Supporting information for:

Metabolites from the induced expression of cryptic single operons found in the genome of *Burkholderia pseudomallei*.

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Operon cloning: Operons and genes of interest (Table S1) were PCR amplified from either *Burkholderia pseudomallei* K96243 and *Burkholderia thailandensis* E264 genomic DNA using Phusion High-Fidelity DNA polymerase (NEB; Cambridge, MA) and either an optimized FailSafe buffer (Epicentre; Madison, WI) or Phusion HF buffer. Forward and reverse primers were designed to contain XbaI and PmeI restriction sites, respectively (Table S2). PCR cycling conditions were as follows: 98°C for 30s, 30 cycles of 98°C for 30s; annealing for 30s (see Table S2 for annealing temperatures); 30s per kb extension at 72°C; 72°C, 10 min. Reactions that yielded a single amplicon of the correct predicted size were passed through a QIAquick PCR purification column (Qiagen). PCR reactions that yielded more than one PCR product were separated on a 1.0% agarose gel and the amplicon of the correct predicted size was recovered from gel (Qiagen, QIAquick Gel extraction kit). The purified amplicons were double digested with XbaI and PmeI. The resulting XbaI/PmeI cut amplicons were purified and ligated (Fast-link DNA ligation kit, Epicentre; Madison, WI) with pMMB67EXPH that had been digested with XbaI and PmeI. Ligation reactions were electroporated into *E. coli* EC100 (Epicentre; Madison, WI) and selected on LB/agar/ampicillin (50 μg-mL^{-1}). In each case the correct product was verified by end-sequencing and restriction mapping. The vector used here, pMMB67EXPH, was constructed from pMMB67EH (ATCC# 77289) by the following transformations: 1. digest with EcoRI and XbaI, blunt-end with End-It (Epicentre) and ligate to yield pMMB67EXH, 2. digest pMMB67EXH with SbfI, blunt-end with End-It (Epicentre), CIP treated and ligate to a PmeI linker (p-GGGTTTAAACCC) to yield pMMB67EXPH.

Table S1. Cloned putative biosynthetic operons

	Amplified Operon (B.P. equivalent)	Description of ORF (1)	Description of ORF (2)	Description of ORF (3)	Description of ORF (4)	Description of ORF (5)	Description of ORF (6)	Description of ORF (7)	Description of ORF (8)
	Chromosome I								
1	BPSL0212-0215[a] (BTH_I0174-I0178)	S-adenosylmethionine synthetase	hypothetical	phytanoyl-CoA dioxygenase	hypothetical	aldo/keto reductase family oxidoreductase			
2	BPSL1081-1085 (BTH_I0949-I0953)	polyphosphate kinase	drug/metabolite transporter	aminotransferase	L-PSP family endoribonuclease	phenazine biosynthesis protein PhzF			
3	BPSL1234-1237 (BTH_I1083-I1086)	acyl-CoA dehydrogenase	phosphotransferase	phosphoglycerate mutase	enoyl-CoA hydratase				
4	BPSL1488[b] (BTH_I2209-I2210)	carboxymuconolactone decarboxylase	lipoic acid synthetase						
5	BPSL1846-1849[b] (BTH_I2487-I2491)	Thioesterase family	Aldo/keto oxidoreductase	transporter	hypothetical	hypothetical			
6	BPSL2328-2329 (BTH_I1833-1834)	acyl-CoA dehydrogenase	CaiB/BaiF family CoA transferase						
	Chromosome II								
7	BPSS0023-0028[b] (BTH_II0025-0031)	acyl-CoA dehydrogenase	cytochrome P450-related protein	hypothetical	AP endonuclease	hypothetical	radical SAM domain/B12 binding domain-containing protein	SAM-dependent methyltransferases	
8[c]	BPSS0130-0133 (BTH_II0204-0207)	NRPS	Epimerase	Epoxide Hydrolase	Methyltransferase				
9	BPSS1258-1259	iron-containing alcohol dehydrogenase	oxidoreductase/ dehydrogenase						
10	BPSS1318-1321	methyltransferase	Amidophospho-ribosyltransferase	D-fructose-6-phosphate amidotransferase	D-lactate dehydrogenase				
11	BPSS2037-2044 (BTH_II 0274-0281)	Fatty acid desaturase	PKS	Mycolic acid synthase	Stearoyl-CoA desaturase	amidohydrolase	AMP-dependent synthetase	thioesterase	hydrolase
12[c]	BPSS2111-2113	monooxygenase	pseudogene	hypothetical					
13	BPSS2338-2340[b] (BTH_II2358- 2361)	phytoene synthase	hypothetical	squalene-hopene cyclase	hopanoid-associated phosphorylase				

[a] One gene in reverse direction with other genes in *B. pseudomallei* genome, while being a single operon in *B. thailandensis* genome
[b] One additional gene was included in the operon from *B. thailandensis* compared to corresponding operon in *B. pseudomallei*
[c] Generated clone-specific molecules in *P. aeruginosa*

Table S2. PCR cloning primers and buffers.

Operon	Forward primer (XbaI, TCTAGA)[a]	Reverse primer (PmeI, GTTTAAAC)	PCR Buffer[b]	Annealing temp.(°C)
1	GCGCTCTAGATGCCTCGCTCATTTCTTGTG	GCGCGTTTAAACGAATTCCGCCACAACGTG	J	60
2	GCGCTCTAGATGCGGCGCGGGAATCTGCGCAAG	GCGCGTTTAAACGCAGGATATCGCTAACCGGGGGAAG	G	67
3	GCGCTCTAGATGAACATGCCCAGCGCG	GCGCGTTTAAACCAGTGCGAATGAGACAAACG	J	60
4	GCGCTCTAGATGGAACCACGAATCGAGGAA	GCGCGTTTAAACGGCTCGCGTTCTATCTGC	HF	55
5	GCGCTCTAGATGAACCCGTCTTCAGGCAAG	GCGCGTTTAAACCTTCACGTCGCCATACTCG	J	60
6	GCGCTCTAGATGAACATGATGAAAGAAATCGTCG	GCGCGTTTAAACTCTACTTCGCCTCCTTCAGC	G	55
7	GCGCTCTAGATGCAAACAAGTGGTTTCCTCG	GCGCGTTTAAACTATAGCCCCACCAGATGGA	HF	55
8	GCGCTCTAGATGACCCATCCTCTGTTCA	GCGCGTTTAAACATACGGCGACGCACAGGTA	J	60
9	GCGCTCTAGATGCTGAATTTCGATTTCTACAACC	GCGCGTTTAAACGCGAACTTAGAGCGTGTCG	HF	55
10	GCGCTCTAGATGCGCATCGACAGAATCGG	GCGCGTTTAAACCGTCCAGAACTCAGTCGAA	G	55
11	GCGCTCTAGATGCAGCGCGAACGCGCA	GCGCGTTTAAACGTTTCGATACGAGGCAAGGA	J	60
12	GCGCTCTAGATGAAAAAAATCGTCGTAGTCGG	GCGCGTTTAAACGTCCTTTCGGTCAGTTGTT	HF	55
13	GCGCTCTAGATGTTTTGGAGTGGAGTTCGCCTTC	GCGCGTTTAAACGAAACGGGACCGGCAATG	G	60

[a] Terminus of XbaI site (TCTAGATG…) contains transcription initiating *ATG* codon.
[b] G, J: Epicentre FailSafe buffer mix; HF: 5X NEB Phusion HF buffer

Mating expression constructs into *Pseudomonas aeruginosa*: Expression constructs were transformed into *E. coli* S17.1 and mated into *Pseudomonas aeruginosa* (PA14 or PAK5) using the following bi-parental conjugation protocol. Equal volumes of overnight cultures of *E. coli* S17.1 and *P. aeruginosa* were mixed (50 μl each), spotted upon LB/agar plate, allowed to air dry and then incubated 6h at 30 °C. The resulting mixed-colonies were scraped from the agar plates, distributed onto LB/agar/irgasan (25 $\mu g\text{-}mL^{-1}$)/carbenicillin (200 $\mu g\text{-}mL^{-1}$) plates and incubated at 30 °C. Exconjugates typically appeared after 24-48 hours.

Screening for clone-specific molecules: Representative exconjugates transformed with each expression construct were inoculated into 2 ml LB/carbenicillin (200 $\mu g\text{-}mL^{-1}$) and grown at 30 °C for 12-18 h with shaking (200 rpm). 0.5 mL of this culture broth (1/100 dilution) was used to inoculate 50 mL cultures of LB/carbenicillin and these cultures were shaken at 30 °C until they reached an OD_{600} of 0.1-0.2. IPTG was then added to reach a final concentration of 0.1 mM. After an additional 36 h at 30°C (250 rpm) cultures were extracted with an equal volume of ethyl acetate. Ethyl acetate was collected and dried *in vacuo* after phase separation by centrifugation (4,000 x*g*, 20 min). Extracts were eventually resuspended in 1 μL of methanol per mL of starting culture broth and examined by normal phase (90:10 $CHCl_3/CH_3OH$) TLC. Extracts that appeared to contain metabolites that were not in extracts from vector control cultures were further analyzed by reversed-phase HPLC-MS (linear gradient from 20:80 $CH_3OH:H_2O$ with 0.1% formic acid to 100% CH_3OH with 0.1% formic acid over 20 min; 1.5 $mL\text{-}min^{-1}$; Waters XBridge C18, 5 μm, 4.6 x 150 mm). Six-liter batches of cultures grown under the same conditions used in our initial screen for molecule production were used to generate extracts for molecule isolation and structure determination.

