

Discovery and Heterologous Production of Sarubicins and Quinazolinone C-Glycosides with Protecting Activity for Cardiomyocytes

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Experiment Section

Tab. S1. Bacterial strains and plasmids used in this study

Strain and plasmid	Description	References or sources
<i>E. coli</i>		
DH5 α	Host strain for cloning	Invitrogen
BW25113/pIJ790	Host strain for PCR-targeting	Ref. 1
ET12567/pUZ8002	Host strain for conjugation between <i>E. coli</i> and <i>Streptomyces</i>	Ref. 2
<i>Streptomyces</i>		
XL1-blueMR	Host strain for genomic library	Agilent Technologies
DH5 α /BT340	Host strain for in-frame deletion	Ref. 3
<i>Streptomyces</i>		
<i>S. sp.</i> KIB-H91	Wild type sarubicins producing strain	This study
<i>S. coelicolor</i> M1154	Host strain for heterologous expression	Ref. 4
<i>S. coelicolor</i> M1154/p18F3	<i>Streptomyces coelicolor</i> M1154 integrated with plasmid 18F3 which contains <i>sar</i> biosynthetic gene cluster	This study
<i>S. coelicolor</i> 18F3 Δ <i>sarS3</i>	<i>sarS3</i> inactivation mutant of <i>S. coelicolor</i> 18F3	This study
<i>S. coelicolor</i> 18F3 Δ <i>sarS5</i>	<i>sarS5</i> inactivation mutant of <i>S. coelicolor</i> 18F3	This study
<i>S. coelicolor</i> 18F3 Δ <i>sarO</i>	<i>sarO</i> inactivation mutant of <i>S. coelicolor</i> 18F3	This study
Cosmid		
pJTU2554	Apr ^r , Cosmid vector for genomic library construction	Ref. 1
pJTU6722	Ery ^r , Vector for PCR targeting	Constructed by Prof. Meifeng Tao
p18F3	Apr ^r , Cosmid which contains <i>sar</i> biosynthetic gene cluster	This study
p18F3_ Δ <i>sarS3</i>	Apr ^r , gene inactivation clone used for <i>sarS3</i> mutant	This study
p18F3_ Δ <i>sarS5</i>	Apr ^r , gene inactivation clone used for <i>sarS5</i> mutant	This study
p18F3_ Δ <i>sarO</i>	Apr ^r , gene inactivation clone used for <i>sarO</i> mutant	This study

Tab. S2. The primers used in this study

gene	Sequence (5' to 3')
For gene Screening	
<i>gra-Orf14</i>	14F: GCTGGAGCACATCGCGTTCA 14R: CACGAGCTTGGGCATGTTGG
<i>gra-Orf17</i>	17F: TCGTGGTGAAGAGCGGGATC 17R: GCGGCTGACCTTGGTGAAA
For gene disruption	
<i>sarS3</i>	5152F: GCCACGCGCCGACGGAACGCGGGGCACCAGGTCTTCATGattccggggatcc gtcgac 5152R: TCTCGCAGGCGCCACCACGGCGTCCTGCGAGTCCCTCCttaggctggag ctgcttc
<i>sarS5</i>	5158F: CGCCACCGTGGAAGTGGACGCCGTGTACGTCCCGTGCCattccggggatcc gtcgac 5158R: CGGGTGAACGCCCGGTCCACCCGGACCGTGCCGTGTGCCcttaggctggag ctgcttc
<i>sarO</i>	5154F: TTGGAGACCCAGAACCTCATGGTCAAGACAGGCATCTCGattccggggatcc gtcgac 5154R: CTCAGGTGCCCGGCCGGGCTCCGCCGTTTGTATCCCGCGCttaggctggag ctgcttc
For gene confirmation	
<i>sarS3</i>	5152YF: GAAGTTCCTGGGTATTCC 5152YR: CGCCCCGGCCAGCGCTTCG
<i>sarS5</i>	5158YF: CGCCGATGCTGCCGGCC 5158YR: GGTGCGCACGGCCGTAC
<i>sarO</i>	5154YF: CCGTCGGAAGGTATTGAC 5154YR: CATGTACGTGATAGCCAAC

Tab. S3. Deduced functions of ORFs in *sar* BGC

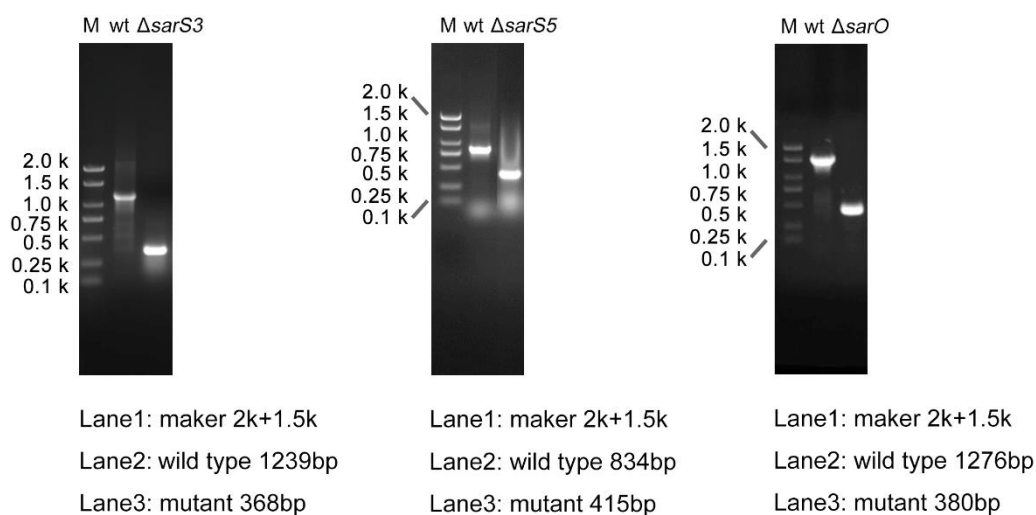
Gene NO.	Size aa	Proposed function by a BLAST search (blastp) Protein, Origin	Positives/ Identities	Accession No.
<i>sarN</i>	610	ATP-dependent amidotransferase, TtmN [<i>Streptomyces afghaniensis</i>]	66/55	ALJ49932.1
<i>sarR1</i>	391	MFS transporter, partial, <i>Streptomyces acidiscabies</i>	74/62	WP_040837276.1
<i>sarA1</i>	390	DHAP synthase, PhzC [<i>Streptomyces tendae</i>]	72/62	WP_028424979.1
<i>sarA2</i>	269	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase DhbX [<i>Klebsiella oxytoca</i>]	61/46	AFS18549.1
<i>sarA3</i>	228	Isochorismatase, PhzD [<i>Streptomyces tendae</i>]	71/64	AFS18573.1
<i>sarA4</i>	623	2-Amino-2-desoxyisochorismate Synthase PhzE [<i>Streptomyces tendae</i>]	73/62	AFS18572.1
<i>sarU1</i>	116	hypothetical protein, <i>Streptomyces gancidicus</i> BKS 13-15	74/63	WP_006135773.1
<i>sarH</i>	396	3-hydroxybenzoate-6-hydroxylase,3HB6H [<i>Rhodococcus jostii</i>]	46/33	WP_011594786.1
<i>sarS1</i>	323	dTDP-glucose 4,6 dehydratase, StrE [<i>Streptomyces griseus</i>]	72/66	WP_030951601.1
<i>sarS2</i>	329	dTDP-1-glucose thymidyltransferase, StrD [<i>Streptomyces griseus</i>]	69/61	CAH94331.1
<i>sarS3</i>	344	C-glycosyltransferase,UrdGT2 [<i>Streptomyces fradiae</i>]	61/52	AAF00209.1
<i>sarR2</i>	200	SARP family transcriptional regulator, SrrZ [<i>Streptomyces sp.</i> F-3]	61/48	WP_003951745.1
<i>sarO</i>	311	Dehydrogenase, <i>Nocardia sp.</i> BMG51109	71/57	WP_036571061.1
<i>sarU2</i>	101	Hypothetical protein, <i>Amycolatopsis sp.</i> MJM2582	61/43	WP_037347854.1
<i>sarR3</i>	492	Arabinose ABC transporter permease, <i>Bacillus megaterium</i>	78/59	WP_034679354.1
<i>sarS4</i>	434	Putative NDP-deoxyglucose-2,3-dehydratase, SpnO [<i>Saccharopolyspora spinosa</i>]	60/47	AAG23276.1
<i>sarS5</i>	326	dTDP-3,4-diketo-2,6-dideoxy-D-glucose 3-ketoreductase, SpnN [<i>Saccharopolyspora spinosa</i>]	61/49	AAG23275.1
<i>sarR4</i>	151	MerR family transcriptional regulator, <i>Microbispora sp.</i> ATCC PTA-5024	80/67	WP_036330534.1
<i>sarR5</i>	384	Transposase, <i>Streptomyces himastatinicus</i>	61/51	WP_039941984.1
<i>sarR6</i>	652	Transcriptional regulator, <i>Streptomyces sp.</i> NRRL F-5065	62/49	WP_037858633.1

Tab. S4. Protective effects of compounds **1** and **2** against H₂O₂-induced cardiomyocytes injury

Compound	Concentration ^a	
	0 μM	10 μM
1	49.03±3.89	81.36±4.84**
2	49.03±3.89	89.43±2.58**
Carvedilol ^b	49.03±3.89	85.98±6.18**

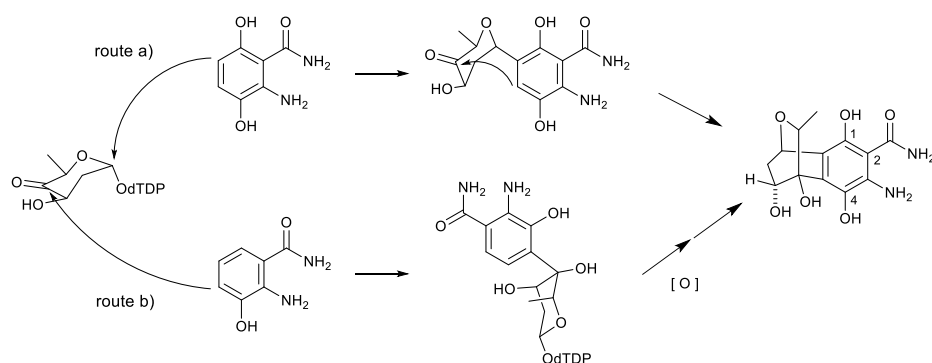
Data expressed as means ±SD (n = 3). **p<0.01 vs 0 μM group. ^b Positive control.

Fig. S1. Confirmation of mutants by PCR using the primers.



Confirmation of mutants *S. coelicolor* p18F3 *ΔsarS3*, p18F3 *ΔsarS5*, and p18F3 *ΔsarO* by PCR using the primers listed in supplementary Tab.S2.

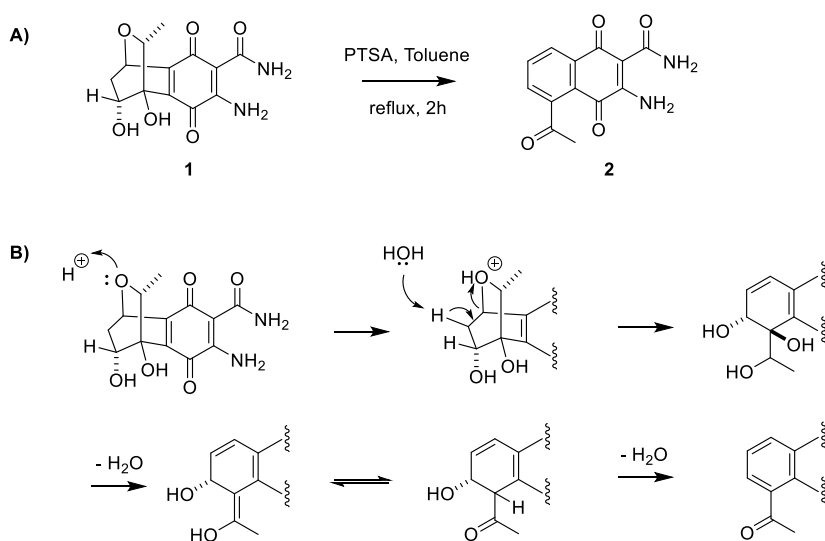
Fig. S2. Two possible routes of C-glycosylation in the biosynthesis of sarubicin A



Route a): glycosyl transfer at C-6 of hydroquinone; then intramolecular aldol condensation between C-5 and C-4'.

