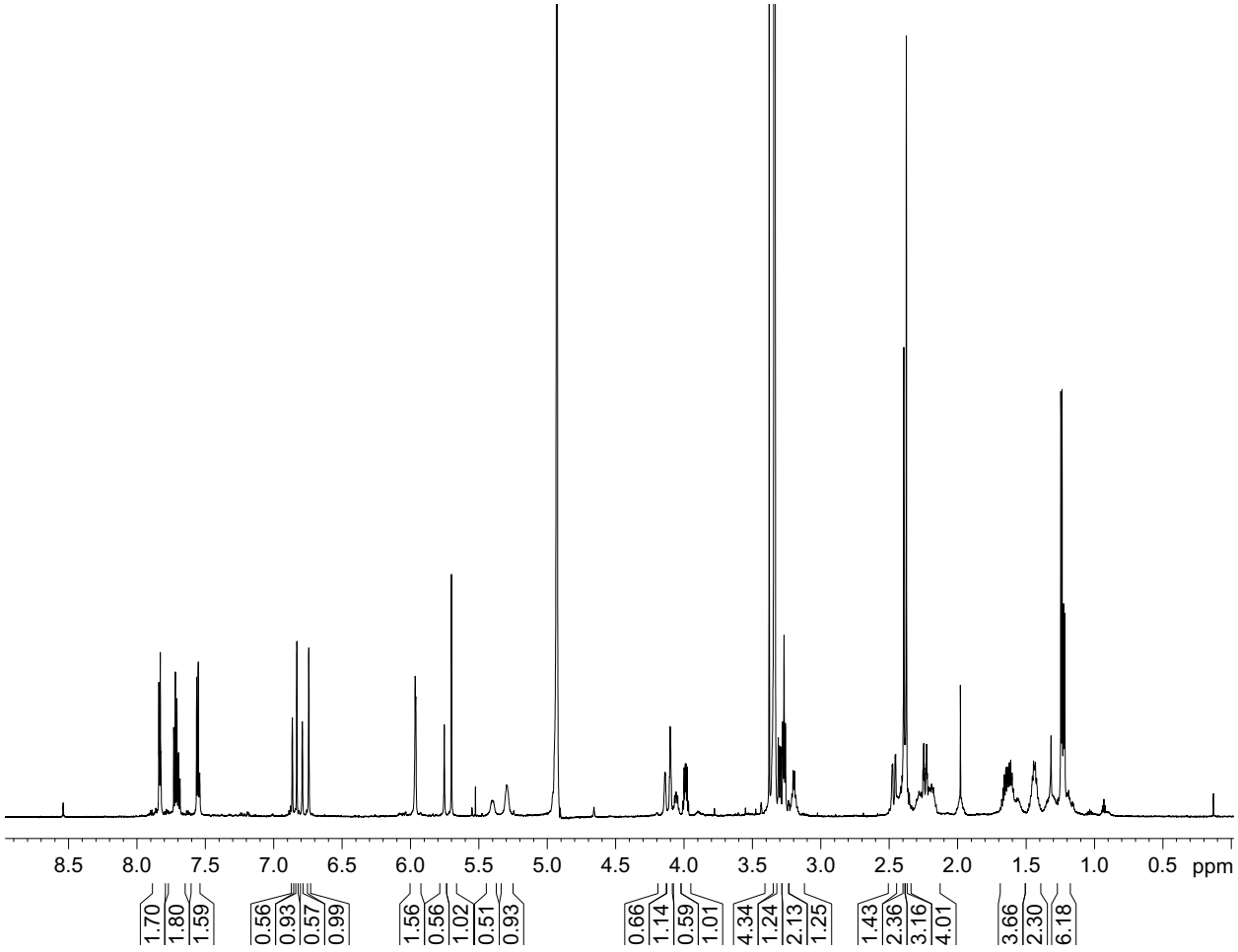


Figure S12. $^{13}C\{^{1}H\}$ spectrum of **1** (176 MHz, methanol-*d4*).

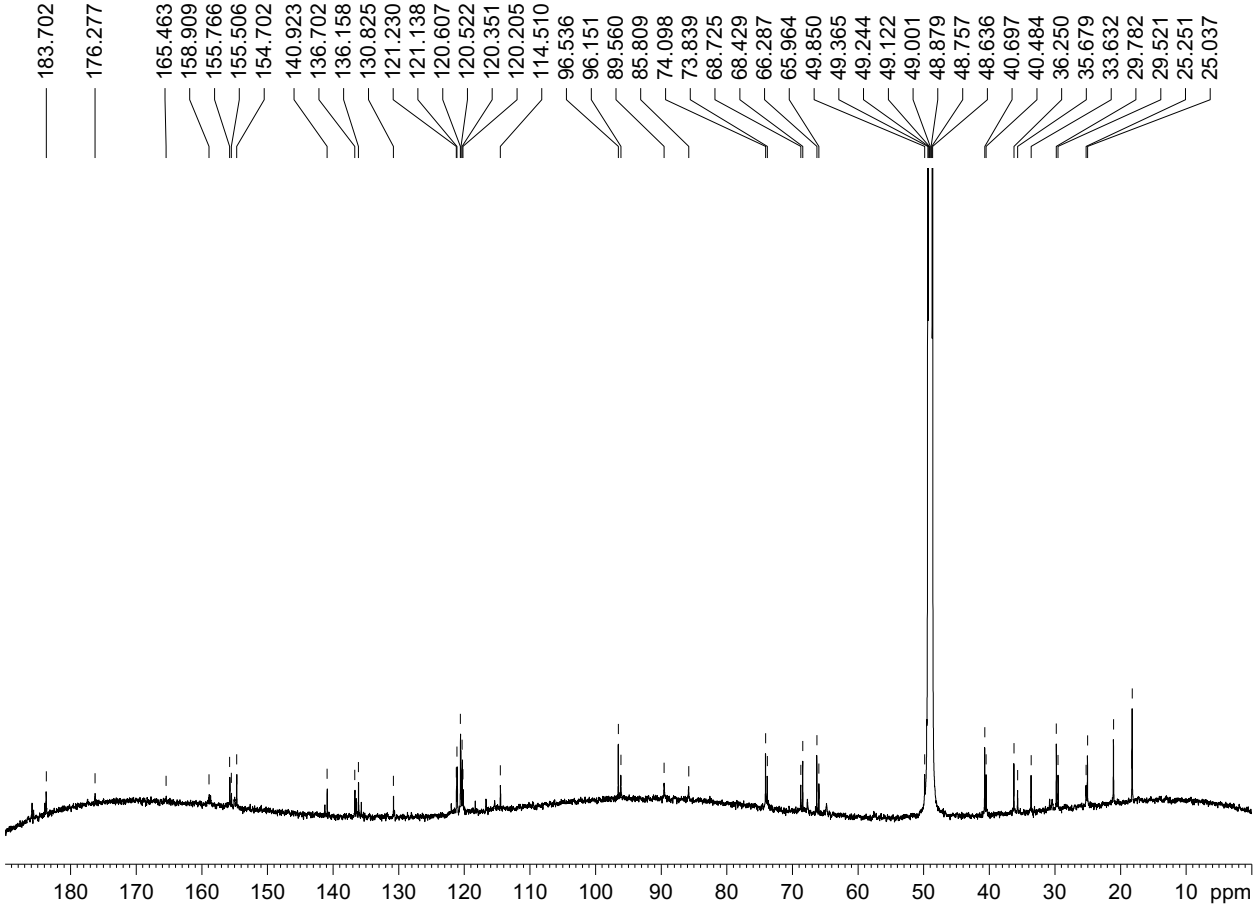


Figure S13. COSY NMR spectrum of **1** in (700 MHz, methanol-*d4*).

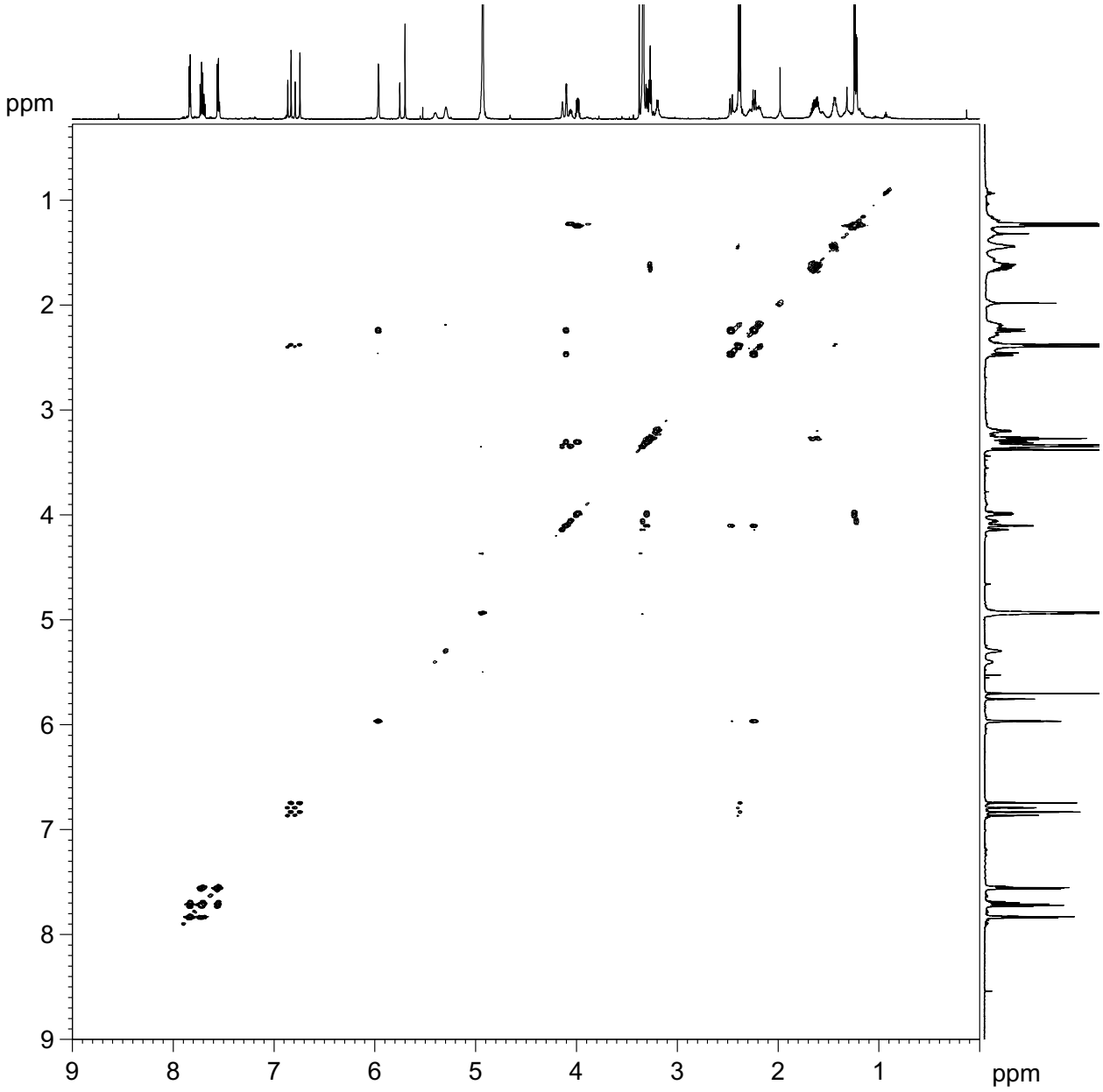


Figure S14. HSQC NMR spectrum of **1** in (700 MHz, methanol-*d4*).

