Supplementary Material for:

Isolation, Synthesis and Biological Activity of Aphrocallistin, an Adenine Substituted Bromotyramine metabolite from the Hexactinellida Sponge *Aphrocallistes beatrix*

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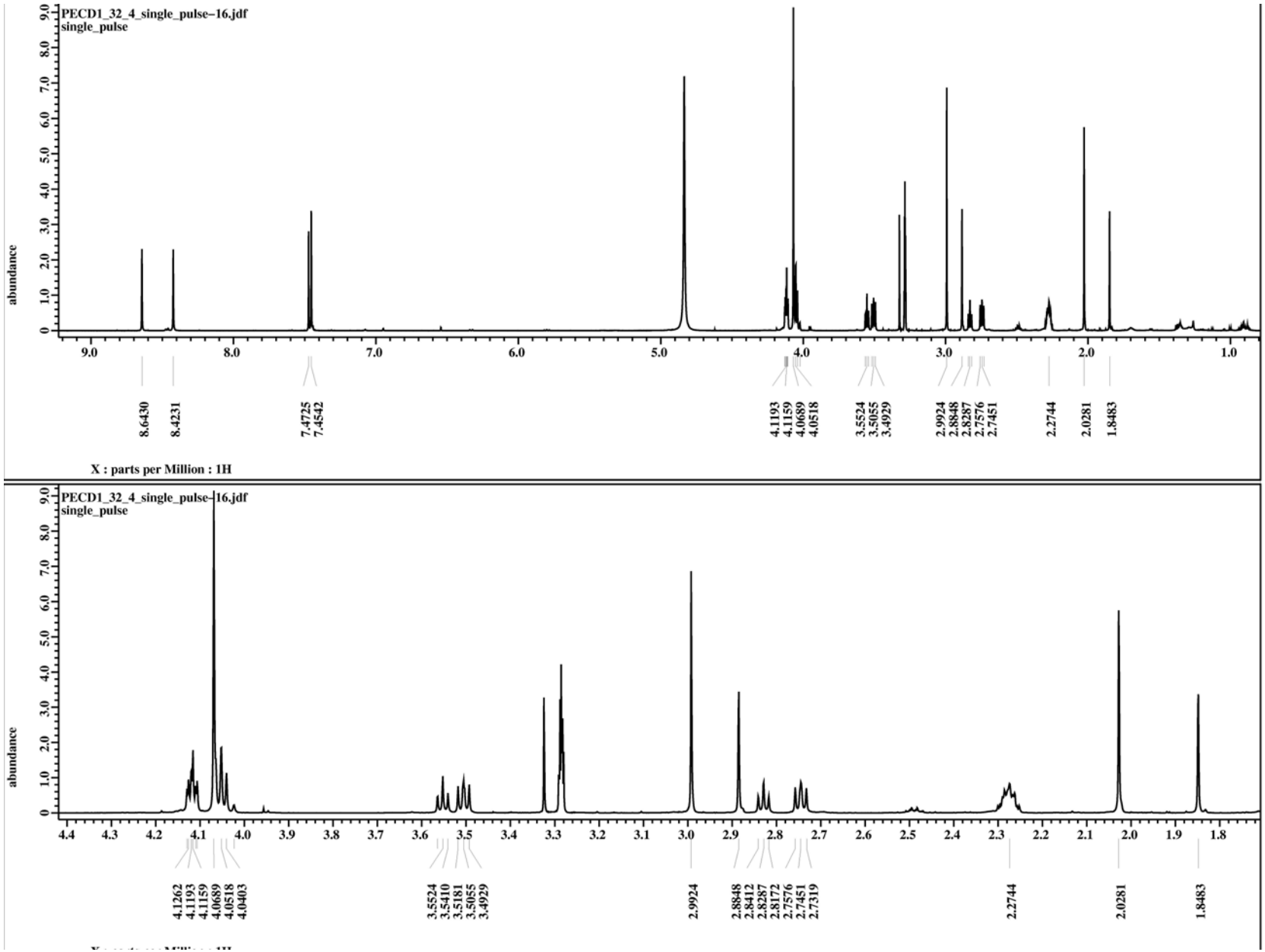
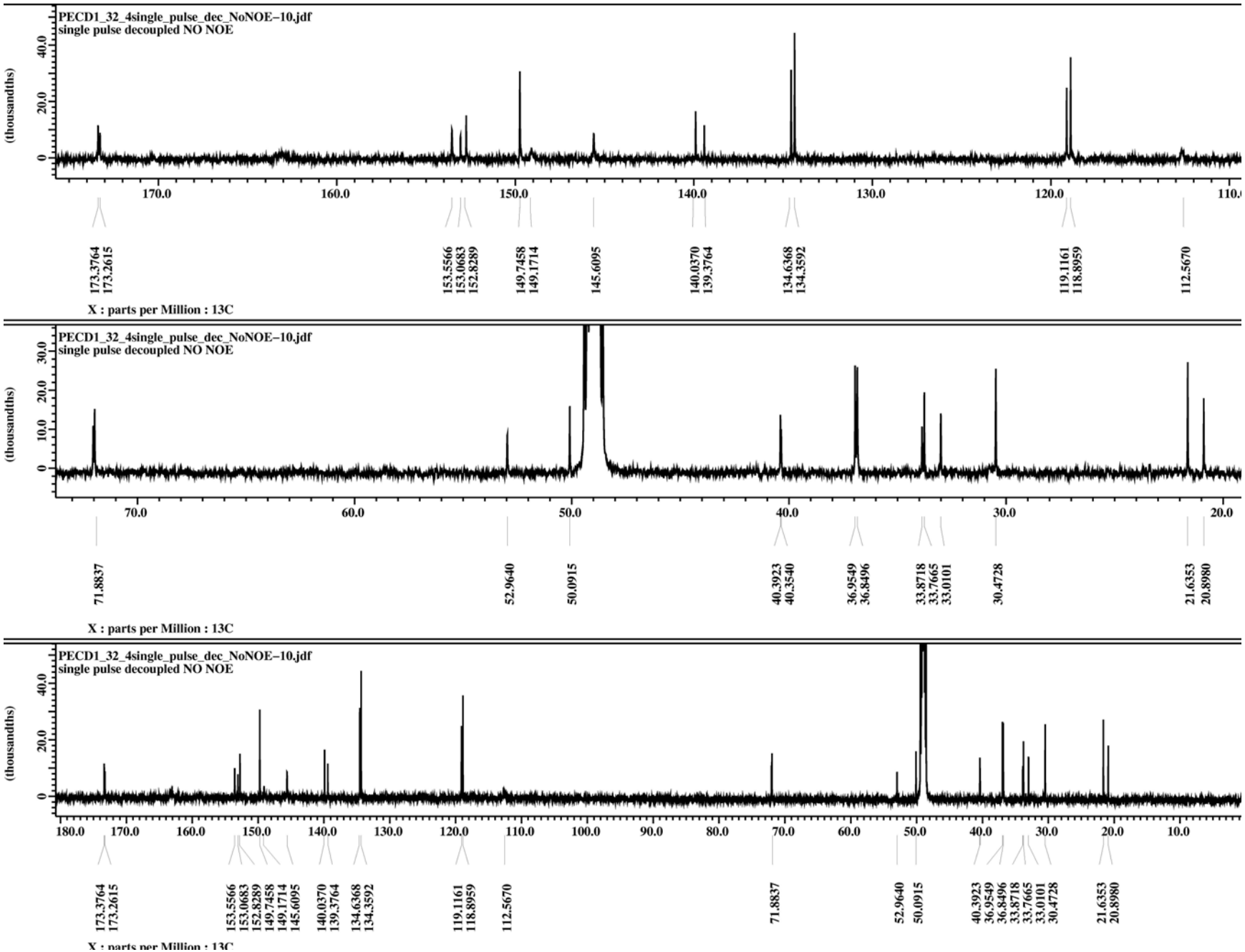
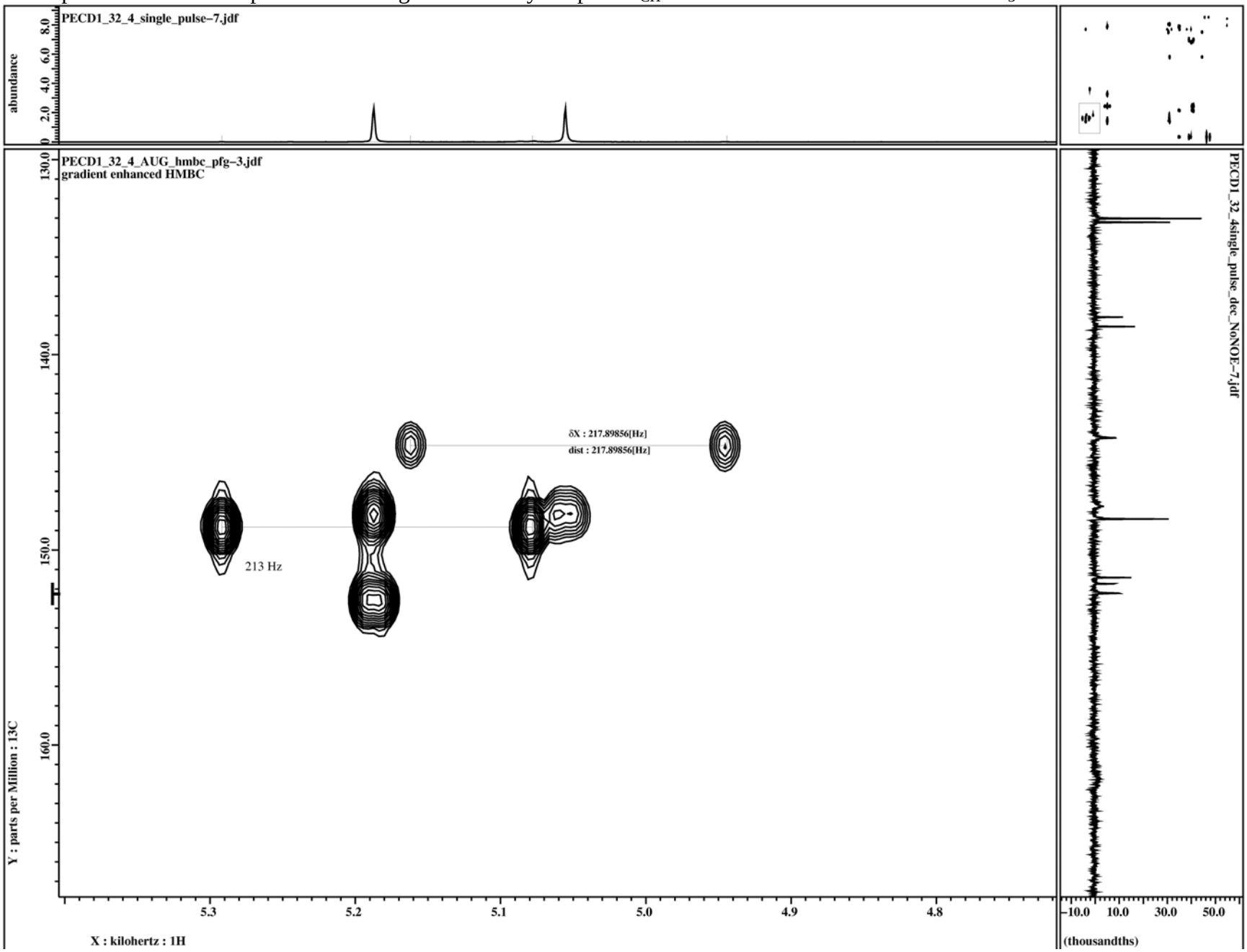
S.1 ^{1}H NMR Spectrum of Aphrocallistin **(1).** (600 MHz CD_3OD).