Route b): intermolecular aldol condensation between C-5 and C-4'; followed by hydroxylation at C-1 of 3HAA; then cyclization between C-6 and C-1'.

Scheme S1. A) Transformation of **1** to **2**; B) Proposed mechanism of transformation from **1** to **2**.



Tab. S5. 1D and 2D NMR data of compound **3** (H9101)

H9101				
Position	C Signals	H Signals	HMBC Correlations	ROESY Correlations
	δ_C , type	δ_H , (int. mult. <i>J</i> in Hz)		
2	152.9 (s)			
2-Me	21.5 (q)	2.36 (3H, s)	2	
4	167.3 (s)			
4a	102.6 (s)			
5	147.1 (s)			
6	124.3 (s)			
7	117.9 (d)	7.18 (1H, s)	1', 5, 6, 8, 8a	2'-H _{aiX}
8	143.4 (s)			
8a	135.5 (s)			
1'	70.2 (d)	4.68 (1H, d, 11.4)	5', 5, 6, 7	5', 3'
2'-aix		1.31 (1H, pseudo-q, 12.6)	1', 3', 7'	4', 7
2'-eq	40.5 (t)	2.09 (1H, dd, 12.6, 3.0)	3', 4'	
3'	71.8 (d)	3.48 (1H, overlapped)		1'
4'	77.2 (d)	2.85 (1H, t, 9.0)	3', 5', 6'	2'-H _{aiX} , 6'
5'	76.1 (d)	3.32 (1H, dq, 9.0, 6.0)		1'
6'	18.5 (q)	1.22 (3H, d, 6.0)	5'	4'
Exchangeable Protons		4.72-5.30 (2H) 11.58 (5-OH, s)		

a) Data were recorded at 600 MHz for ¹H and 150 MHz for ¹³C in DMSO-*d*₆ using the corresponding solvent residual signal as internal standard.

Tab. S6. 1D and 2D NMR data of compound **4** (H9102)

H9102 ^a				
Position	C Signals δ_c , type	H Signals δ_H , (int. mult. <i>J</i> in Hz)	HMBC Correlations	ROESY Correlations
2	152.1 (s)			
2-CH ₃	21.4 (q)	2.37 (3H, s)	2	
4	167.3 (s) ^b			
4a	106.1 (s)			
5	147.0 (s)			
6	125.0 (s)			
7	118.5 (d)	7.28 (1H, s)	1', 5, 6, 8, 8a	2'-H _{aix}
8	144.1 (s)			
8a	135.5 (s) ^b			
1'	70.4 (d)	4.63 (1H, d, 11.4)	2', 3', 5', 5, 6, 7	5', 3'
2'-aix	34.7 (t)	1.71 (1H, dd, 12.0, 2.4)	3'	7
2'-eq		1.56 (1H, brd, 12.0)	1', 6	
3'	69.3 (d)	3.69 (1H, brd, 11.4)		1'
4'	69.9 (d)	3.41 (1H, overlapped)	2', 3'	6'
5'	74.1 (d)	3.58 (1H, q, 6.0)		1'
6'	17.6 (q)	1.22 (3H, d, 6.0)	4', 5'	4'
Exchangeable		4.12 (1H, brs)		
Protons		4.30 (1H, brs)		
3-NH _b		8.93 (1H, brs)		
5-OH		11.50 (1H, s)		
3-NH _a		12.48 (1H, brs)		

a) Data were recorded at 600 MHz for ¹H and 150 MHz for ¹³C in DMSO-*d*₆ using the corresponding solvent residual signal as internal standard; b) The signals were detected by the HMBC spectrum.

Tab. S7. 1D and 2D NMR data of compound 5 (H9103)

H9103 ^a				
Position	C Signals δ_c , type	H Signals δ_H , (int. mult. <i>J</i> in Hz)	HMBC Correlations	ROESY Correlations
2	152.5 (s) ^b			
2-Me	21.4 (q)	2.37 (3H, s)	2	
4	167.1 (s)			
4a	106.4 (s)			
5	147.2 (s)			
6	122.7 (s)			
7	117.8 (d)	7.32 (1H, s)	1', 5, 8a	2'-H _{aiX}
8	144.0 (s)			
8a	136.3 (s) ^b			
1'	70.2 (d)	4.92 (1H, dd, 12.0, 1.2)	5', 5, 6, 7	5'
2'-aix	47.0 (t)	2.73 (1H, pseudo-t, 12.6)	1', 3', 7'	4', 7
2'-eq		2.56 (1H, dd, 12.6, 1.2)	3'	
3'	206.4 (s)			
4'	78.4 (d)	3.88 (1H, d, 9.6)	3', 5', 6'	2'-H _{aiX} , 6' (w)
5'	78.3 (d)	3.58 (1H, dq, 9.6, 6.0)		1'
6'	19.3 (q)	1.37 (3H, d, 6.0)	5'	4' (w)
Exchangeable Protons		4.72-5.30 (2H) 11.56 (5-OH, s)		

a) Data were recorded at 600 MHz for ¹H and 150 MHz for ¹³C in DMSO-*d*₆ using the corresponding solvent residual signal as internal standard; b) The signals were detected by the HMBC spectrum.

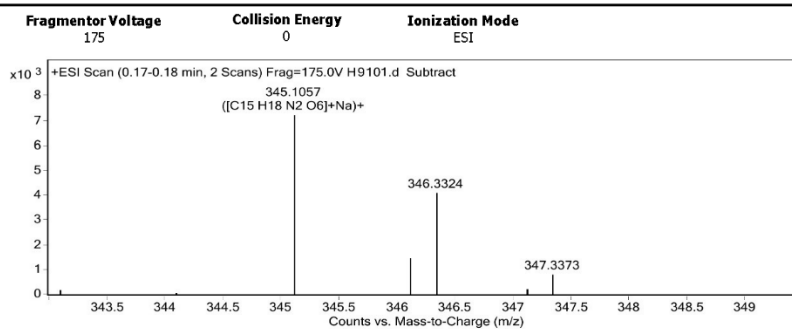
NMR and HRESIMS spectra of compounds 3-5 and 8-10

Fig. S3. HRESIMS spectrum of **3**

Qualitative Analysis Report

Data Filename	H9101.d	Sample Name	H9101
Sample Type	Sample	Position	P1-A3
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	3/11/2019 11:45:01 AM
IRM Calibration Status	Success	DA Method	Default.m
Comment			
Sample Group		Info.	
Acquisition SW	6200 series TOF/6500series		
Version	Q-TOF B.05.01(B5125.2)		

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
169.0111	1	8736.38		
274.2746	1	26216.83		
302.3058	1	6251.86		
318.301	1	19078.77		
345.1057	1	7243.87	C15 H18 N2 O6	(M+Na)+
437.1942	1	23759.44		
438.1971	1	5972.08		
453.1677	1	23368.36		
454.1712	1	4665.22		
659.289	1	4408.28		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	5

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C15 H18 N2 O6	322.1165	345.1057	345.1057	0.00	0.00	8.0000

--- End Of Report ---

Fig. S4. ¹H NMR (600 MHz) spectrum of **3** in DMSO-*d*₆

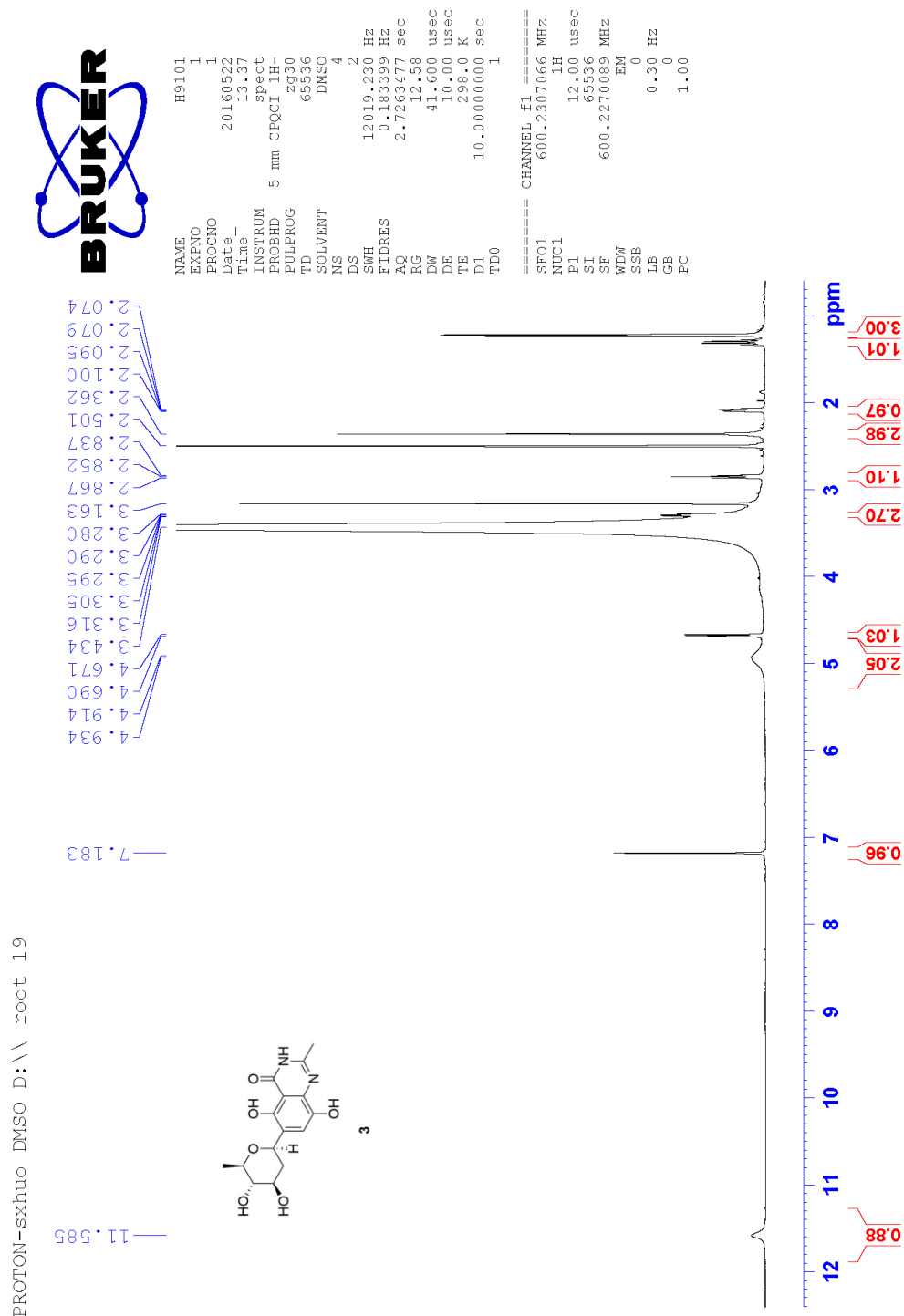


Fig. S5. ¹³C NMR (150 MHz) spectrum of **3** in DMSO-d₆



```

NAME      HS101
EXPNO     4
PROCNO    1
Date_     20160522
Time_     14.10
INSTRUM   spect
PROBHD    5 mm CPQCI 1H-
PULPROG   zgpg30
TD         65536
SOLVENT   DMSO
NS         650
DS         4
SWH        36231.883 Hz
FIDRES     0.4522855 Hz
AQ         0.3044468 sec
RG         199.06
DE         13.800 usec
TE         298.0 K
D1         2.00000000 sec
D11        0.03000000 sec
TD0        1

===== CHANNEL f1 =====
SFO1      150.9438010 MHz
NUC1      13C
P1        11.20 usec
SI        131072
SF        150.9272581 MHz
WDW       EM
SSB       0
LB        1.00 Hz
GB        0
PC        1.40
    
```