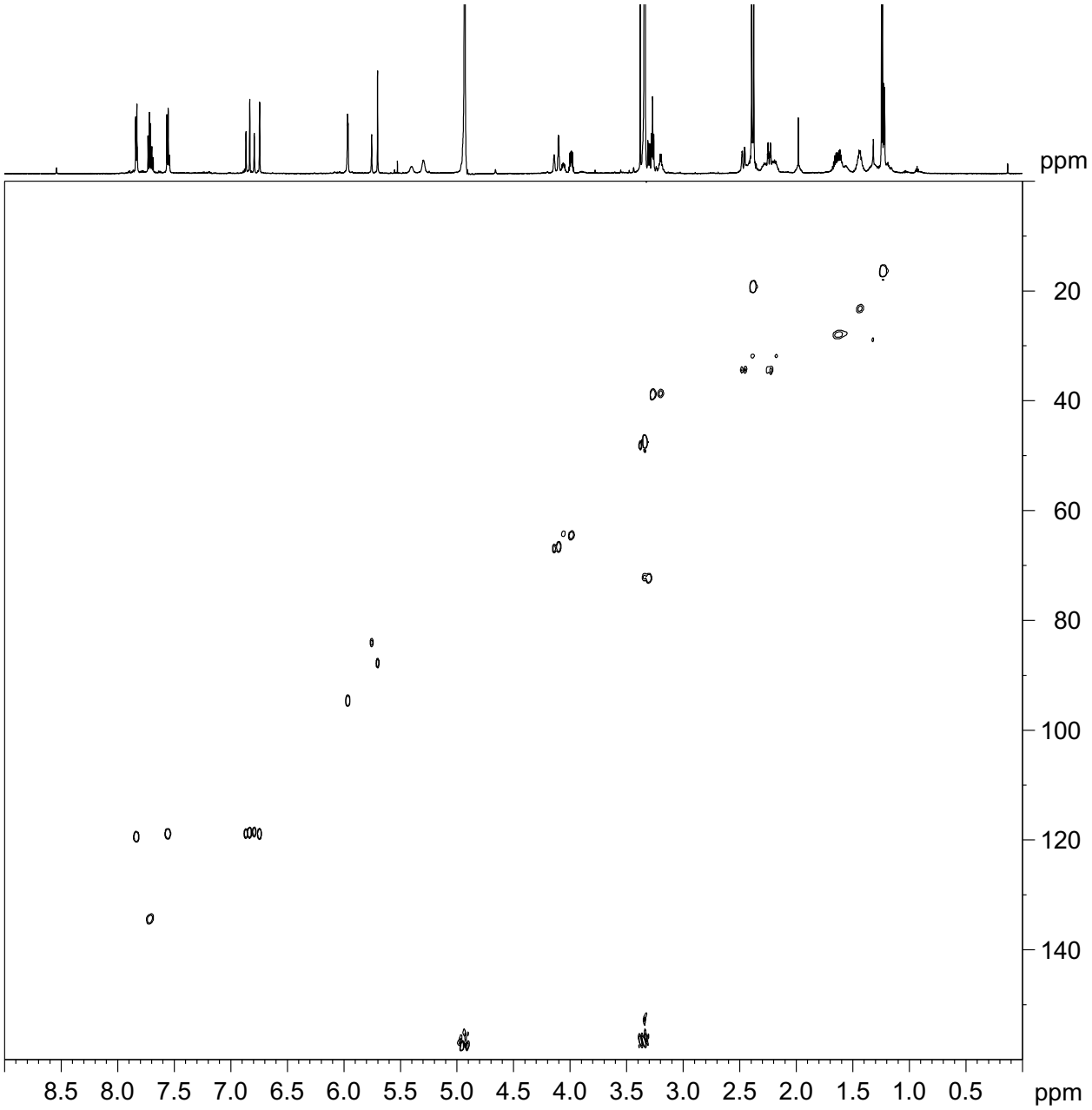


Figure S15. HMBC NMR spectrum of **1** in (700 MHz, methanol-*d4*).

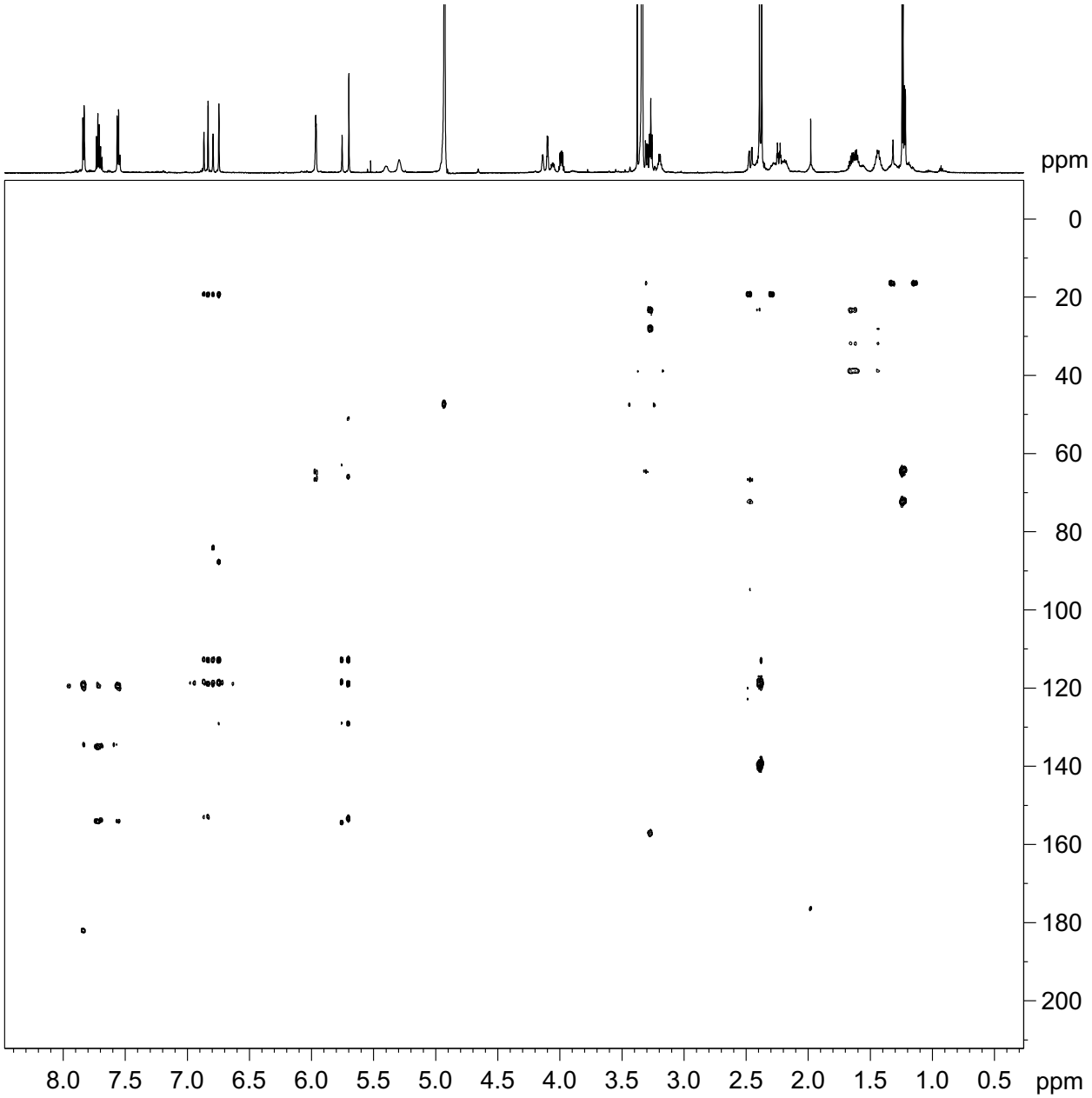


Figure S16. $^{19}F\{^{1}H\}$ NMR spectrum of **1** in (470 MHz, methanol-*d4*).