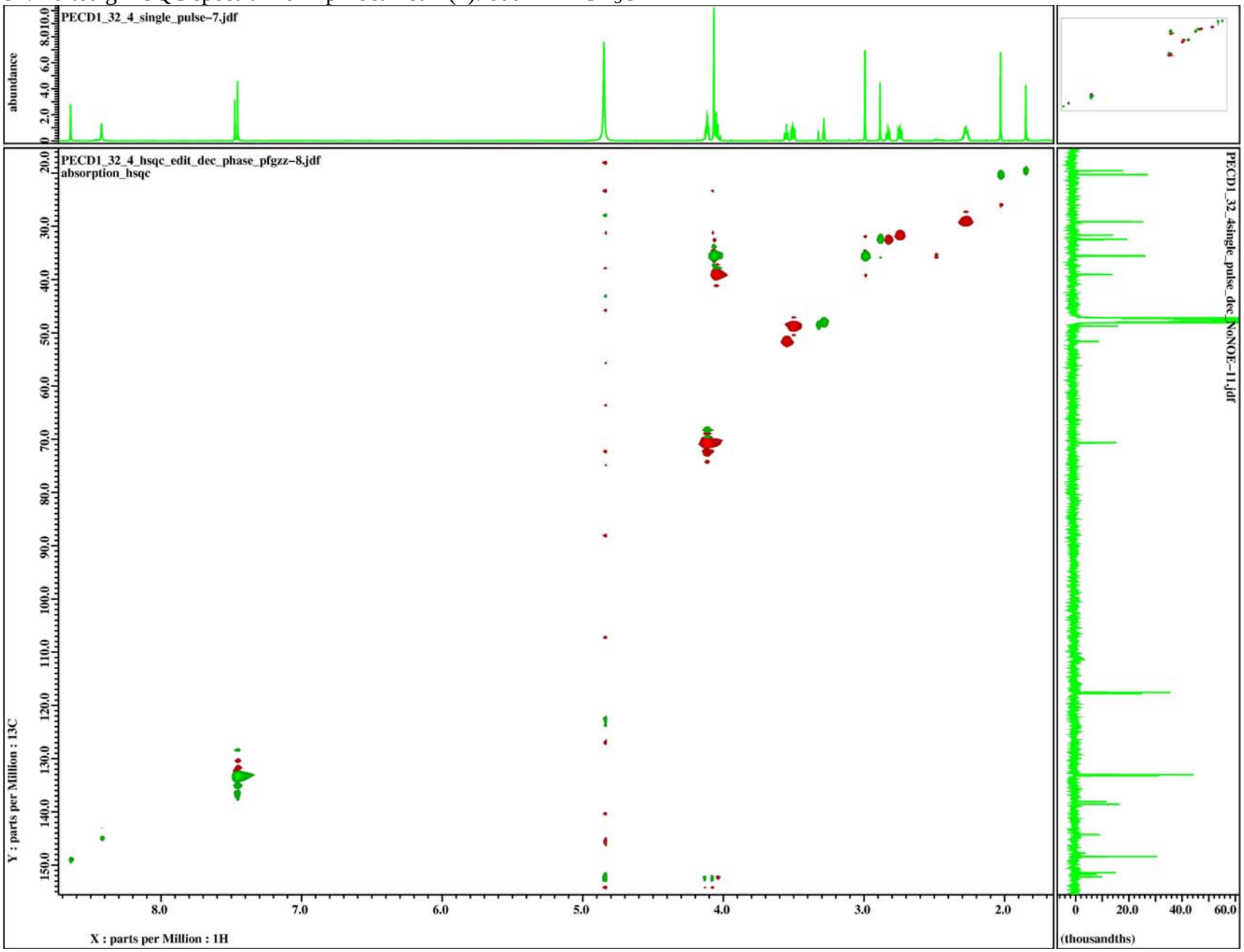
S2. ^{13}C NMR Spectrum of Aphrocallistin (**1**). 600 MHz CD_3OD