ZGDC-sxhuo DMSO D:\ root 19

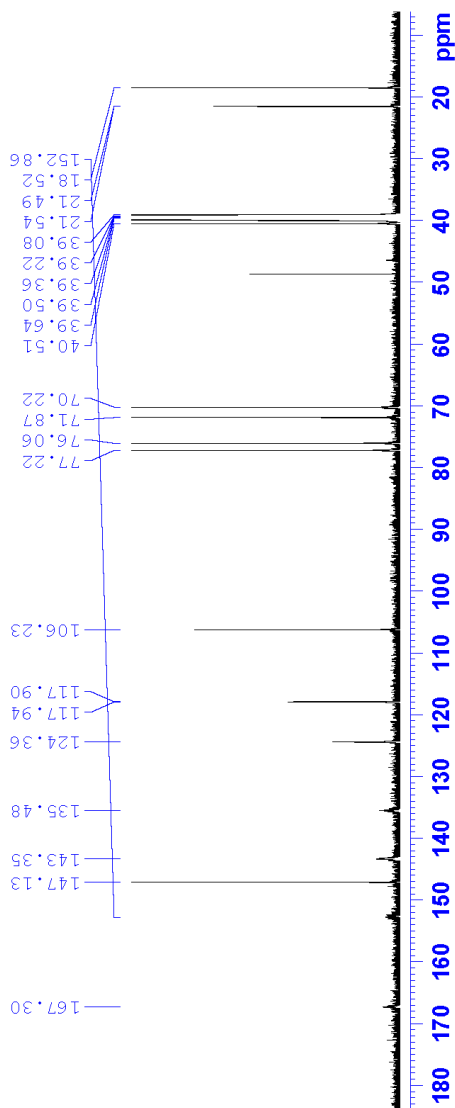
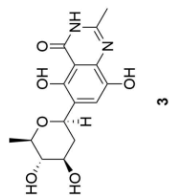


Fig. S6. HSQC (600 MHz) spectrum of **3** in DMSO-*d*₆

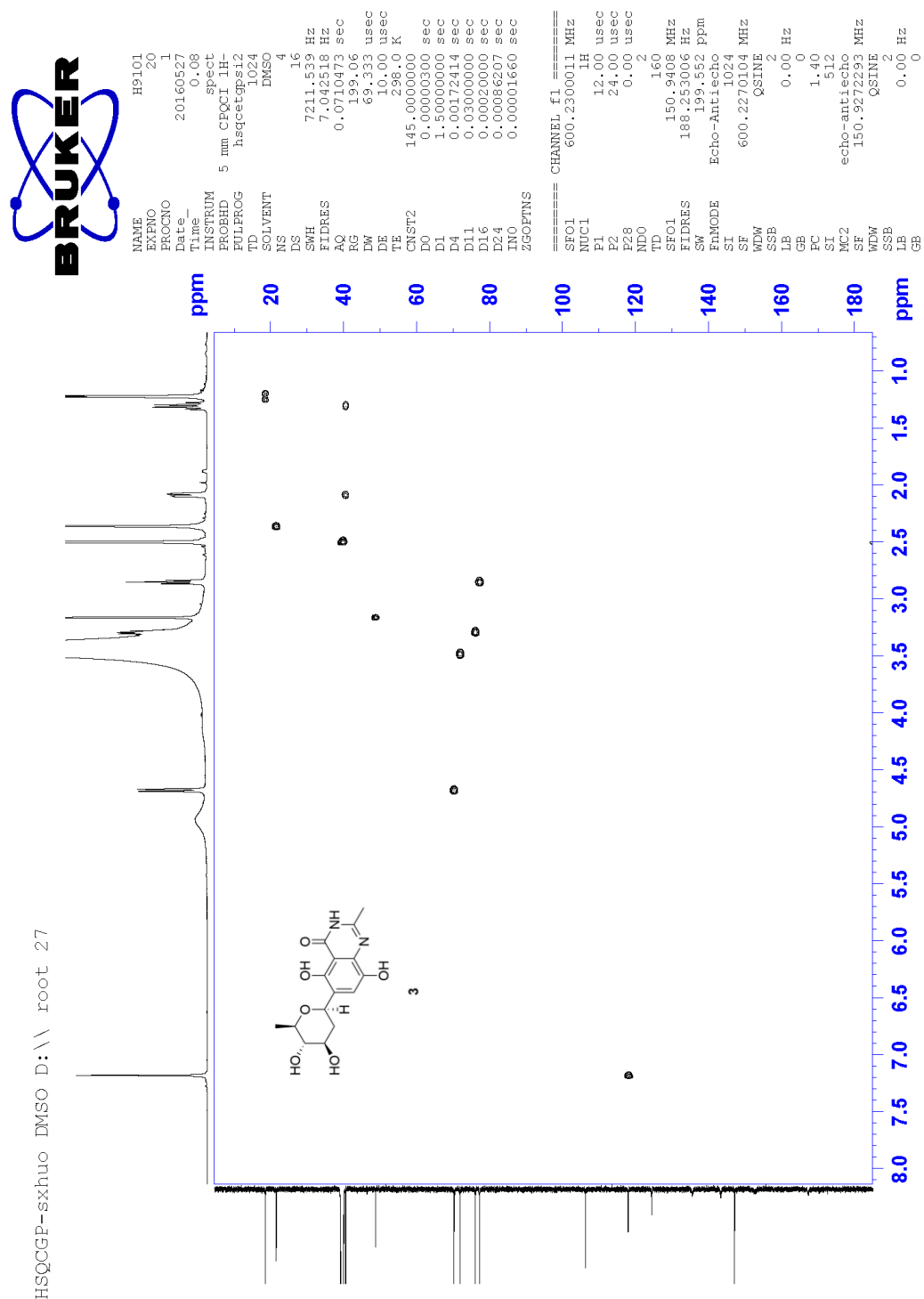


Fig. S7. HMBC (600 MHz) spectrum of **3** in DMSO-*d*₆

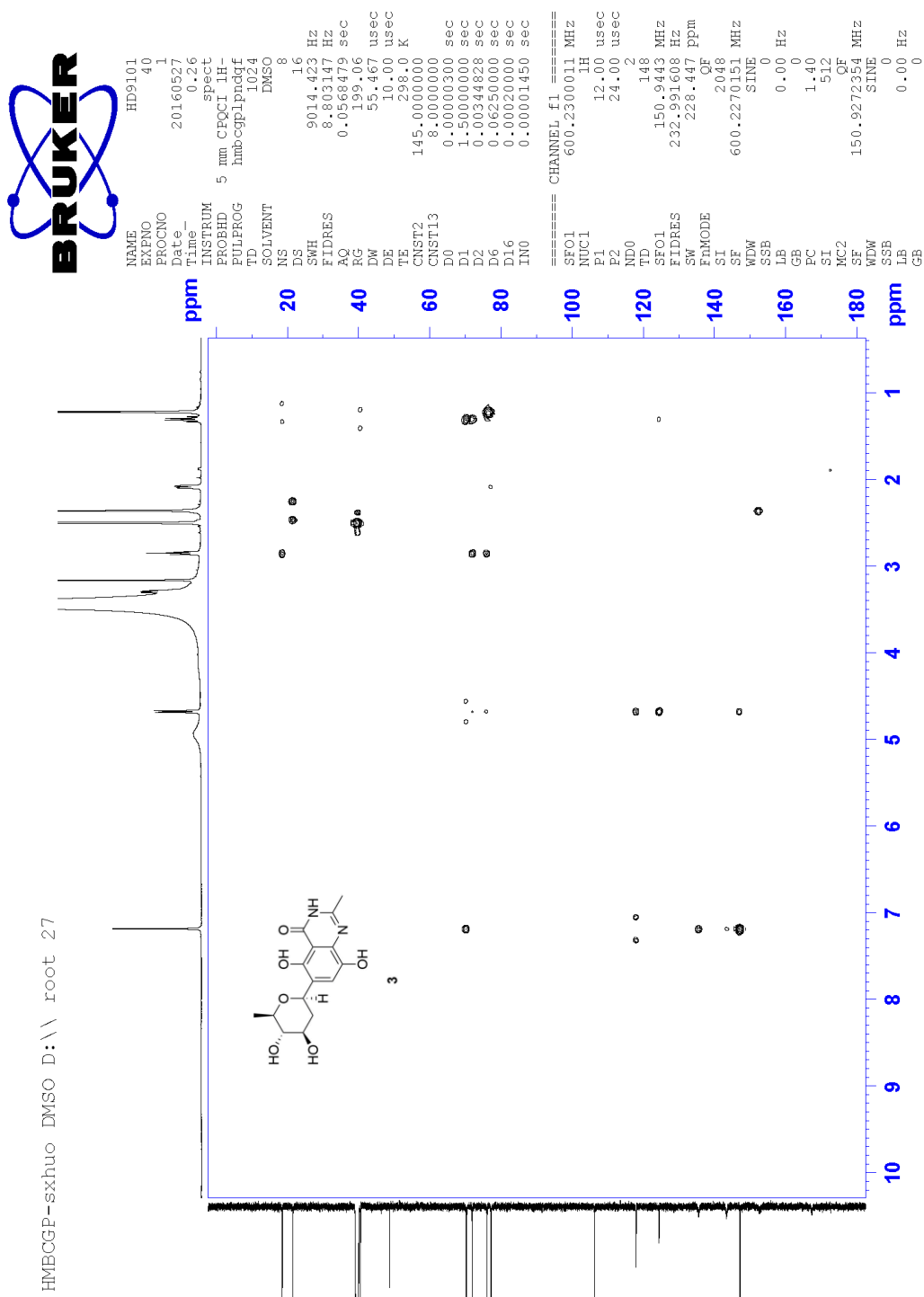


Fig. S8. ROESY (600 MHz) spectrum of **3** in DMSO-*d*₆

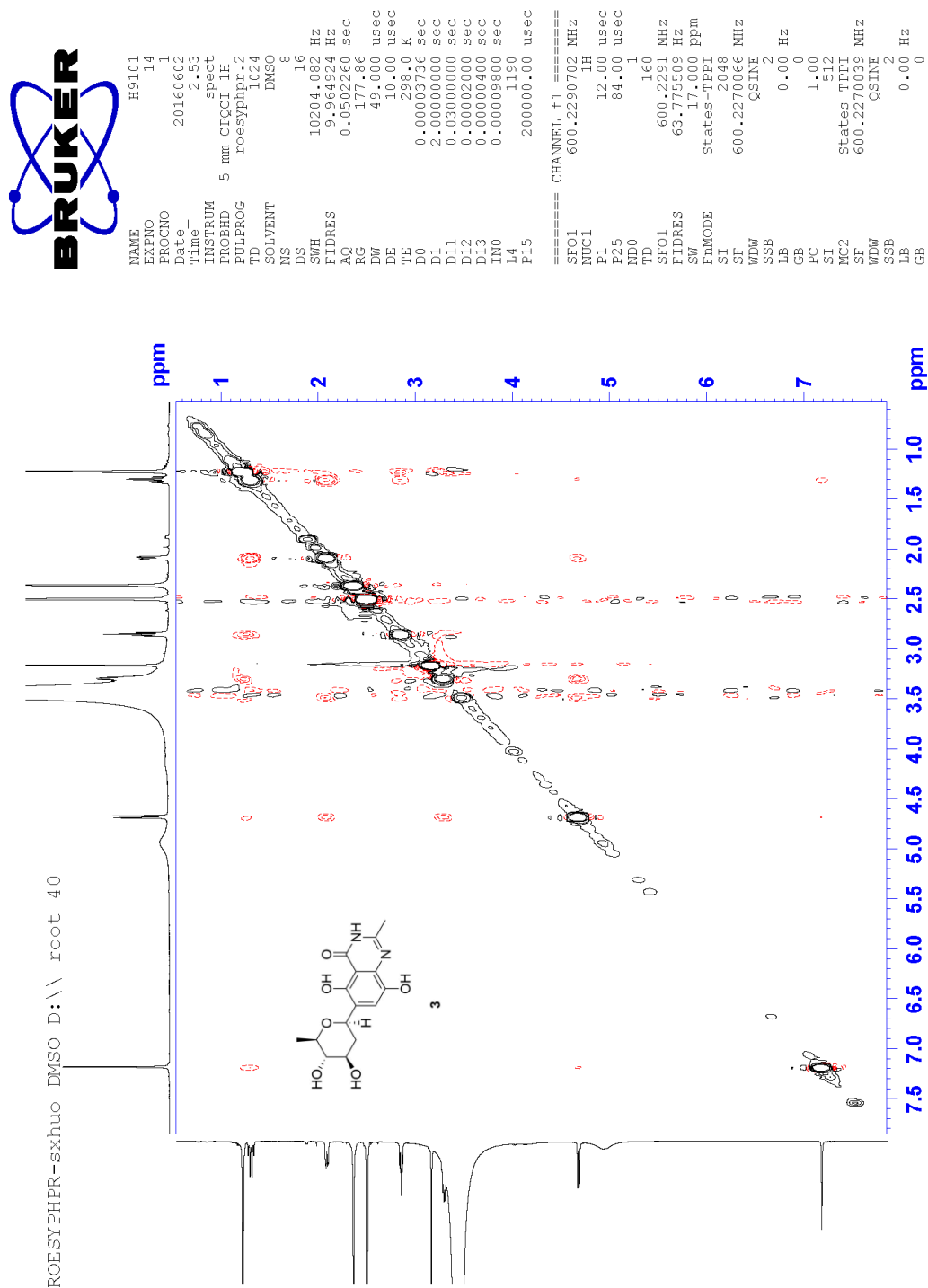


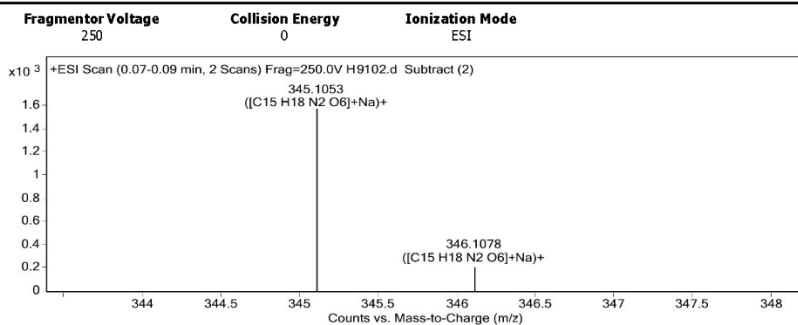
Fig. S9. HRESIMS spectrum of 4

Qualitative Analysis Report

Data Filename	H9102.d	Sample Name	H9102
Sample Type	Sample	Position	P1-C2
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	3/11/2019 2:29:41 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group		Info.
Acquisition SW	6200 series TOF/6500series	
Version	Q-TOF B.05.01(B5125.2)	