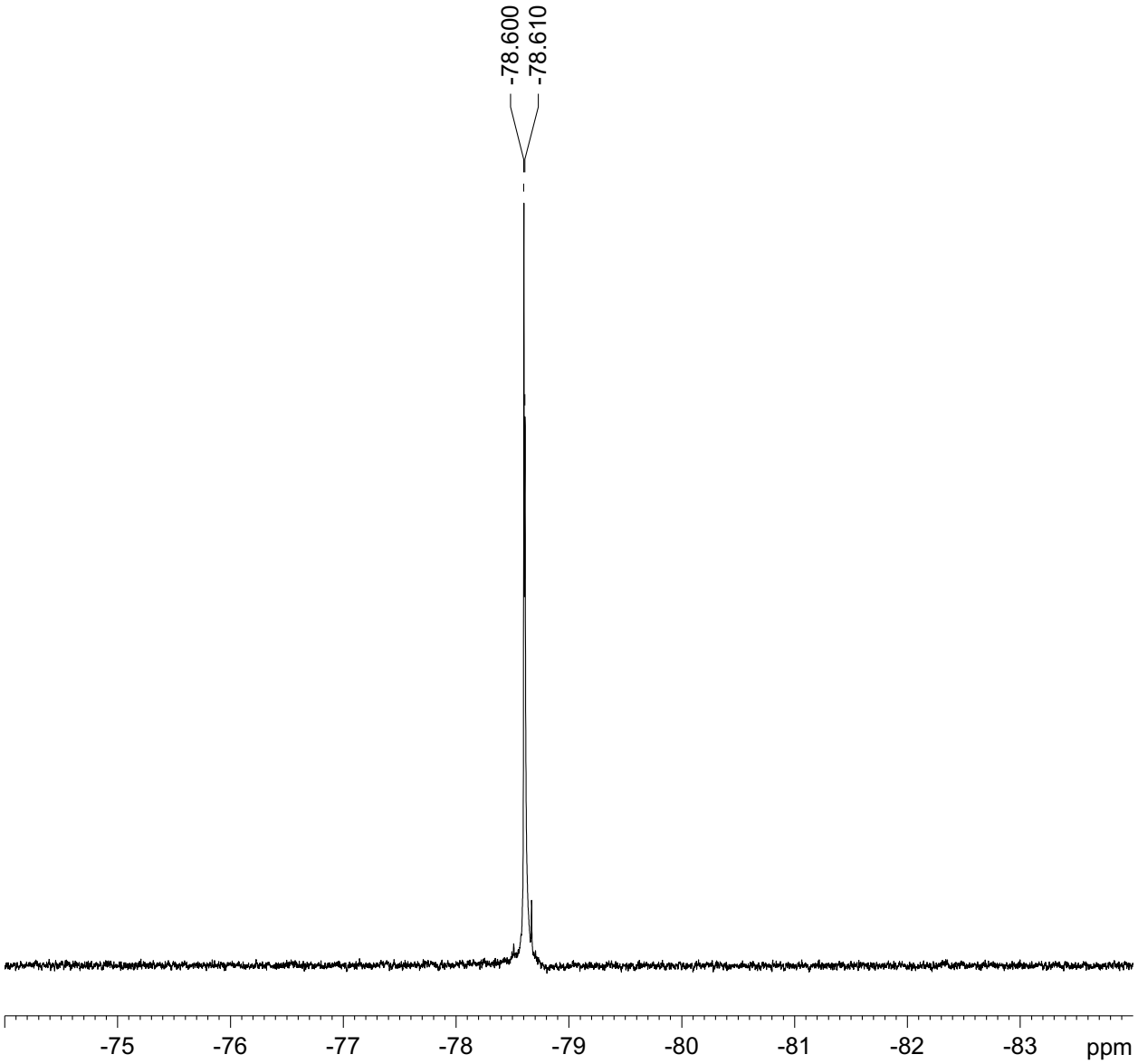


Figure S17. ID NOESY experiment to determine the stereochemistry of position 3a in compound **1**. Irradiation of the 3a proton at 5.67 ppm for the major compound and at 5.72 ppm in the minor compound (500 MHz, methanol-*d4*).

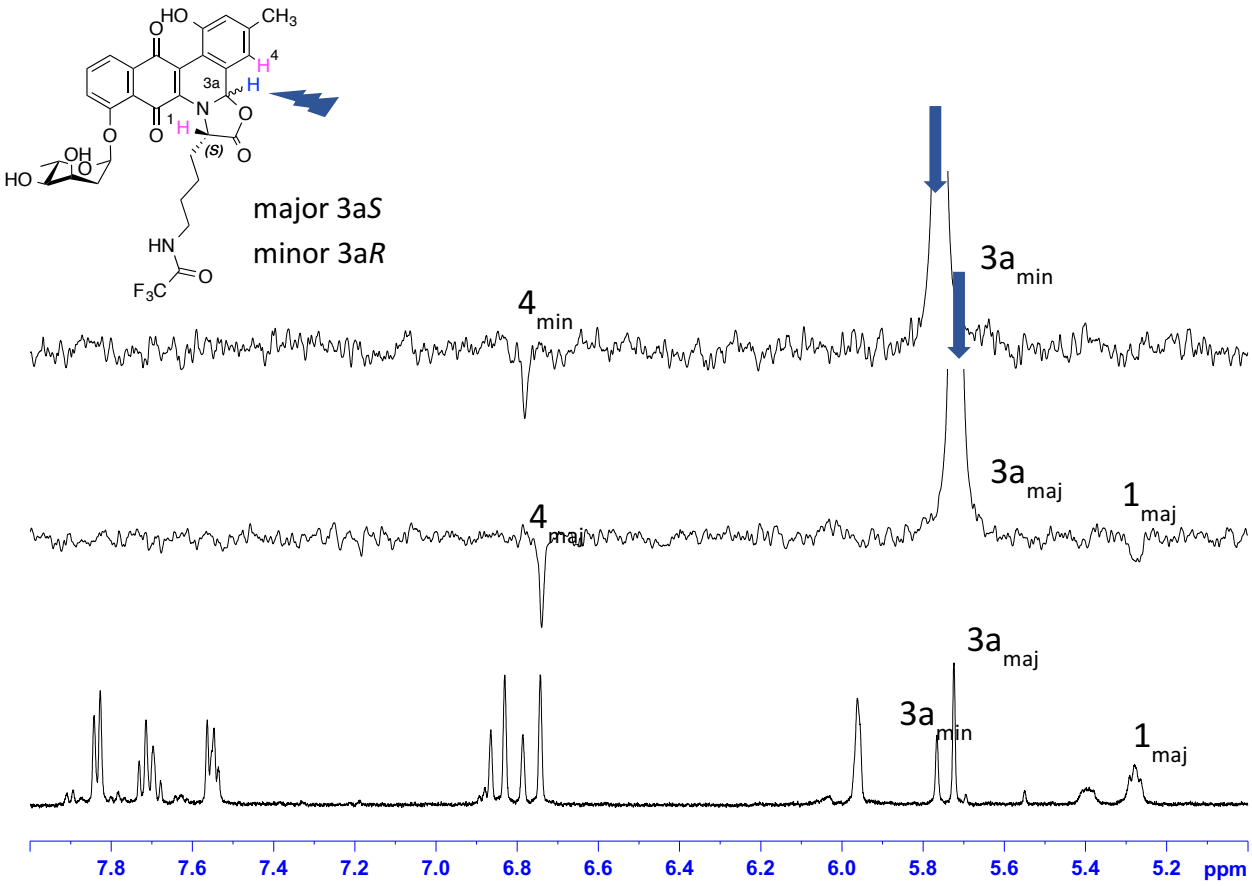


Figure S18. ^{1}H NMR spectrum of **2** (700 MHz, methanol-*d4*).

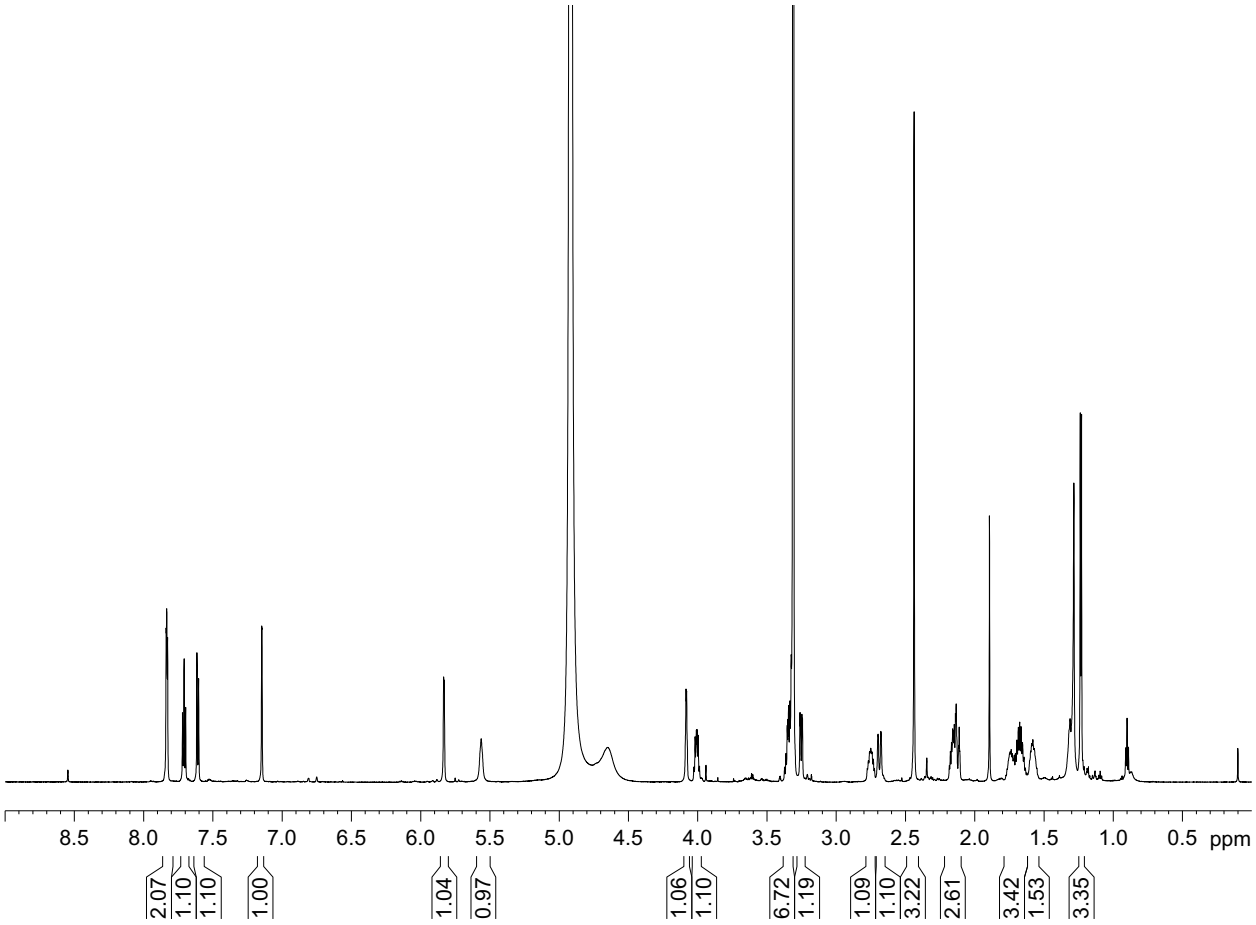


Figure S19. $^{13}C\{^{1}H\}$ NMR spectrum of **2** supplemented with ^{13}C-1 glucose production (125 MHz, methanol-*d4*). Key assignments are indicated. *Due to the reversibility of glycolysis (**Scheme S1**), some labeling of C5"-CH_3 is evident.