Bioassay: Overnight cultures of *Sacchromyces cereviseae* W303, *Bacillus subtilis* BR151, *Staphylococcus aureus* ATCC 6538 P, and *Escherichia coli* strains EC100, BAS849 and DRC639 were diluted 1:10,000 in LB (*S. cereviseae* was grown in YPD). Individual wells of a sterile 96-well plate were filled with 100 μL of these dilute cultures. 15 μL of a methanol stock solution containing a pure compound was added to the first well in each row and then serially diluted in three-fold increments to yield concentrations from 0.4 to 100 $\mu g\text{-}mL^{-1}$. Plates were incubated overnight at 30°C. Identical concentrations were screened against HeLa carcinoma cells as follows: cells were plated at 1000 cells per well overnight, compound added Day 2, and incubated 60 hours at 37°C, 5% CO_2. DMEM medium plus 10% fetal bovine serum used. Following treatment, wells were aspirated, washed with PBS, fixed with 1% paraformaldehyde, and stained with 0.1% crystal violet. Relative cell-count was quantified by extracting stained crystal violet from individual wells with 10% acetic acid (100 μL) and measuring at 590 nm via UV/Vis plate reader.

Phosphodiesterase Assay: Phosphodiesterase (PDE) screening was provided by BPS Biosciences (San Diego, CA). The assay, as per protocol of PDE assay kit (Cat # 60300-60400), is based on the

binding of a fluorescent nucleotide monophosphate (FAM-cAMP) generated by PDEs to the binding agent, then screened by fluorescence polarization. Assay conditions: test compounds were prepared with 100% DMSO in assay buffer and 0.5 µL of the dilution was added to a 50 µL reaction so that the final concentration of DMSO is 1% in all of reactions. The enzymatic reactions were conducted at room temperature for 60 minutes (180 minutes for PDE6C) in a 50 µL mixture containing PDE assay buffer, 100nM FAM-cAMP, a PDE and the test compound. After the enzymatic reaction, 100 µL of a binding solution (1:100 dilution of the binding agent with the binding agent diluent) was added to each reaction and the reaction was performed at room temperature for 60 minutes. Fluorescence intensity was measured at an excitation of 485 nm and an emission of 528 nm using a Tecan Infinite M1000 microplate reader. PDE activity assays were performed in duplicate at each concentration. Data analysis: fluorescence intensity is converted to fluorescence polarization using the Tecan Magellan6 software. The fluorescence polarization data were analyzed using the computer software, Graphpad Prism. The fluorescence polarization (FP_t) in absence of the compound in each data set was defined as 100% activity. In the absence of PDE and the compound, the value of fluorescent polarization (FP_b) in each data set was defined as 0% activity. The percent activity in the presence of the compound was calculated according to the following equation: % activity = $(FP-FP_b)/(FP_t-FP_b)$ x 100%, where FP = the fluorescence polarization in the presence of the compound.

Gene Deletions: Non-ribosomal peptide synthase-encoding gene BTH-II0204 from *B. thailandensis* E264 was deleted from the genome following protocol detailed by Thongdee *et al*. (ref. 14). Briefly, a two stage PCR was performed to incorporate a tetracycline-resistance cassette, amplified from plasmid pJWC1 (Craig, J. W.; Chang, F. Y.; Brady, S. F. *ACS Chem Biol* **2009**, *4*, 23-8.), flanked by regions of genomic DNA adjacent to BTH-II0204. The final PCR construct (100 ng) was then incubated with *B. thailandensis* E264 in Medium DM for 48 hours (2mL, 250 rpm, 37°C) and selected upon TSB/agar/tetracycline (50 µg-mL^{-1}). A colony chosen with successful gene deletion, herein designated as strain *B. thailandensis* E264(ΔII0204), was verified by PCR amplification as described (ref. 14). Operon BTH-II0204-207 was rendered functionally inactive, verified by elimination of *in vivo* monomethylated terphenyl compound production, illustrated in Figure S1. Primers for gene deletion protocol listed in Table S4.

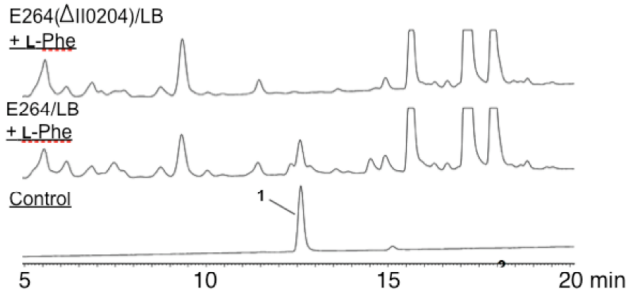
Table S3. Gene deletion PCR primers

BTH-0204 deletion

1F[a]	CCAGTTCGTCGCCCGGTGCGATTCGATATGAAATC GAGCATTTCGGATCG*TCAGCGATCGGCTCGTTGCC*[b]
1R	GACACTGCCATGAGCCTCTTCTCCTTGTTCATCGTG AAGTCGGATATCAG*TCAATCGTCACCCTTTCTCGGTC*
2F	CGGCGGATTCTTTCATGAAG
2R	CGATCCGAAATGCTCGATTTC
3F	CTGATATCCGACTTCACGATG
3R	GACGTATTGCAGGCAATCCTG
4F	ACGATCGGCTCTGGCTCTAT
4R	GCGATCTATGCGGCTTCTAT
5F	GGTGATTGTCGATGGATTGG
5R	TCGTGTTGTCGAACGCATAG

[a] Primer nomenclature detailed in Thongdee *et al*. (ref. 14)
[b] Underlined region corresponds to tetracycline resistance cassette primer

Figure S1. HPLC trace comparison of wild-type *B. thailandensis* E264 (middle) and non-ribosomal peptide synthase deletion *B. thailandensis* E264(ΔII0204) strains (top), wherein monomethylated terphenyl biosynthesis is eliminated with NRPS deletion. Compound **1** control trace is on bottom.

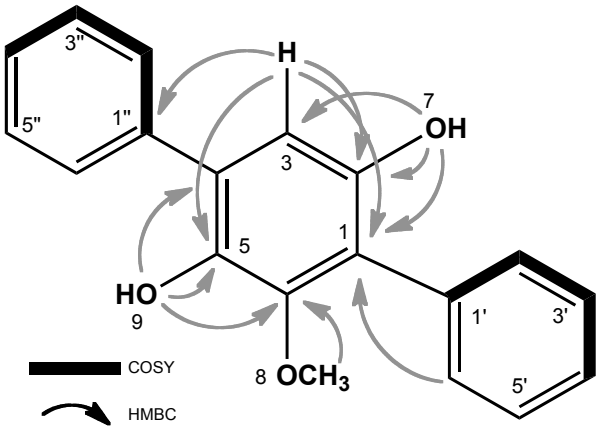


Operon BTH-II0204-207/BPSS0130-133. Crude ethyl acetate extracts from cultures of *P. aeruginosa* (PA14) transformed with pMMB67EXPH:BTH-II0204-207 were initially partitioned using a modified Kupchan Scheme. The extract was resuspended in 100 ml of 90:10 CH_3OH:H_2O and then extracted 4 times with 100 ml of hexanes. The remaining methanolic mixture was diluted to 60:40 CH_3OH:H_2O and extracted 2 times with 100 ml CH_2Cl_2. Each fraction (hexanes, CH_2Cl_2 and CH_3OH:H_2O) was dried *in vacuo* and analyzed by reversed phase HPLC-MS. Compounds **3** and **4** were found in the hexanes fraction (3.5 mg-L^{-1}) while compounds **1** and **2** were found in the CH_2Cl_2 fraction (6.0 mg-L^{-1}). The major metabolites **1** and **3** appear in these extracts at approximately 6- and 20-fold higher concentrations than the minor compounds **2** and **4**, respectively. The hexanes and CH_2Cl_2 fractions were each partitioned by silica gel flash chromatography using hexanes:ethyl acetate step gradients. Compounds **3** and **4** co-eluted with 90:10 hexanes:ethyl acetate and compounds **1** and **2** co-eluted with 80:20 hexanes:ethyl acetate. Compounds **3** and **4** were separated by preparative reversed-phase HPLC using 52:48 CH_3OH:H_2O (Waters XBridge, C18, 5 μm, 10 x 150 mm; isocratic 52:48 CH_3OH:H_2O with 0.1% formic acid; 7 mL-min^{-1}). Under these conditions compounds **3** and **4** eluted at 50 and 52 min, respectively. Compounds **1** and **2** were separated by preparative reversed-phase HPLC using 28:72 CH_3CN:H_2O (Waters XBridge, C18, 5 mm, 10 x 250 mm; isocratic 28:72 CH_3CN:H_2O with 0.1% acetic acid; 5 mL-min^{-1}). Under these conditions compounds **1** and **2** eluted at 117 and 122 min, respectively. X-ray diffraction analysis of single crystals grown from compounds **3** and **4** revealed them to be dimethylated terphenyls that differ in methyl substitution patterns. A comparison of the ^{1}H and ^{13}C spectra for compounds **3** and **4** with those for compounds **1** and **2** indicated **1** and **2** are also terphenyl structures but they each only contain one methyl substituent. Extensive HMBC correlations shown below allowed us to define the position of the methoxy substituents in each metabolite.

Table S4**: BTH-II0204-207:A** (**1**) NMR assignment table, in CD_3COCD_3

C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
1			122.9	
2			148.2	
3	6.81	s	112.9	C1, C2, C5, C1"
4			128.7	
5			141.4	
6			147.0	
7 (OH)	7.65	s		C1, C2, C3
8 (OCH_3)	3.37	s	60.7	C6
9 (OH)	7.37	s		C4, C5, C6
1'			135.2	
2'/6'	7.55	d (7.5)	131.7	C1, C2'/C6', C4'
3'/5'	7.47	t (7.5)	128.8	C1', C3'/C5'
4'	7.38	t (7.5)	127.7	C2'/C6"
1"			139.5	
2"/6"	7.70	d (7.5)	130.1	C2"/C6", C4"
3"/5"	7.45	t (7.5)	128.9	C1", C3"/C5"
4"	7.35	t (7.5)	127.8	C2"/C6"

HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{16}O_3$, 293.1178; found 293.1182.