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
285.0241		218.58		
330.8971		287.57		
337.0899		270.67		
345.1053	1	1573.57	C15 H18 N2 O6	(M+Na)+
346.1078	1	215.08	C15 H18 N2 O6	(M+Na)+
367.0887		294.34		
399.1062		238.65		
437.1944	1	2455.96		
438.1994	1	618.82		
659.2844	1	302.24		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	60
O	0	10
N	0	3

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C15 H18 N2 O6	322.1165	345.1057	345.1053	0.40	1.16	8.0000

--- End Of Report ---

Fig. S10. ¹H NMR (600 MHz) spectrum of **4** in DMSO-*d*₆

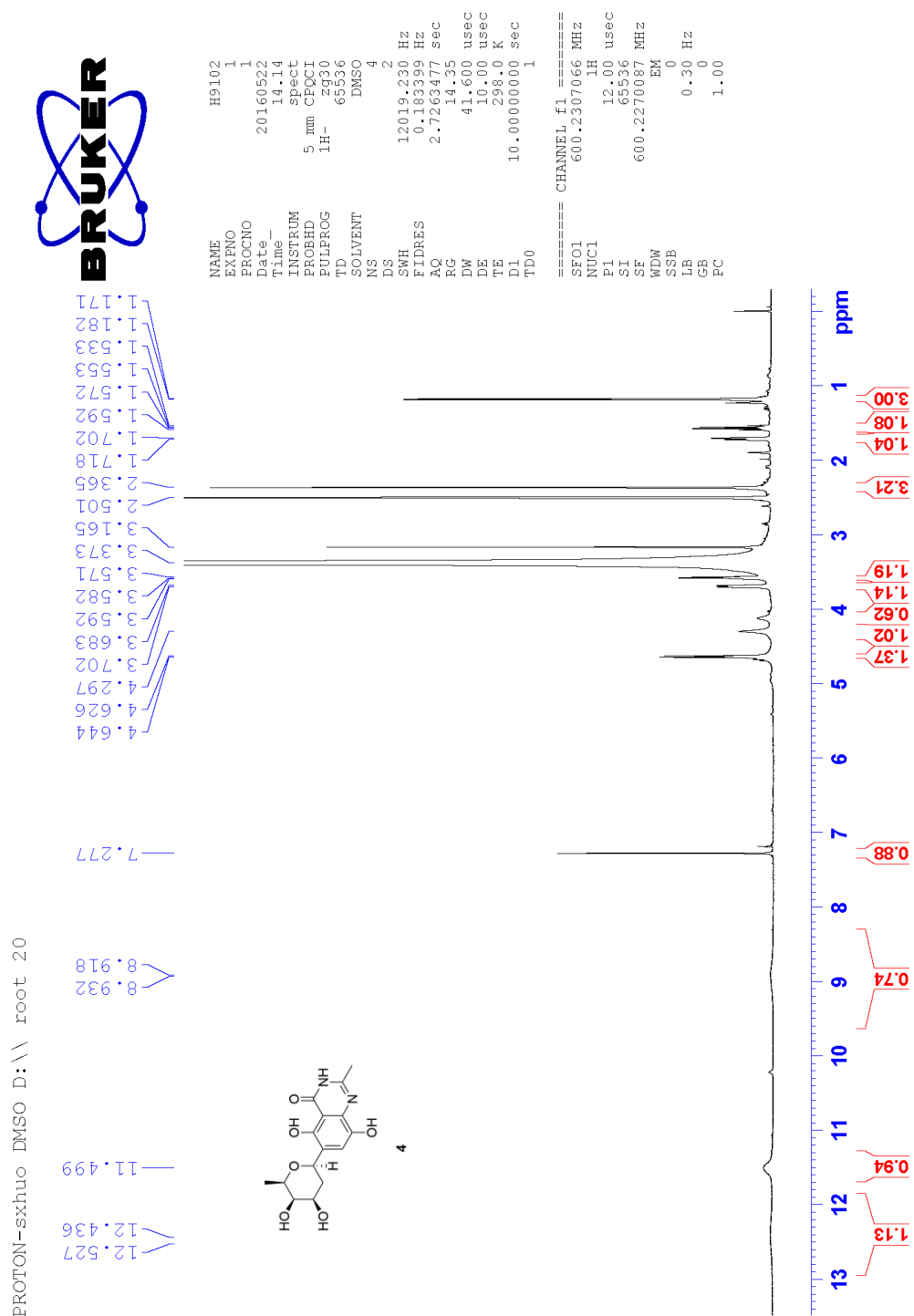


Fig. S11. ^{13}C NMR (150 MHz) spectrum of **4** in $\text{DMSO-}d_6$



```

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EXPNO         4
PROCNO        1
Date_         20160522
Time_        14.47
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PROBHD        5 mm CQCI 1H-
PULPROG       zgpg
TD            65536
SOLVENT       DMSO
NS            650
DS            4
SWH           36231.883 Hz
FIDRES        0.552855 Hz
AQ            0.9044468 sec
RG            199.06
DW            13.800 usec
DE            18.00 usec
TE            298.0 K
D1            2.0000000 sec
D11           0.03000000 sec
TD0           1
===== CHANNEL f1 =====
SFO1          150.9438010 MHz
NUC1          13C
P1            11.20 usec
SI            131072
SF            150.9272672 MHz
WDW           EM
SSB           0
LB            1.00 Hz
GB            0
PC            1.40
  
```

ZGDC-sxihu DMSO D:\ root 20

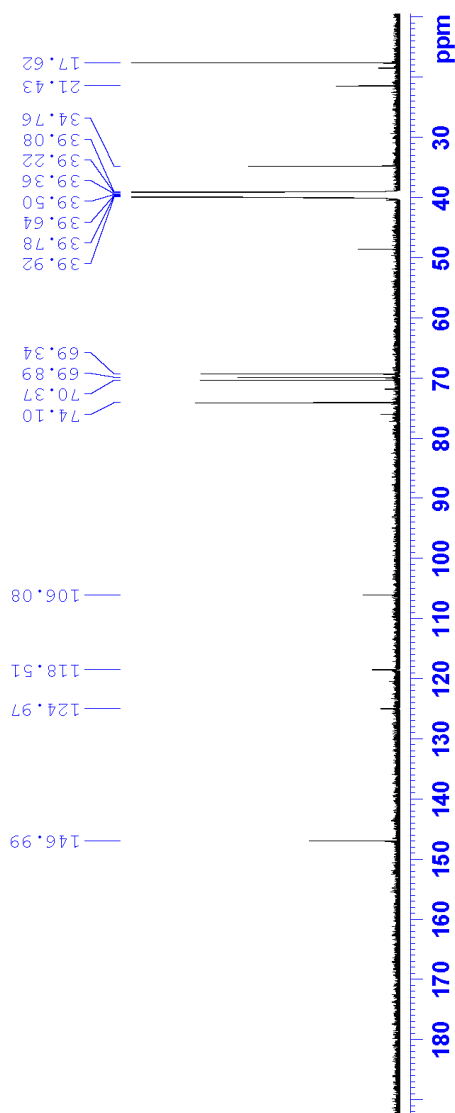
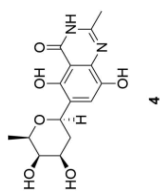