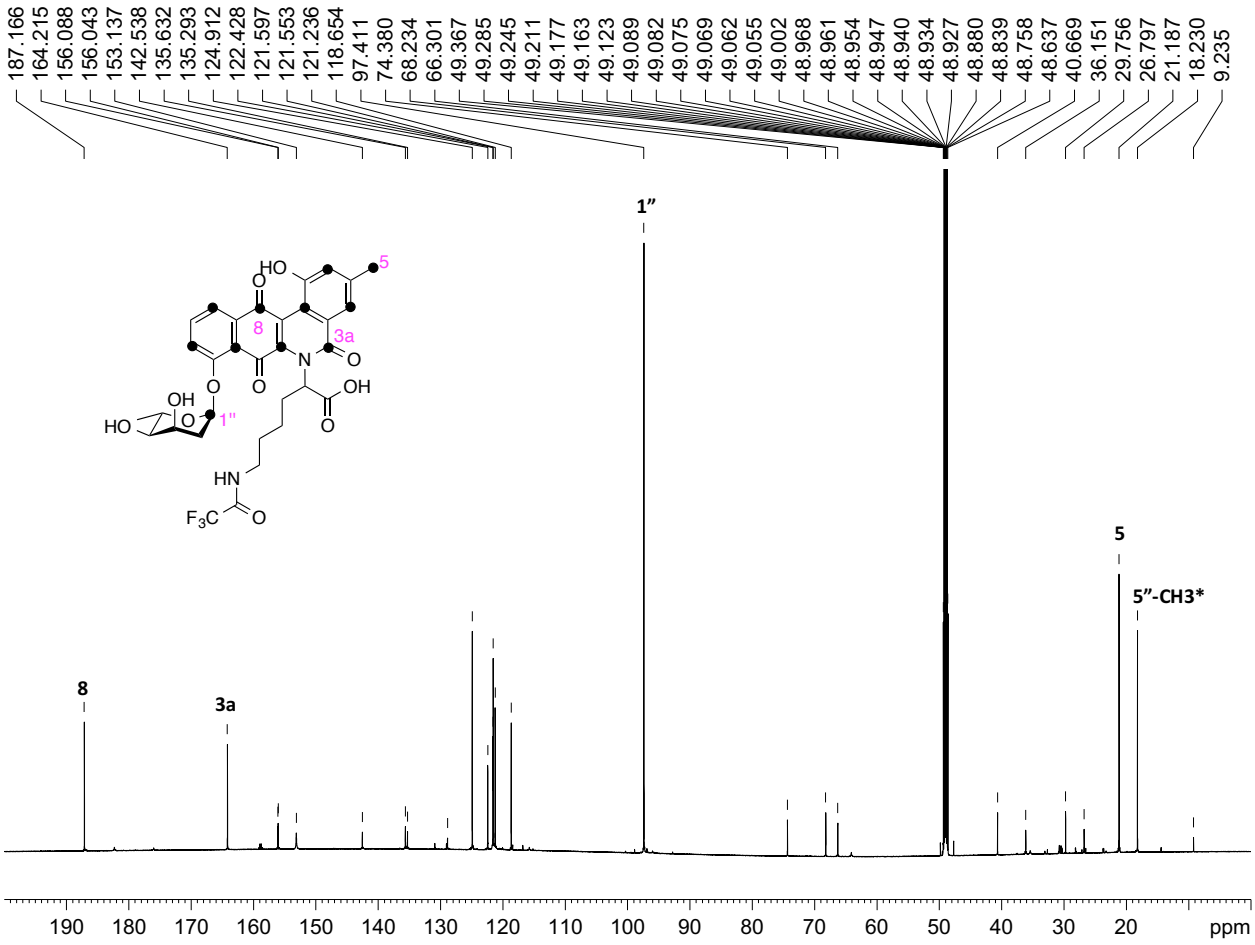


Figure S20. COSY spectrum of **2** (700 MHz, methanol-*d4*).

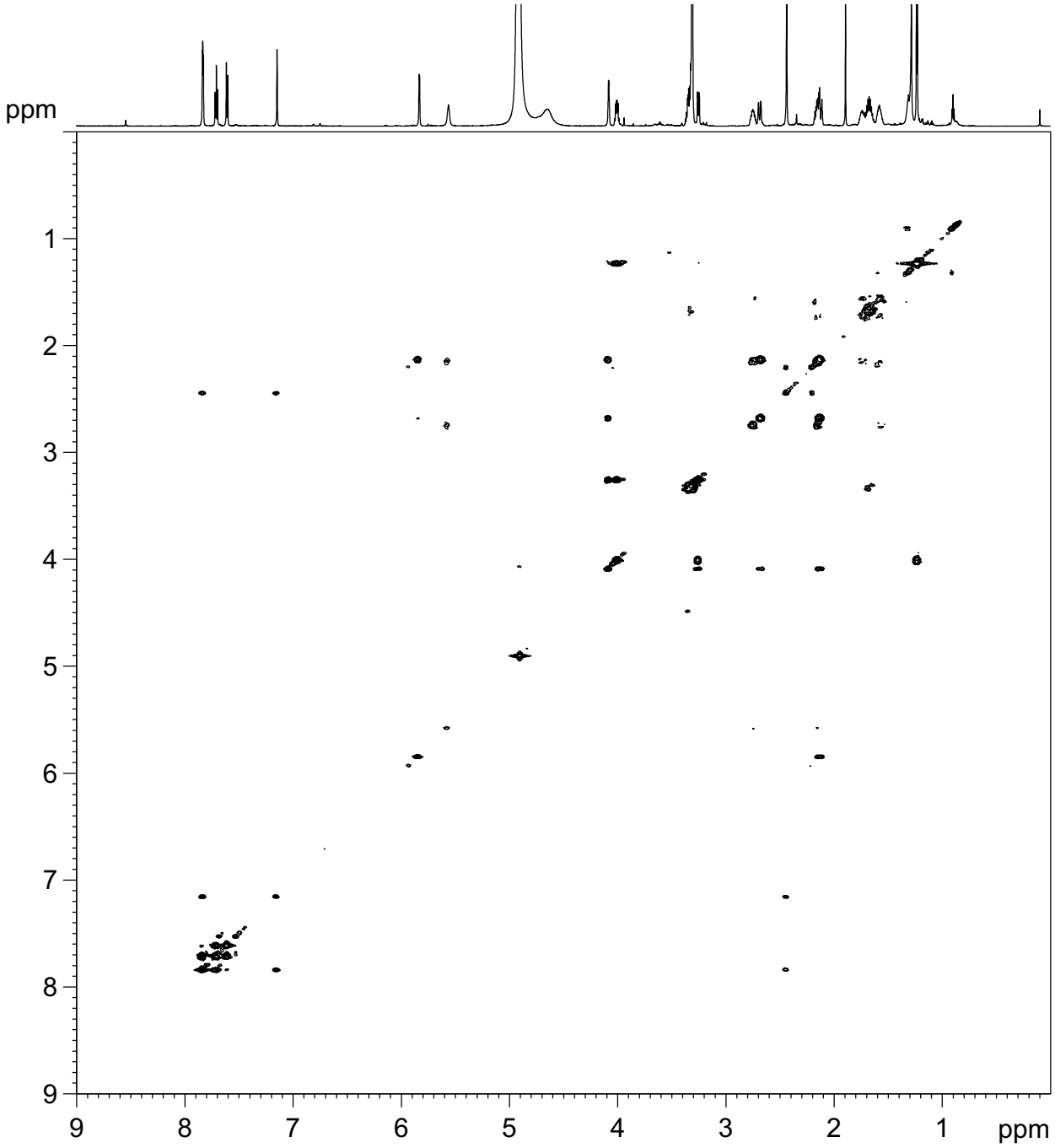


Figure S21. HSQC spectrum of **2** in (700 MHz, methanol-*d4*).