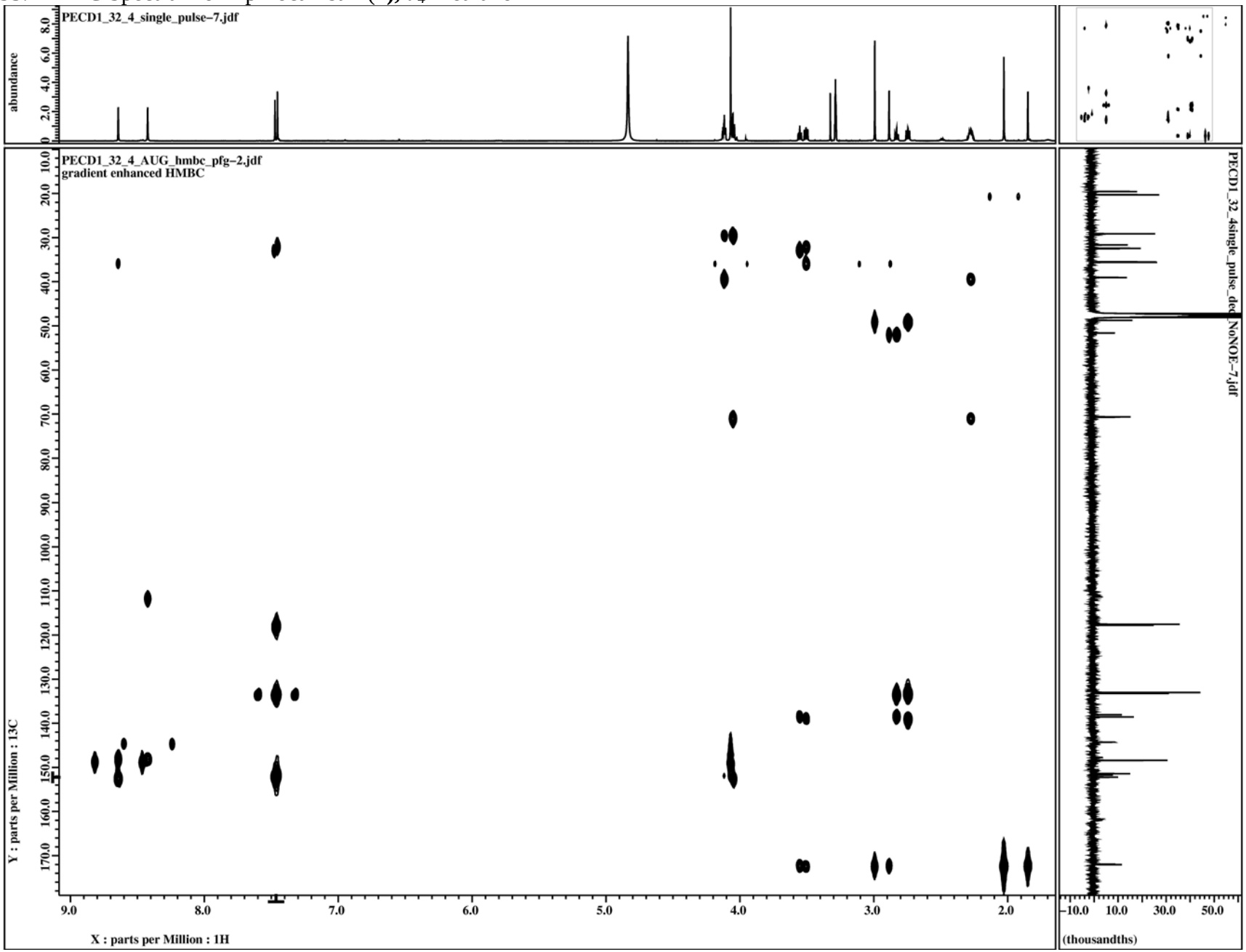
S3. Expansion of HMBC Spectrum showing residual fully coupled $^{1}J_{CH}$ for Carbons 2’ and 8’. 600 MHz CD_3OD



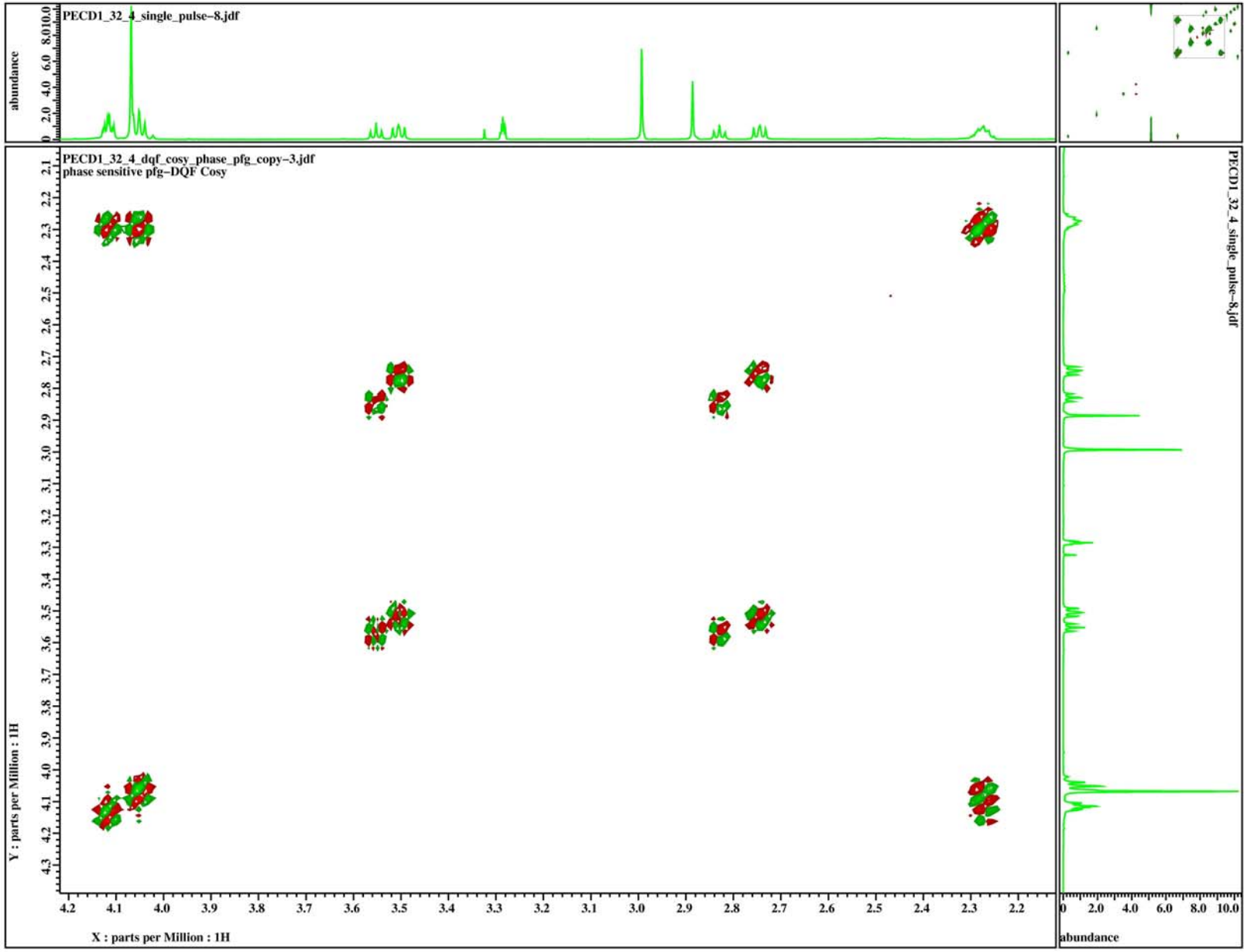
S4. Edited g-HSQC spectrum of Aphrocallistin (**1**). 600 MHz CD_3OD