Fig. S12. HSQC (600 MHz) spectrum of **4** in DMSO-*d*₆

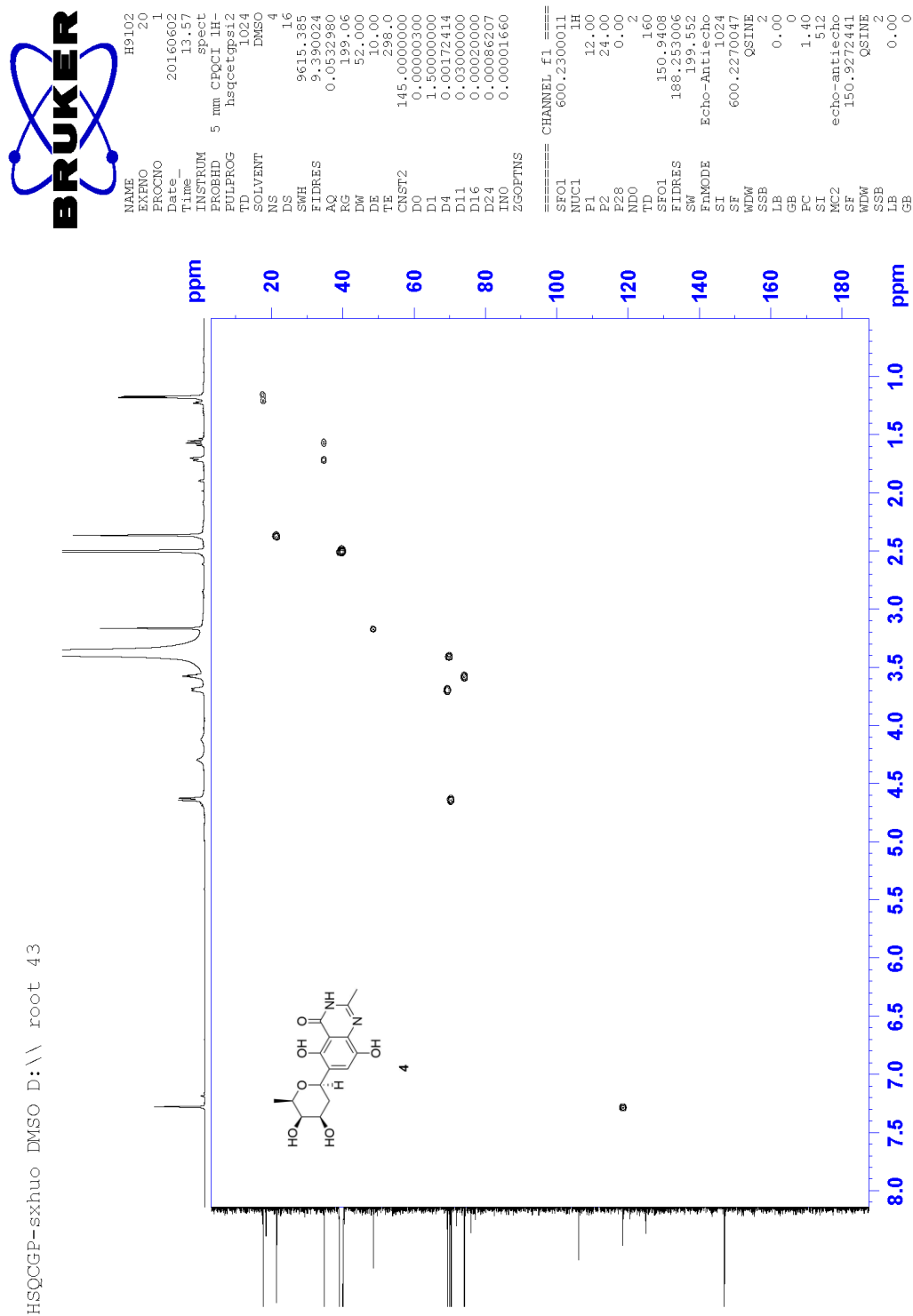


Fig. S13. HMBC (600 MHz) spectrum of **4** in DMSO-*d*₆

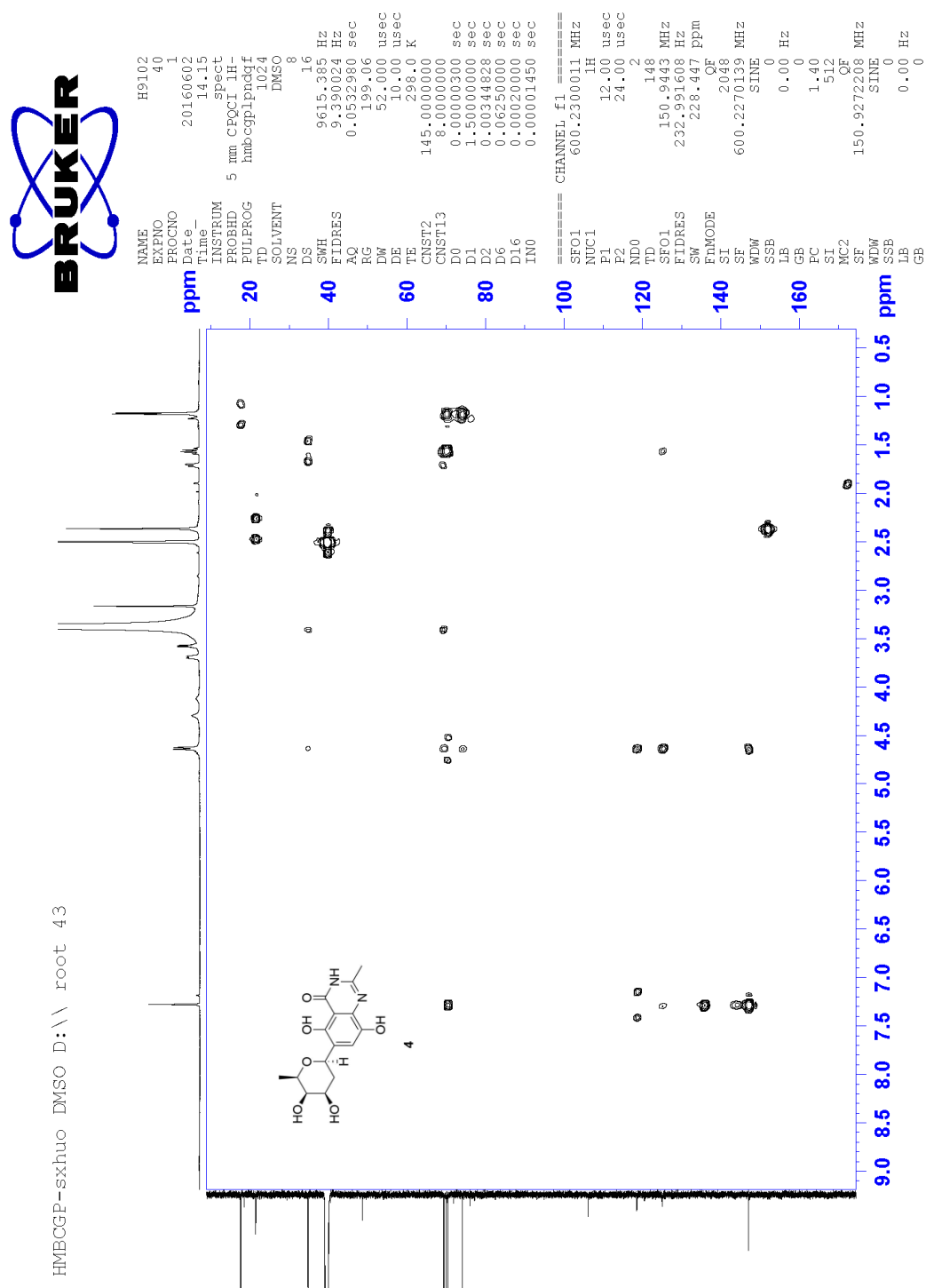


Fig. S14. ROESY (600 MHz) spectrum of **4** in DMSO-*d*₆



```

NAME      HS102
EXPNO     14
PROCNO    1
Date_     20160602
Time_     13:07
INSTRUM   spect
PROBHD    5 mm CPOCI 1H-
PULPROG   roesyphpr.2
TD         1024
SOLVENT   DMSO
NS         8
DS         16
SWH        9615.365 Hz
FIDRES     9.1390024 Hz
AQ         0.0532980 sec
RG         199.06
DW         52.000 usec
DE         10.00 usec
TE         298.0 K
D0         0.00004046 sec
D1         2.00000000 sec
D11        0.03000000 sec
D12        0.00002000 sec
D13        0.00000400 sec
IN0        0.00010420 sec
L4         1190
P15        200000.00 usec

===== CHANNEL f1 =====
SF01      600.2290330 MHz
NUC1       1H
P25        12.00 usec
ND0         84.00 usec
TD          160
SF01      600.229 MHz
FIDRES     59.980804 Hz
SW         15.989 ppm
FnmODE     States-TPPI
SI         2048
SF         600.2270056 MHz
WDW        QSINE
SSB         2
LB         0.00 Hz
GB         1.00
PC         1.00
SI         1512
MC2        States-TPPI
SF         600.2270051 MHz
WDW        QSINE
SSB         2
LB         0.00 Hz
GB         0
    
```

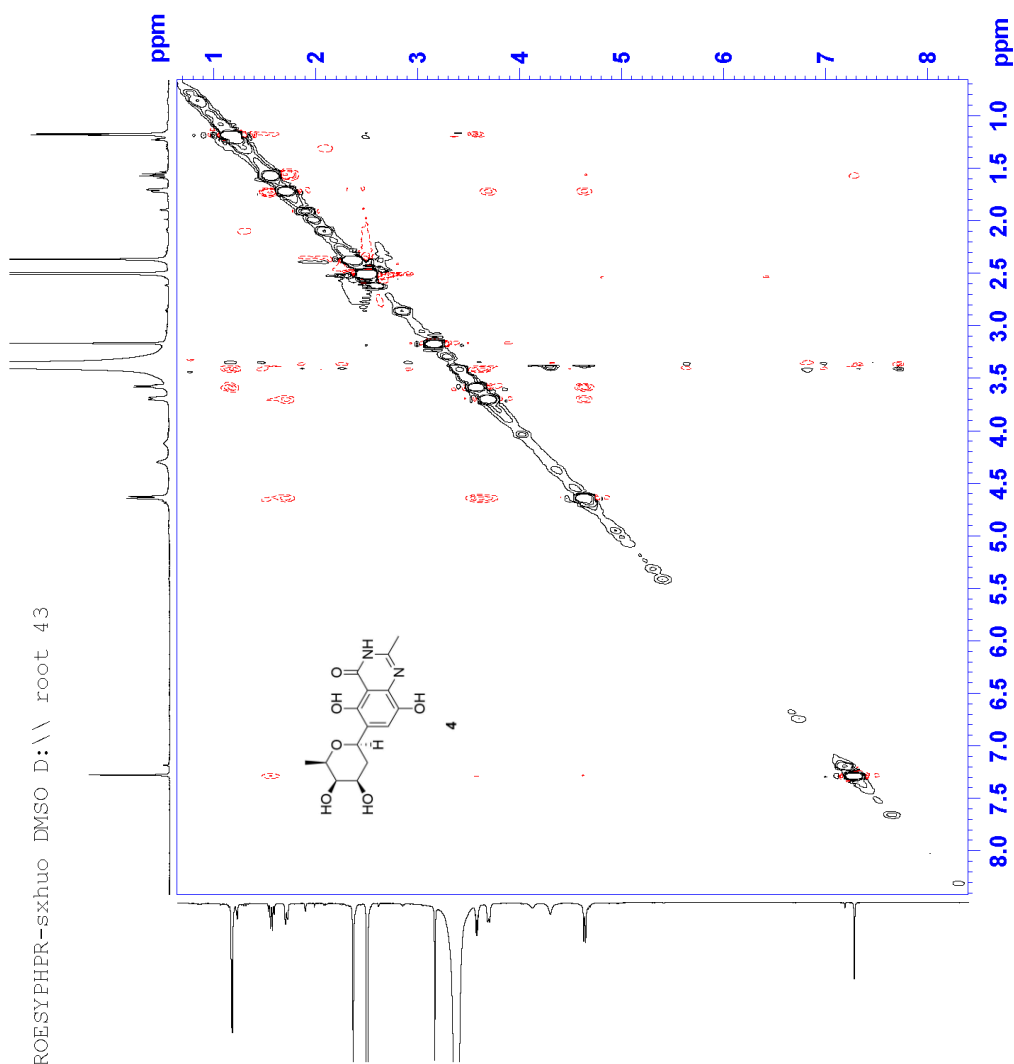


Fig. S15. HRESIMS spectrum of 5

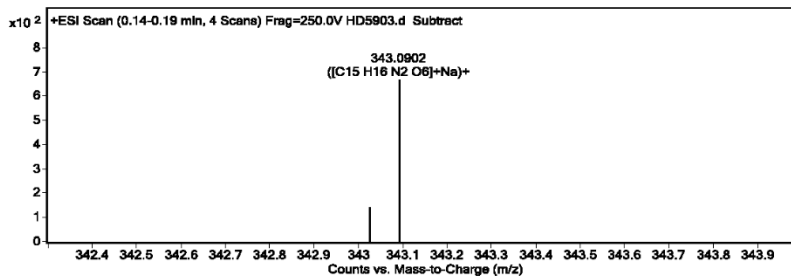
Qualitative Analysis Report

Data Filename	H9103.d	Sample Name	H9103
Sample Type	Sample	Position	P1-A1
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	3/20/2019 4:08:33
IRM Calibration Status	Success	DA Method	PM Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
250	0	ESI



Peak List

<i>m/z</i>	<i>z</i>	Abund
285.0217	1	2646.84
299.1254	1	2326.36
345.1056	1	19489.03
346.1087	1	2973.38
371.1583	1	2529.09
437.1937	1	54439.48
438.1968	1	14210.92
453.1679	1	2502.27
659.2874	1	2891.54
675.2608	1	2747.1

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	60
O	0	10
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C15 H16 N2 O6	320.1008	343.0901	343.0902	-0.10	-0.29	9.0000

--- End Of Report ---

Fig. S16. ¹H NMR (600 MHz) spectrum of **5** in DMSO-*d*₆

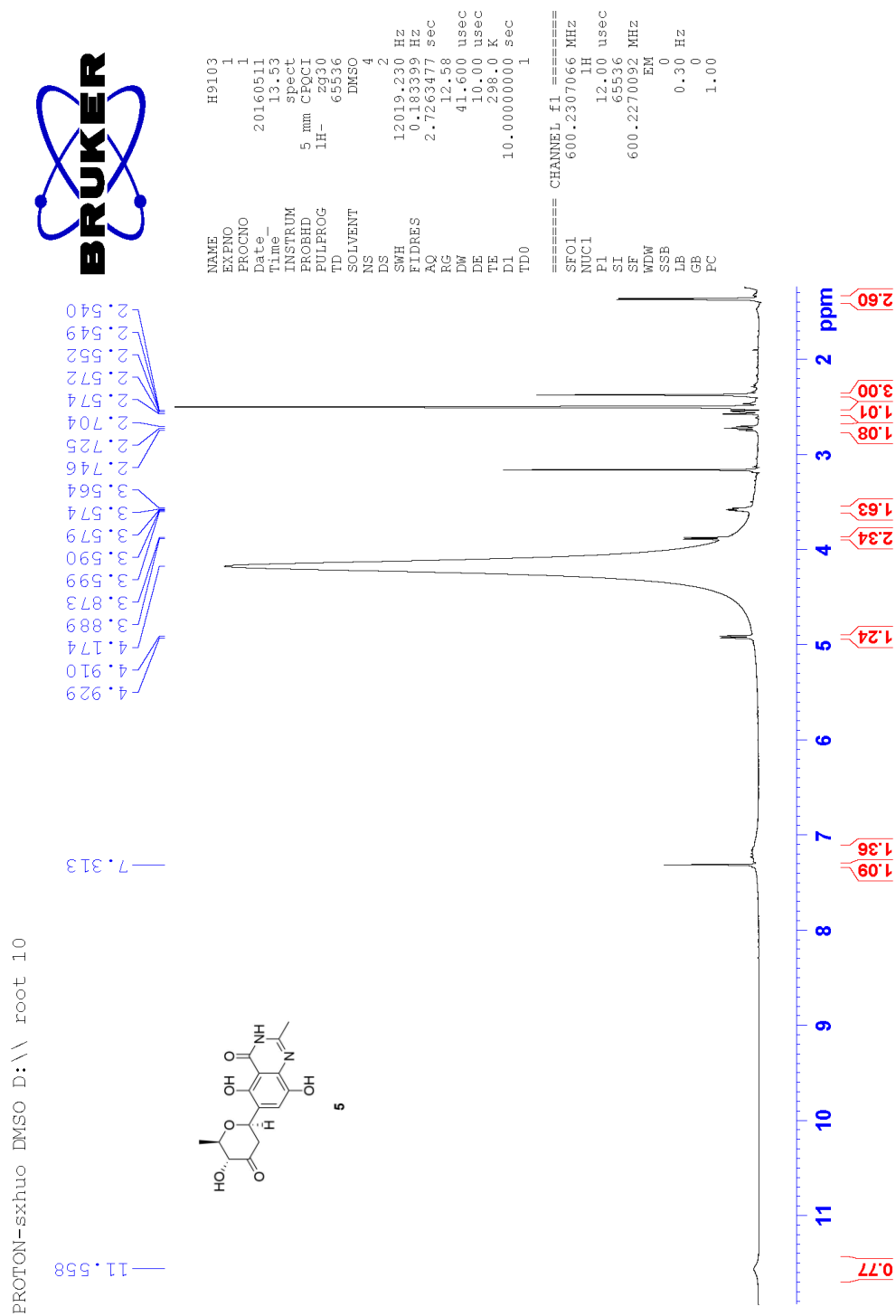
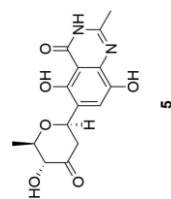