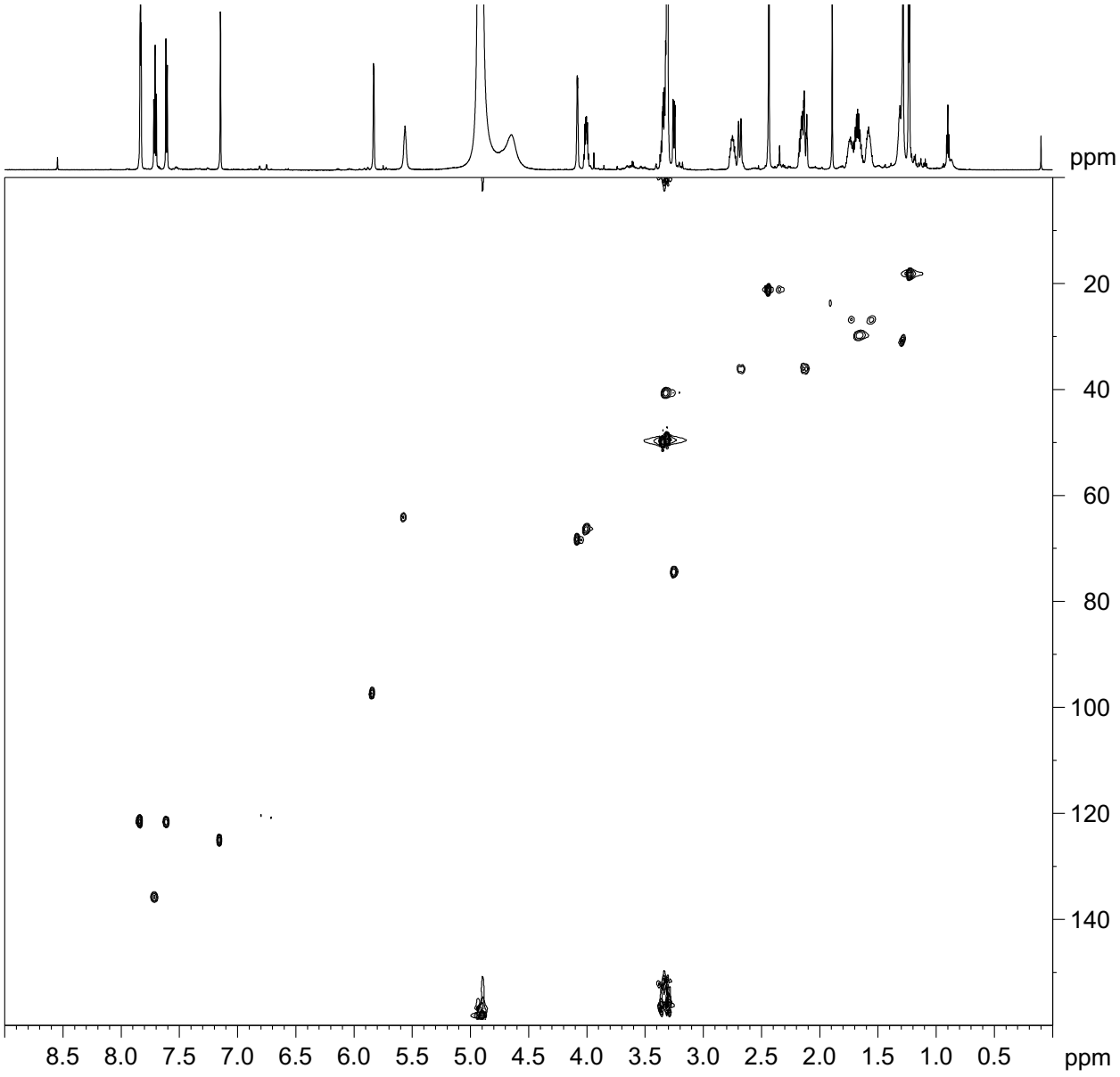


Figure S22. HMBC spectrum of ^{13}C-1 supplemented **2** in (700 MHz, methanol-*d4*).

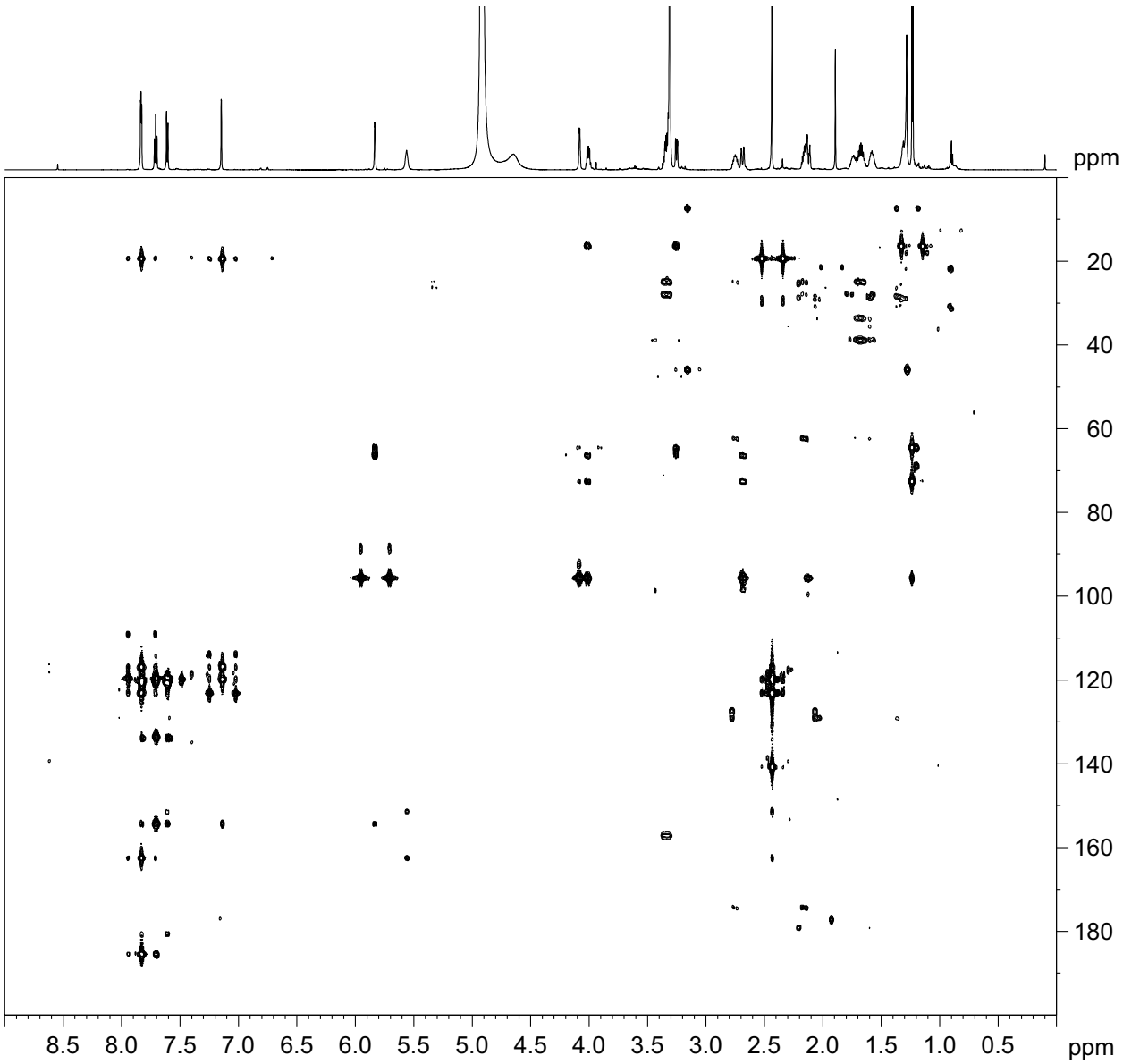

ppm
20
40
60
80
100
120
140
160
180
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

Figure S23. $^{19}F\{^{1}H\}$ spectrum of ^{13}C-1 supplemented **2** (470 MHz, methanol-*d4*).

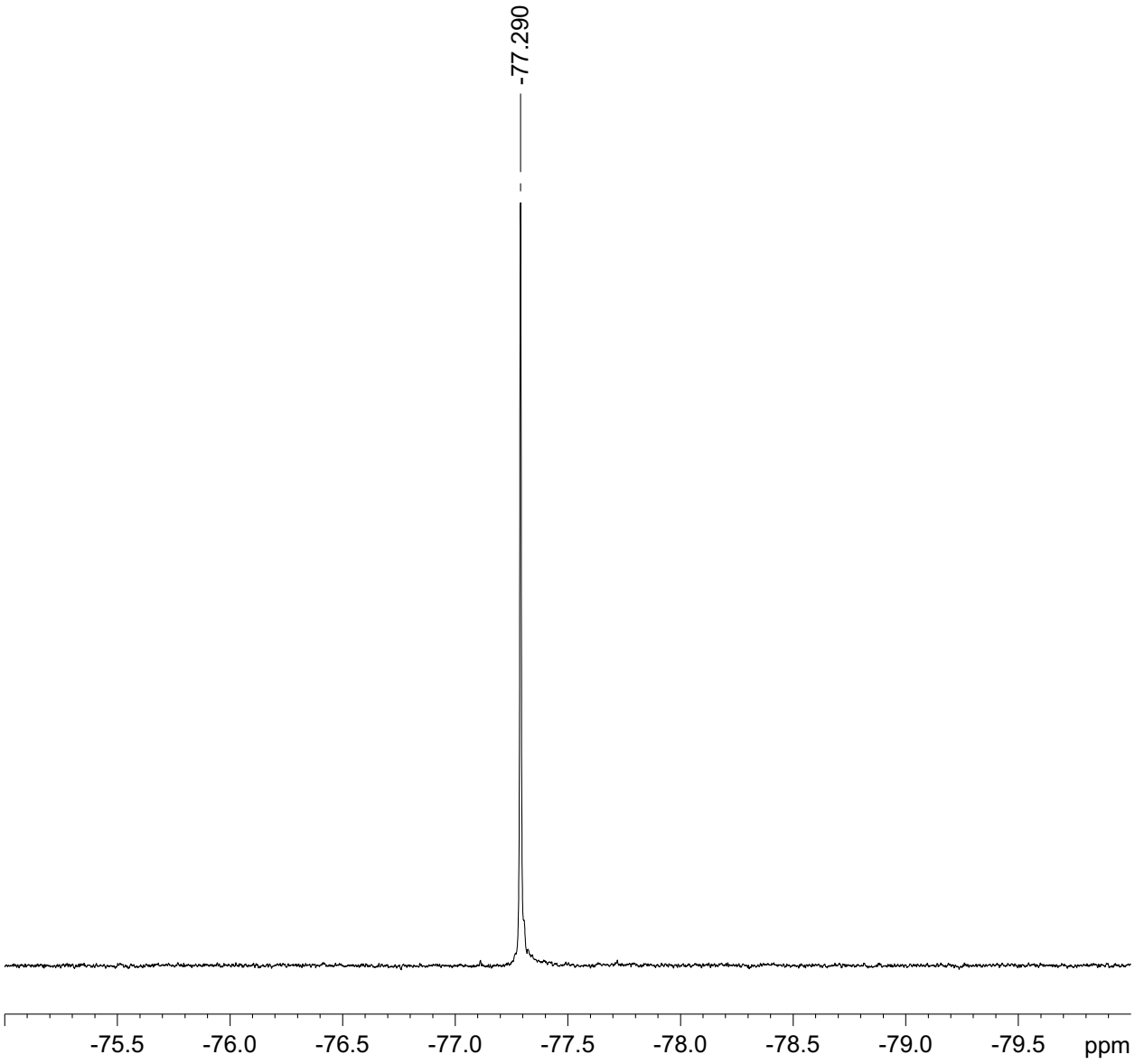


Figure S24. ^{1}H NMR spectrum of **3** and **4** (700 MHz, $CDCl_3$).

