

Supporting Information

Table of Contents

Supplementary tables	3
Table S1 Proposed function of genes in <i>obl_{Au}</i> gene cluster	3
Table S2 Primers used in this study	3
Table S3 Putative FAD dependent oxidoreductase in <i>A. ustus</i> 094102.....	5
Table S4 ¹ H NMR (400 MHz) data of compound 1-7 and 10 in CDCl ₃ [1-3].....	6
Table S5 ¹³ C NMR (100 MHz) data of compound 1-7 and 10 in CDCl ₃ [1-3].....	8
Table S6 Plasmids used in this study	8
Supplementary figures	10
Figure S1 <i>obl</i> gene cluster from <i>A. ustus</i> , <i>A. stellatus</i> , <i>A. clavatus</i> and <i>Bipolaris maydis</i> [4, 5]	10
Figure S2 Gene expression level analysis of <i>oblA_{Au}-D_{Au}</i> and <i>oblR_{Au}</i> by RNA-Seq (a) and RT-qPCR (b).....	10
Figure S3 Screening of mutants.....	11
Figure S4 PCR verification of heterologous expression strains	12
Figure S5 HPLC analysis of crude extracts from <i>A. oryzae</i> expressing <i>oblA_{Au}-D_{Au}</i>	13
Figure S6 HPLC analysis of cultural extracts of mutants Δ <i>oblA_{Au}</i> fed with compound 1 and 10	13
Figure S7 The inhibition test of 0-50 μ M of compound 2 and 3 on <i>A. oryzae</i>	14
Figure S8 Docking of the modelled OblC with different substrates	14
NMR and HR-ESI-MS spectra	15
Figure S9 ¹ H NMR spectrum of compound 1 (400 MHz, CDCl ₃)	15
Figure S10 ¹³ C NMR spectrum of compound 1 (100 MHz, CDCl ₃)	15
Figure S11 ¹ H NMR spectrum of compound 2 (400 MHz, CDCl ₃)	16
Figure S12 ¹³ C NMR spectrum of compound 2 (100 MHz, CDCl ₃)	16
Figure S13 ESI-HRMS spetrum of compound 2	17
Figure S14 ¹ H NMR spectrum of compound 3 (400 MHz, CDCl ₃)	17
Figure S15 ¹³ C NMR spectrum of compound 3 (100 MHz, CDCl ₃)	18
Figure S16 ESI-HRMS spetrum of compound 3	18
Figure S17 ¹ H NMR spectrum of compound 4 (400 MHz, CDCl ₃)	19
Figure S18 ¹³ C NMR spectrum of compound 4 (100 MHz, CDCl ₃)	19
Figure S19 ESI-HRMS spetrum of compound 4	20
Figure S20 ¹ H NMR spectrum of compound 5 (400 MHz, CDCl ₃)	20
Figure S21 ¹³ C NMR spectrum of compound 5 (100 MHz, CDCl ₃)	21
Figure S22 ESI-HRMS spetrum of compound 5	21
Figure S23 ¹ H NMR spectrum of compound 6 (400 MHz, CDCl ₃)	22
Figure S24 ¹³ C NMR spectrum of compound 6 (100 MHz, CDCl ₃)	22
Figure S25 ESI-HRMS spetrum of compound 6	23
Figure S26 ¹ H NMR spectrum of compound 7 (400 MHz, CDCl ₃)	23
Figure S27 ¹³ C NMR spectrum of compound 7 (100 MHz, CDCl ₃)	24
Figure S28 ESI-HRMS spetrum of compound 7	24
Figure S29 ¹ H NMR spectrum of compound 10 (400 MHz, CDCl ₃)	25

Figure S30 ^{13}C NMR spectrum of compound 10 (100 MHz, CDCl_3)	25
Figure S31 ESI-HRMS spectrum of compound 10	26
Figure S32 DEPT135 of compound 10 (100 MHz, CDCl_3).....	26
Figure S33 ^1H - ^1H COSY spectrum of compound 10 (400 MHz, CDCl_3)	27
Figure S34 HMBC spectrum of compound 10 (400 MHz, CDCl_3)	27
Figure S35 HSQC spectrum of compound 10 (400 MHz, CDCl_3)	28
Supplementary references	29

Supplementary tables

Table S1 Proposed function of genes in *obl_{Au}* gene cluster

Gene	Size (aa)	Proposed function	Species	Identity (%)	Sequence ID
<i>oblA_{Au}</i>	725	Ophiobolin F synthase	<i>A. stellatus</i>	82.28	A0A1V1FVQ6.1
		OblA	<i>A. clavatus</i> NRRL 1	65.34	XP_001276070.1
			<i>B. maydis</i> C5	61.37	M2V8C1.1
<i>oblB_{Au}</i>	544	Cytochrome P450	<i>A. stellatus</i>	79.38	A0A1V1FNM9.1
		monooxygenase OblB	<i>A. clavatus</i> NRRL 1	66.22	XP_001276069.1
			<i>B. maydis</i> C5	66.79	M2V933.1
<i>oblD_{Au}</i>	1464	ABC transporter OblD	<i>A. stellatus</i>	85.64	A0A1V1GB10.1
			<i>A. clavatus</i> NRRL 1	78.04	XP_001276075.1
			<i>B. maydis</i> C5	74.90	M2UCE5.1
<i>oblR_{Au}</i>	426	Putative Zn (II) ₂ Cys ₆ transcription factor	<i>A. nidulans</i> FGSC	21.96	CBF83040.1
			A4		
			<i>A. stellatus</i>	50.54	BAX09284.1

Table S2 Primers used in this study

Purpose	Primers	Sequence (5' to 3')
For qPCR	q- <i>oblA</i> -s	ATATTGTGGCTCTCAGCCTCG
	q- <i>oblA</i> -a	CTGAGCCATGTGTTGAGCG
	q- <i>oblB</i> -s	CCGTGACAGGCTACTACGAG
	q- <i>oblB</i> -a	GTCGCAGATGGAGAGTTCAA
	q- <i>oblC</i> -s	TGTCAGACCCTCGACCTCA
	q- <i>oblC</i> -a	CCAAGGCACCAGCGTAGTAA
	q- <i>oblD</i> -s	CAATTATCAAGGCCGACTGC
	q- <i>oblD</i> -a	GTATCTCCAACGGTGAGTCTGG
	q- <i>oblR</i> -s	GGAACCTCCAGACTACAGCG
	q- <i>oblR</i> -a	GGCGGATCAAGCTCTGCTAA
<i>sh ble</i>	<i>sh ble</i> -up	ATCGAT GGGG ACTAGT GATTAAGTGAGACCTTCGTTGTGC
	<i>sh ble</i> -down	CTCACATGTTGGTCTCCAGCTTG
<i>hph</i>	<i>hph</i> -s	AAATTGACGCTTAGACAACCTAA
	<i>hph</i> -a	GCAGCTTGCCAACATGGTG
<i>oblA</i> gene deletion	Δ <i>oblA</i> -L-s	GAGACCAACATGTGAGAAC TCTAGA CGAGTTGATGGTGGAGACG
	Δ <i>oblA</i> -L-a	AGTTGTCTAACGCTCAATTGTCGAGTACTTATACTCCAT
	Δ <i>oblA</i> -R-s	CCACCATGTTGGCAAGCTGCTGGAGCTGCTGAAGGTTGA
	Δ <i>oblA</i> -R-a	GTTGTAAAACGACGCCAGT GAATTCTGCCGAGT ATTAGTATGGA
	Δ <i>oblA</i> -yz-s	GTCTCAGCTCGACACCACA
	Δ <i>oblA</i> -yz-a	GCACCCAATCCAATGTTT
	Δ <i>oblB</i> -L-s	GTCGACGATTATCGATGGGG ACTAGT CACATCTCCACCTCCCTC
<i>oblB</i> gene deletion	Δ <i>oblB</i> -L-a	TACGGGACGGACGAGTCGGA
	Δ <i>oblB</i> -hph-s	TCCGACTCGTCCGTCCGTAAAATTGACGCTTAGACAACTTAA

	$\Delta oblB$ -R-s	CCACCATGTTGGCAAGCTGCCGCCTGGGATGGTAGAAG
	$\Delta oblB$ -R-a	GTTGTAAAACGACGCCAGT GAATT CCTGGCATCAGCGTGGTA
	$\Delta oblB$ -yz-s	TCCACCCACGAACTCATC
	$\Delta oblB$ -yz-a	CCGACGCATCCATTGTAG
<i>oblC</i> gene deletion	$\Delta oblC$ -L-s	GCTATGACCATTACGCC AAGCTT ATTGAGAAGGAGGAGTTGG
	$\Delta oblC$ -L-a	AGTTGTCTAACGCTCAATTGGCTGGACGAAGTAAGTAGA
	$\Delta oblC$ -R-s	CCACCATGTTGGCAAGCTGCAATCTTGTTGGCTTTGGC
	$\Delta oblC$ -R-a	GTTGTAAAACGACGCCAGT GAATT CACAGACCTGTTGCTCC
	$\Delta oblC$ -yz-s	CCCATTCTTAGTCAGTTCTTG
	$\Delta oblC$ -yz-a	CCTCTCGGTCTTGTGGTA
<i>oblD</i> gene deletion	$\Delta oblD$ -L-s	GAATCTCTAGAGGATCCCCG GGTACCGTGGATAAGCAGAGGCAAT
		A
	$\Delta oblD$ -L-a	AGTTGTCTAACGCTCAATTGACGGATGTCAAGAAAGAGC
	$\Delta oblD$ -R-s	CCACCATGTTGGCAAGCTGCTACCGTCGTCGAAATCGT
	$\Delta oblD$ -R-a	GTTGTAAAACGACGCCAGT GAATT CGAGGGAGGTGGAGATGTG
	$\Delta oblD$ -yz-s	AG
<i>oblR</i> gene deletion	$\Delta oblR$ -yz-s	GAGGCTATGCGTATGTTGC
	$\Delta oblR$ -yz-a	CTTCTGCCGTTGGATGG
	$\Delta oblR$ -L-s	GTCGACGATTATCGATGGGG ACTAG TTGAGGAAGGTGGAGCAGC
	$\Delta oblR$ -L-a	AGTTGTCTAACGCTCAATTGGTGGAGACGAGCCGTGTT
	$\Delta oblR$ -R-s	CCACCATGTTGGCAAGCTGCTTGAATTGCATCGAATAGGGG
	$\Delta oblR$ -R-a	GTTGTAAAACGACGCCAGT GAATT CGCCGTATGAAGGACTAT
For heterologous expression in <i>A. oryzae</i>	$\Delta oblR$ -yz-s	GCTGAGGAACGACCAATC
	$\Delta oblR$ -yz-a	GACGTTGGTCGCTGATC
	AO- <i>oblA</i> -F	TAAACCCCACAGCAAGCTCC GAATT CATGGAGTATAAGTACTCGA
		C
	AO- <i>oblA</i> -R	TTCACGAGCTACTACAGATC CCCGGGTCAAACCTTCAGCAGCTCC
		A
	AO- <i>oblB</i> -F	TAAACCCCACAGCAAGCTCC GAATT CATGGAGGCCTACCTGCC
		AA
	AO- <i>oblB</i> -T _{oblB} -R	AAATGATCAAAACACCATGAAGATGTTCAGGCCACGT
	Pamy-F	TGACGTGGCGCTGAACATCTTGTGGTTTGATCAT
	Pamy-R	GTCGAGTACTTATACTCCATCTGTGGGTTATTGTTTCAG
	Adea-Pamy-F	GTTGCGCAGATATCCATAT ACTAGT TCATGGTGGTTGATCAT
	Pamy-R'	TTCACGAGCTACTACAGATCTCACTCCTTGTGCCATCGC
	Tamy-F	GCGATGGCAAGAAGGAGTGAGATCTGTAGTAGCTCGTGA
	Adea-Tamy-R	TTGCATGCCTGCAGGTCGAC TCTAGA CCATCGATGGATCTC
		TTTTGATC
	AO- <i>oblD</i> -F	TAAACCCCACAGCAAGCTCC GAATT CATGGCACATCAAACGATT
		TC
	AO- <i>oblD</i> -R	TTCACGAGCTACTACAGATCTCACTCCTTGTGCCATCGC
	AO- <i>oblC</i> -F	GTTGCGCAGATATCCATAT ACTAGT TCATGGTGGTTGATCAT
	AO- <i>oblC</i> -T _{oblC} R	TTGCATGCCTGCAGGTCGAC TCTAGA AGGATGCACAAGGCAACGG
		T