Fig. S17. ¹³C NMR (150 MHz) spectrum of **5** in DMSO-*d*₆



ZGDC-sxhuo DMSO D:\ root 10



```

NAME          H9103
EXPNO         4
PROCNO        1
Date_         20160511
Time_        21.25
INSTRUM       spect
PROBHD        5 mm CPQCI 1H-
PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            650
DS            4
SWH           36231.883 Hz
FIDRES        0.552855 Hz
AQ            0.9044468 sec
RG            199.06
DE            13.800 usec
TE            298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0           1
===== CHANNEL f1 =====
SFO1          150.9438010 MHz
NUC1          13C
P1            11.20 usec
SI            131072
SF            150.9272586 MHz
WDW           EM
SSB           0
LB            1.00 Hz
GB            0
PC            1.40
    
```

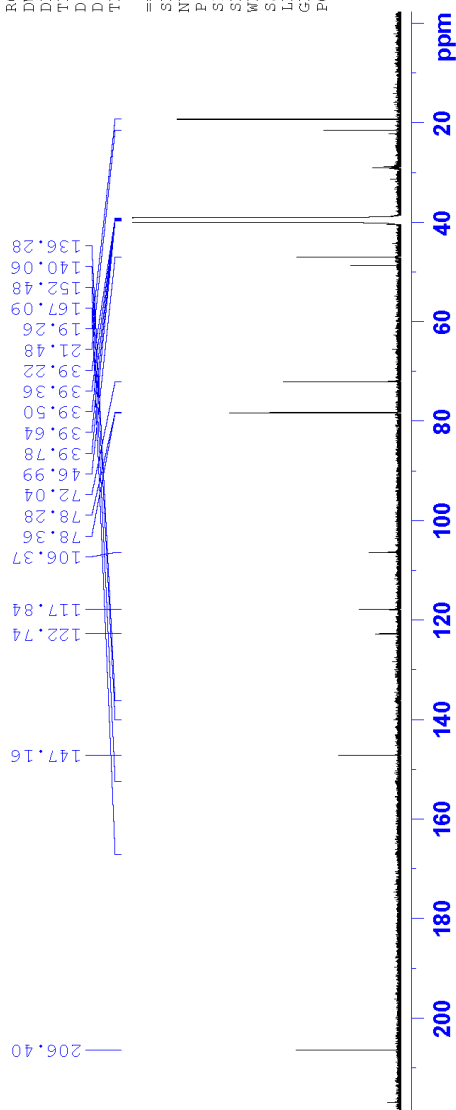


Fig. S18. HSQC (600 MHz) spectrum of **5** in DMSO-*d*₆

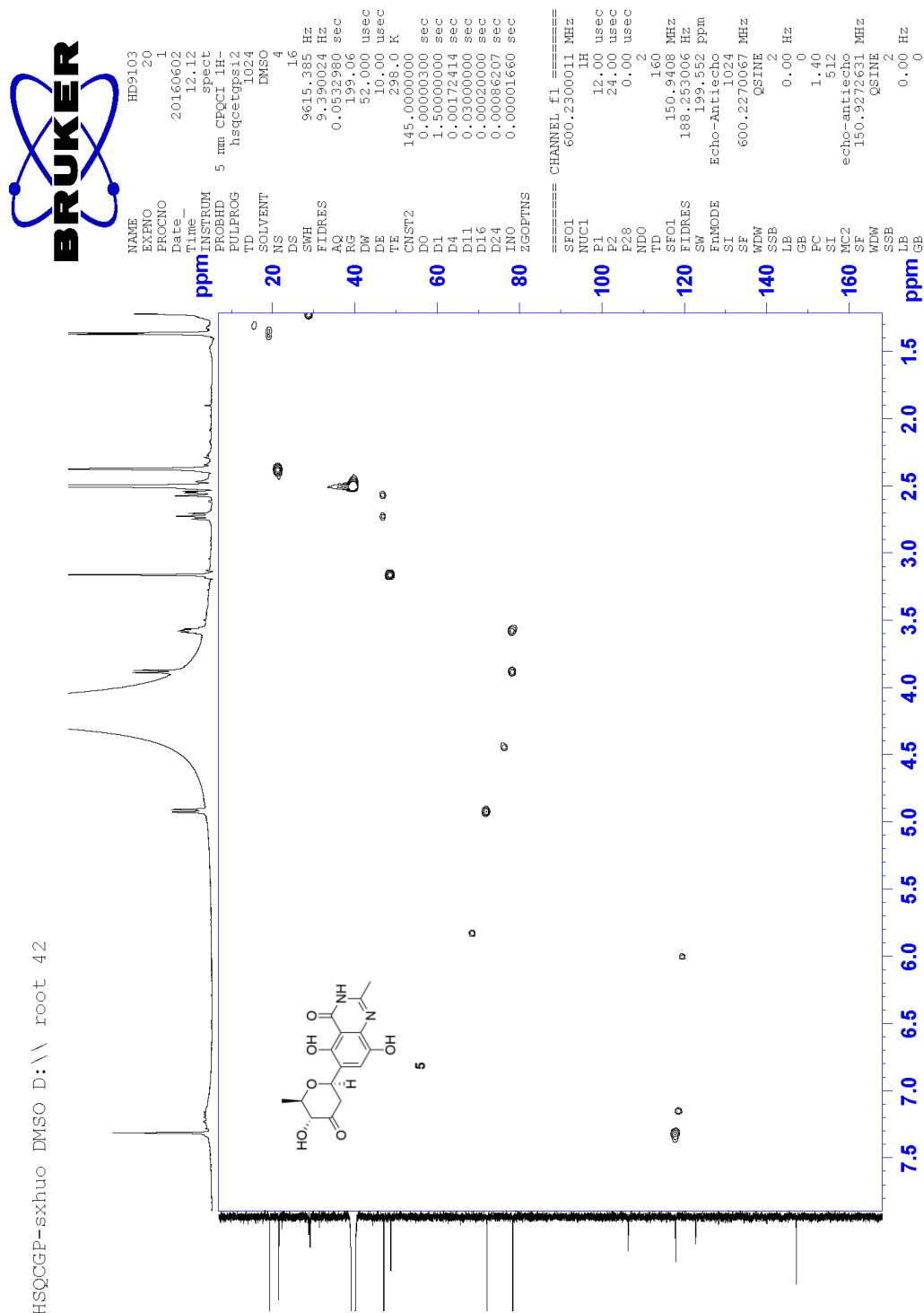


Fig. S19. HMBC (600 MHz) spectrum of **5** in DMSO-*d*₆

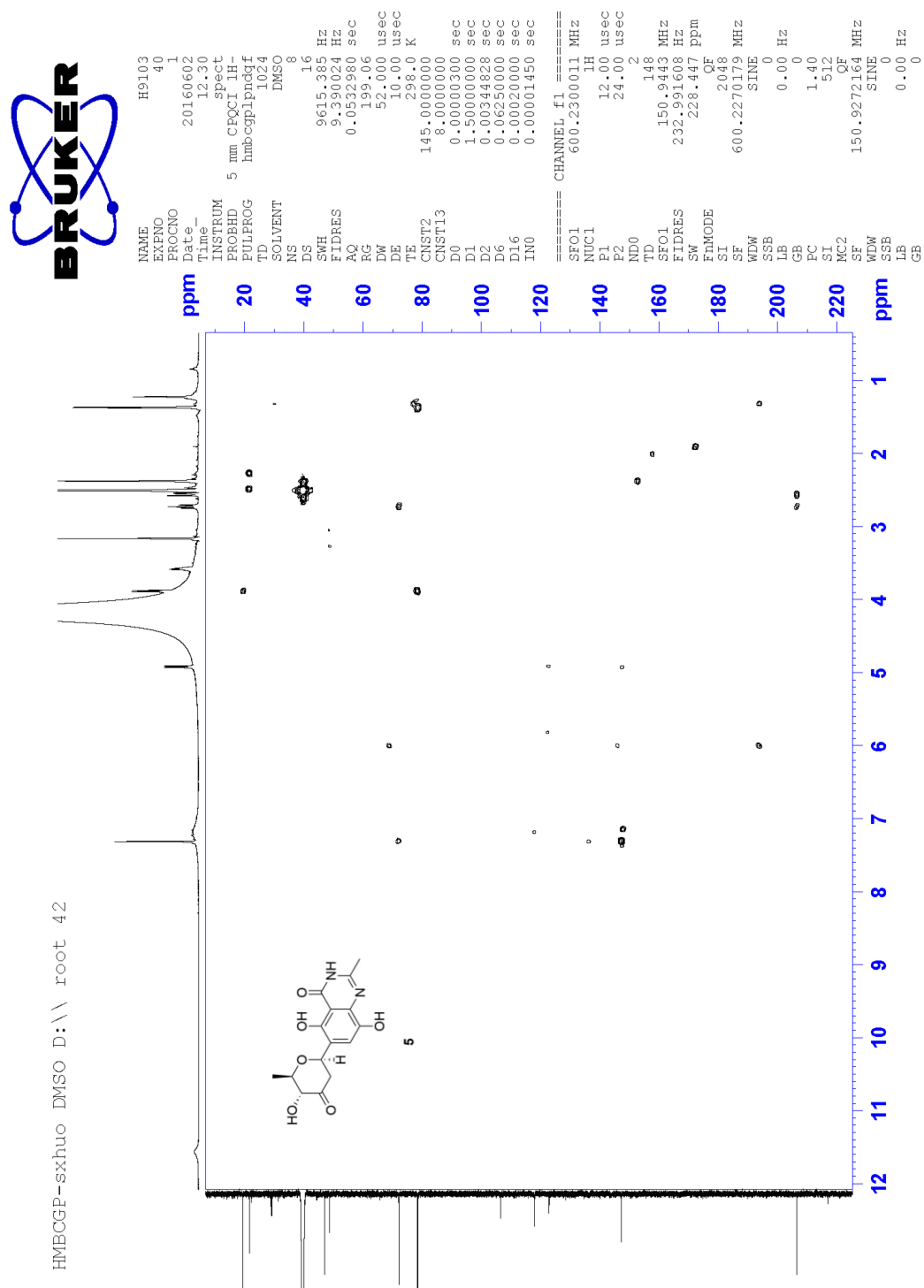


Fig. S20. ROESY (600 MHz) spectrum of **5** in DMSO-*d*₆



```

NAME      H9103
EXNO     L4
PROCNO   1
Date     20160602
Time     11.22
INSTRUM spect
PROBHD   5 mm CPOCI 1H-
PULPROG roesyphpr.2
TD       1024
SOLVENT  DMSO
NS       8
DS       16
SWH      9615.385 Hz
FIDRES   9.390024 Hz
AQ        0.0532980 sec
RG        157.96
DM        52.000 usec
DE        10.00 usec
TE        298.0 K
D0        0.00004046 sec
D1        2.00000000 sec
D11       0.03000000 sec
D12       0.00002000 sec
D13       0.00000400 sec
IN0       0.00010420 sec
L4        1190
P15       2000000.00 usec

===== CHANNEL f1 =====
SF01     600.2295156 MHz
NUC1     1H
P1       12.00 usec
E25      84.00 usec
ND0      1
TD        160
SF01     600.2295 MHz
FIDRES   59.980804 Hz
SW        15.989 ppm
FhMODE   States-TPPI
SI        2048
SF       600.2270074 MHz
WDW      QSINE
SSB      2
LB       0.00 Hz
GB       0
PC       1.00
RC       512
SI       States-TPPI
MC2      600.2263944 MHz
SF       600.2263944 MHz
WDW      QSINE
SSB      2
LB       0.00 Hz
GB
  