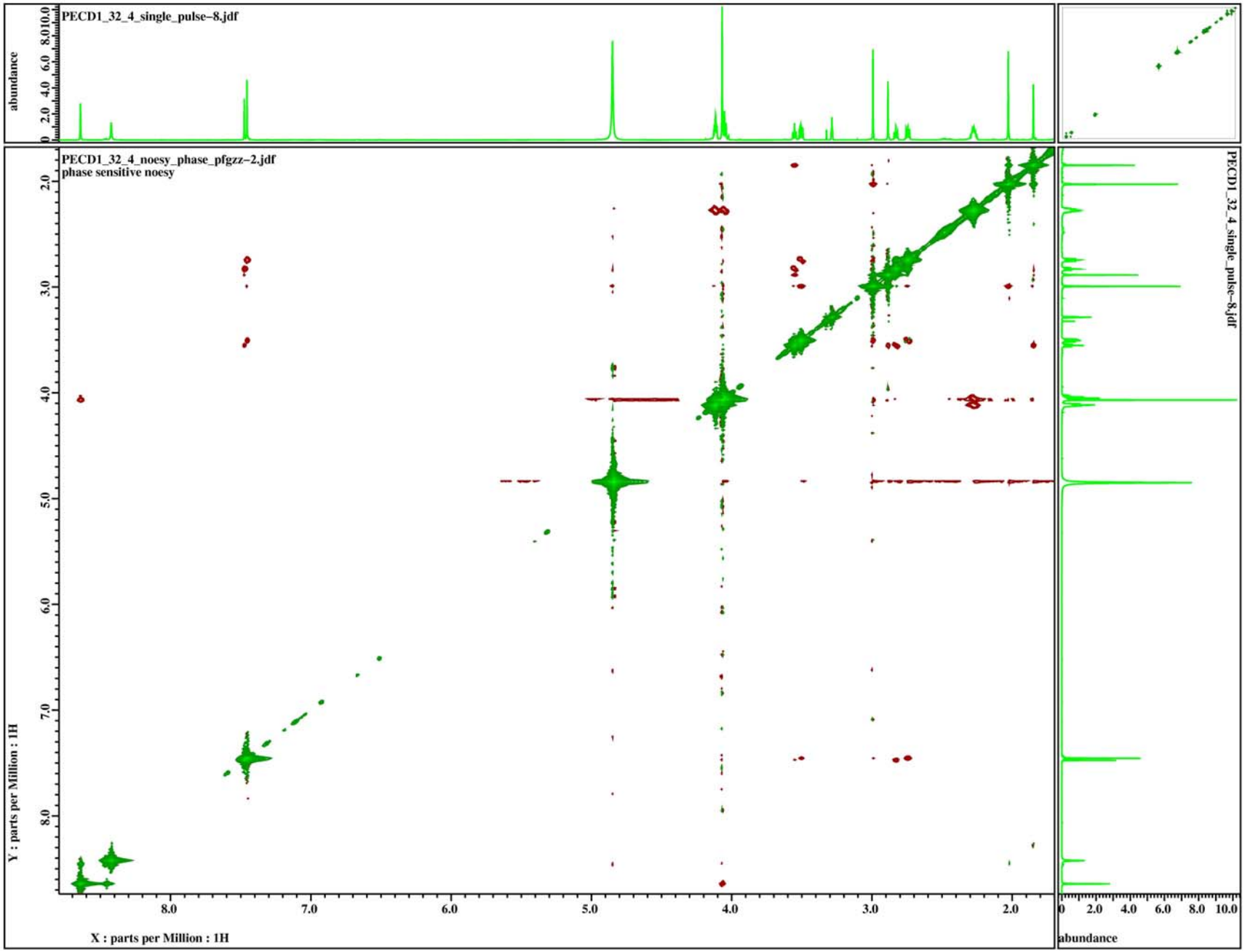
S5. HMBC Spectrum of Aphrocallistin (**1**), d_4-methanol



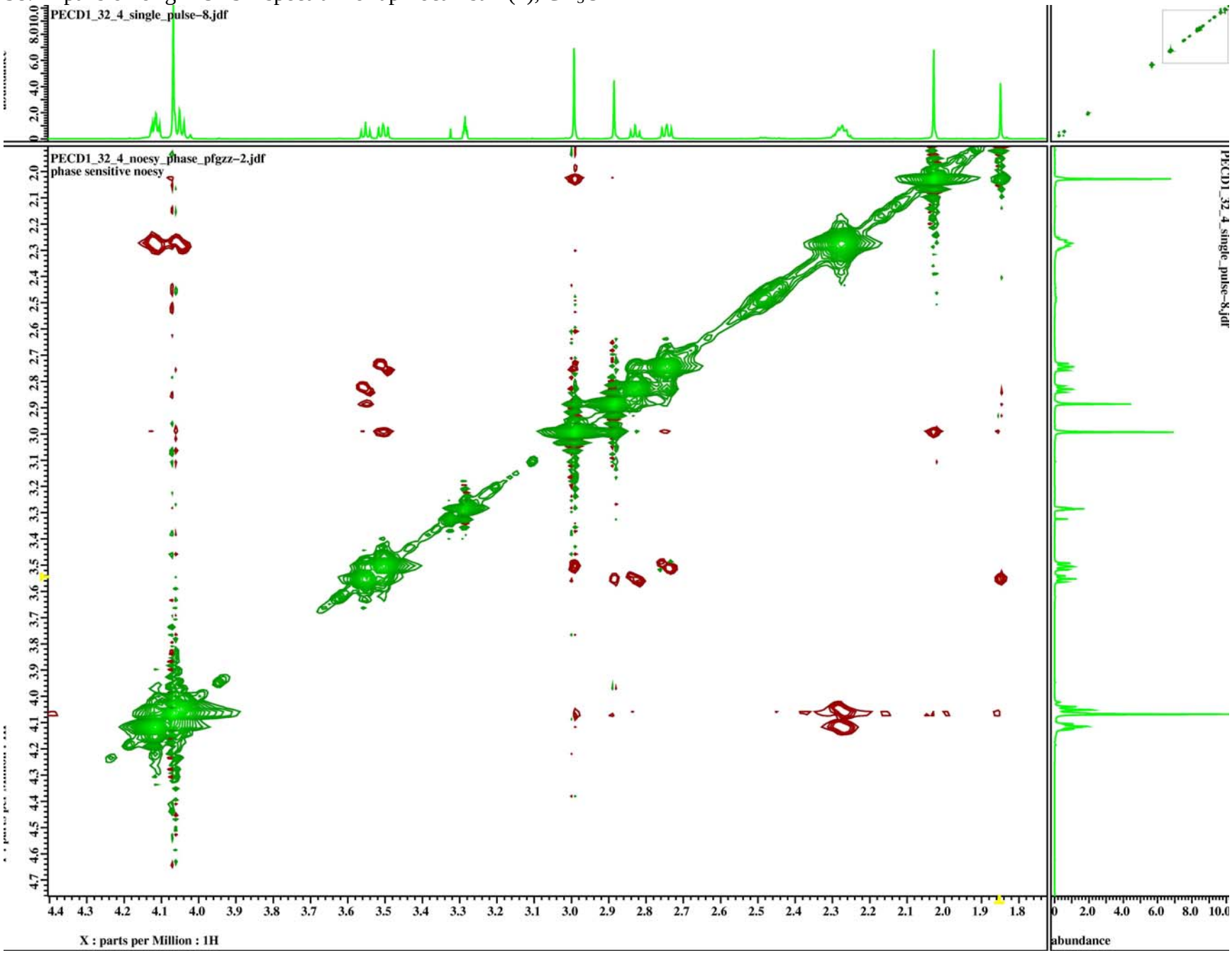
S6-Expansion of aliphatic region of g-DQF-COSY spectrum of aphrocallistin (**1**), d_4-methanol