BTH-II0204-207:A (**1**) ^{1}H NMR ; 600MHz in CD_3COCD_3

BTH-II0204-207:A (**1**) ^{13}C NMR; 150 MHz in CD_3COCD_3

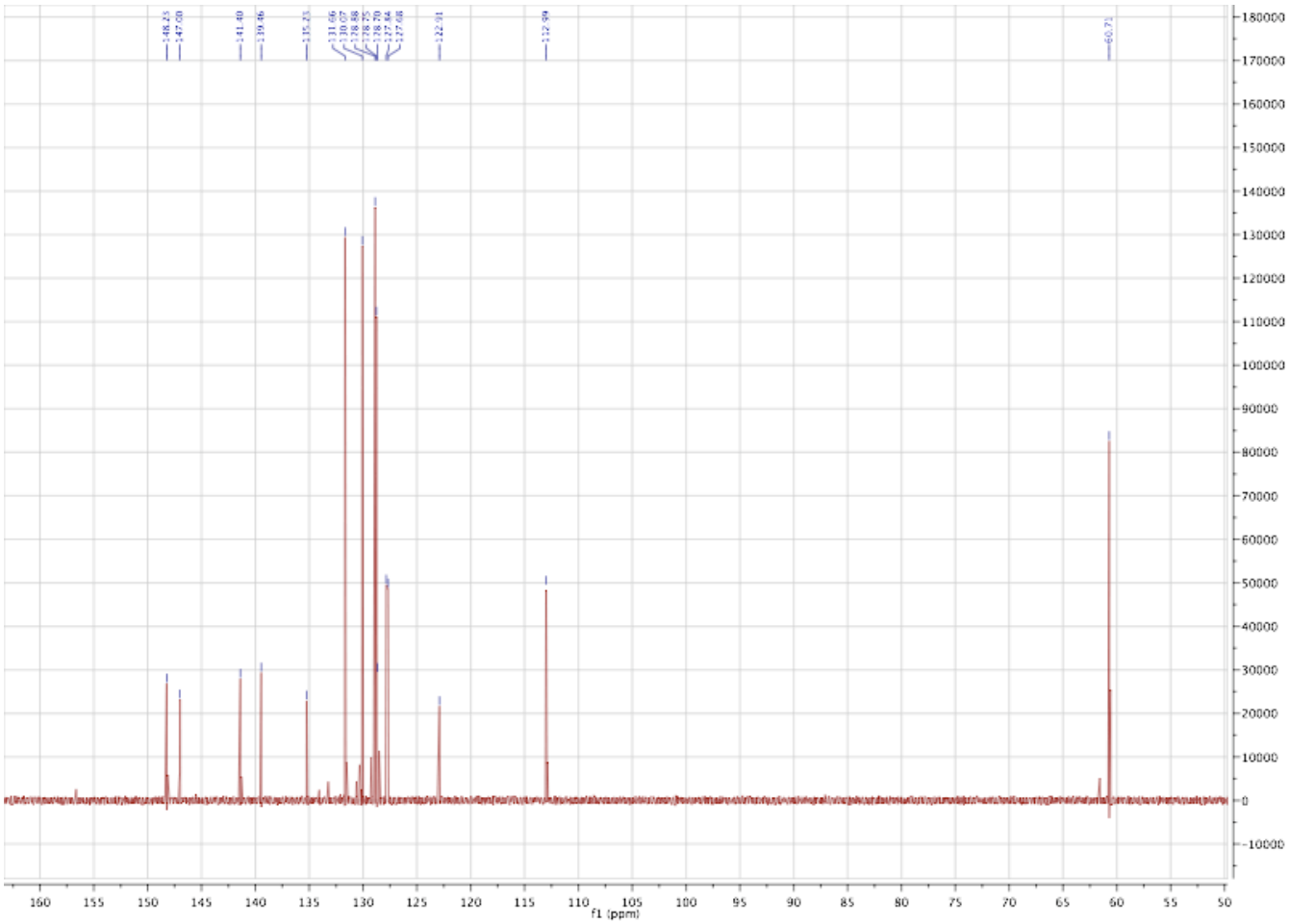
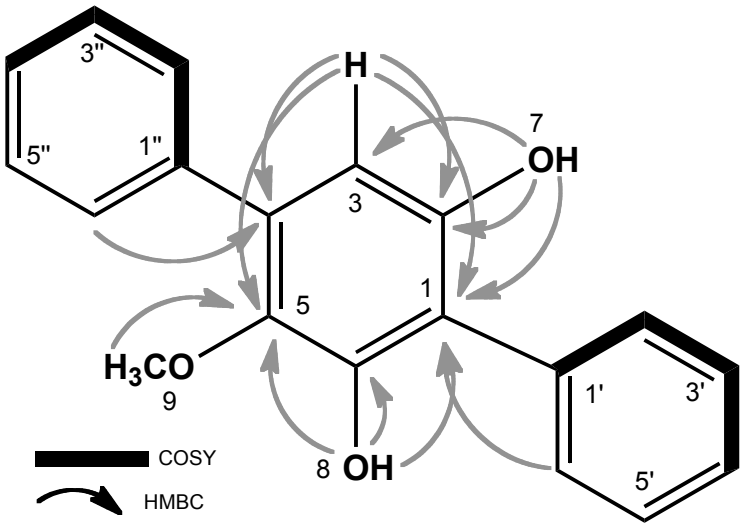


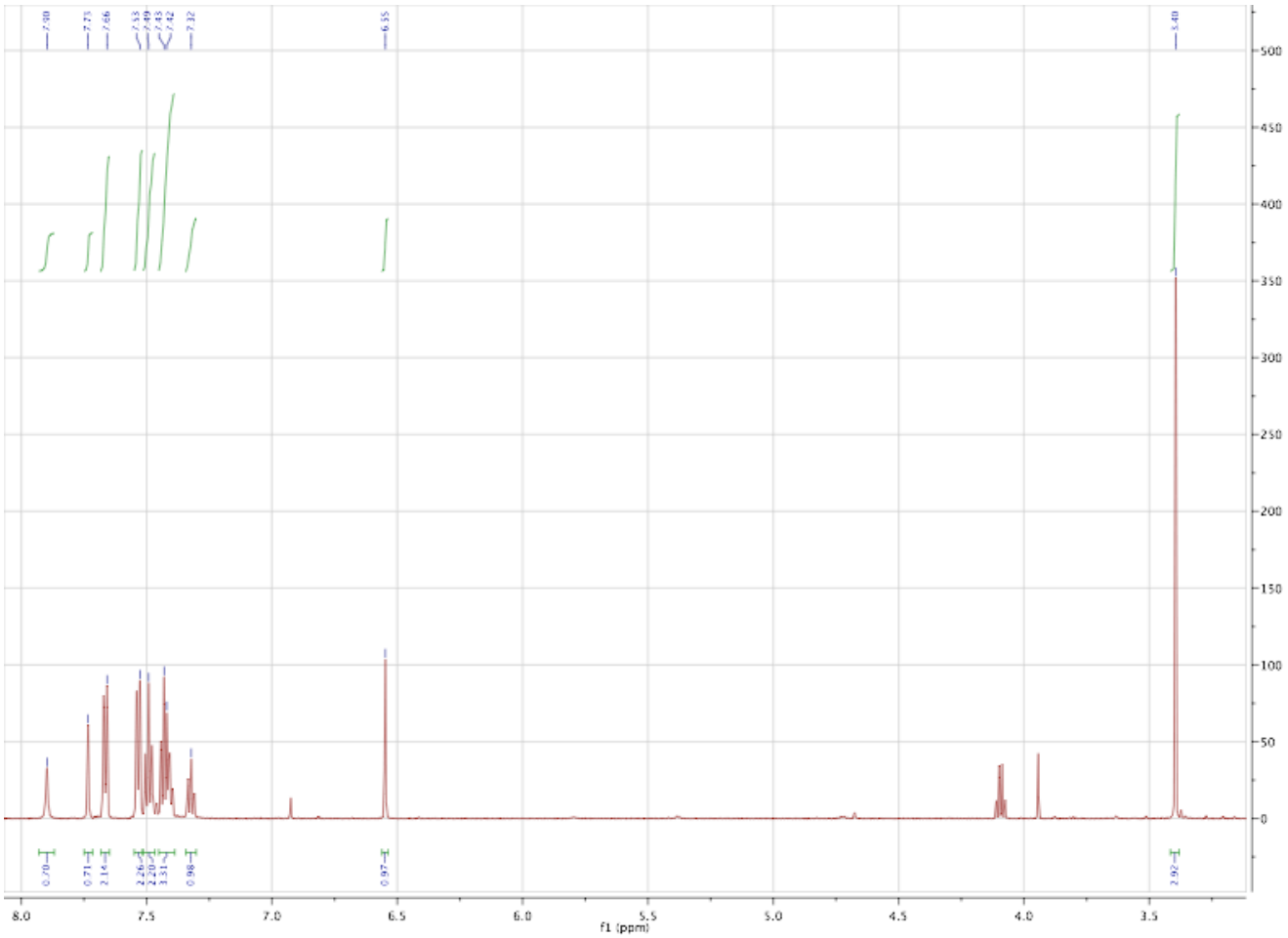
Table S5: **BTH-II0204-207:B** (**2**) NMR assignment table, in CD_3COCD_3

C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
1			117.1	
2			152.0	
3	6.55	s	108.3	C1, C2, C4, C5
4			134.4	
5			139.3	
6			149.3	
7 (OH)	7.90	s		C1, C2, C3
8 (OH)	7.73	s	61.0	C1, C5, C6
9 (OCH_3)	3.40	s		C5
1'			135.3	
2'/6'	7.53	d (7.5)	132.0	C1, C2'/C6', C4'
3'/5'	7.43	t (7.5)	128.5	C1', C3'/C5'
4'	7.32	t (7.5)	127.5	C2'/C6"
1"			139.3	
2"/6"	7.66	d (7.5)	129.6	C4, C1"/C6", C4"
3"/5"	7.49	t (7.5)	129.3	C1", C3"/C5"
4"	7.42	t (7.5)	128.2	C2"/C6"

HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{16}O_3$, 293.1178; found 293.1180.