	Pamy- <i>oblC</i> -F	AAAAGGAGATCCATCGATGGCTAGATCATGGTGTGATCAT
	Pamy- <i>oblC</i> -R	AAAAGAAACCACAAAAGATTTCATAGGCCCTAACAAAG
<i>eGFP</i>	eGFP-F	TAAACCCCACAGCAAGCTCC GAATT CATGGTGAGCAAGGGCGAG
	eGFP-A	GA
		CTTGTACAGCTCGTCCATGC
<i>oblA</i>	eGFP- <i>oblA</i> -F	GCATGGACGAGCTGTACAAGATGGAGTATAAGTACTCGAC
	eGFP- <i>oblA</i> -R	GATCCCCGGTACCGAGCTC GAATT CTCAAACCTTCAGCAGCTCC
		A
<i>oblB</i>	eGFP- <i>oblB</i> -F	GCATGGACGAGCTGTACAAGATGGAGGCCTACCTGCCCAA
	eGFP- <i>oblB</i> -R	GATCCCCGGTACCGAGCTC GAATT CTCAATGGTACACAACGCGA
		A
<i>oblC</i>	eGFP- <i>oblC</i> -F	GCATGGACGAGCTGTACAAGATGCCCTCCCTAAATCAT
	eGFP- <i>oblC</i> -R	GATCCCCGGTACCGAGCTC GAATT CTCATAGGCCCTAACAAAGC
		G
<i>oblD</i>	eGFP- <i>oblD</i> -F	GCATGGACGAGCTGTACAAGATGCCACATCAAACGATT
	eGFP- <i>oblD</i> -R	GATCCCCGGTACCGAGCTC GAATT CTCACTCCTCTGCCATCGC
Partial of <i>arg</i> gene	Arg-S	ATCTCATACCGTCAACTCA
	Arg-A	ACTGCCTCATCCGTCA
Partial of <i>ade</i> gene	Ade-S	AATGACTTGGTAAGACGG
	Ade-A	CTGAATGGCTAAGGAGA

Table S3 Putative FAD dependent oxidoreductase in *A. ustus* 094102

Genes	Identity	FPKM in ophs (+)	FPKM in ophs (-)
<i>au-orf1</i>	58%	378.81	2.35
<i>au-orf2</i>	37%	3.26	3.56
<i>au-orf3</i>	33%	4.11	34.4

Note: oph(+), ophiobolin-producing strain; oph(-), ophiobolin-nonproducing strain; FPKM, Fragments Per Kilobase of exon model per Million mapped fragments.

Table S4 ^1H NMR (400 MHz) data of compound **1-7** and **10** in CDCl_3 [1-3]

Position	δH (J in Hz)							
	1	2	3	4	5	6	7	10
1	1.86 m,	1.20 (d, 14.6),	1.24 m,	1.56 m,	1.16 m,	1.59 m,	2.04 (d, 3.6),	1.19 m, 1.87 m
	1.94 m	1.79 (d, 2.9)	1.81 (dd, 14.6, 3.2)	1.76 m	2.04 m	1.80 m	1.18 (t, 13.1)	
2	1.60 m	2.37 (dt, 19.0, 4.4)	2.39 m	2.14 m	2.67 m	2.12 m	2.68 m	1.67 m
4	1.68 m	2.51 (d, 19.2),	2.50 (d, 19.2),	2.43 (dd, 16.8, 1.5),	6.03 (t, 1.6)	2.42 (dd, 16.7, 1.6)	6.06 s	1.74 m, 1.91 m
		2.81 (d, 19.2)	2.80 (d, 19.3)	3.08 (d, 16.7)				
5	1.39 m, 1.52 m							1.36 m, 1.53 m
6	3.17 (t, 8.2)	3.28 (d, 10.5)	3.27 (d, 10.5)	3.35 (d, 10.8)	3.44 (d, 4.2)	3.30 (d, 10.6)	3.41 (d, 3.8)	3.14 (t, 8.4)
8	5.53 (tt, 8.2, 1.5)	7.14 (t, 8.6)	7.22 (t, 8.5)	6.89 (dd, 6.7, 2.2)	6.84 (dd, 6.3, 2.4)	6.87 (dd, 6.8, 2.3)	6.84 (d, 4.3)	6.00 (dt, 11.5, 1.3)
9	1.99 m	2.97 (dd, 12.6, 8.3),	2.29 m,	2.65 m,	2.70 m,	2.17 (d, 14.6),	2.94 (d, 20.4),	1.83 m,
		2.13 (dd, 12.5, 7.6)	2.44 m	2.21 m	2.26 m	2.83 (dt, 20.5, 3.2)	2.25 m	2.02 (qd, 9.6, 6.9)
10	1.68 m	1.57 m	-	2.60 m	2.70 m	2.51 m	2.64 m	1.93 m
12	1.33 m, 1.49 m	1.42 m,	1.39 m,	1.41 m,	1.40 m,	1.47 m,	1.55 m, 1.46 (td,	1.44 (dd, 13.6, 3.2),
		1.44 m	1.46 m	1.47 m	1.49 m	1.50 m	12.3, 4.9)	1.52 m
13	1.25 m, 1.49 m	1.25 m,	1.43 m,	1.16 (qd, 12.2, 5.8),	1.24 m,	1.20 m,	1.27 m,	1.33 m, 1.74 m
		1.61 m	1.54 m	1.56 m	1.58 m	1.68 m	1.69 m	
14	2.27 (dtd, 13.3,	2.08 (t, 9.5)	2.35 m	1.76 m	1.73 m	1.89 m	1.91 m	2.27 (dd, 13.9, 8.1)
	8.8, 4.4)							
15	1.68 m	2.71 (dt, 15.9, 8.0)	1.65 m	1.49 m	1.40 m	2.55 m	2.58 m	2.70 (ddt, 9.6,
								6.6, 4.8)
16	1.21 m, 1.33 m	5.20 (t, 10.1)	1.16 m, 1.24 m	0.98 (tt, 9.2, 4.8),	0.98 m, 1.40 m	5.13 (t, 10.0)	5.13 (t, 10.0)	5.21 (t, 9.7)
				1.38 m				
17	1.91 m, 1.94 m	6.05 (t, 11.0)	1.95 m, 2.00 m	1.91 m, 2.05 m	1.92 m, 1.92 m	6.09 (t, 11.1)	6.12 (t, 11.1)	5.96 m
		5.98 (d, 11.7)	5.09 (tt, 7.0, 1.6)	5.10 m	5.10 m	6.00 (d, 11.6)	6.03 (d, 12.1)	5.47 (tt, 8.3, 1.5)

	7.2, 2.9, 1.5)							
20	1.27 s	1.37 s	1.36 s	1.44 s	2.06 s	1.44 s	2.09 s	1.26 s
21	1.80 s	9.22 s	9.23 s	9.19 s	9.30 s	9.16 s	9.29 s	1.71 s
22	0.87 s	0.98 s	0.90 s	0.83 s	0.85 s	0.84 s	0.88 s	0.92 s
23	0.76 (d, 6.8)	0.92 (d, 6.6)	0.78 (d, 6.8)	0.89 (d, 6.5)	0.89 (d, 6.5)	0.97 (d, 6.7)	0.99 (d, 6.7)	0.87 (d, 6.7)
24	1.60 s	1.75 s	1.60 s	1.60 s	1.60 s	1.76 s	1.79 s	1.77 s
25	1.68 s	1.82 s	1.69 s	1.69 s	1.69 s	1.82 s	1.85 s	1.80 s

Table S5 ^{13}C NMR (100 MHz) data of compound **1-7** and **10** in CDCl_3 [1-3]

Position	δC							
	1	2	3	4	5	6	7	10
1	26.24	35.09	36.21	41.39	45.90	41.40	45.95	26.60
2	54.29	50.28	51.05	49.62	49.08	49.68	49.23	53.32
3	81.28	76.89	76.97	76.76	177.68	76.87	177.62	80.97
4	42.05	54.91	54.99	55.01	130.33	55.05	130.39	41.65
5	23.78	217.64	217.73	217.34	207.56	217.40	207.67	25.73
6	42.51	48.54	48.66	48.96	50.09	48.92	50.07	41.93
7	136.60	141.33	141.65	141.83	140.46	141.41	140.08	138.25
8	129.67	164.06	164.36	159.93	157.16	160.96	158.08	134.78
9	23.70	25.44	24.99	31.06	31.11	30.95	31.00	24.31
10	56.00	53.56	53.64	43.14	43.19	43.85	43.93	55.77
11	44.09	43.85	44.09	44.44	45.15	44.81	45.50	43.59
12	43.69	42.59	42.69	45.59	44.68	45.36	44.42	43.30
13	36.90	26.55	23.03	27.31	27.21	27.82	27.87	35.77
14	45.87	47.13	45.46	51.66	51.60	52.17	52.18	47.38
15	33.41	35.85	32.96	32.03	31.95	32.69	32.69	35.53
16	37.78	137.11	37.09	37.00	37.30	135.70	135.81	121.55
17	26.71	122.44	26.20	25.87	25.83	124.09	124.13	120.54
18	125.46	119.95	124.65	124.57	124.51	120.08	120.08	129.02
19	131.58	136.06	131.58	131.69	131.72	136.57	136.65	136.02
20	29.49	25.67	25.68	25.92	17.36	25.99	17.34	29.05
21	21.97	196.28	196.49	194.45	193.17	194.43	193.13	21.58
22	19.25	18.73	19.18	23.56	23.19	23.43	23.04	20.54
23	17.39	20.42	16.68	18.75	18.72	21.43	21.35	18.22
24	18.14	18.15	17.80	17.84	17.83	18.34	18.32	18.59
25	26.31	26.55	25.87	25.83	25.88	26.69	26.65	26.63