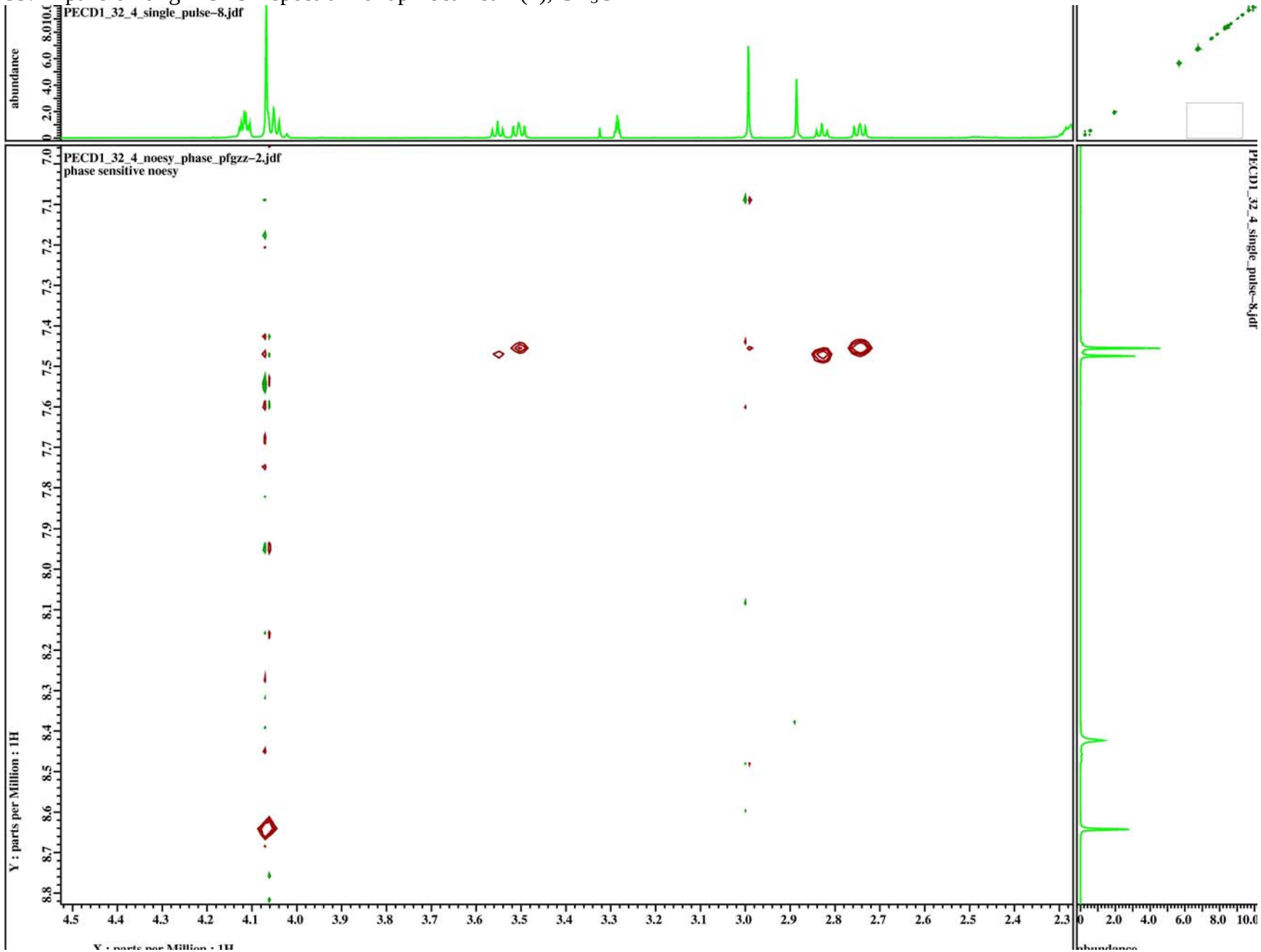
S7. g-NOESY Spectrum of Aphrocallistin (**1**), CD_3OD



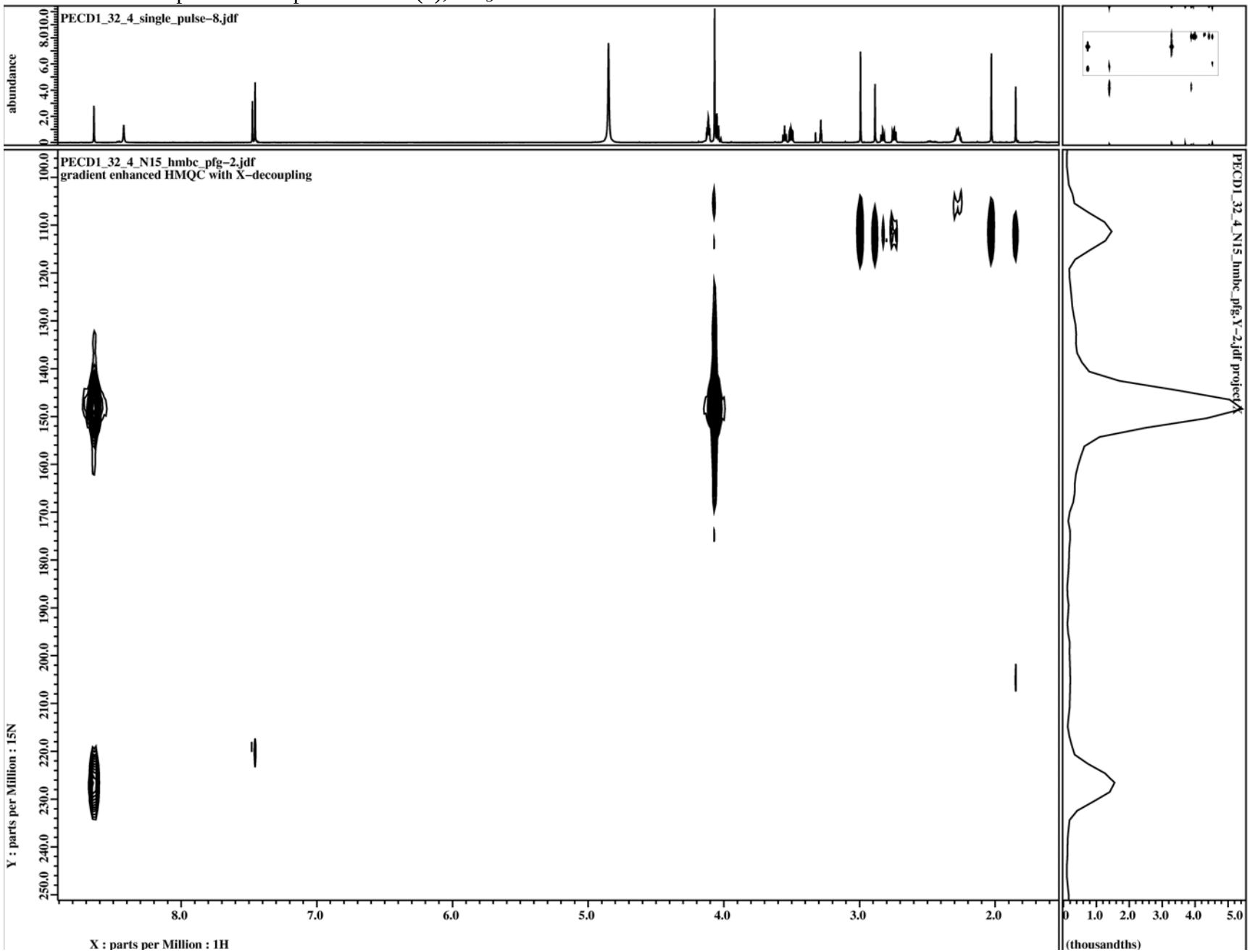
S8. Expansion of g-NOESY spectrum of aphrocallistin (**1**), CD_3OD