```

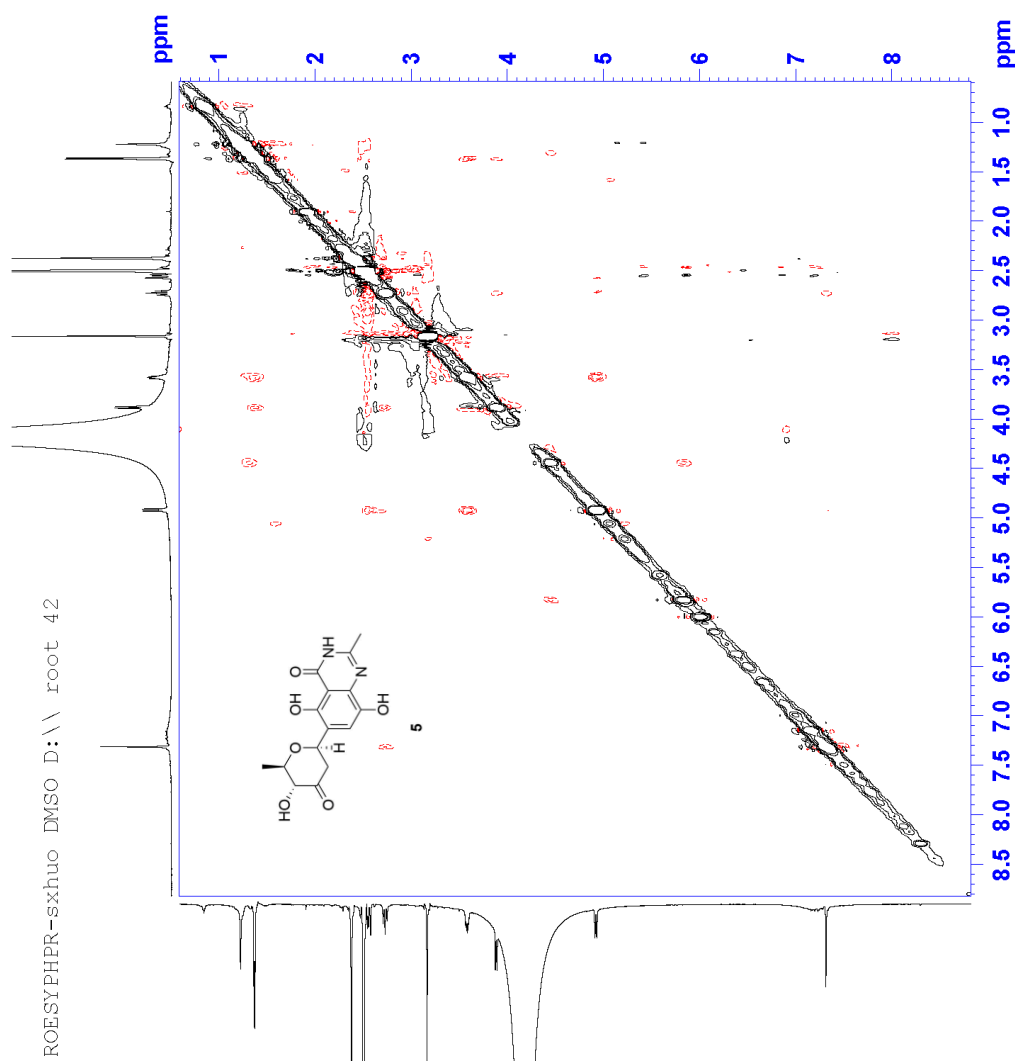


Fig. S21. ¹H NMR (600 MHz) spectrum of **8** in DMSO-*d*₆

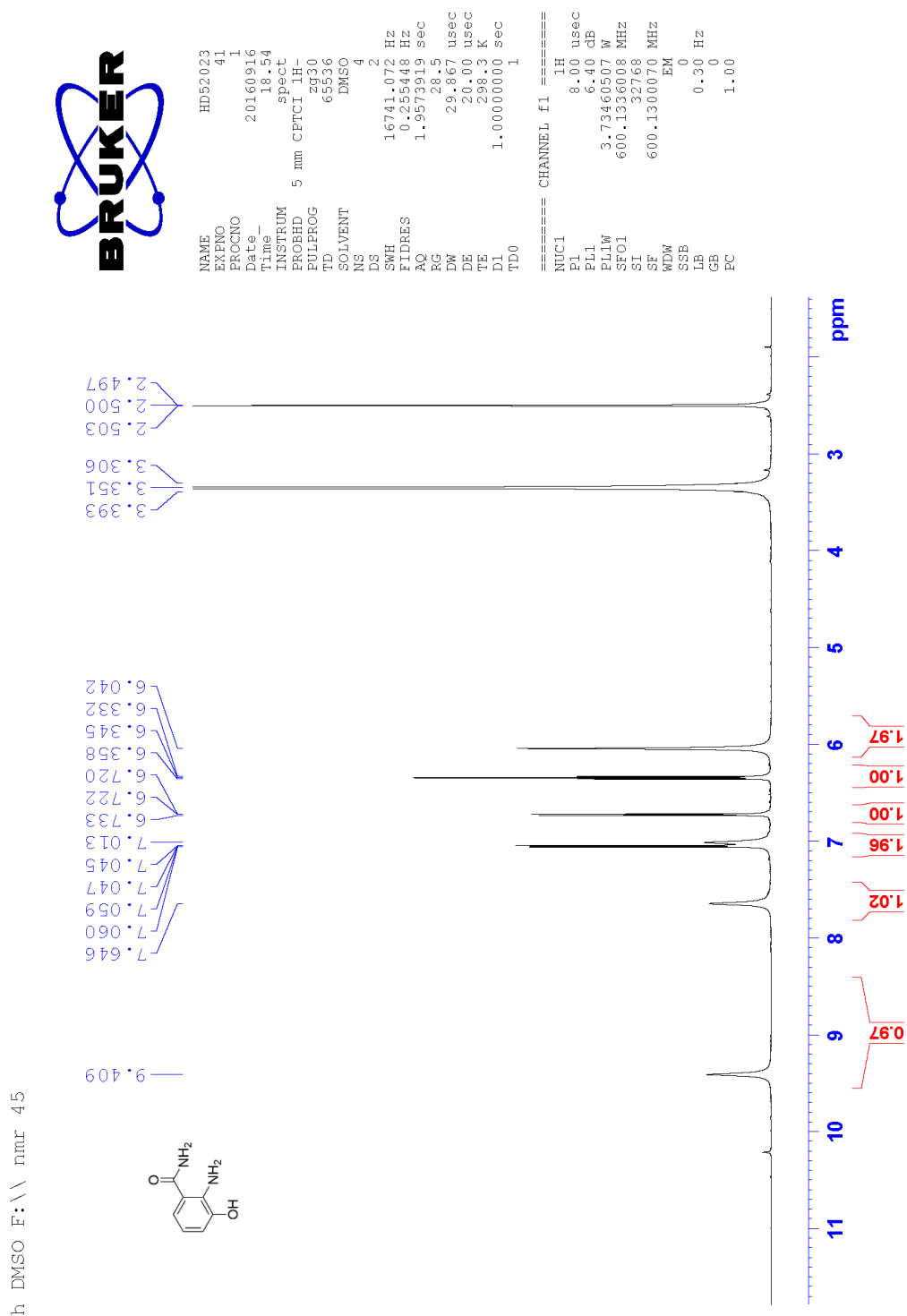
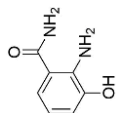


Fig. S22. ^{13}C NMR (150 MHz) spectrum of **8** in $\text{DMSO-}d_6$



c13 DMSO F:\n nmr 45



```

NAME HD52023
EXPNO 2
PROCNO 1
Date_ 20160918
Time 13.18
INSTRUM spect
PROBHD 5 mm CPYX
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SFR 39062.500 Hz
FIDRES 0.596046 Hz
AQ 0.8385108 sec
RG 2050
DQ 12.800 usec
DE 0.0000000 usec
TE 298.5 K
D1 2.00000000 sec
D11 0.03000000 sec
TDO 1
===== CHANNEL f1 =====
NUC1 13C
P1 12.00 usec
PL1 0.00 dB
PL12 93.443 dB
SFO1 150.9194083 MHz
===== CHANNEL f2 =====
NAME waltz16
PROCNO 1
PCPD2 80.00 usec
PL2 9.20 dB
PL12 25.68 dB
PL29 1.95994878 W
PLD29 0.04400001 M
SFO2 600.152768 MHz
SI 32768
SF 150.9028733 MHz
WDW EM
SFB 0
GB 0
PC 1.40
  