Table S6 Plasmids used in this study

Plasmids	Features
pGAPZ α A	<i>ble</i> ^r selection marker
pCAMBIA1301	<i>hph</i> selection marker; CAMV35S promoter
pTAex3-rev-1022	<i>argB</i> auxotrophic gene; α -amylase promoter and terminator
pAdeA-R	<i>adeA</i> auxotrophic gene
peGFP-C1	<i>kan</i> ^r and <i>neo</i> ^r selection marker; <i>egfp</i> gene
pMD19T- <i>sh ble</i>	Introducing <i>SpeI</i> and <i>Clal</i> into multiple cloning sites of pMD19T
pMD19T- <i>oblAL-hph-oblAR</i>	<i>oblA</i> gene-targeting cassette inserted into <i>Xba</i> I and <i>Eco</i> RI
pMD19T- <i>oblBL-hph-oblBR</i>	<i>oblB</i> gene-targeting cassette inserted into <i>Spe</i> I and <i>Eco</i> RI
pMD19T- <i>oblCL-hph-oblCR</i>	<i>oblC</i> gene-targeting cassette inserted into <i>Hind</i> III and <i>Eco</i> RI
pMD19T- <i>oblDL-hph-oblDR</i>	<i>oblD</i> gene-targeting cassette inserted into <i>Kpn</i> I and <i>Eco</i> RI
pMD19T- <i>oblRL-hph-oblRR</i>	<i>oblR</i> gene-targeting cassette inserted into <i>Spe</i> I and <i>Eco</i> RI
pTAex3- <i>oblA</i>	<i>oblA</i> was introduced into between PamY and TamY

pAdeA- <i>oblB</i>	Introducing gene-expression cassette Pamy- <i>oblB</i> -Tamy
pAdeA- <i>oblC</i>	Introducing gene-expression cassette Pamy- <i>oblC</i> -Tamy
pAdeA- <i>oblD</i>	Introducing gene-expression cassette Pamy- <i>oblD</i> -Tamy
pTAex3- <i>oblA-oblB</i>	Introducing gene-expression cassette Pamy- <i>oblB</i> -T _{oblB} -Pamy- <i>oblA</i> -Tamy
pAdeA- <i>oblC-oblD</i>	Introducing gene-expression cassette Pamy- <i>oblC</i> -T _{oblC} -Pamy- <i>oblD</i> -Tamy
pTAex3-eGFP	<i>egfp</i> inserted into site of <i>EcoR I</i>
pTAex3-eGFP <i>oblA</i>	<i>egfp-oblA</i> inserted into site of <i>EcoR I</i>
pTAex3-eGFP <i>oblB</i>	<i>egfp-oblB</i> inserted into site of <i>EcoR I</i>
pTAex3-eGFP <i>oblC</i>	<i>egfp-oblC</i> inserted into site of <i>EcoR I</i>
pTAex3-eGFP <i>oblD</i>	<i>egfp-oblD</i> inserted into site of <i>EcoR I</i>

Supplementary figures

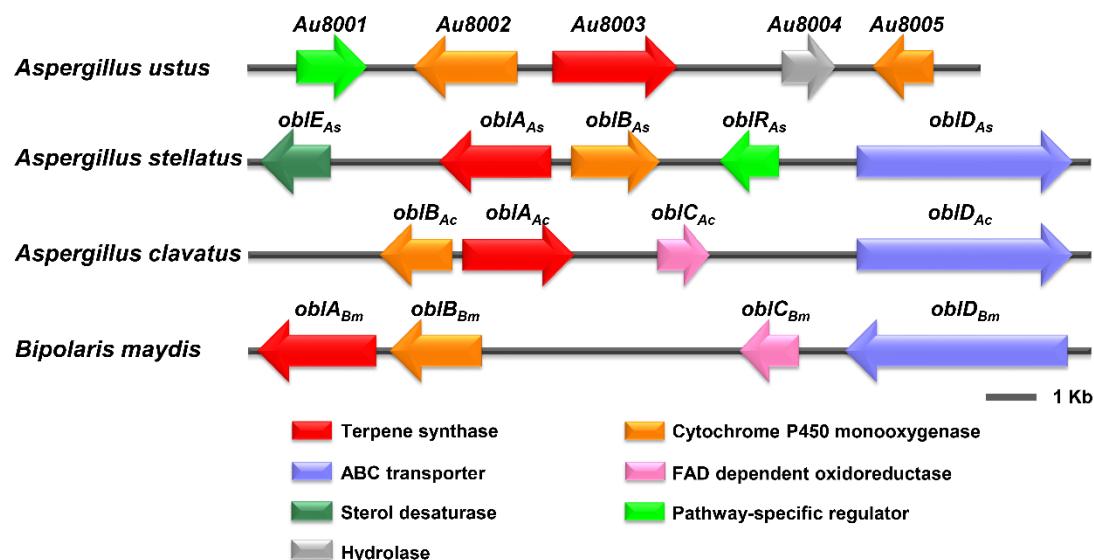


Figure S1 *obl* gene cluster from *A. ustus*, *A. stellatus*, *A. clavatus* and *Bipolaris maydis* [4, 5]

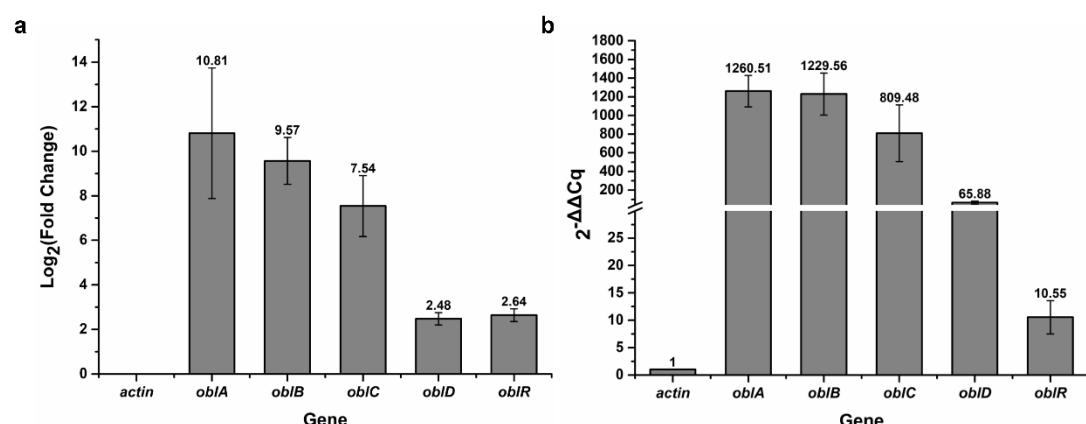


Figure S2 Gene expression level analysis of *oblA_{Au}-D_{Au}* and *oblR_{Au}* by RNA-Seq (a) and RT-qPCR (b). Log₂ (Fold Change) and 2^{-ΔΔCq} were calculated according to the expression ratio of each gene. The housekeeping gene *actin* was used for normalization. Data are means ± SE of three independent measurements.

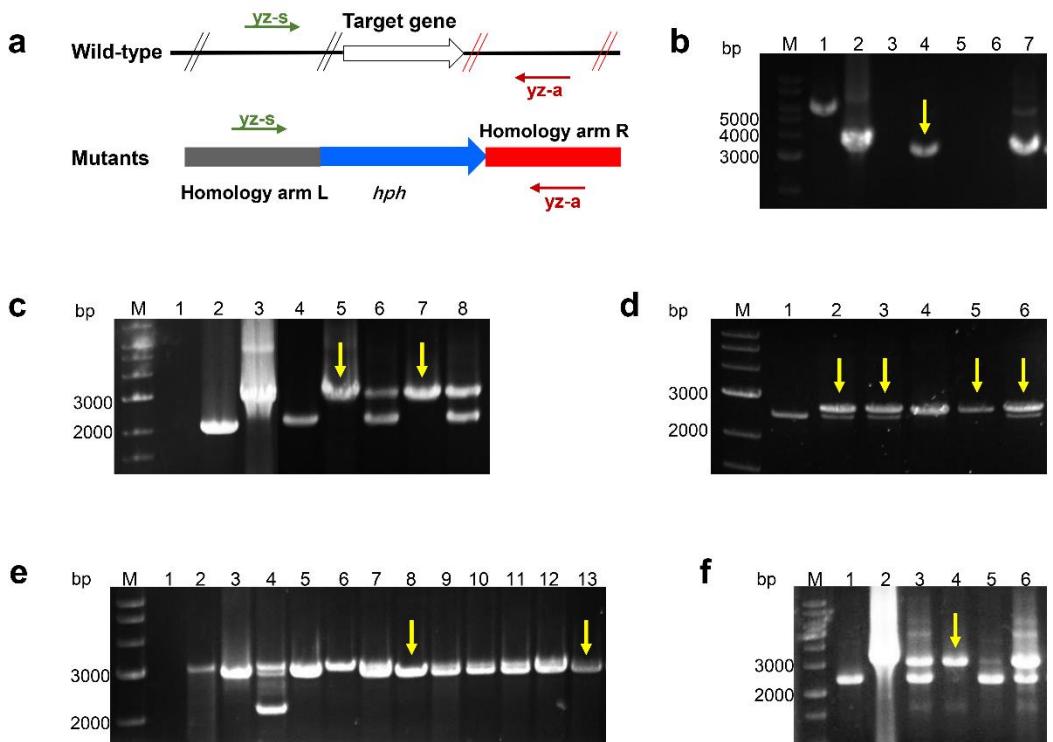


Figure S3 Screening of mutants. (a) The schematic diagram of screening of wild-type and mutants using primers *yz-s/a*. (b) PCR verification of ΔoblD transformants with primers *oblD-*yz-s/a**. Lane 1: wild-type strain (5478 bp); lane 2: pMD19T-*oblDL-hph-oblDR* (3205 bp); lane 3: blank control; lane 4-7: four transformants. (c) PCR verification of ΔoblR transformants with primers *oblR-*yz-s/a**. Lane 1: blank control; lane 2: wild-type strain (1999 bp); lane 2: pMD19T-*oblRL-hph-oblRR* (2805 bp); lane 4-8: five transformants. (d) PCR verification of ΔoblB transformants with primers *oblB-*yz-s/a**. Lane 1: wild-type strain (2508 bp); lane 4: pMD19T-*oblBL-hph-oblBR* (2718 bp); lane 2-3 and 5-6: four transformants. (e) PCR verification of ΔoblA transformants with primers *oblA-*yz-s/a**. Lane 1: blank control; lane 2: wild-type strain (3110 bp); lane 3: pMD19T-*oblAL-hph-oblAR* (2906 bp); lane 4-13: ten transformants. (f) PCR verification of ΔoblC transformants with primers *oblC-*yz-s/a**. Lane 1: wild-type strain (2406 bp); lane 2: pMD19T-*oblCL-hph-oblCR* (3103 bp); lane 3-6: four transformants. M: 1 kb DNA ladder; the yellow arrows indicate positive mutants.