BTH-II0204-207:B (**2**) ^{1}H NMR ; 600MHz in CD_3COCD_3



BTH-II0204-207:B (**2**) ^{13}C NMR ; 150 MHz in CD_3COCD_3

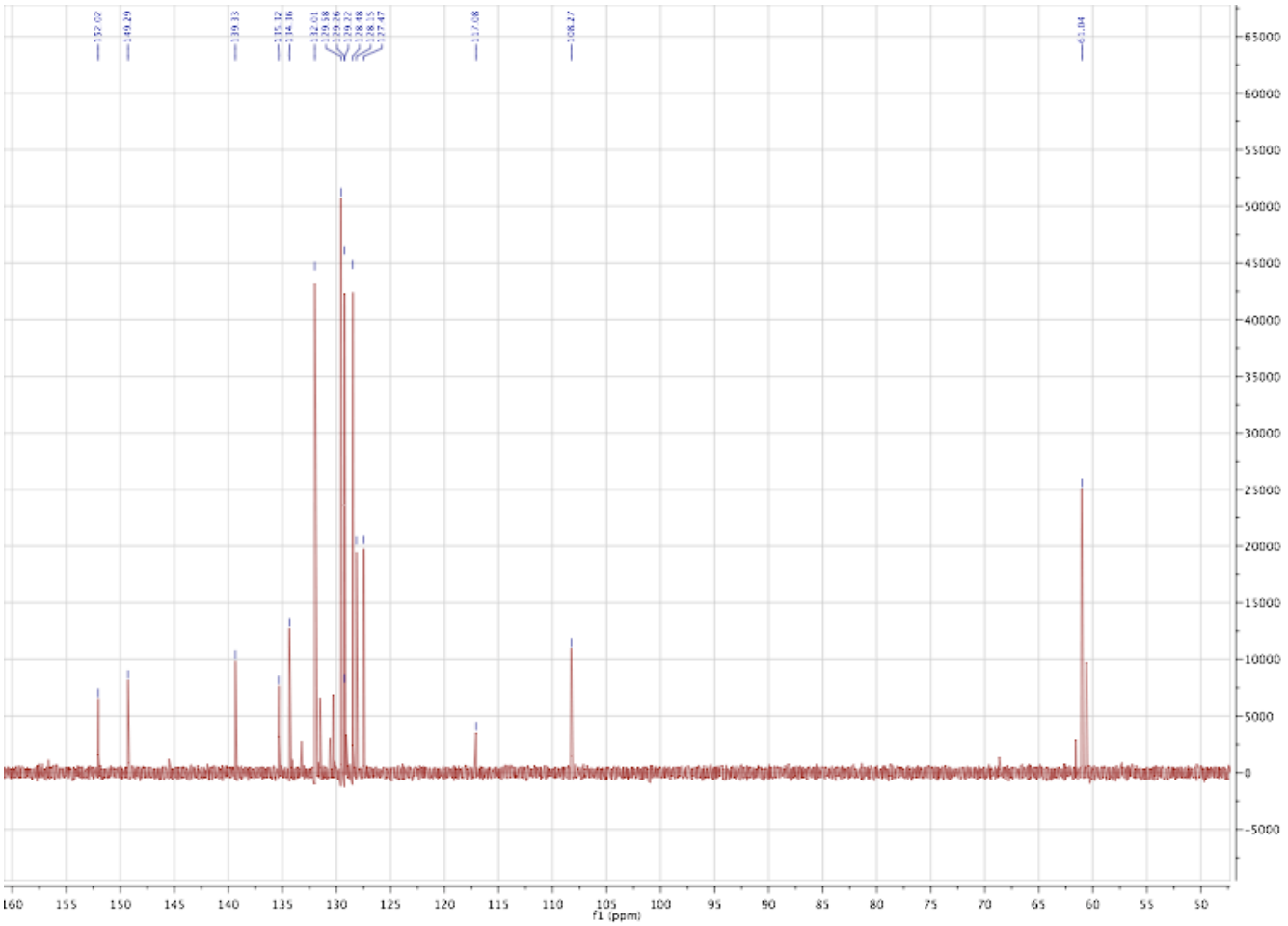
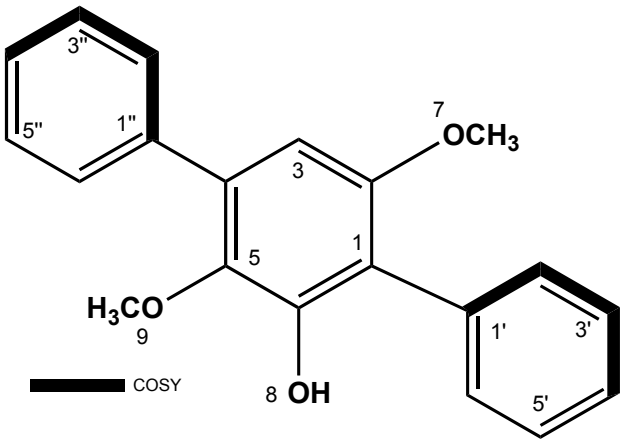


Table S6: **BTH-II0204-207:C** (**3**) NMR assignment table, in CD_3COCD_3

C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
1			118.5	
2			154.6	
3	6.56	s	104.5	C1, C2, C4, C5
4			134.2	
5			140.4	
6			149.2	
7 (OCH_3)	3.74	s	56.3	C2
8 (OH)	7.75	s		C1, C5
9 (OCH_3)	3.37	s	61.1	C5
1'			135.3	
2'/6'	7.42	d (7.5)	132.0	C1, C2'/C6', C4'
3'/5'	7.38	m	128.3	C1', C3'/C5'
4'	7.28	t (7.5)	127.4	C2'/C6'
1"			139.5	
2"/6"	7.68	d (7.5)	129.7	C4, C2"/C6", C4"
3"/5"	7.47	t (7.5)	129.3	C1", C3"/C5"
4"	7.39	m	128.4	C2"/C6"

HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{18}O_3$, 307.1335; found 307.1325.



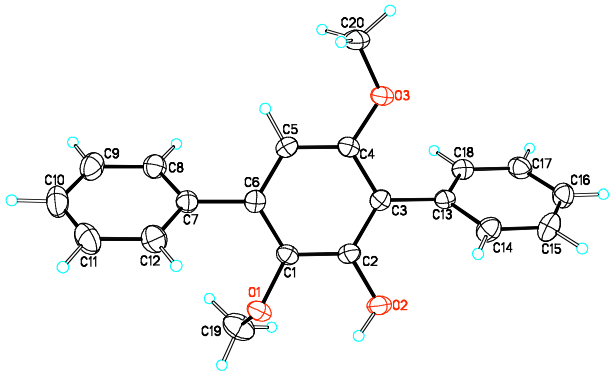
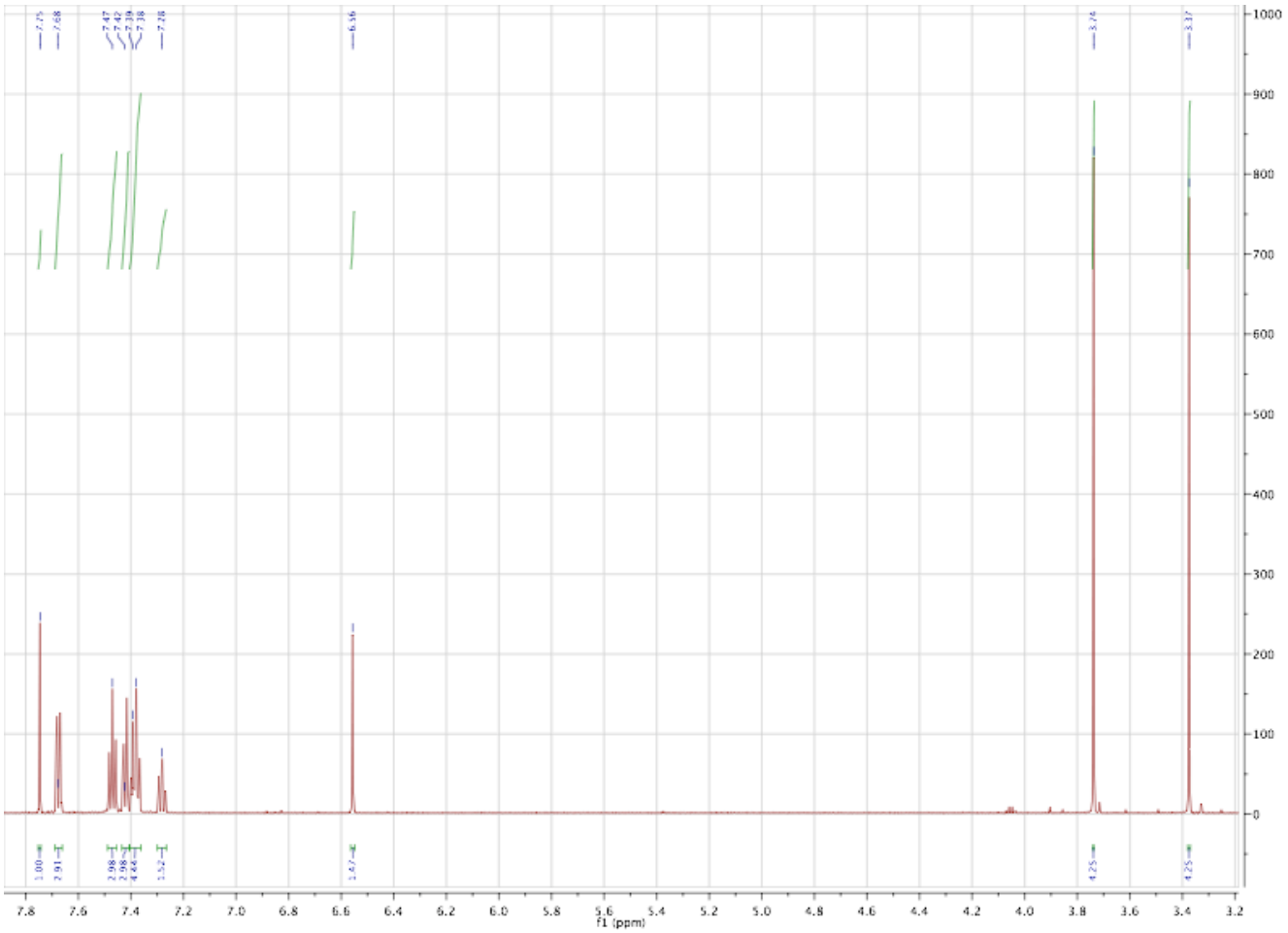


Table S7. Crystal data and structure refinement for BTH-II0204-207:C (**3**).