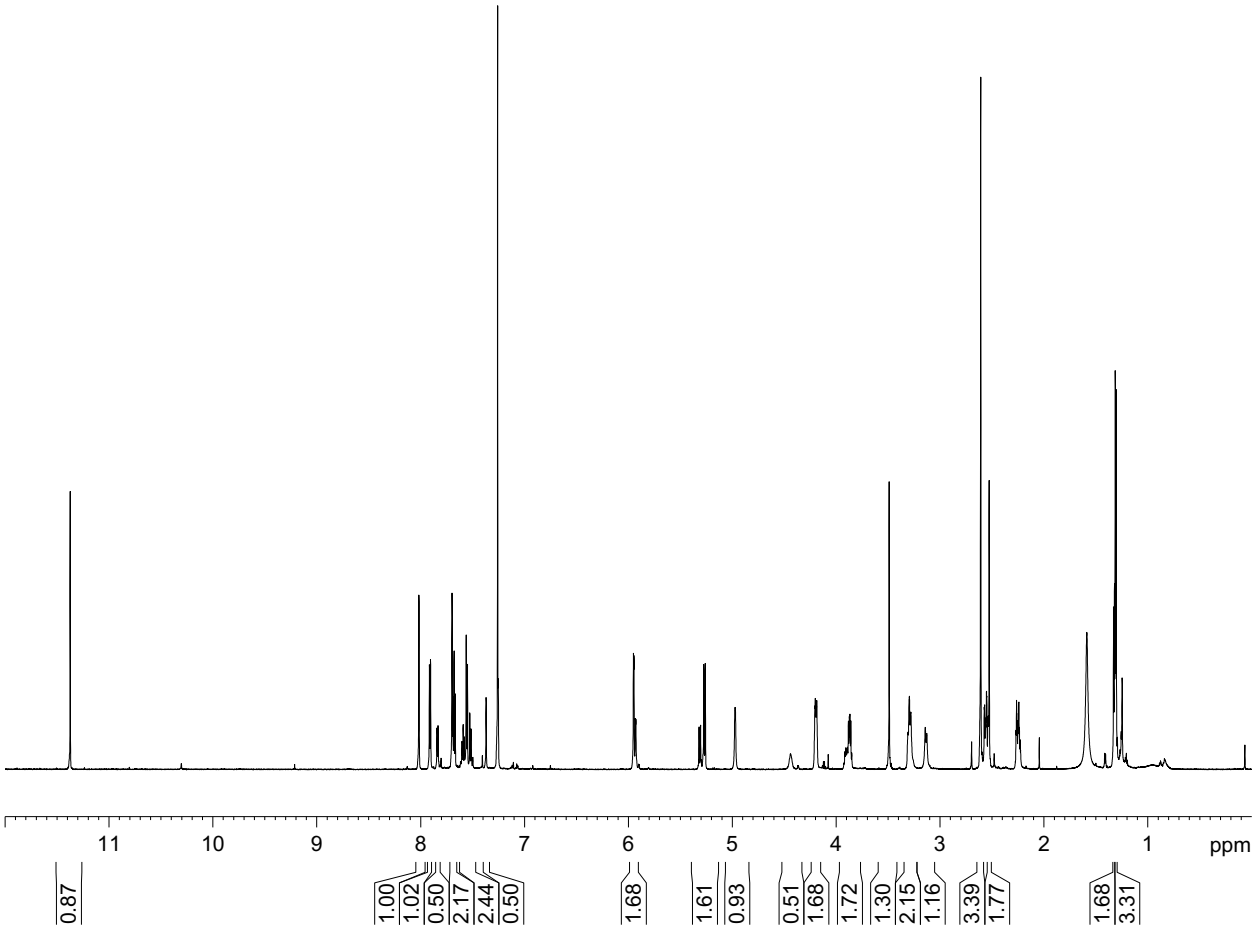


Figure S25. COSY NMR spectrum of **3** and **4** in (700 MHz, $CDCl_3$).

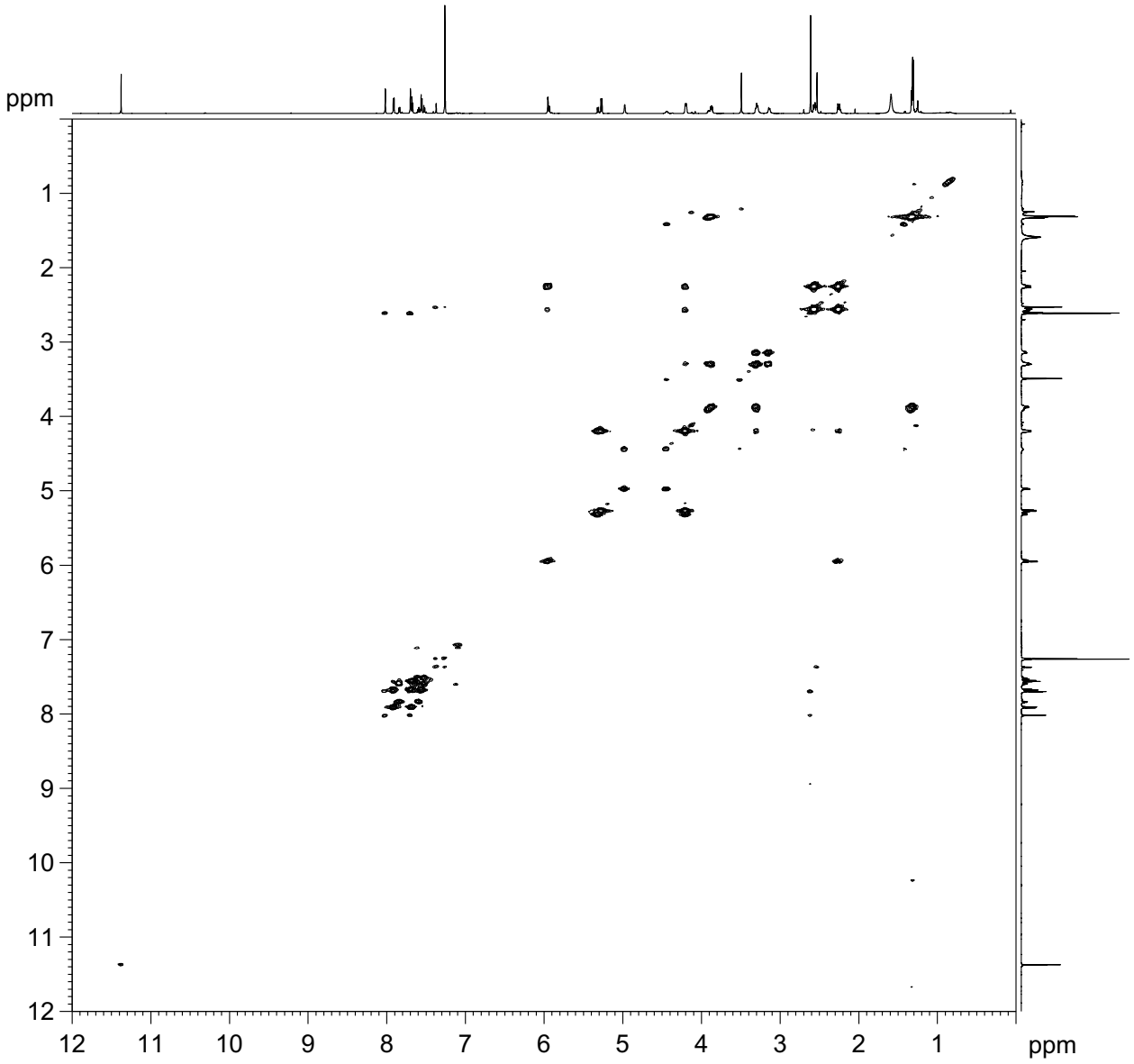


Figure S26. HSQC spectrum of **3** and **4** in (700 MHz, $CDCl_3$).