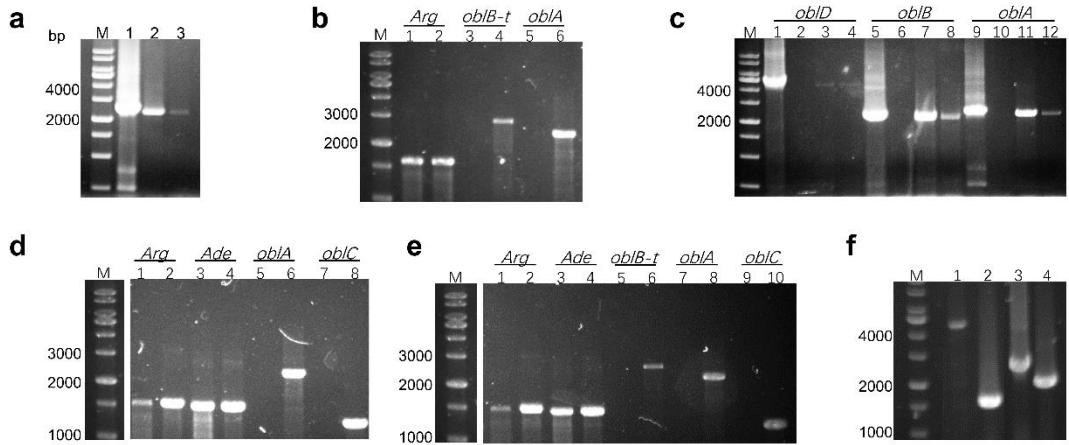


Figure S4 PCR verification of heterologous expression strains. (a) Ao-*oblA*. Lane 1, 2 and 3: *oblA* gene amplified from plasmid of pTAex3-*oblA*, gDNA of Ao-*oblA*-1 and gDNA of Ao-*oblA*-2, respectively; (b) Ao-*oblA*-*oblB*. Lane 1, 3 and 5: gDNA of Ao-pTAex3; Lane 2, 4 and 6: gDNA of Ao-*oblA*-*oblB*; (c) Ao-*oblA*-*oblB*-*oblD*. Lane 1: plasmid of pAdeA-*oblD*; Lane 5 and 9: plasmid of pTAex3-*oblA*-*oblB*; Lane 2, 6 and 10: gDNA of Ao-pTAex3-pAdeA; Lane 3, 7 and 11: gDNA of Ao-*oblA*-*oblB*-*oblD*-1; Lane 4, 8 and 12: gDNA of Ao-*oblA*-*oblB*-*oblD*-2. (d) Ao-*oblA*-*oblC*. Lane 1, 3, 5 and 7: gDNA of Ao-pTAex3-pAdeA; Lane 2, 4, 6 and 8: gDNA of Ao-*oblA*-*oblC*; (e) Ao-*oblA*-*oblB*-*oblC*. Lane 1, 3, 5, 7 and 9: gDNA of Ao-pTAex3-pAdeA; Lane 2, 4, 6, 8 and 10: gDNA of Ao-*oblA*-*oblB*-*oblC*; (f) Ao-*oblA*-*oblB*-*oblC*-*oblD*. Lane 1, 2, 3 and 4: *oblD*, *oblC*, *oblB-t* and *oblA* gene amplified from gDNA of Ao-*oblA*-*oblB*-*oblC*-*oblD*, respectively. M: 1 kb DNA ladder.

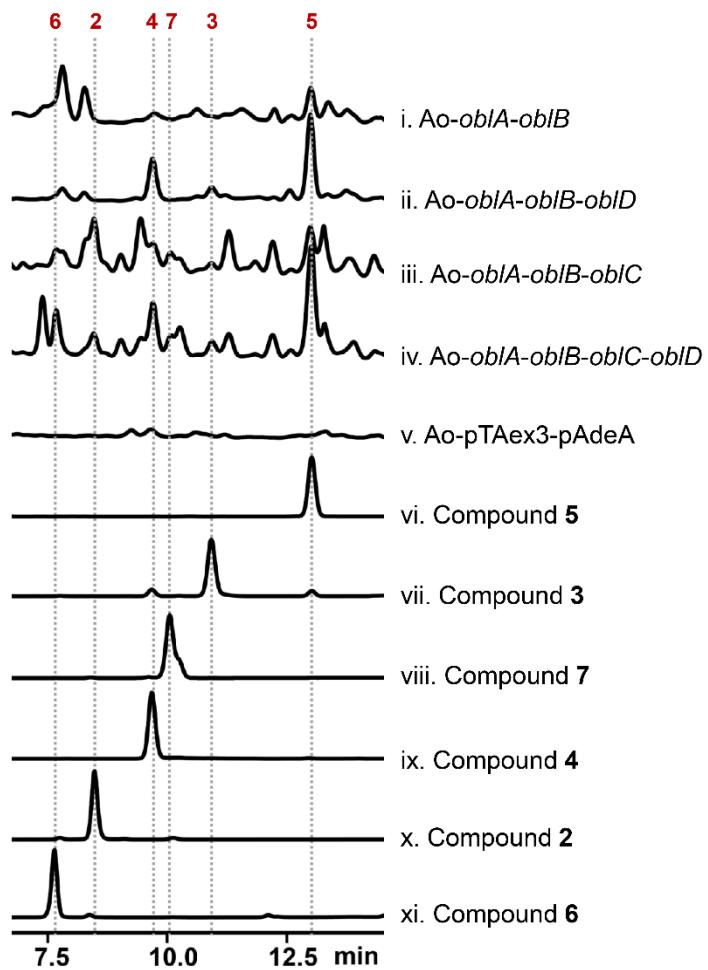


Figure S5 HPLC analysis of crude extracts from *A. oryzae* expressing *oblIA_{Au}*-*D_{Au}*

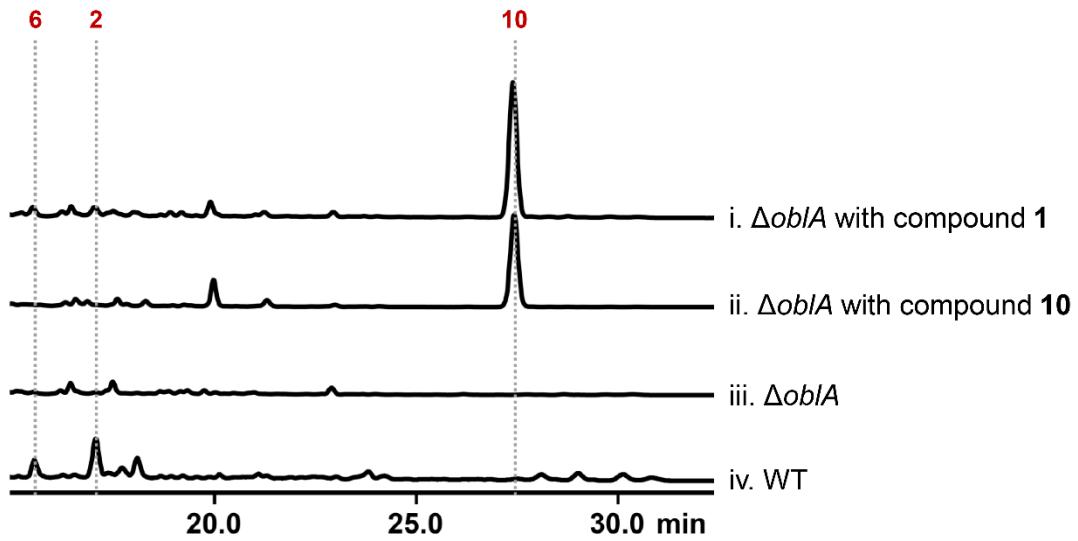


Figure S6 HPLC analysis of cultural extracts of mutants $\Delta\text{oblIA}_{\text{Au}}$ fed with compound 1 and 10

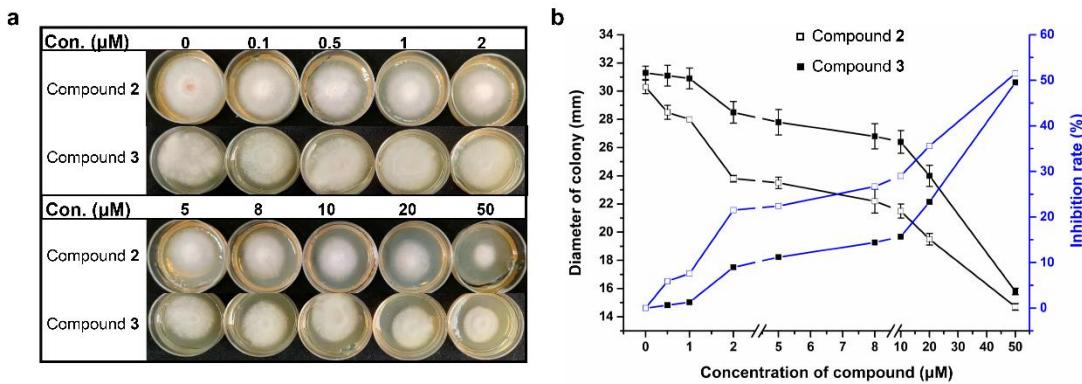


Figure S7 The inhibition test of 0-50 μM of compound **2** and **3** on *A. oryzae*. (a) The colony of *A. oryzae* growing in CD medium with 0-50 μM of compound **2** and **3**, respectively. (b) The diameter of colony of *A. oryzae* on CD medium with 0-50 μM of compound **2** and **3** and inhibition rate of 0-50 μM of compound **2** and **3** against *A. oryzae*, respectively.