Identification code	sean8	
Empirical formula	C20 H18 O3	
Formula weight	306.34	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 10.6588(8) Å	α= 90°.
	b = 20.2223(14) Å	β= 90.946(3)°.
	c = 7.4519(6) Å	γ = 90°.
Volume	1606.0(2) $Å^3$	
Z	4	
Density (calculated)	1.267 Mg/m^3	
Absorption coefficient	0.084 mm^{-1}	
F(000)	648	
Crystal size	0.20 x 0.15 x 0.05 mm^3	
Theta range for data collection	2.16 to 27.86°.	
Index ranges	-8<=h<=13, -26<=k<=26, -9<=l<=9	
Reflections collected	14907	
Independent reflections	3790 [R(int) = 0.0323]	
Completeness to theta = 27.86°	99.4 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9958 and 0.9833	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3790 / 0 / 280	
Goodness-of-fit on F^2	1.009	
Final R indices [I>2sigma(I)]	R1 = 0.0374, wR2 = 0.0903	
R indices (all data)	R1 = 0.0604, wR2 = 0.1043	
Largest diff. peak and hole	0.272 and -0.198 $e.Å^{-3}$	

BTH-II0204-207:C (**3**) ^{1}H NMR ; 600MHz in CD_3COCD_3



BTH-II0204-207:C (**3**) ^{13}C NMR ; 150 MHz in CD_3COCD_3

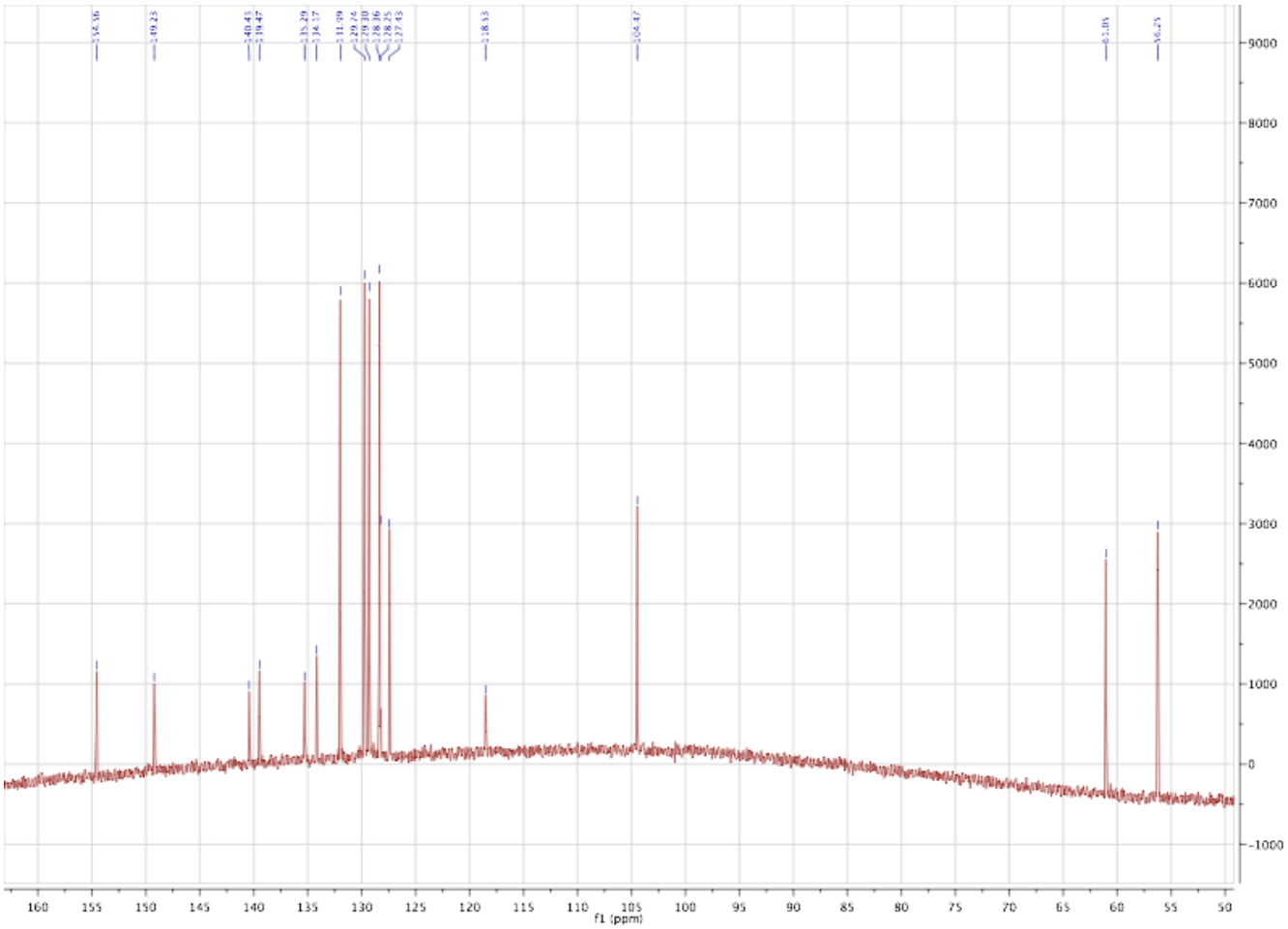
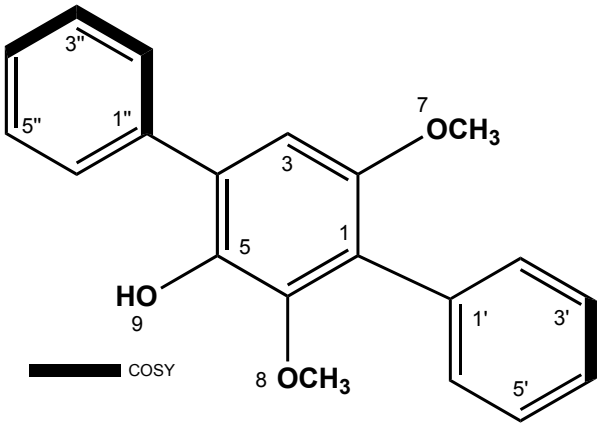


Table S8: **BTH-II0204-207:D** (**4**) NMR assignment table, in CD_3COCD_3

C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
1			124.7	
2			151.0	
3	6.83	s	109.6	C1, C2, C5, C6, C1”
4			128.3	
5			142.4	
6			147.1	
7 (OCH_3)	3.72	s	56.7	C2
8 (OCH_3)	3.33	s	60.8	C6
9 (OH)	7.49	s		C4, C5, C6
1'			135.1	
2'/6'	7.44	m	131.7	C1, C2'/C6'
3'/5'	7.43	m	128.9	C1’
4'	7.35	t (7.5)	127.8	C2’/C6’
1"			139.6	
2"/6"	7.71	d (7.5)	130.2	C4, C2"/C6", C4"
3"/5"	7.43	m	128.7	C1”
4"	7.33	t (7.5)	127.9	C2”/C6”

HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{18}O_3$, 307.1335; found 307.1317.



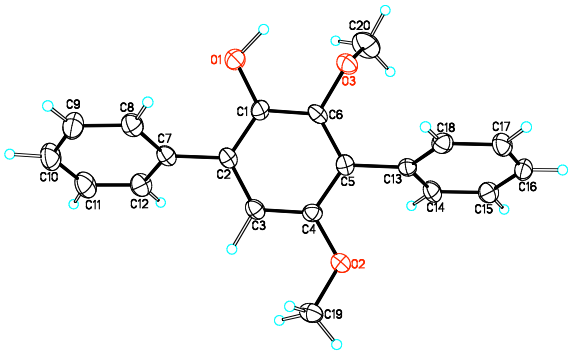
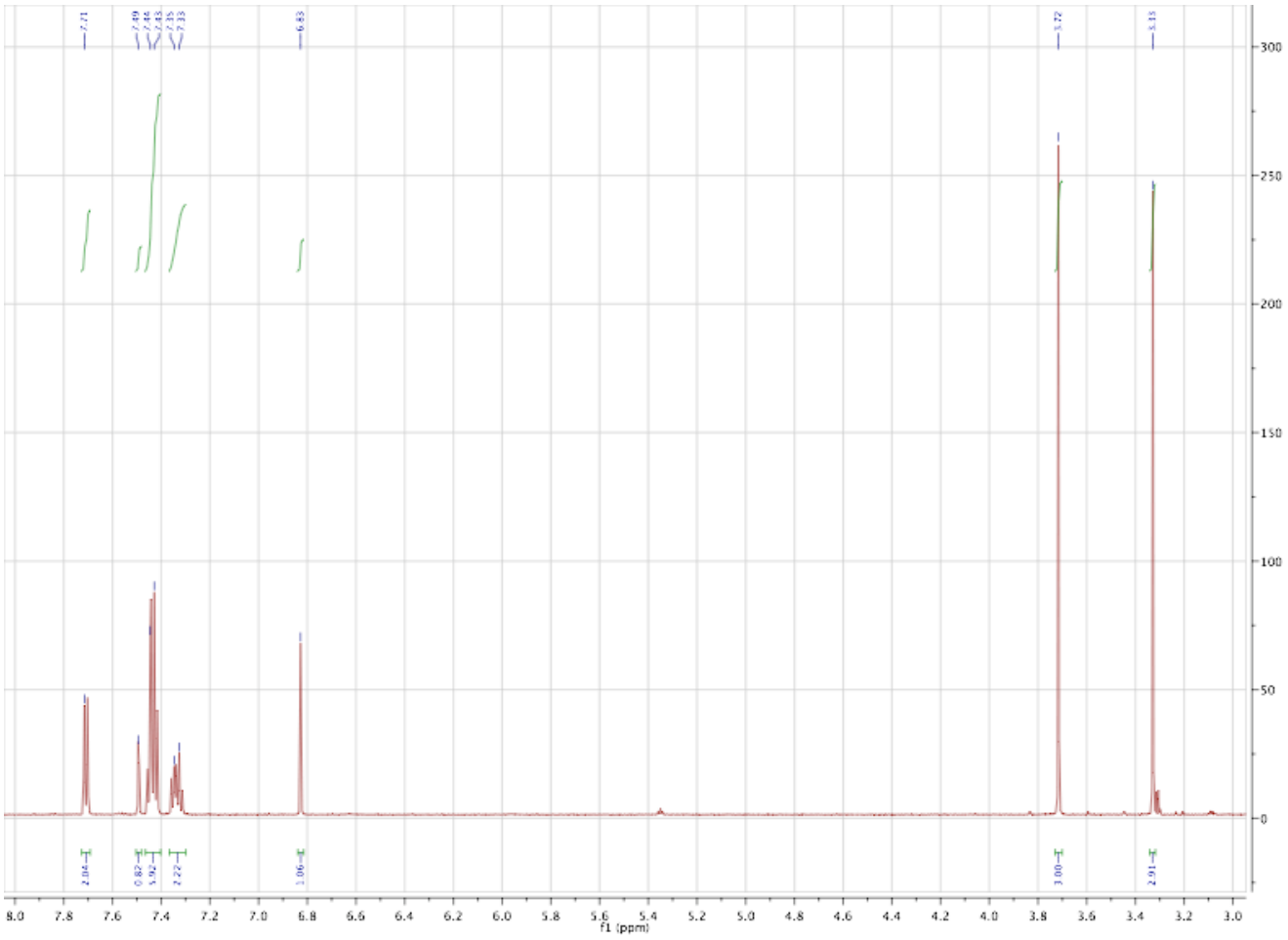