S9. Expansion of g-NOESY spectrum of aphrocallistin (**1**), CD_3OD



S10. ^{15}N HMBC Spectrum of aphrocallistin (**1**), CD_3OD



S.11 In Vitro Pharmacology of Aphrocallistin

The pharmacological properties of aphrocallistin were evaluated in various *in vitro* assays (Table S2). The solubility of the compound was assessed to be high at three pH levels (Table S2A). It exhibited moderate permeability at pH 6.2 and 7.4 and poor-to-moderate permeability at pH 5.0 (Table S2B). Aphrocallistin showed extensive protein binding at both test concentrations in human plasma – 95.6% at 1 µM and 89.7% at 10 µM. In mouse plasma, high protein binding was observed at 1 µM (85.0% bound) and moderate at 10 µM (69.1% bound) (Table S2C). Aphrocallistin was observed to remain stable in both human and mouse plasma at t=3.0 hours and in PBS (Table S2D). However, it was metabolized 96.92% and 98.56% in human and mouse liver microsomes, respectively, which suggests that aphrocallistin will be a subject to significant hepatic metabolism in vivo (Table S2E).

Table S2 *In vitro* pharmacology data for aphrocallistin (**1**).

S2A. Solubility.

pH	**5.0**	**6.2**	**7.4**
Avg. Sol (µg/mL)	>267	>267	>267
Limit (µg/mL)	267	267	267

S2B. Permeability.

Permeability of Compound at 50 µM	**pH**	**Avg. P_e ($\times 10^{-6}$ cm/s)**	**SD P_e**	**-log P_e**
Poor to moderate	5.0	33	2	4.5
Moderate	6.3	201	2	3.7
Moderate	7.4	321	29	3.5

S2C. Plasma Protein Binding.

Plasma Protein Binding (Test Concentration, µM)		**% Free**	**% Bound**
Human	Strong (10 µM)	10.3	89.7
	Strong (1 µM)	4.4	95.6
Mouse	Moderate (10 µM)	30.9	69.1
	Strong (1 µM)	15.0	85.0

S2D. Plasma Stability.

Test Compound at 40 µM	**Plasma/PBS % Remaining**		**Plasma/PBS/PI % Remaining**		**PBS/PI % Remaining**		**PBS % Remaining**	
	Human	Mouse	Human	Mouse	Human	Mouse	Human	Mouse
Plasma Stability	103.1	96.3	76.6	79.6	76.5	85.8	103.8	99.7

S2E. Microsomal Stability.

Microsomal Stability	**Final Assay Concentration**	**NADPH**	**% Remaining at 60 min**	**% Metabolized**
Human Liver Microsomes	1 µM	+	3.08	96.92
Human Liver Microsomes	1 µM	-	117.73	
Mouse Liver Microsomes	1 µM	+	1.44	98.56
Mouse Liver Microsomes	1 µM	-	103.32	

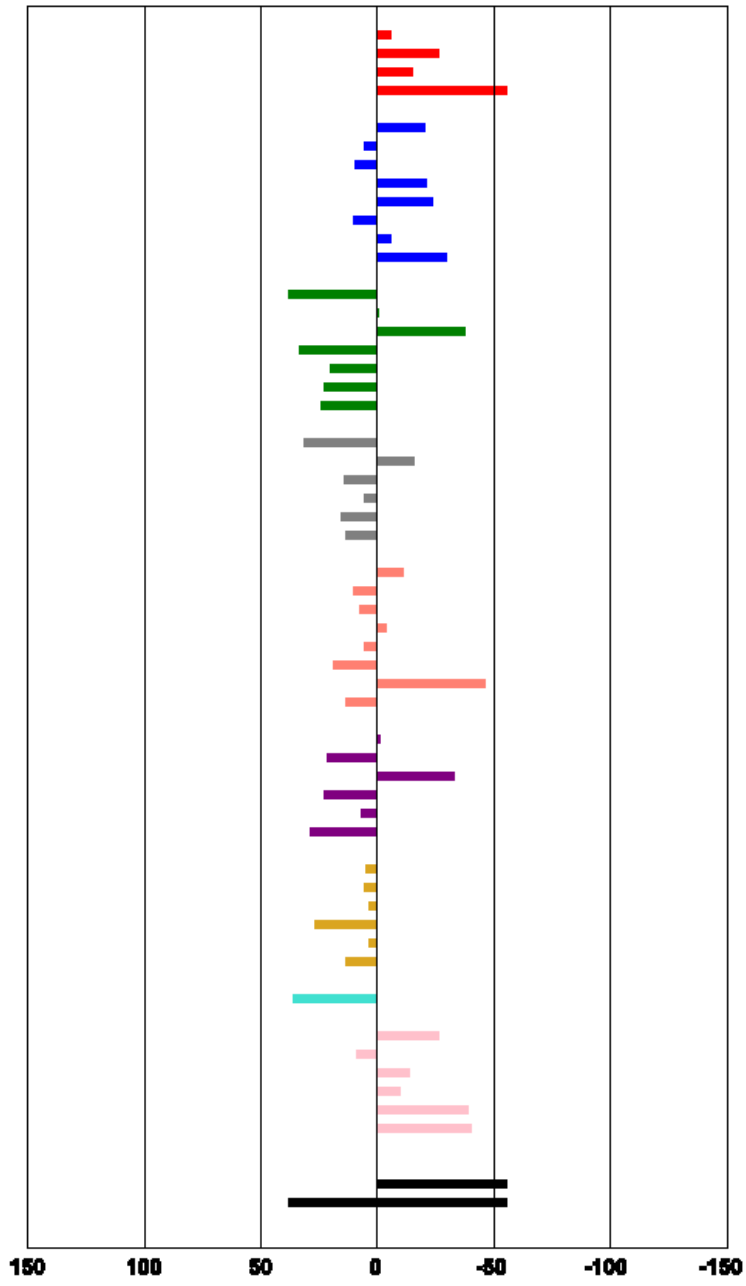
S12. NCI 60 cell line panel data for Aphrocallistin