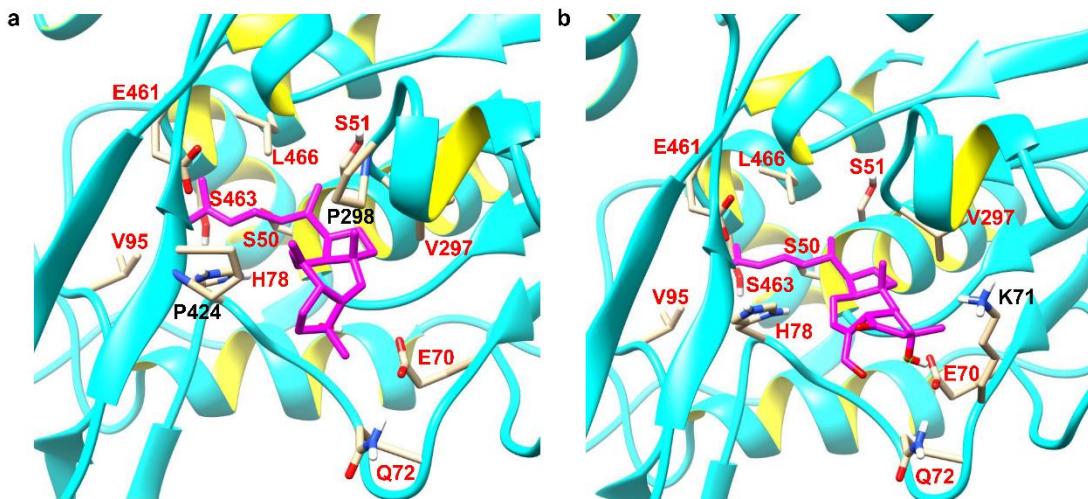


Figure S8 Docking of the modelled ObIC with different substrates. (a) Docking of ObIC with compound **1** (as sticks in lavender). The residues (S50, S51, E70, Q72, H78, V95, V297, V298, P424, E461, S463 and L466) are shown as sticks. (b) Docking of ObIC with compound **3** (as sticks in lavender). The residues (S50, S51, E70, K71, Q72, H78, V95, V297, E461, S463 and L466) are shown as sticks. The residues in red are consensus residues in both models.

Supplementary NMR and HR-ES spectra

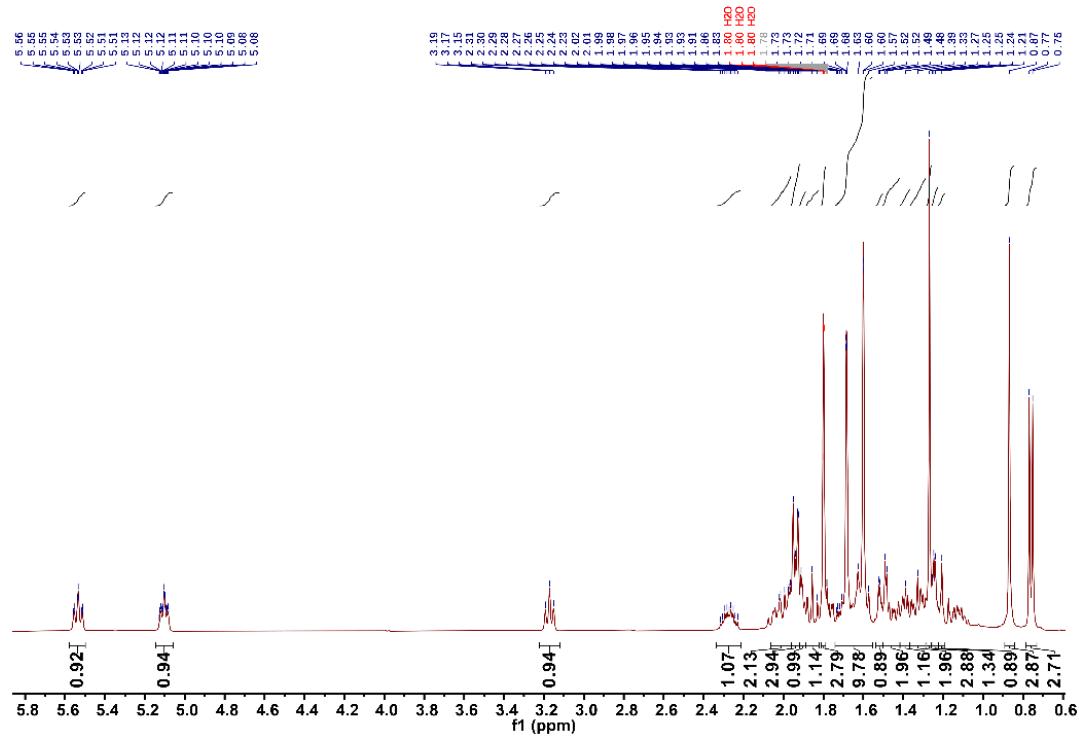


Figure S9 ^1H NMR spectrum of compound **1** (400 MHz, CDCl_3)

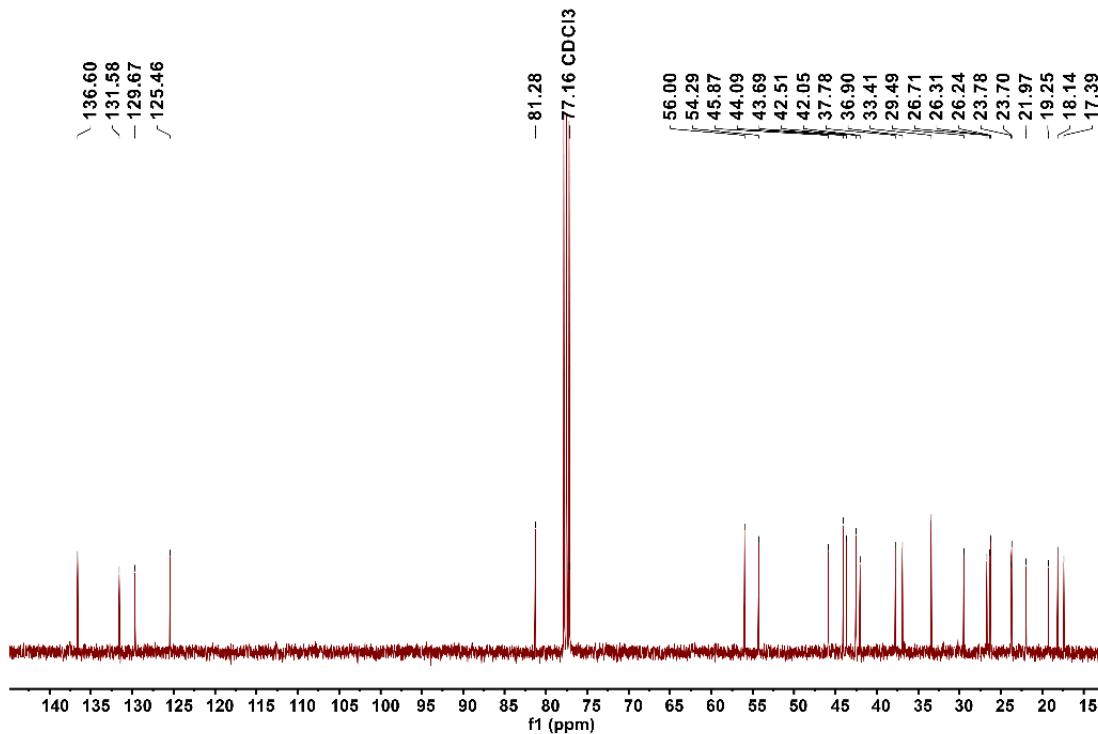


Figure S10 ^{13}C NMR spectrum of compound 1 (100 MHz, CDCl_3)

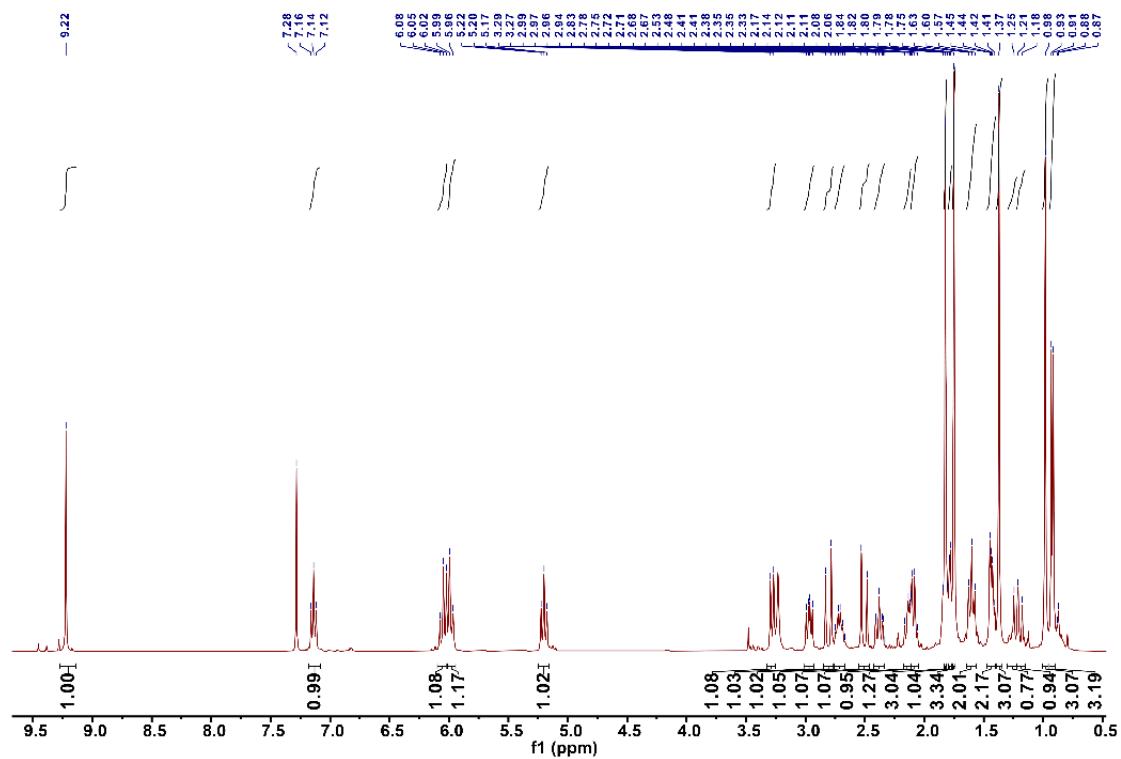


Figure S11 ^1H NMR spectrum of compound **2** (400 MHz, CDCl_3)