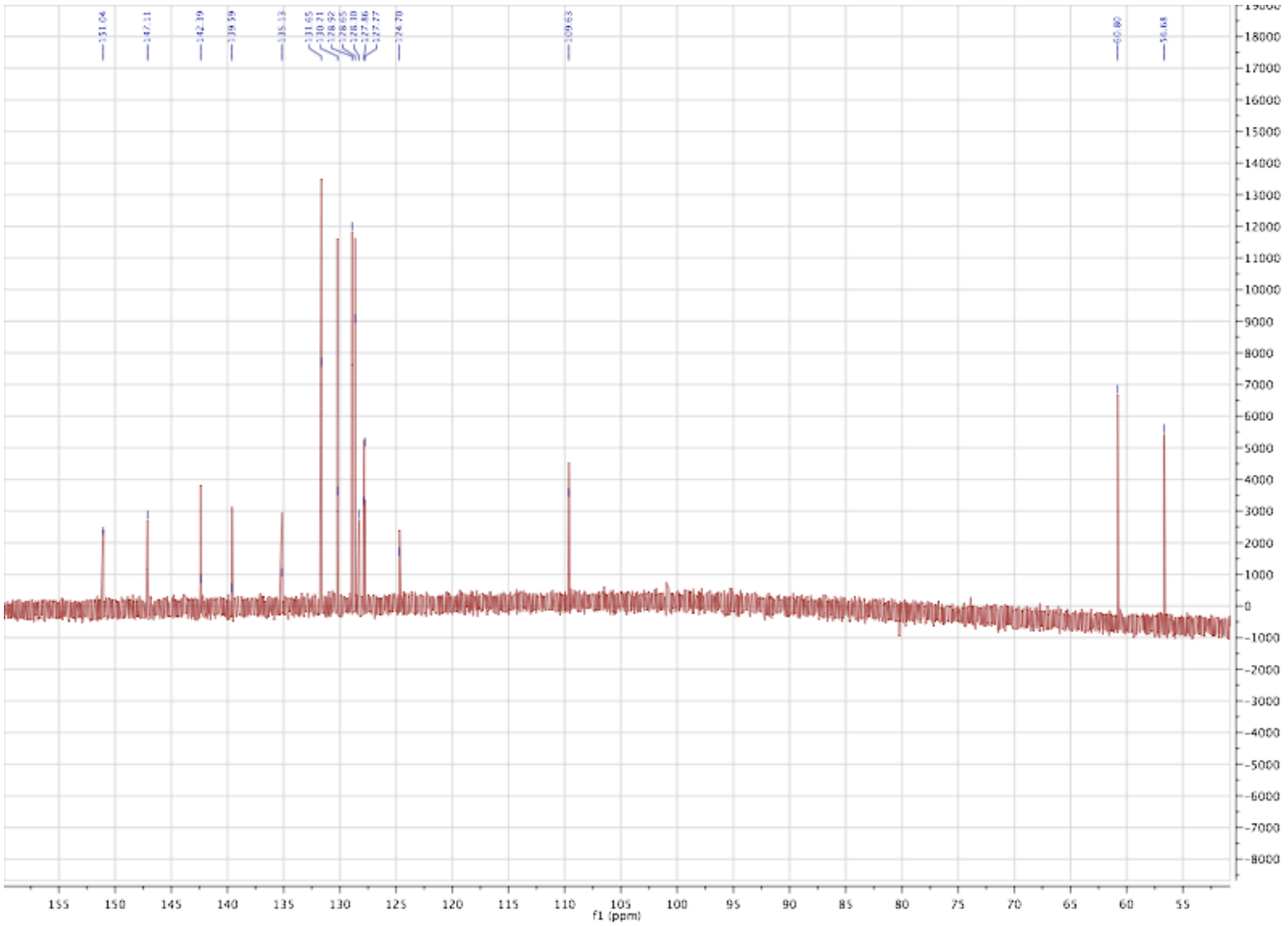
Table S9. Crystal data and structure refinement for BTH-II0204-207:D (**4**).

Identification code	sean10	
Empirical formula	C20 H18 O3	
Formula weight	306.34	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	Pbcn	
Unit cell dimensions	a = 18.1916(15) Å	α= 90°.
	b = 8.3327(7) Å	β= 90°.
	c = 20.9582(17) Å	γ = 90°.
Volume	3177.0(5) $Å^3$	
Z	8	
Density (calculated)	1.281 Mg/m^3	
Absorption coefficient	0.085 mm^{-1}	
F(000)	1296	
Crystal size	0.60 x 0.10 x 0.10 mm^3	
Theta range for data collection	1.94 to 23.26°.	
Index ranges	-20<=h<=19, -9<=k<=8, -14<=l<=23	
Reflections collected	9484	
Independent reflections	2286 [R(int) = 0.0439]	
Completeness to theta = 23.26°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9915 and 0.9506	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2286 / 0 / 257	
Goodness-of-fit on F^2	1.055	
Final R indices [I>2sigma(I)]	R1 = 0.0520, wR2 = 0.1281	
R indices (all data)	R1 = 0.0853, wR2 = 0.1499	
Largest diff. peak and hole	0.247 and -0.200 $e.Å^{-3}$	

BTH-II0204-207:D (**4**) ^{1}H NMR ; 600MHz in CD_3COCD_3



BTH-II0204-207:D (**4**) ^{13}C NMR ; 150 MHz in CD_3COCD_3



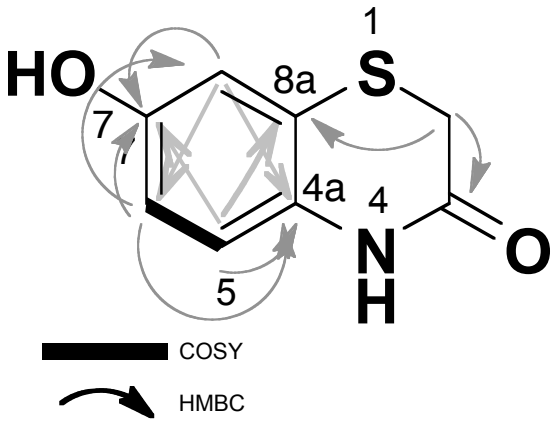
Operon BPSS2111-2113. Crude ethyl acetate extracts from cultures of *P. aeruginosa* (PAK5) transformed with pMMB67EXPH:BPSS2111-2113 were partitioned by silica gel flash chromatography using a $CHCl_3$:CH_3OH step-gradient (100:0, 99:1, 97:3, 95:5, 90:10, 80:20, and 0:100). Compounds **5**, **6** and **7** eluted with 97:3, 100:0 and 100:0 $CHCl_3$:CH_3OH, respectively. Preparative reversed HPLC yielded pure **5** (0.6 mg-L^{-1}), **6** (0.4 mg-L^{-1}) and **7** (0.3 mg-L^{-1}) (Waters XBridge, C18, 5 μm, 10 x 150 mm; gradient 20:80 CH_3OH:H_2O with 0.1% formic acid to 100% CH_3OH with 0.1% formic acid over 25 min; 7 mL-min^{-1}; retention times: **5**, 6.27 min; **6**, 12.25 min; **7**, 14.9 min).

The structure of compound **7** was solved by X-ray crystallography. The X-ray structure of **7** showed it to be a tri-methylthio substituted bicyclic structure. HRMS data together with 1 and 2D NMR indicates that compounds **5** and **6** resemble **7,** expect **5** contains no methylthio substituents and **6** contains only two methylthio substituents. The positions of the two methylthio substituents found in compound **6** were defined by HMBC correlations from the remaining two methyl groups, the lone aromatic proton singlet and the phenolic proton to aromatic carbons in the structure.