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 749266 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 08, 2008
Experiment ID: 0812OS07		Report Date: Jan 07, 2009

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	80.24
HL-60(TB)	39.94
MOLT-4	50.93
SR	10.72
Non-Small Cell Lung Cancer	
EKVX	45.36
HOP-62	71.36
HOP-92	75.25
NCI-H226	44.72
NCI-H23	42.16
NCI-H322M	76.48
NCI-H460	80.08
NCI-H522	36.60
Colon Cancer	
COLO 205	104.32
HCC-2998	85.57
HCT-116	28.57
HCT-15	89.13
HT29	86.11
KM12	88.97
SW-620	89.87
CNS Cancer	
SF-268	87.67
SF-295	50.01
SF-539	80.09
SNB-19	71.64
SNB-75	81.17
U251	79.39
Melanoma	
LOX IMVI	55.23
MALME-3M	76.40
M14	73.49
MDA-MB-435	82.57
SK-MEL-2	71.47
SK-MEL-28	84.56
SK-MEL-5	20.00
UACC-62	79.32
Ovarian Cancer	
IGROV1	84.84
OVCAR-3	87.30
OVCAR-4	33.32
OVCAR-5	88.85
NCI/ADR-RES	72.89
SK-OV-3	94.44
Renal Cancer	
786-0	70.85
A498	71.71
ACHN	89.23
SN12C	82.68
TK-10	89.47
UO-31	79.45
Prostate Cancer	
DU-145	102.31
Breast Cancer	
MCF7	39.83
MDA-MB-231/ATCC	74.94
HS 578T	52.46
BT-549	56.49
T-47D	28.91
MDA-MB-468	25.46
Mean	66.01
Delta	55.29
Range	89.60



(**1**)

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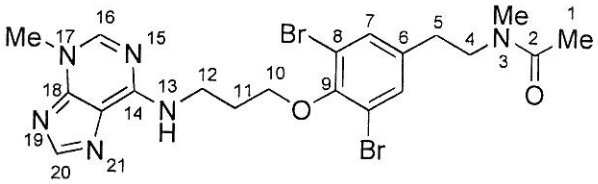
S13. 1H NMR of Synthetic Aphrocallistin (1) (400 MHz CD3OD)

Aphrocallistin 9-19



BRUKER

NAME	Sep19-2008-jkh
EXPNO	10
PROCNO	1
Date_	20080919
Time	14.01
INSTRUM	spect
PROBHD	5 mm QNP 1H/15
PULPROG	zg30
TD	65536
SOLVENT	MeOD
NS	64
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	362
DW	60.400 usec
DE	6.50 usec
TE	293.5 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.12 usec
PL1	-4.00 dB
SFO1	400.1324710 MHz
SI	32768
SF	400.1300477 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

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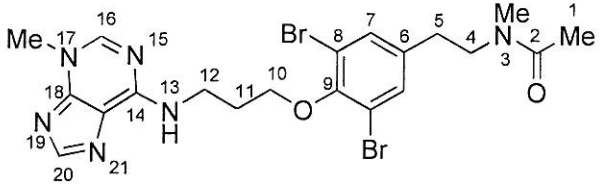
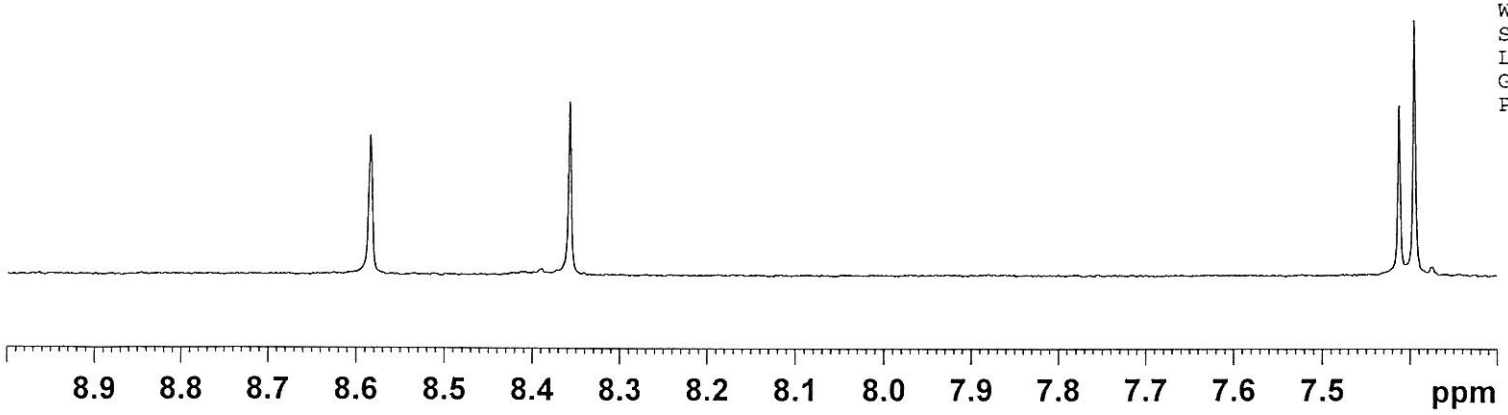
S14. Expansion of 1H NMR of Synthetic Aphrocallistin (1) (400 MHz CD3OD)