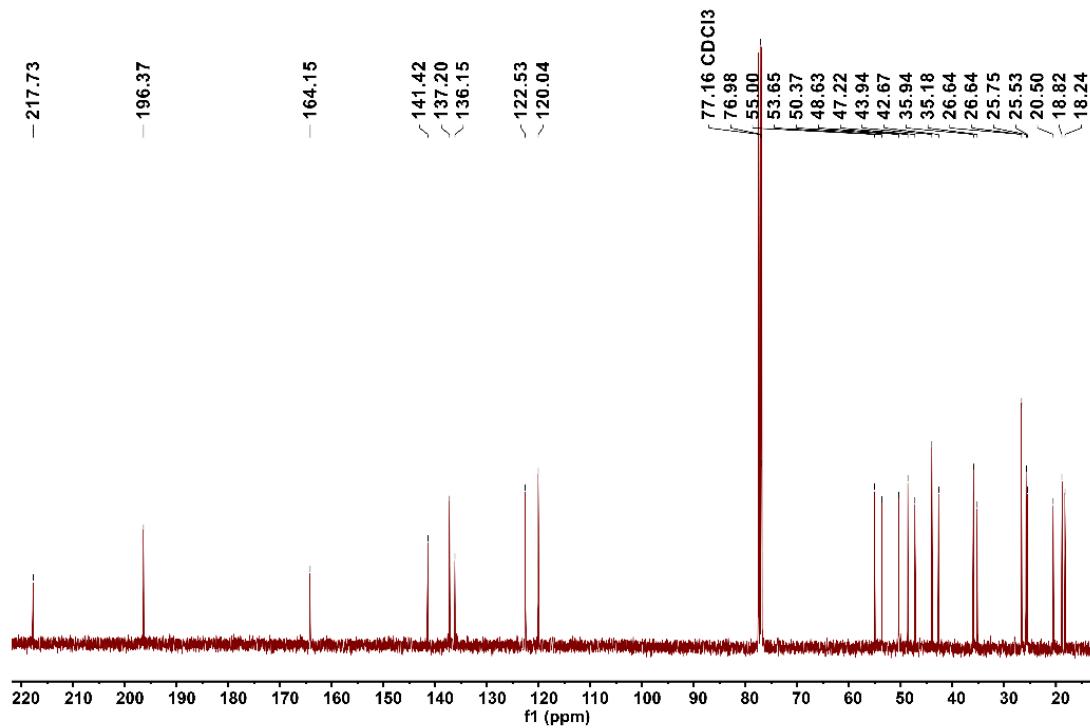


Figure S12 ^{13}C NMR spectrum of compound 2 (100 MHz, CDCl_3)

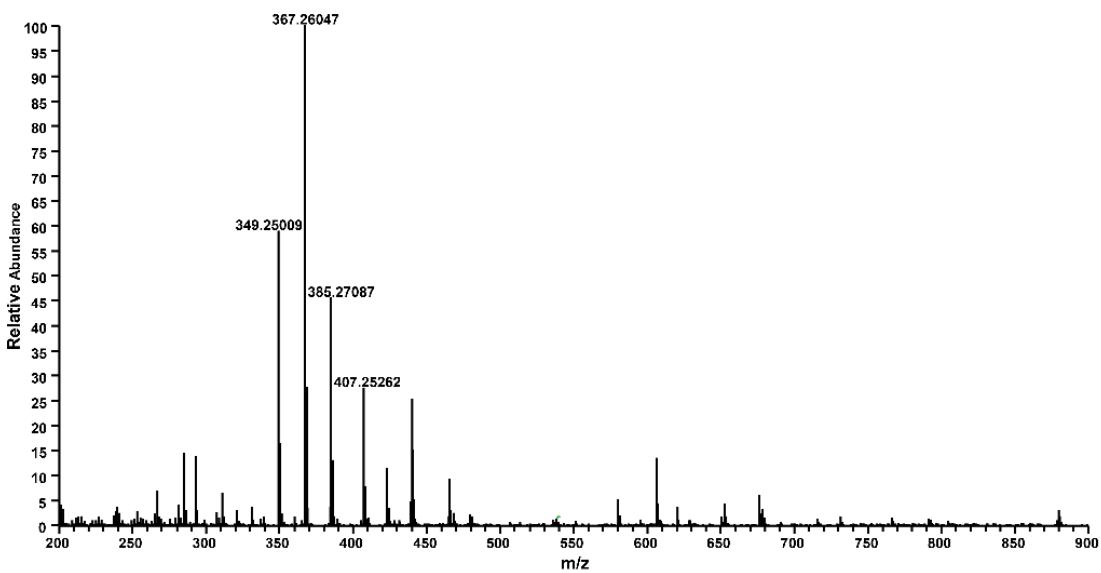


Figure S13 ESI-HRMS spectrum of compound 2

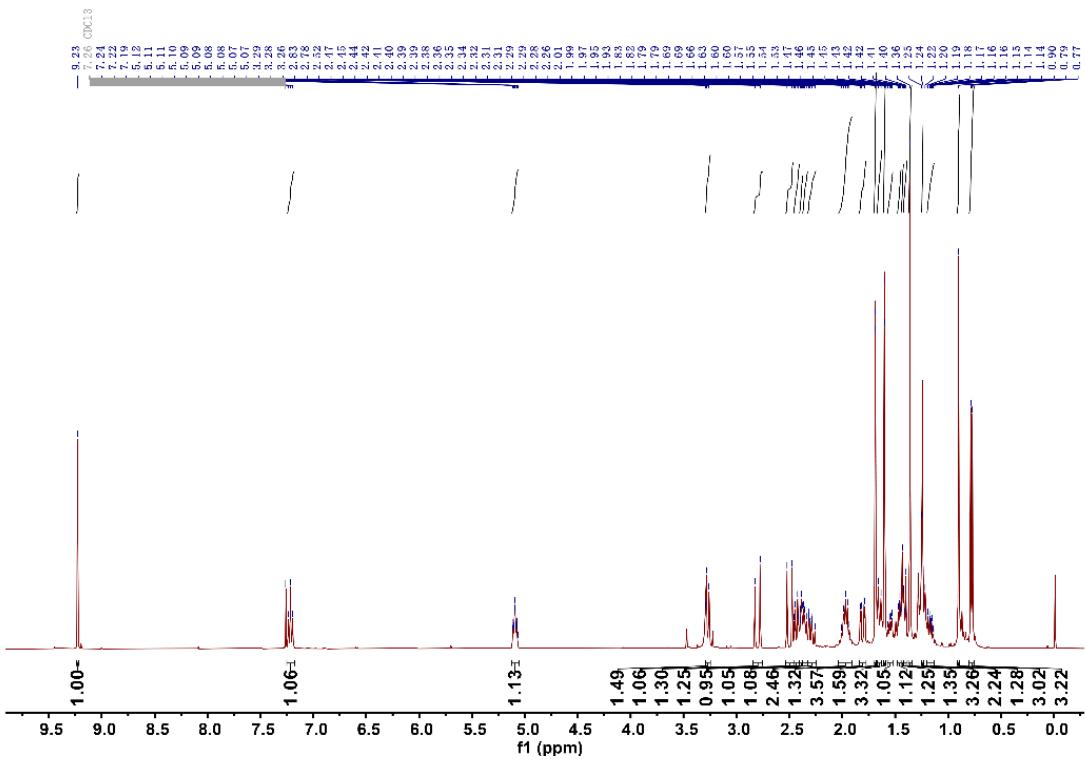


Figure S14 ^1H NMR spectrum of compound 3 (400 MHz, CDCl_3)

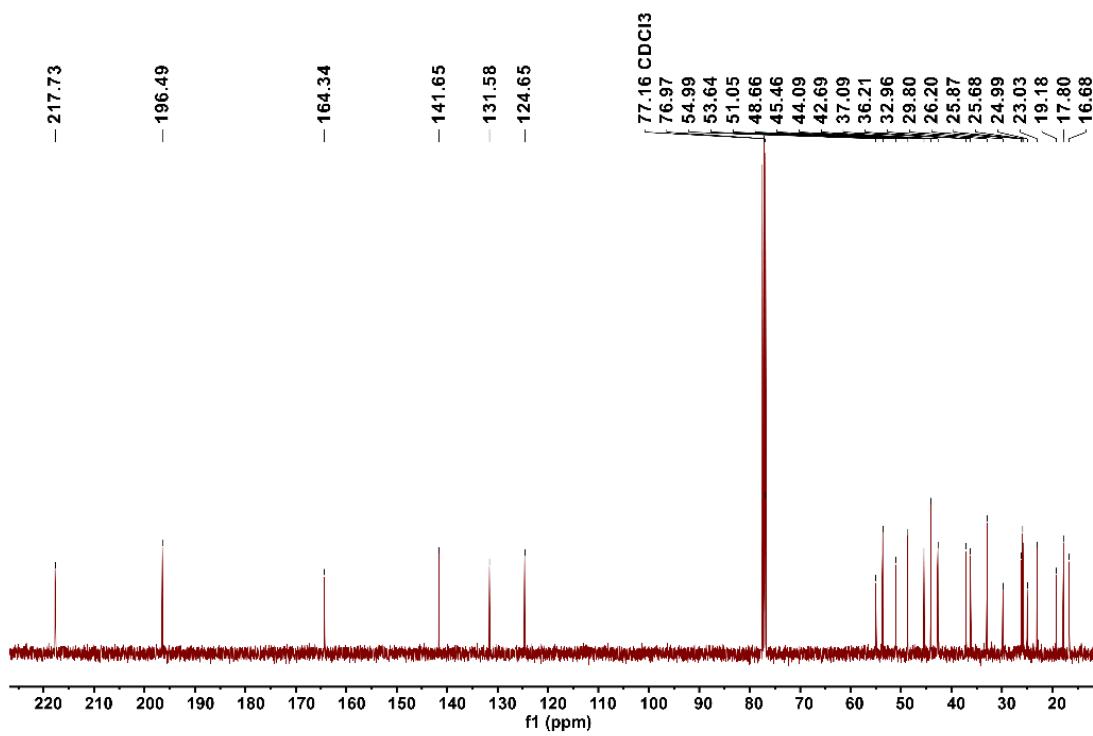


Figure S15 ^{13}C NMR spectrum of compound 3 (100 MHz, CDCl_3)