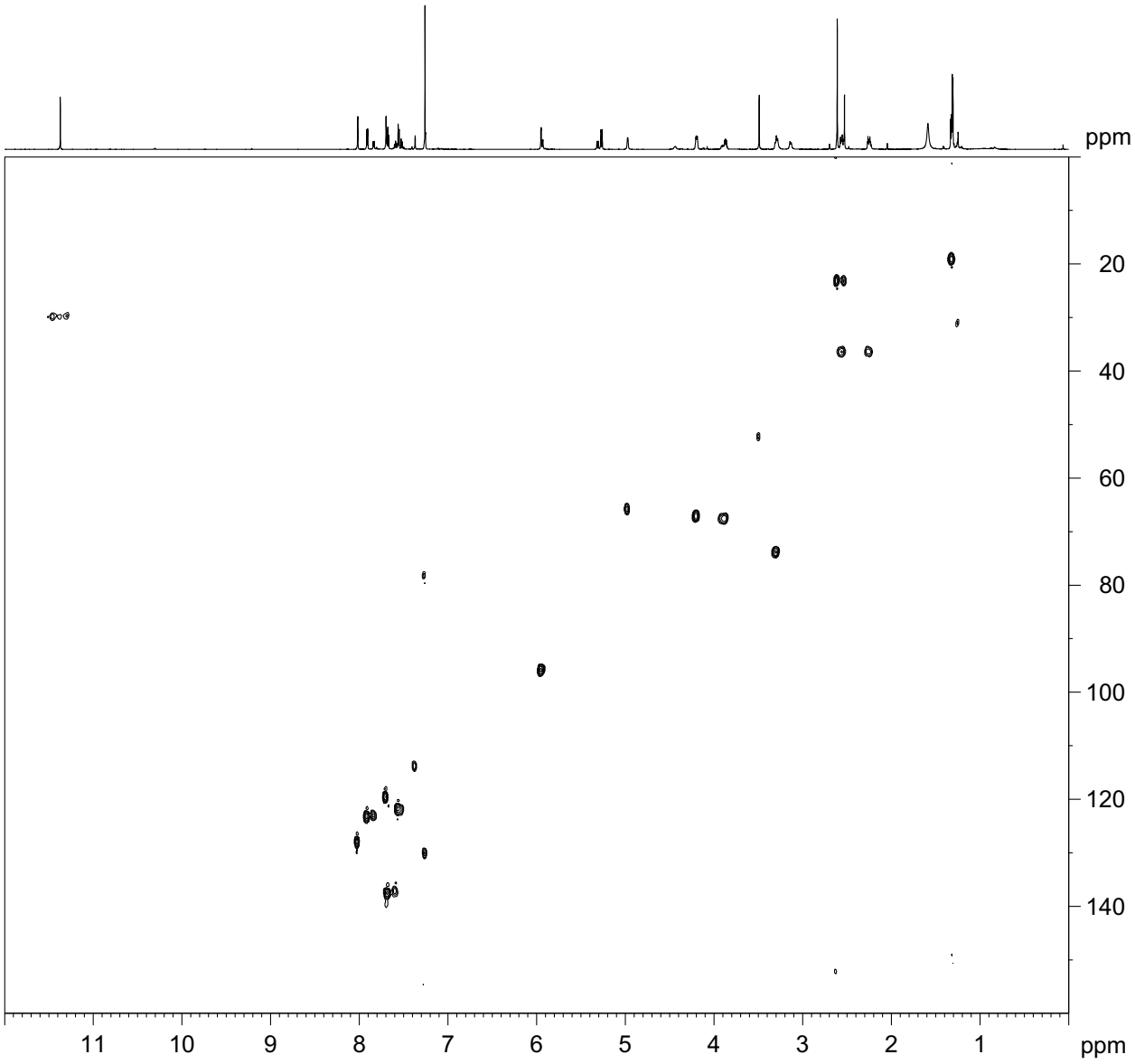
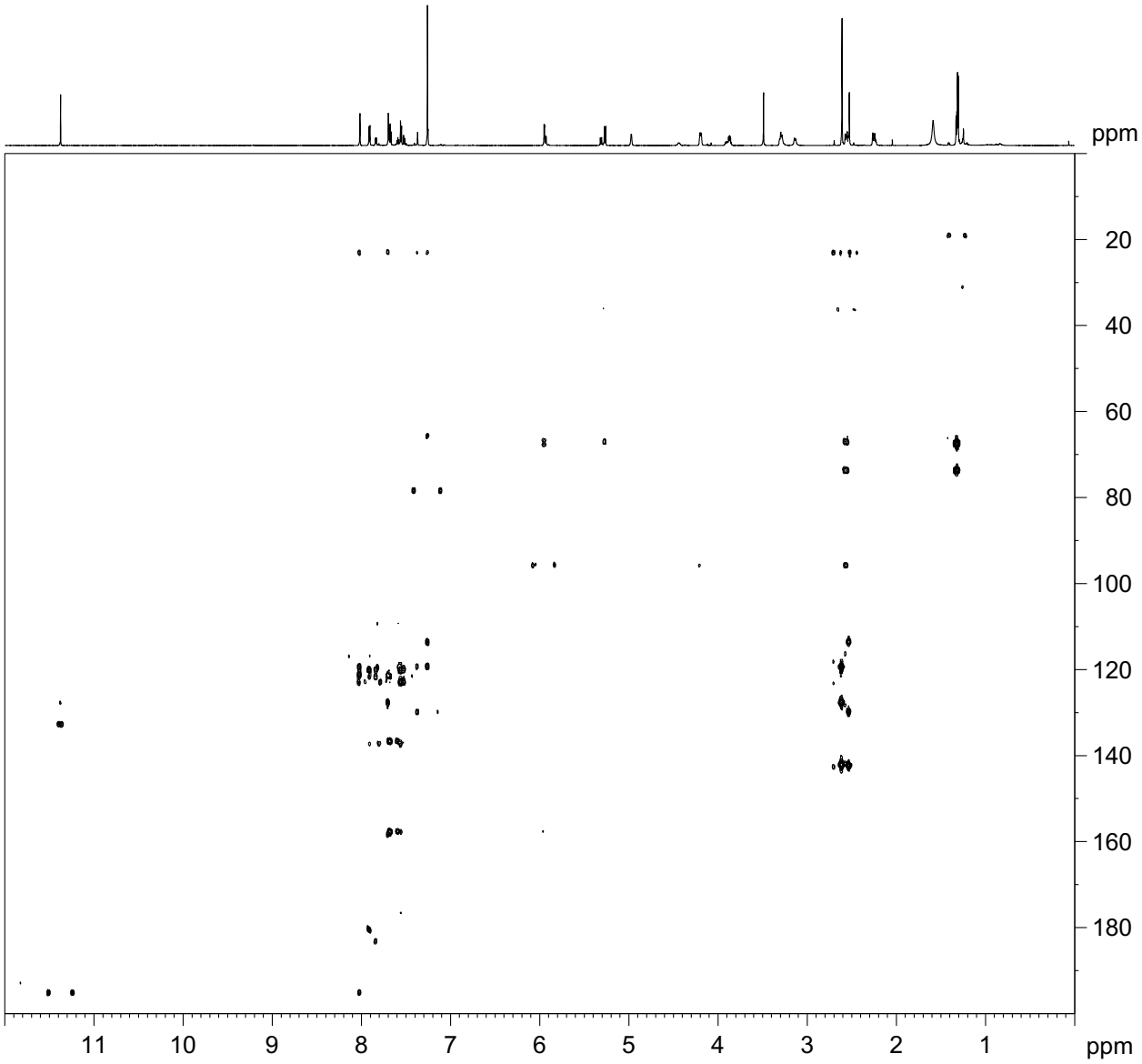
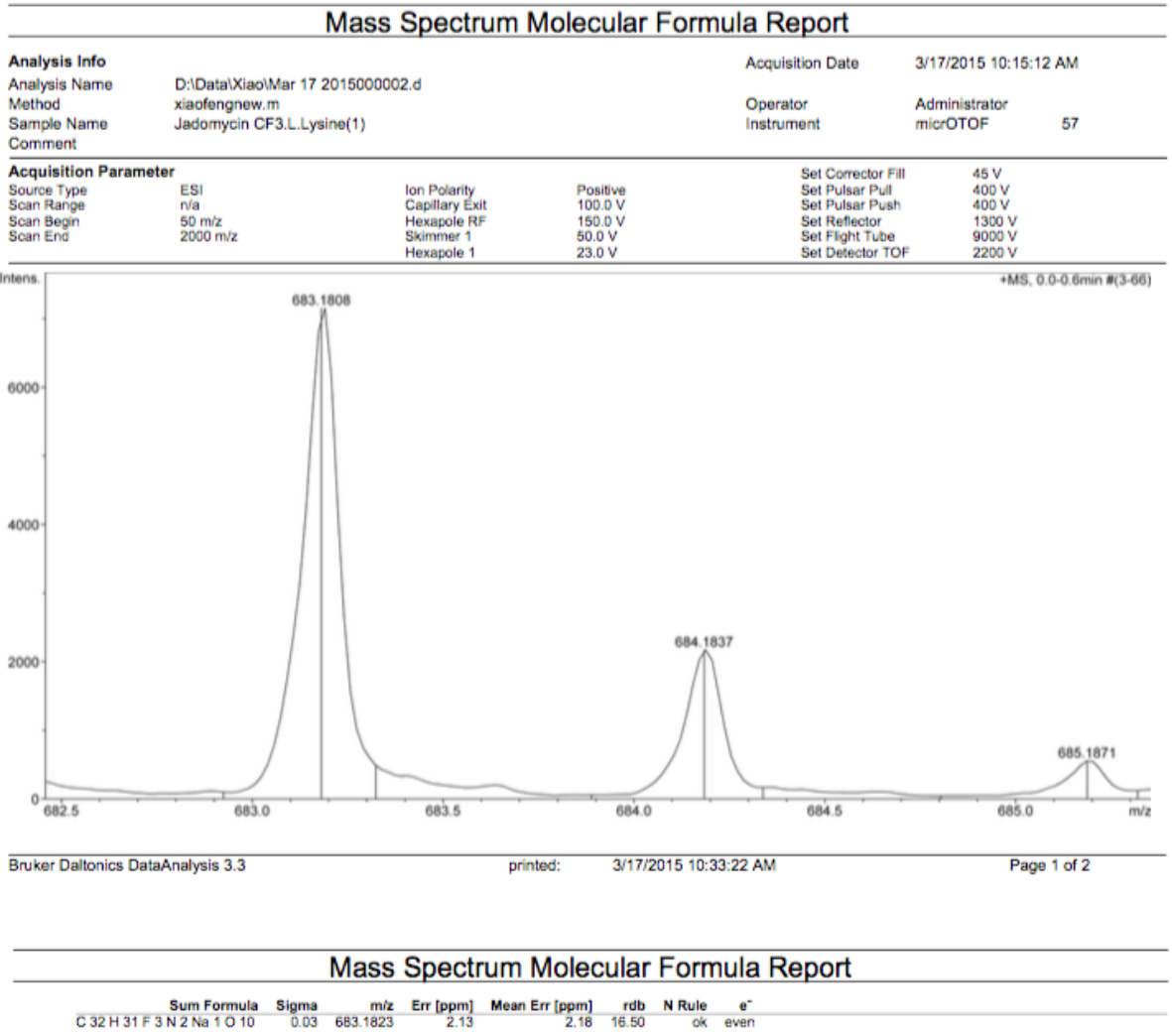


Figure S27. HMBC spectrum of **3** and **4** in (700 MHz, $CDCl_3$)



HRMS data

Figure S28. HRMS data for jadomycin TFAL **1**.



Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Mar 17 2015000002.d	Acquisition Date	3/17/2015 10:15:12 AM
Method	xiaofengnew.m	Operator	Administrator
Sample Name	Jadomycin CF3.L.Lysine(1)	Instrument	micrOTOF 57
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	45 V
Scan Range	n/a	Capillary Exit	100.0 V	Set Pulsar Pull	400 V
Scan Begin	50 m/z	Hexapole RF	150.0 V	Set Pulsar Push	400 V
Scan End	2000 m/z	Skimmer 1	50.0 V	Set Reflector	1300 V
		Hexapole 1	23.0 V	Set Flight Tube	9000 V
				Set Detector TOF	2200 V

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Mass Spectrum Molecular Formula Report

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 32 H 31 F 3 N 2 Na 1 O 10	0.03	683.1823	2.13	2.18	16.50	ok	even

Figure S29. HRMS data for jadomycin TFAL lactam **2**.