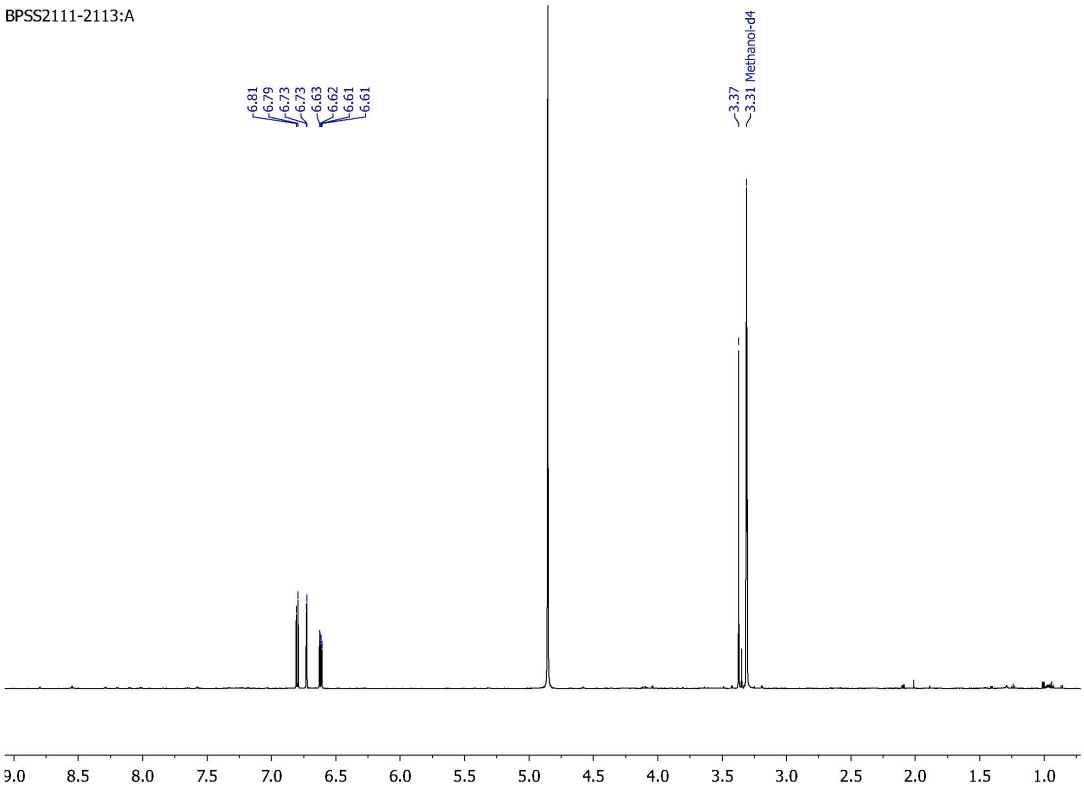
Table S10: **BPSS2111-2113:A** (**5**) NMR assignment table, in CD_3OD

C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
2	3.37	s	29.3	C3, C8a
3			166.4	
4a			129.4	
5	6.80	d (8.6)	118.2	C4a, C7, C8a
6	6.62	dd (8.6, 2.7)	113.9	C4a, C7, C8
7			153.5	
8	6.73	d (2.7)	113.3	C4a, C6, C7
8a			121.2	
OH	nd			
NH	nd			

HRMS-TOF (*m/z*): $[M - H]^-$ calcd for $C_8H_7NO_2S$, 180.0118; found 180.0116.



BPSS2111-2113:A (**5**) ^{1}H NMR ; 600MHz in CD_3OD



BPSS2111-2113:A (**5**) ^{13}C NMR ; 150 MHz in CD_3OD

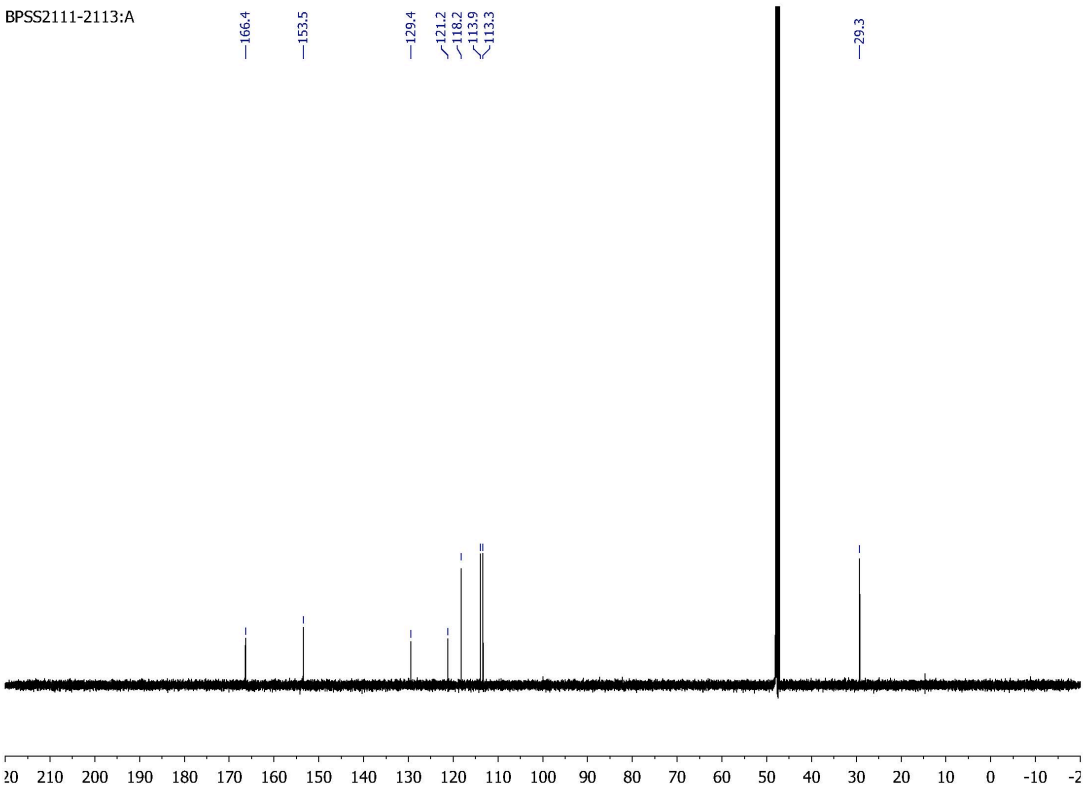
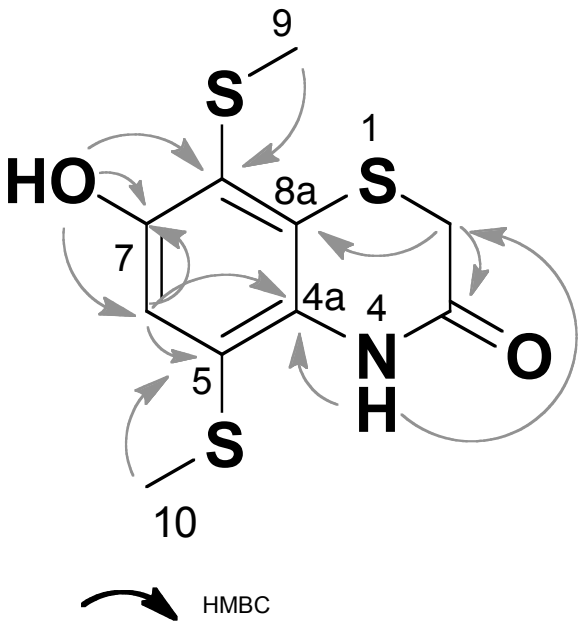


Table S11: **BPSS2111-2113:B** (**6**) NMR assignment table, in CD_2Cl_2

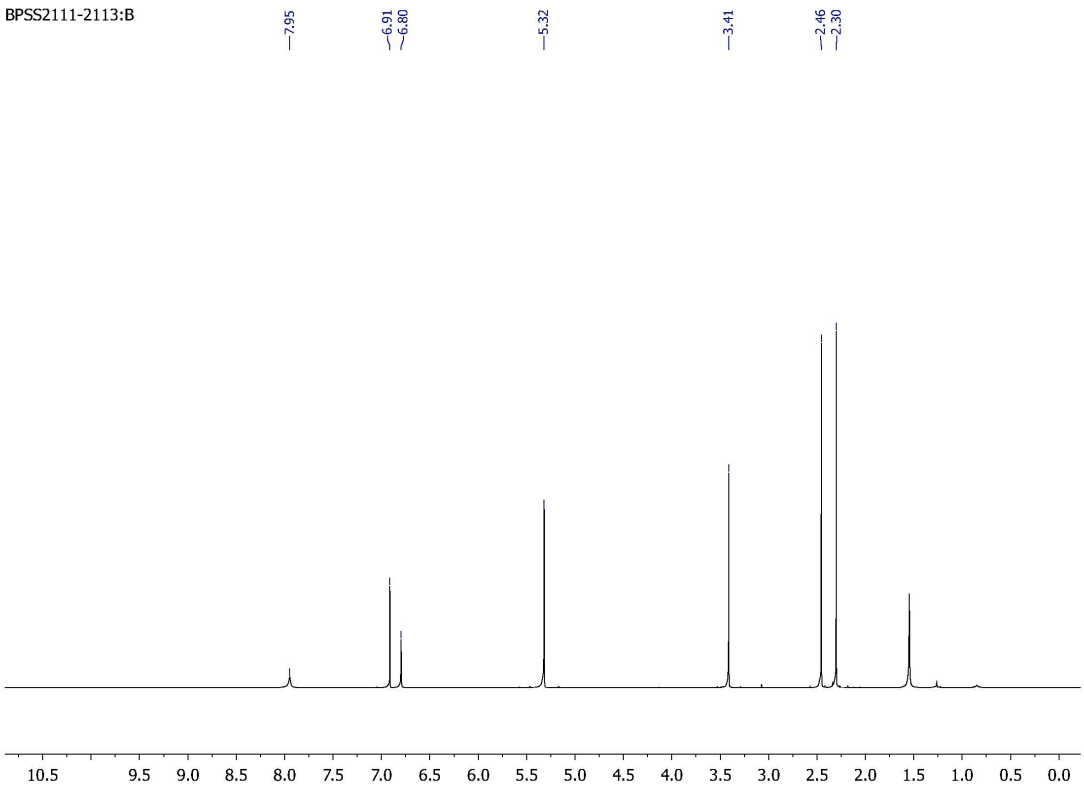
C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
2	3.41	s	30.2	C3, C8a
3			163.8	
4a			128.6 [a]	
5			116.4	
6	6.91	s	113.2	C4a, C5, C7
7			153.3	
8			128.7[a]	
8a			128.5 [a]	
9	2.46	s	17.2	C8
10	2.30	s	18.0	C5
NH	7.95	s		C2, C4a
OH	6.80	s		C6, C7, C8

[a], assignments may be interchangeable.