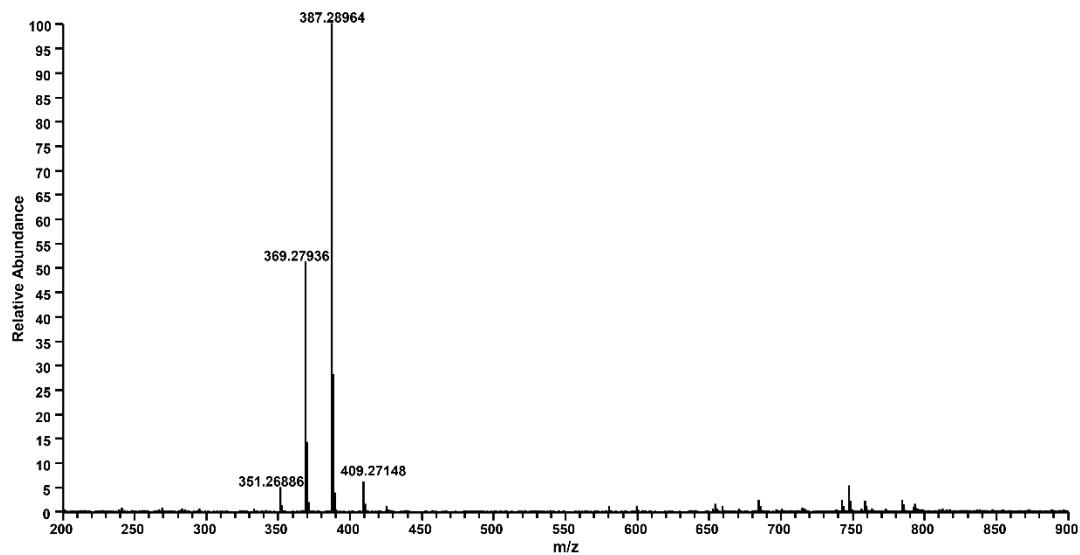


Figure S16 ESI-HRMS spectrum of compound 3

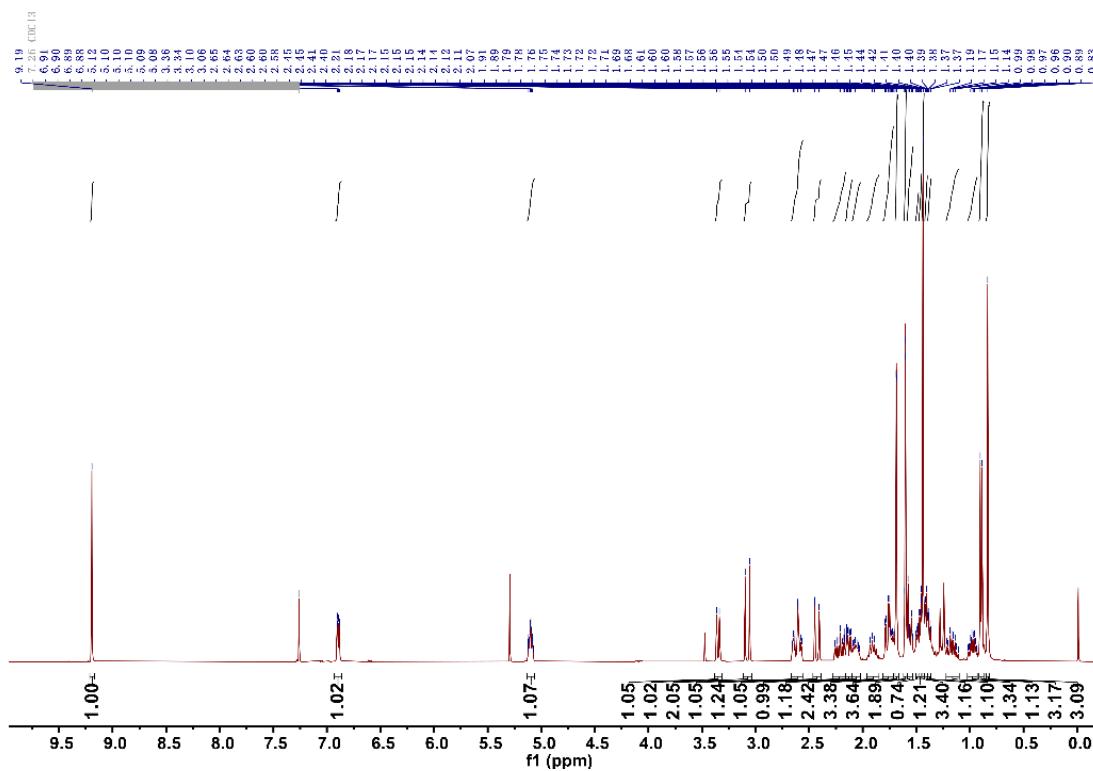


Figure S17 ^1H NMR spectrum of compound **4** (400 MHz, CDCl_3)

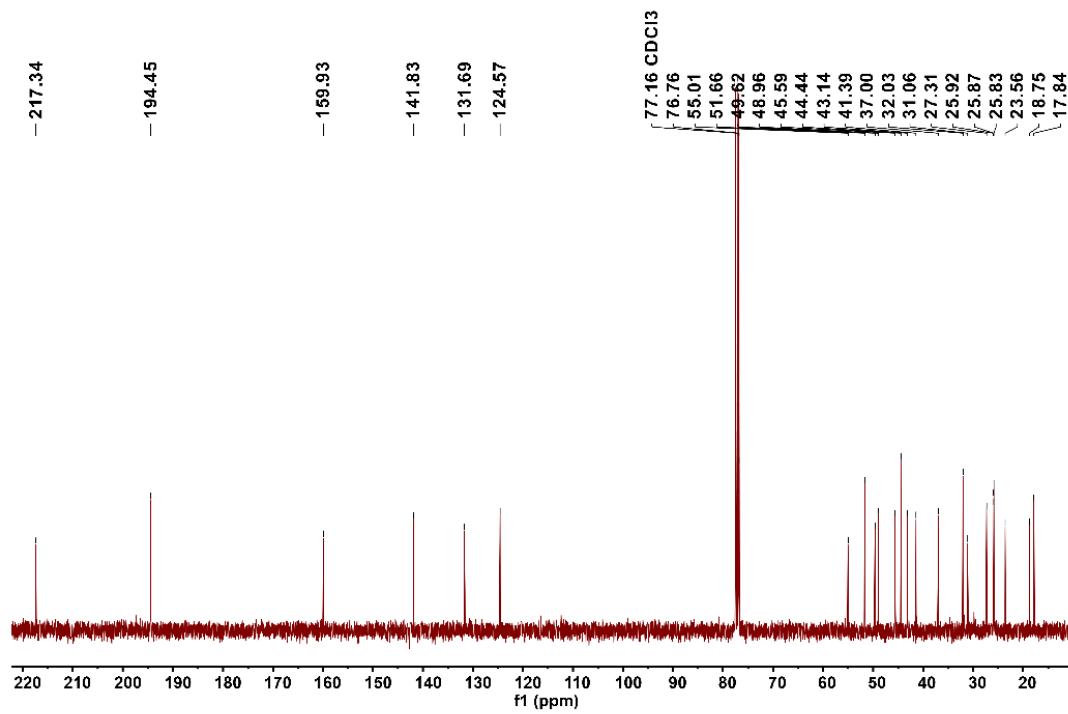


Figure S18 ^{13}C NMR spectrum of compound **4** (100 MHz, CDCl_3)

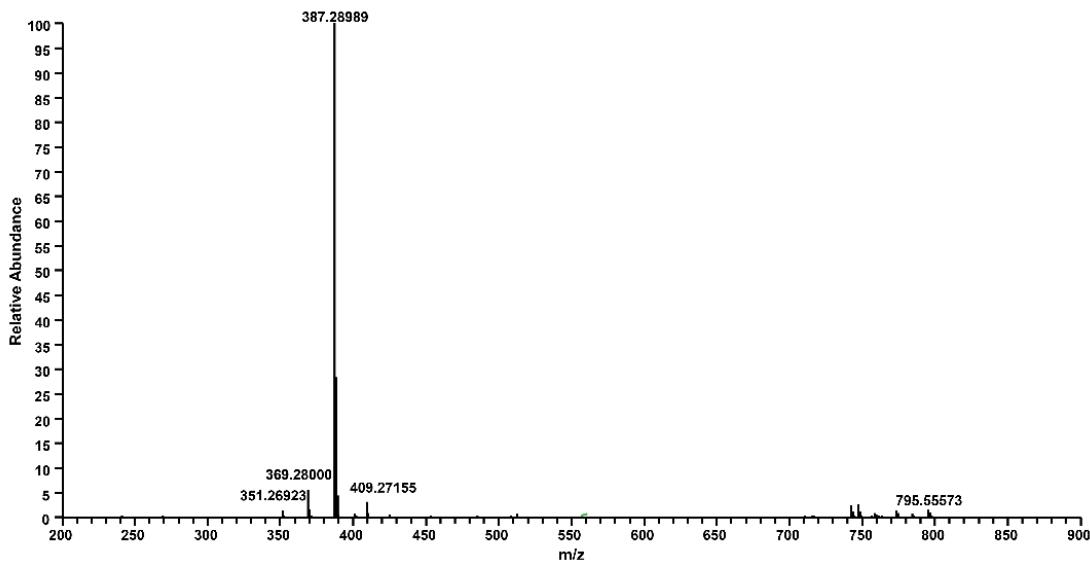


Figure S19 ESI-HRMS spectrum of compound 4

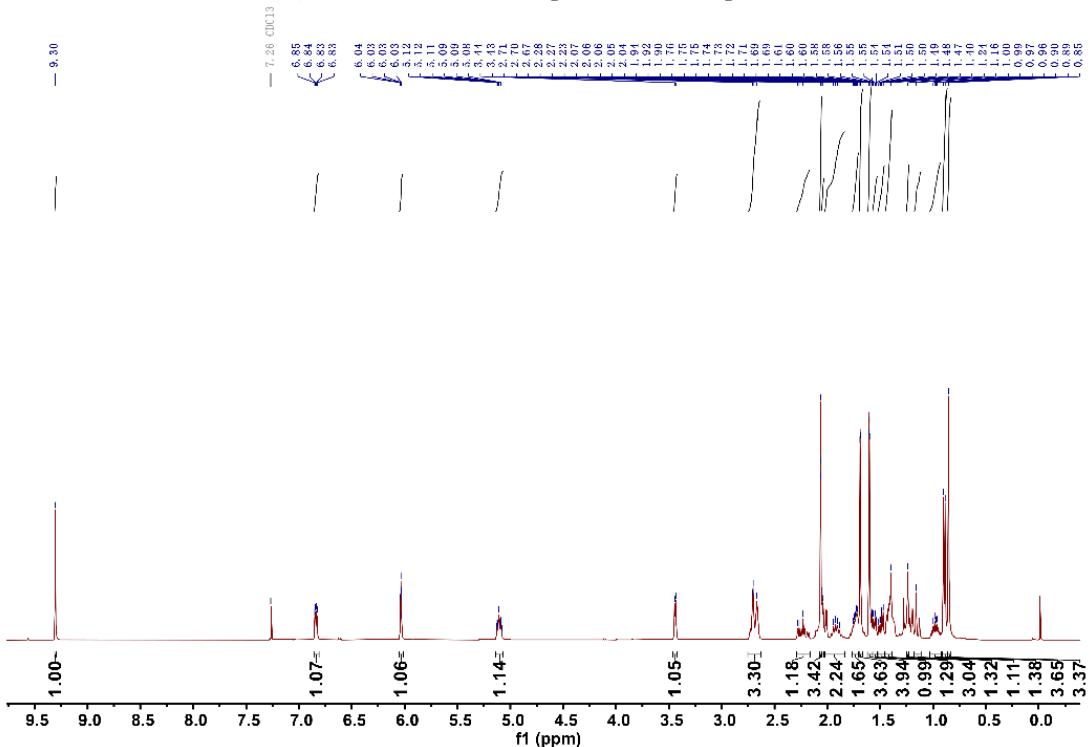


Figure S20 ^1H NMR spectrum of compound 5 (400 MHz, CDCl₃)