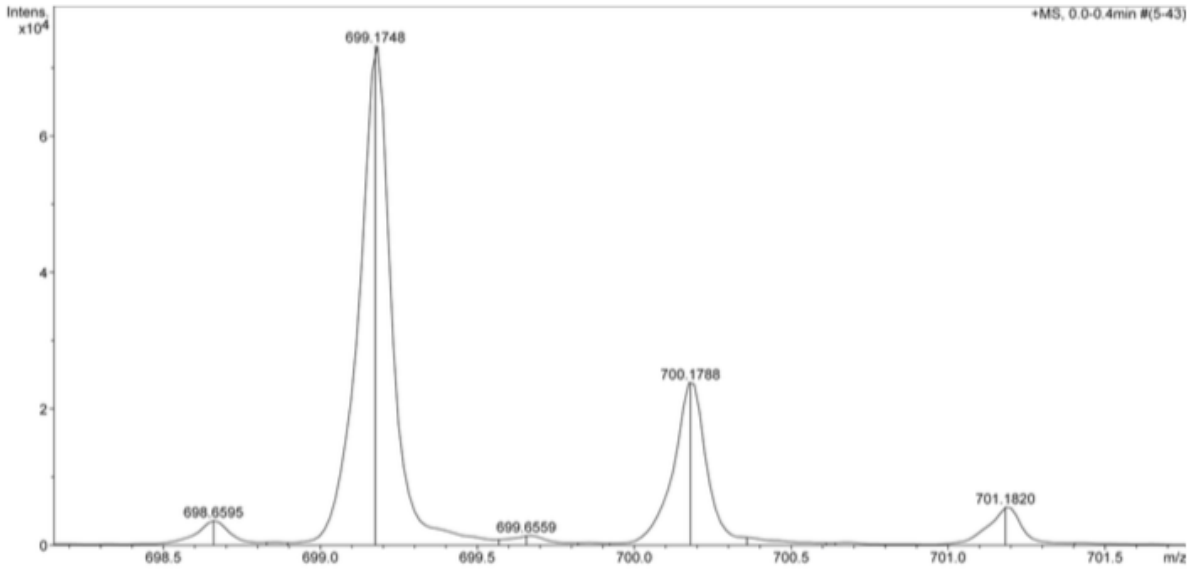
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Mar 17 2015000004.d	Acquisition Date	3/17/2015 10:35:13 AM
Method	xiaofengnew.m	Operator	Administrator
Sample Name	CF3.L.Lys UNKNOWN JADOMYCIN (2)	Instrument	micrOTOF 57
Comment			

Acquisition Parameter

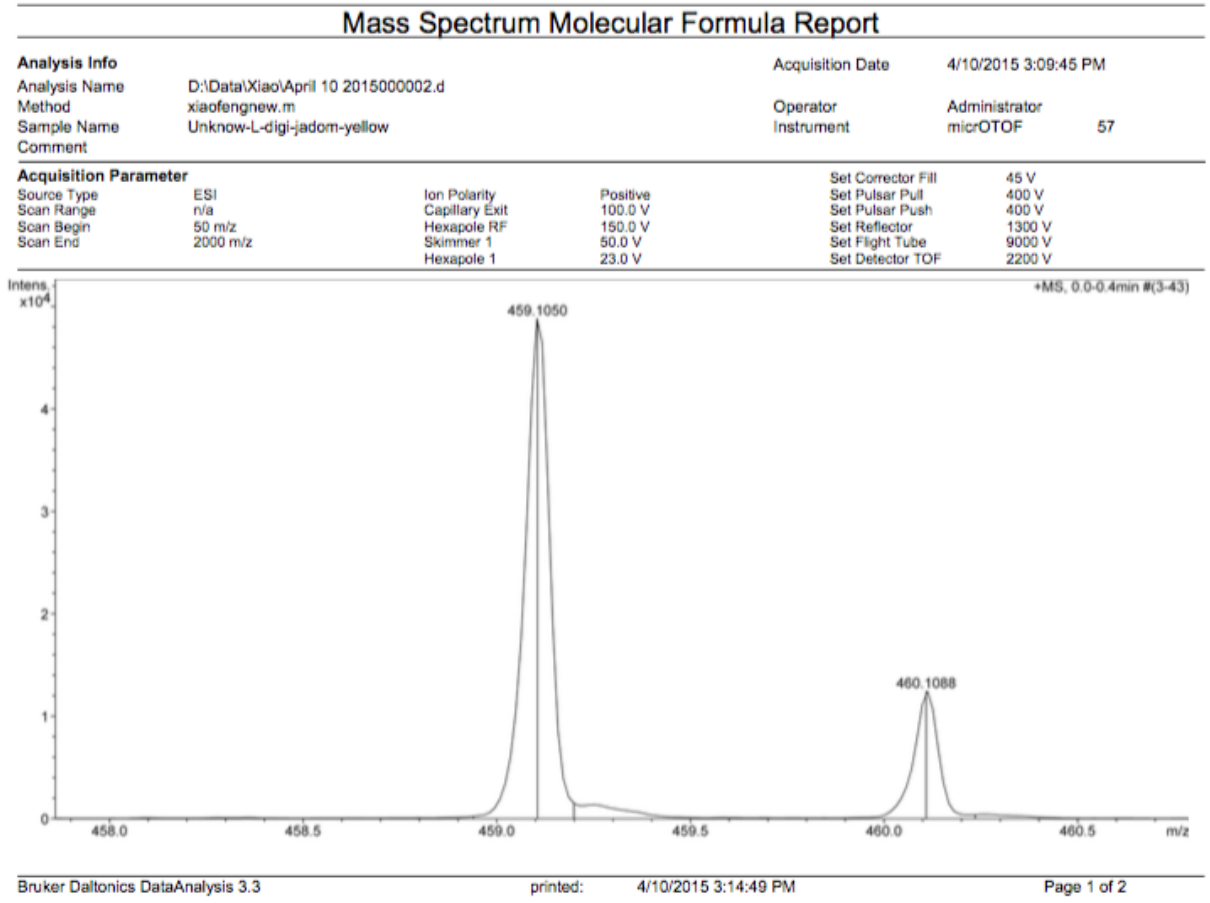
Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	45 V
Scan Range	n/a	Capillary Exit	100.0 V	Set Pulsar Pull	400 V
Scan Begin	50 m/z	Hexapole RF	150.0 V	Set Pulsar Push	400 V
Scan End	2000 m/z	Skimmer 1	50.0 V	Set Reflector	1300 V
		Hexapole 1	23.0 V	Set Flight Tube	9000 V
				Set Detector TOF	2200 V



Mass Spectrum Molecular Formula Report

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 32 H 31 F 3 N 2 Na 1 O 11	0.02	699.1772	3.40	3.31	16.50	ok	even

Figure S30. HRMS data for jadomycin furan **3**.



Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Xiao\April 10 2015000002.d	Acquisition Date	4/10/2015 3:09:45 PM
Method	xiaofengnew.m	Operator	Administrator
Sample Name	Unknow-L-digi-jadom-yellow	Instrument	micrOTOF 57
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	45 V
Scan Range	n/a	Capillary Exit	100.0 V	Set Pulsar Pull	400 V
Scan Begin	50 m/z	Hexapole RF	150.0 V	Set Pulsar Push	400 V
Scan End	2000 m/z	Skimmer 1	50.0 V	Set Reflector	1300 V
		Hexapole 1	23.0 V	Set Flight Tube	9000 V
				Set Detector TOF	2200 V

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Mass Spectrum Molecular Formula Report

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 24 H 20 Na 1 O 8	0.09	459.1050	0.14	-1.58	14.50	ok	even

Figure S31. HRMS data for jadomycin furan **4**.

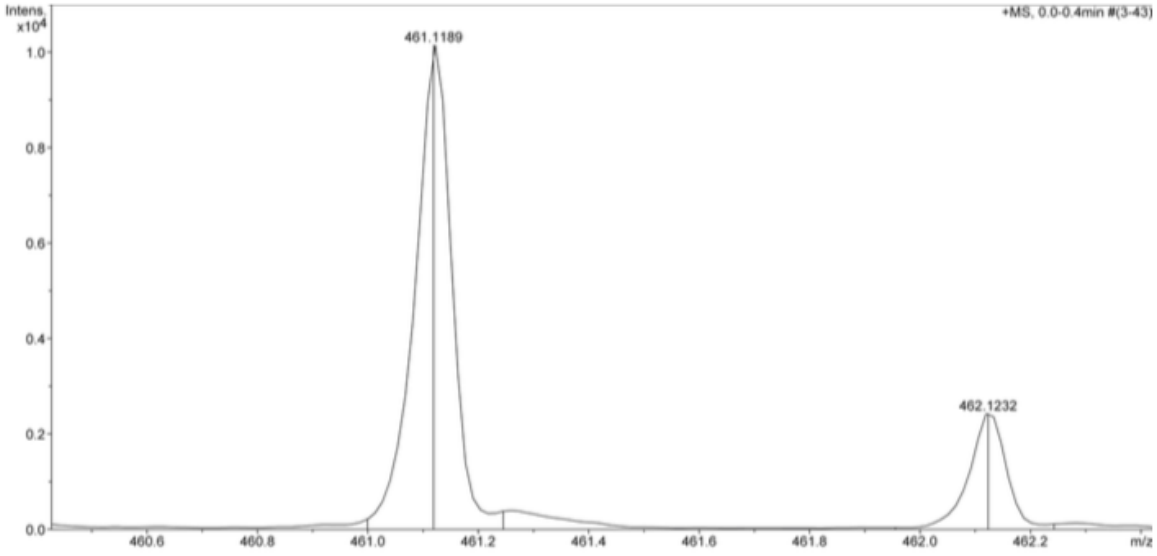
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Xiao\April 10 2015000002.d	Acquisition Date	4/10/2015 3:09:45 PM
Method	xiaofengnew.m	Operator	Administrator
Sample Name	Unknow-L-digi-jadom-yellow	Instrument	micrOTOF 57
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	45 V
Scan Range	n/a	Capillary Exit	100.0 V	Set Pulsar Pull	400 V
Scan Begin	50 m/z	Hexapole RF	150.0 V	Set Pulsar Push	400 V
Scan End	2000 m/z	Skimmer 1	50.0 V	Set Reflector	1300 V
		Hexapole 1	23.0 V	Set Flight Tube	9000 V
				Set Detector TOF	2200 V



Mass Spectrum Molecular Formula Report

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 24 H 22 Na 1 O 8	0.02	461.1207	3.92	3.93	13.50	ok	even