HRMS-TOF (*m/z*): $[M - H]^-$ calcd for $C_{10}H_{11}NO_2S3$, 271.9873; found 271.9885.



BPSS2111-2113:B (**6**) ^{1}H NMR ; 600MHz in CD_2Cl_2



BPSS2111-2113:B (**6**) ^{13}C NMR ; 150 MHz in CD_2Cl_2

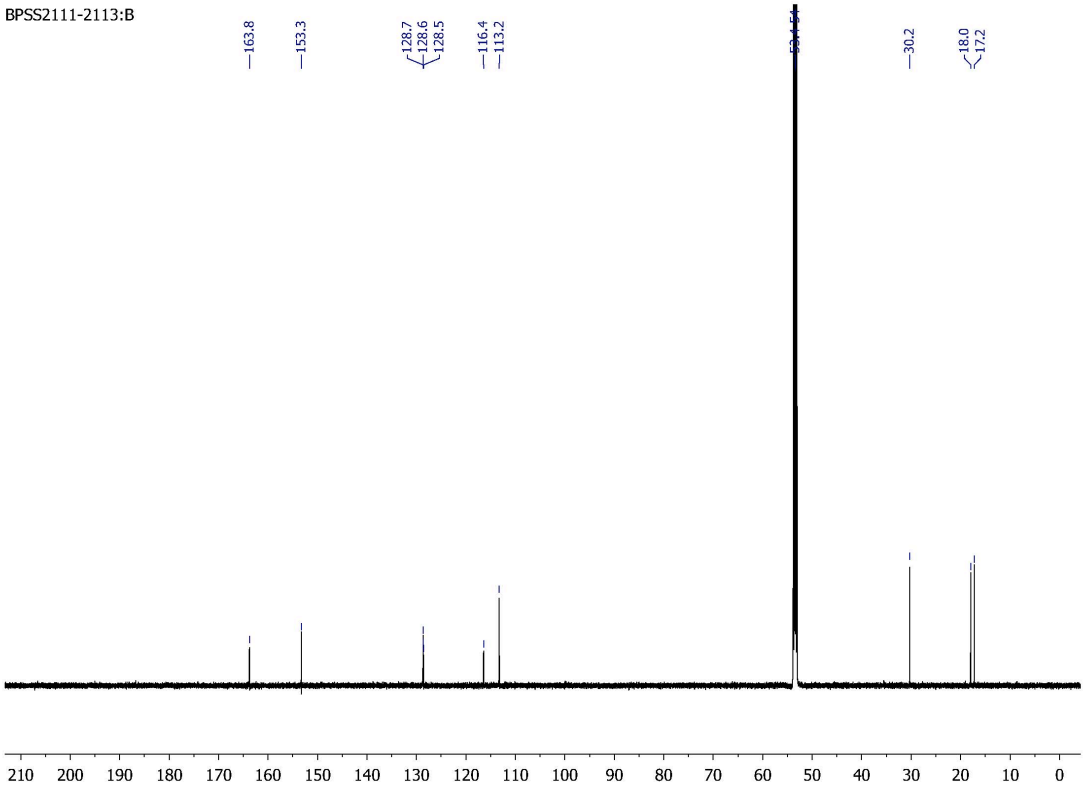
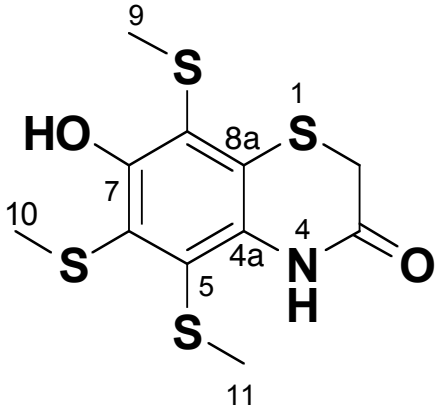


Table S12: **BPSS2111-2113:C** (**7**) NMR assignment table, in CD_2Cl_2

C/H	δ_H	Multiplicity (Hz)	δ_C	HMBC
2	3.43	s	30.8	C3, C8a
3			164.6	
4a			132.2	
5			129.5	
6			120.9[b]	
7			154.7	
8			125.3[b]	
8a			129.7	
9	2.41	s	19.9[c]	C8
10	2.41	s	18.0[c]	C6
11	2.37	s	20.1	C5
NH	8.69	s		C2, C8a
OH	7.45	s		C6, C7, C8

[b, c], assignments may be interchangeable.

HRMS-TOF (*m/z*): $[M - H]^-$ calcd for $C_{11}H_{13}NO_2S_4$, 317.9750; found 317.9754.



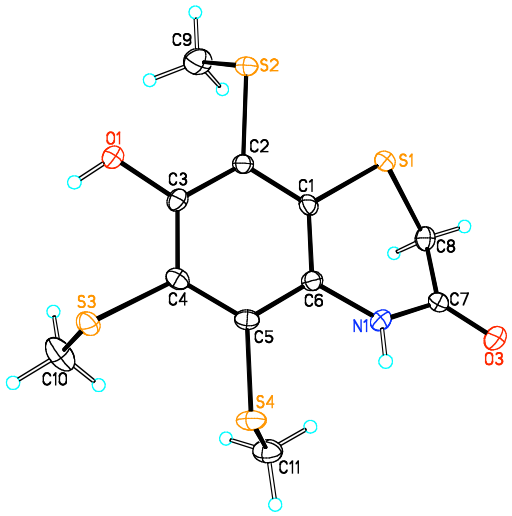
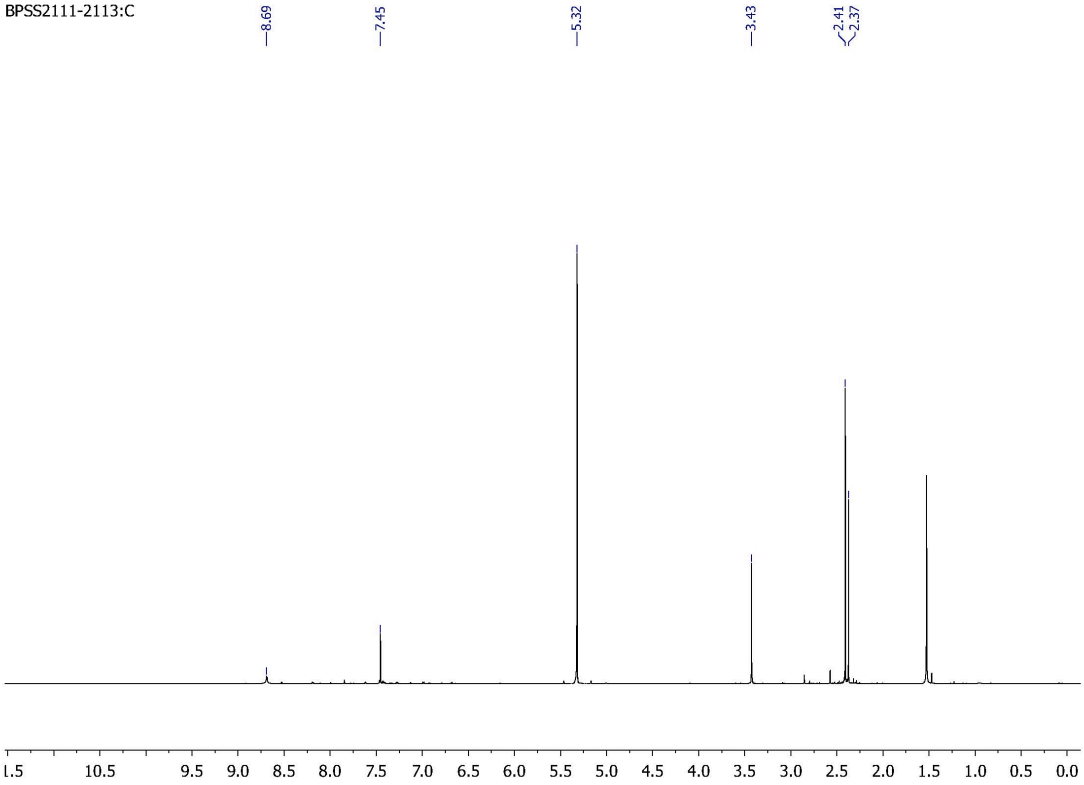


Table S13. Crystal data and structure refinement for BPSS2111-2113:C (**7**).

Identification code	sean9	
Empirical formula	C11 H13 N O2 S4	
Formula weight	319.46	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 9.8403(10) Å	α= 90°.
	b = 17.7022(15) Å	β= 95.805(3)°.
	c = 15.5474(13) Å	γ = 90°.
Volume	2694.4(4) $Å^3$	
Z	8	
Density (calculated)	1.575 Mg/m^3	
Absorption coefficient	0.697 mm^{-1}	
F(000)	1328	
Crystal size	0.15 x 0.10 x 0.05 mm^3	
Theta range for data collection	1.75 to 26.37°.	
Index ranges	-12<=h<=12, -22<=k<=20, -16<=l<=19	
Reflections collected	22991	
Independent reflections	5516 [R(int) = 0.0560]	
Completeness to theta = 26.37°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9660 and 0.9027	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5516 / 0 / 363	
Goodness-of-fit on F^2	1.038	
Final R indices [I>2sigma(I)]	R1 = 0.0421, wR2 = 0.0901	
R indices (all data)	R1 = 0.0726, wR2 = 0.1033	
Largest diff. peak and hole	1.078 and -0.410 $e.Å^{-3}$	

BPSS2111-2113:C (**7**) ^{1}H NMR ; 600MHz in CD_2Cl_2



BPSS2111-2113:C (**7**) ^{13}C NMR ; 150 MHz in CD_2Cl_2