```

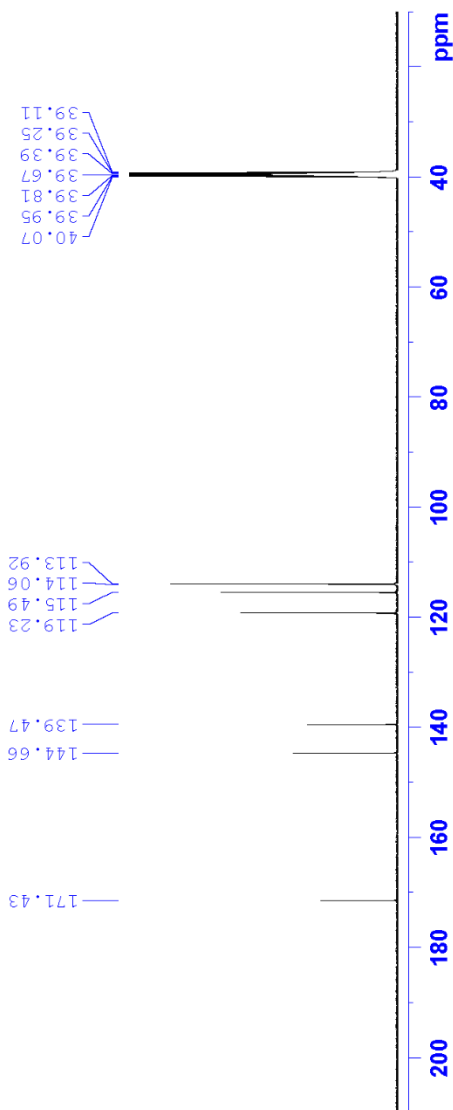


Fig. S23. ^1H NMR (400 MHz) spectrum of **9** in $\text{DMSO-}d_6$

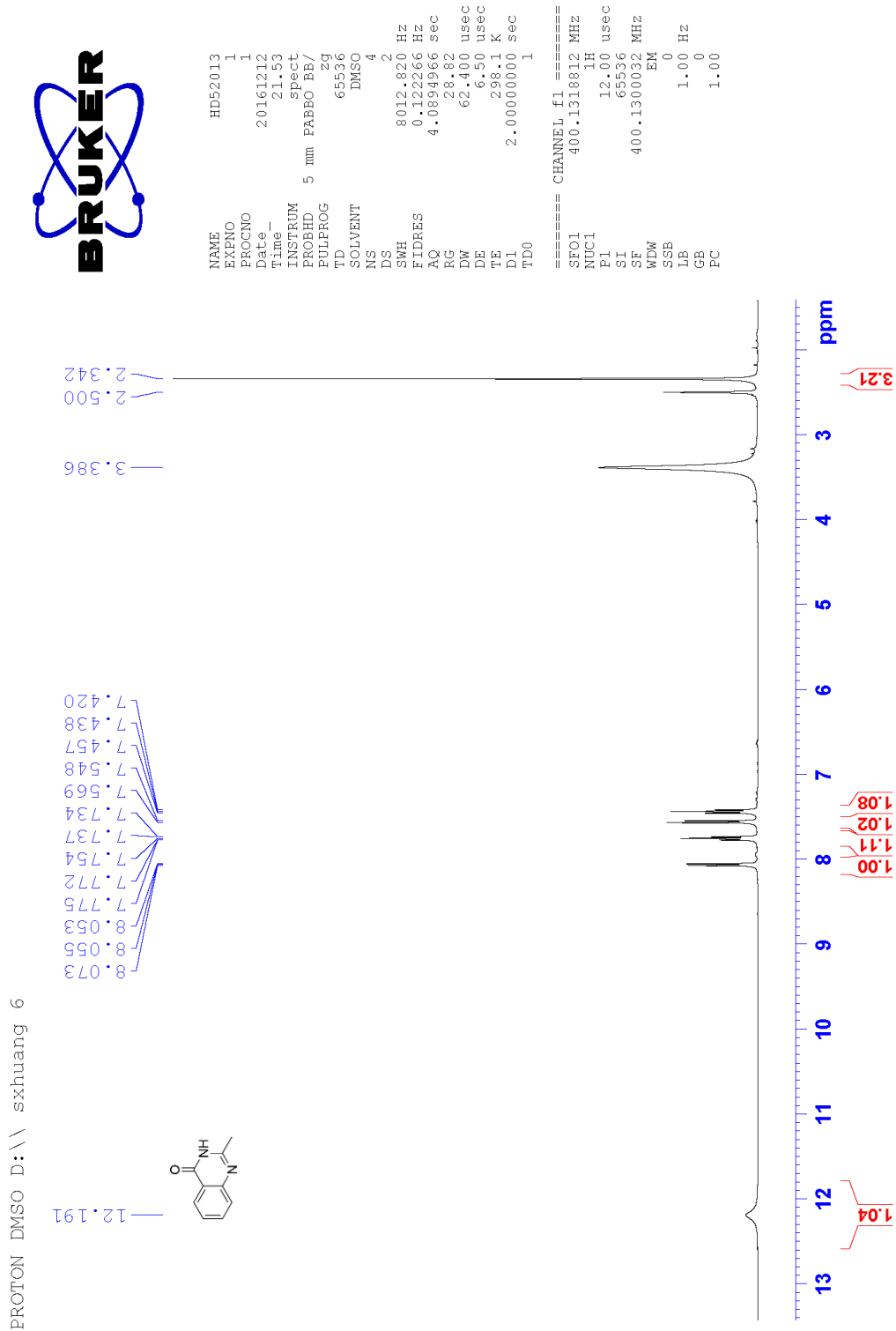
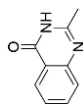


Fig. S24. ^1H NMR (100 MHz) spectrum of **9** in $\text{DMSO-}d_6$



c13 DMSO D:\ \ sxhuang 6



```

NAME          HD52013
EXPNO         4
PROCNO        1
Date_         20161212
Time_         22.21
INSTRUM       spect
PROBHD        5 mm PABBO BB/
PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            350
DS            4
SWH           24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631988 sec
RG            214.83
DW            20.800 usec
DE            6.50 usec
TE            298.1 K
D1            3.0000000 sec
D11           0.0300000 sec
TD0           1
===== CHANNEL f1 =====
SFO1          100.6240376 MHz
NUC1          13C
P1            12.00 usec
SI            32768
SF            100.6128137 MHz
WDW           EM
SSB           0
LB            1.00 Hz
GB            0
PC            1.40
    
```

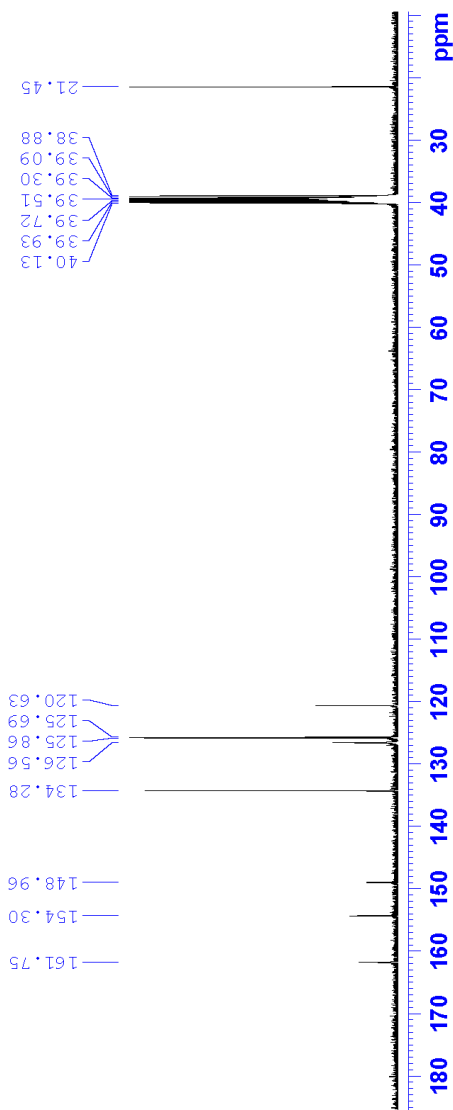


Fig. S25. ¹H NMR (400 MHz) spectrum of **10** in DMSO-d₆

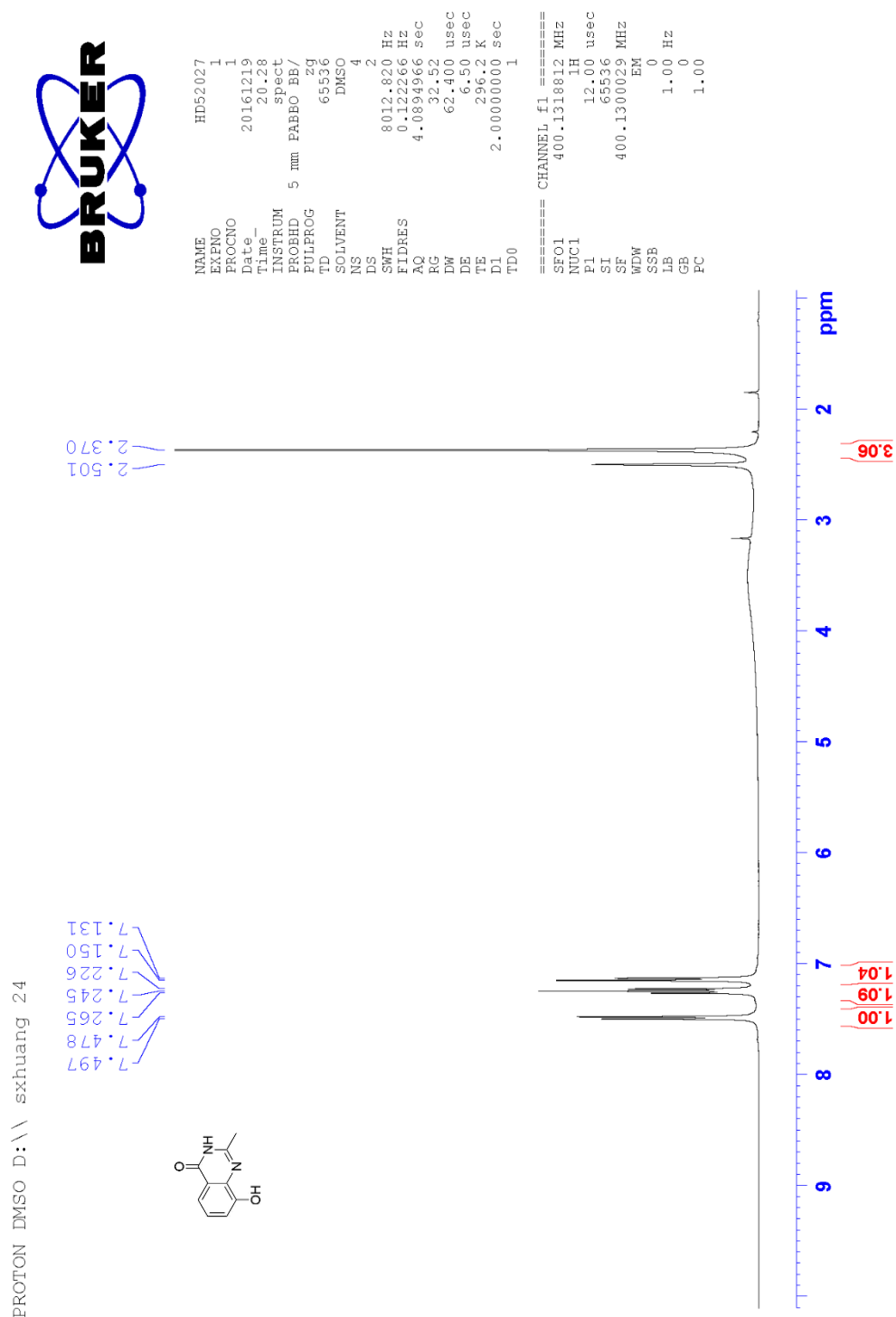


Fig. S26. ^1H NMR (100 MHz) spectrum of **10** in $\text{DMSO}-d_6$

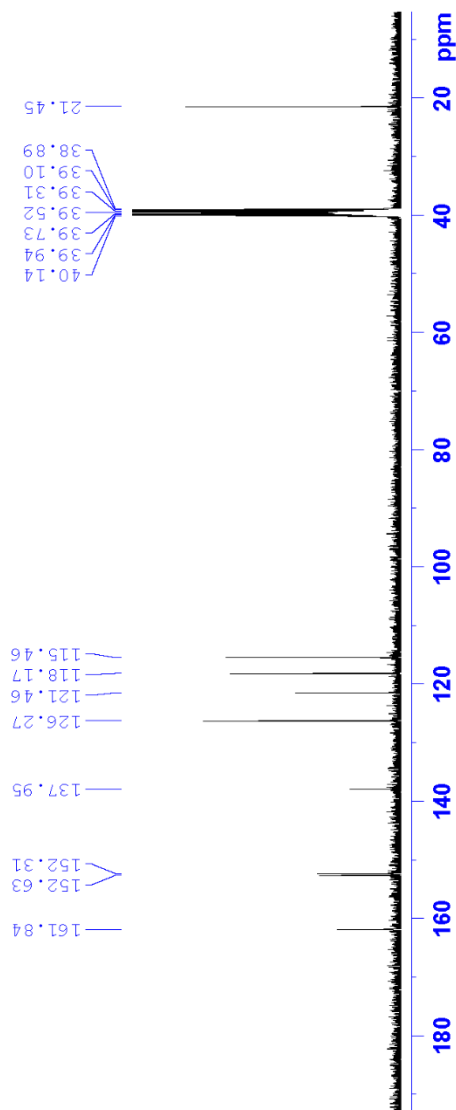
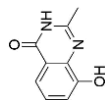


```

NAME HD52027
EXPNO 4
PROCNO 1
Date_ 20161219
Time_ 20.35
INSTRUM spect
PROBHD 5 mm PABBO
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 59
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 214.83
DW 20.800 usec
DE 6.50 usec
TE 296.2 K
D1 3.0000000 sec
D11 0.0300000 sec
TD0 1

===== CHANNEL f1 =====
SF01 100.6240376 MHz
NUC1 13C
P1 12.00 usec
SI 32768
SF 100.6128112 MHz
SSB 0
WDW EM
LB 1.00 Hz
GB 0
PC 1.40
    
```

c13 DMSO D:\ \ sxhuang 24



Supplementary references

1. L. Li, Z. Xu, X. Xu, J. Wu, Y. Zhang, T. Zhang, X. He, T. Zabriskie and Z. Deng, The mildiomycin biosynthesis: initial steps for sequential generation of 5-hydroxymethylcytidine 5'-monophosphate and 5-hydroxymethylcytosine in *Streptovorticillium rimofaciens* ZJU5119. *ChemBioChem.*, 2008, **9**, 1286-1294.
2. M. S. B. Paget, L. Chamberlin, A. Atrih, S. J. Foster and M. J. Buttner, Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2).1999, *J. Bacteriol.*, **181**, 204–211.
3. K. A. Datsenko and B. L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 6640-6645.
4. T. Hosaka, M. Ohnishi-Kameyama, H. Muramatsu, K. Murakami, Y. Tsurumi, S. Kodani, M. Yoshida, A. Fujie and K. Ochi, Antibacterial discovery in actinomycetes strains with mutations in RNA polymerase or ribosomal protein S12. *Nat. Biotechnol.*, 2009, **27**, 462-464.