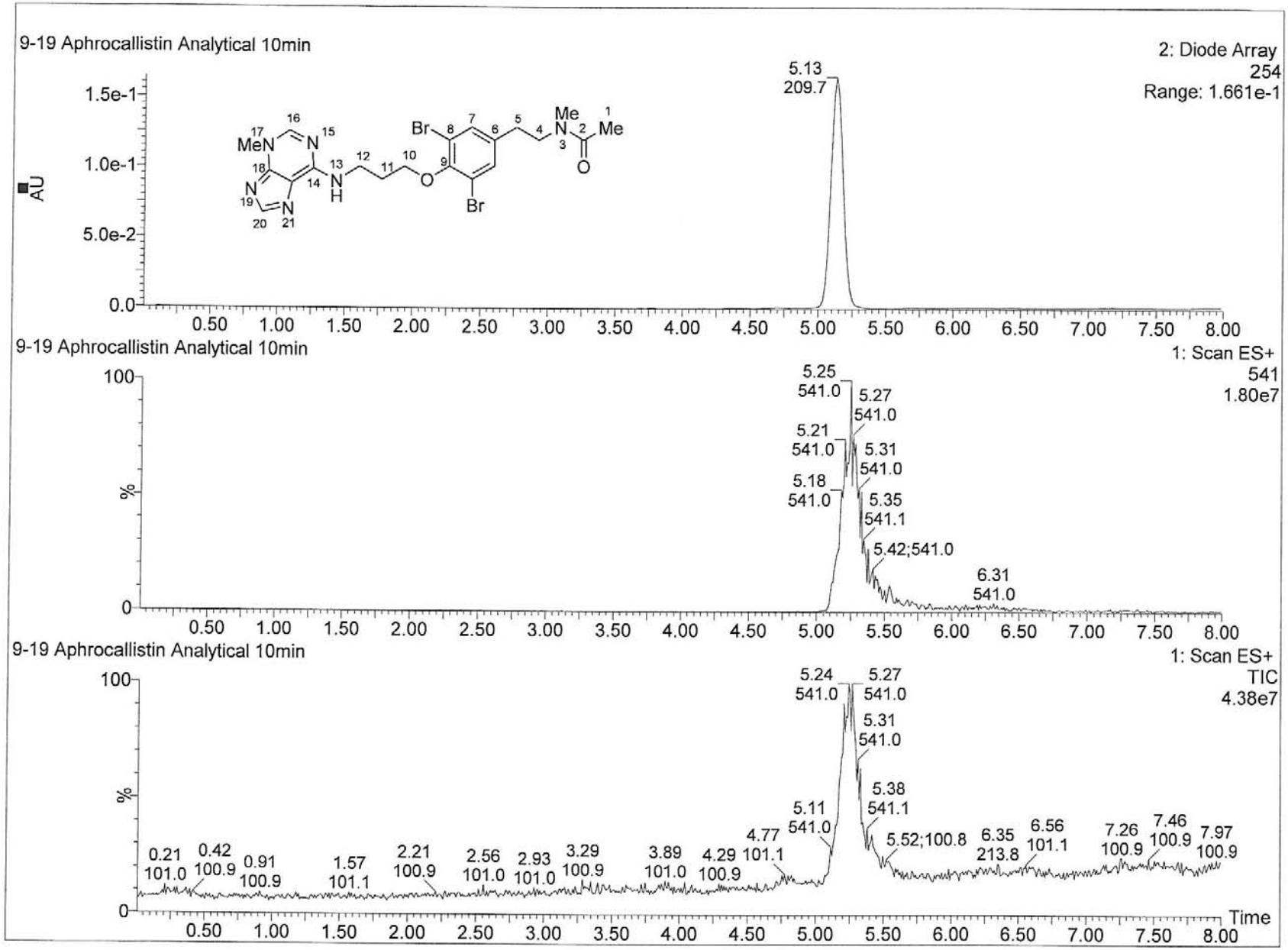
Aphrocallistin 9-19



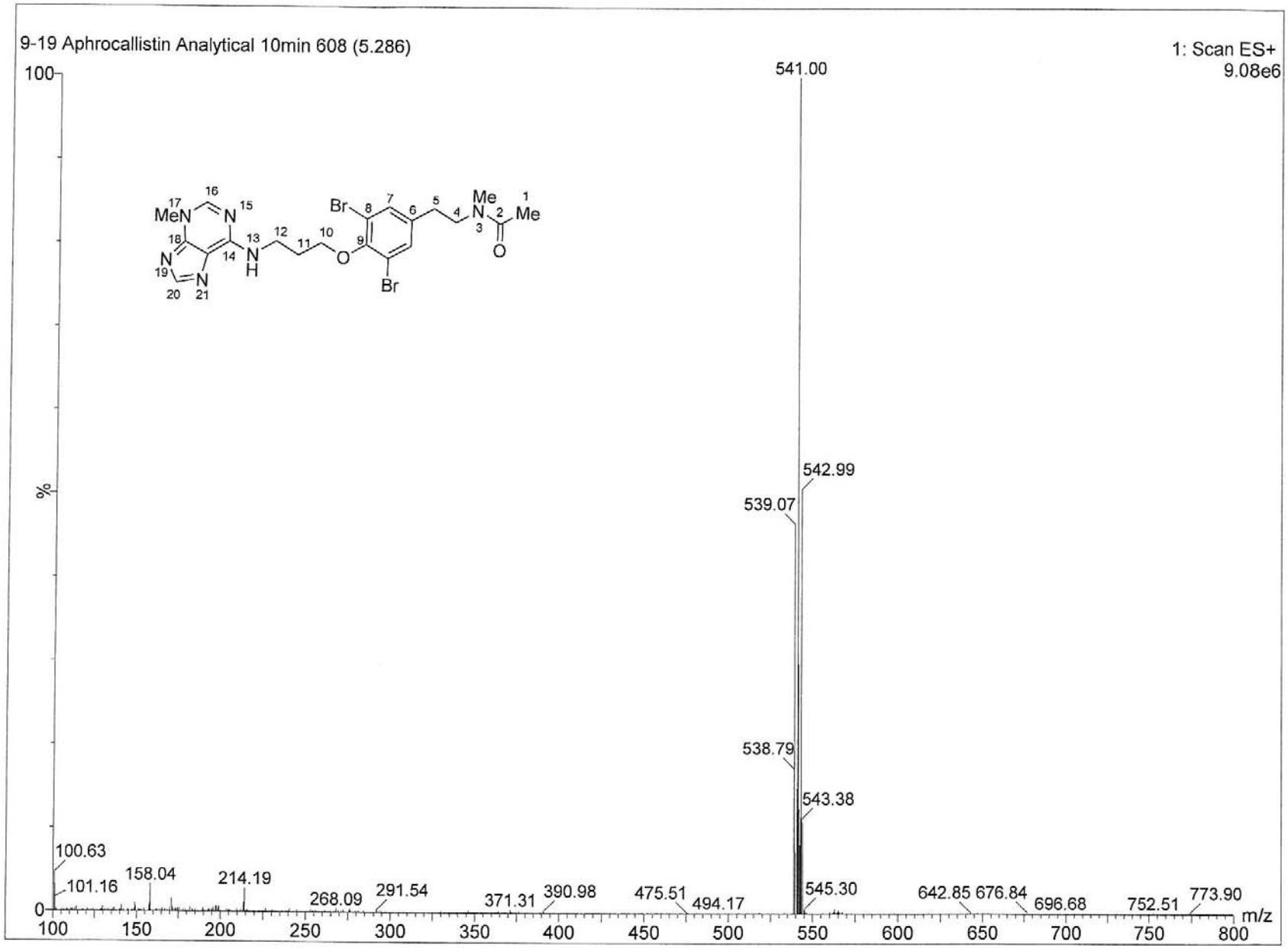


NAME	Sep19-2008-jkh	
EXPNO	10	
PROCNO	1	
Date_	20080919	
Time	14.01	
INSTRUM	spect	
PROBHD	5 mm QNP 1H/15	
PULPROG	zg30	
TD	65536	
SOLVENT	MeOD	
NS	64	
DS	2	
SWH	8278.146	Hz
FIDRES	0.126314	Hz
AQ	3.9584243	sec
RG	362	
DW	60.400	usec
DE	6.50	usec
TE	293.5	K
D1	1.00000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	1H	
P1	10.12	usec
PL1	-4.00	dB
SFO1	400.1324710	MHz
SI	32768	
SF	400.1300477	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

S15. LC-MS trace for Synthetic Aphrocallistin (1).



S15. LC-MS trace for Synthetic Aphrocallistin (1).



S 16. Details of Preparation of *N*-(4-Methoxyphenethyl)acetamide **(6)**

Preparation of *N*-(4-Methoxyphenethyl)acetamide (6). Acetyl chloride (3.52 mL, 49.6 mmol, 1.5 equiv) was added dropwise over a 30-min period to a solution of 2-(4-methoxyphenyl)-ethanamine (**5**) (5.00 g, 33.07 mmol) in pyridine (165 ml) at room temperature. The resulting mixture was stirred for 3 h and worked up following the standard procedure to obtain 5.97 g (93%) of the desired product as an off-white solid. The compound was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 5.53 (br s, 1H), 3.79 (s, 3H), 3.47 (q, *J* = 6.9 Hz, 2H), 2.75 (t, *J* = 6.9 Hz, 2H), 1.93 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 158.2, 130.8, 129.68 (two carbons), 114.1 (two carbons), 55.3, 40.9, 34.7, 23.3.