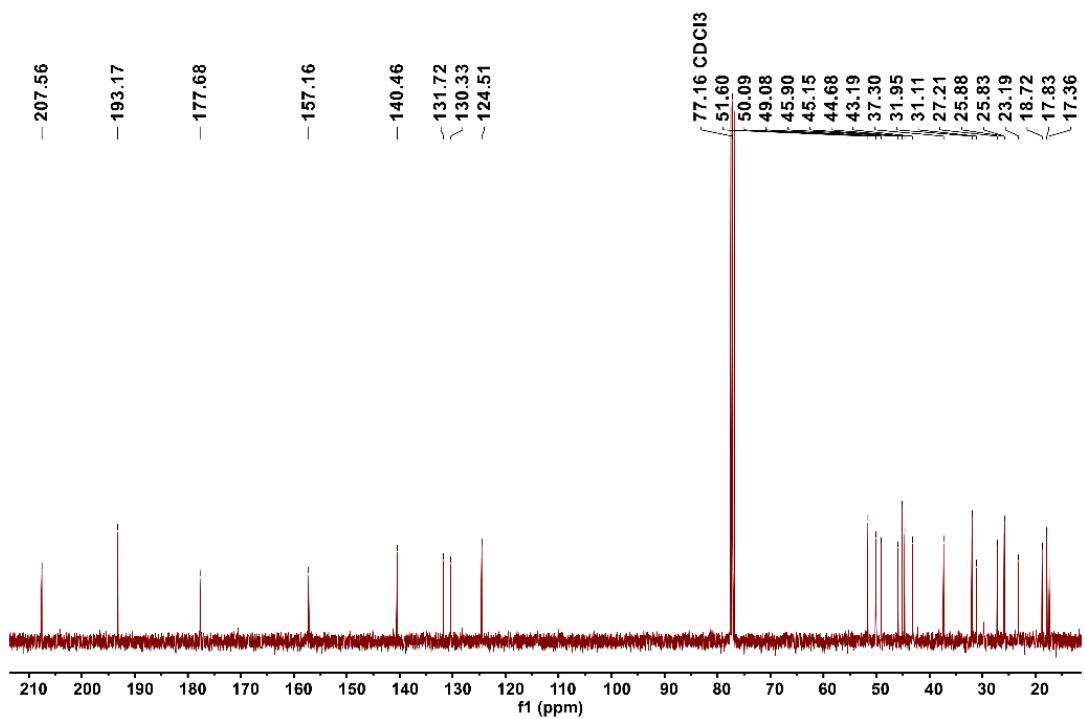


Figure S21 ^{13}C NMR spectrum of compound 5 (100 MHz, CDCl_3)

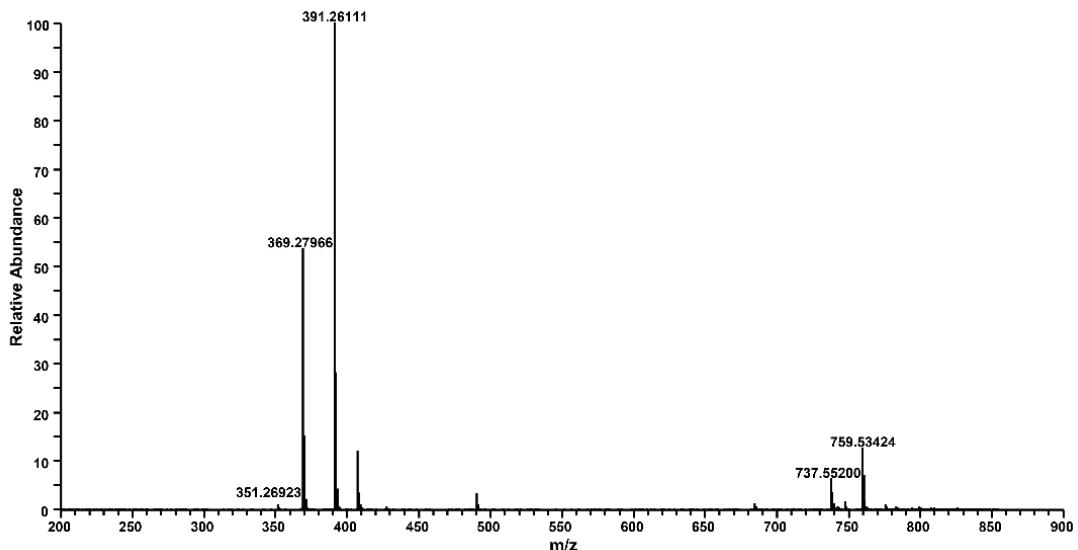


Figure S22 ESI-HRMS spectrum of compound 5

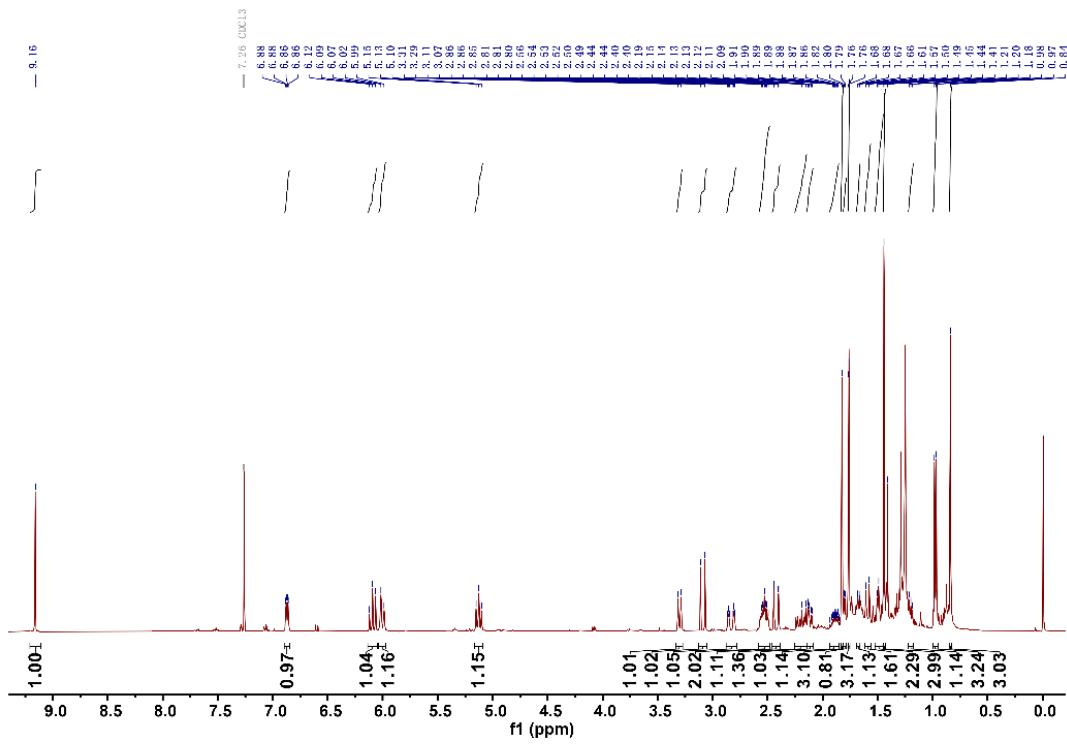


Figure S23 ^1H NMR spectrum of compound **6** (400 MHz, CDCl_3)

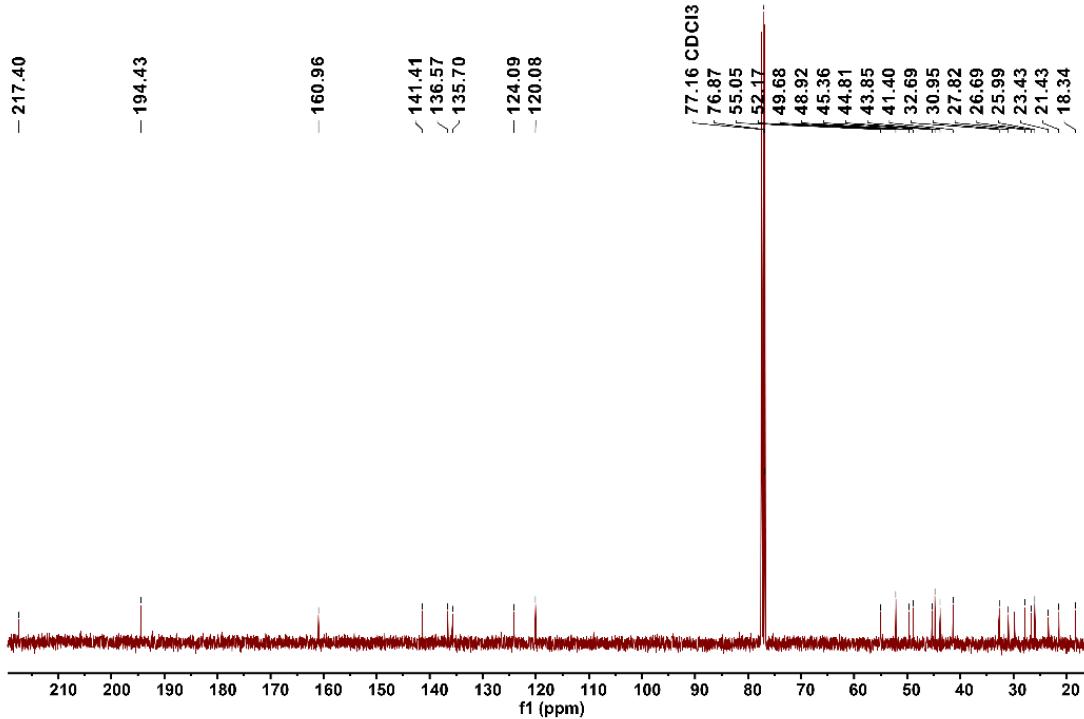


Figure S24 ^{13}C NMR spectrum of compound **6** (100 MHz, CDCl_3)

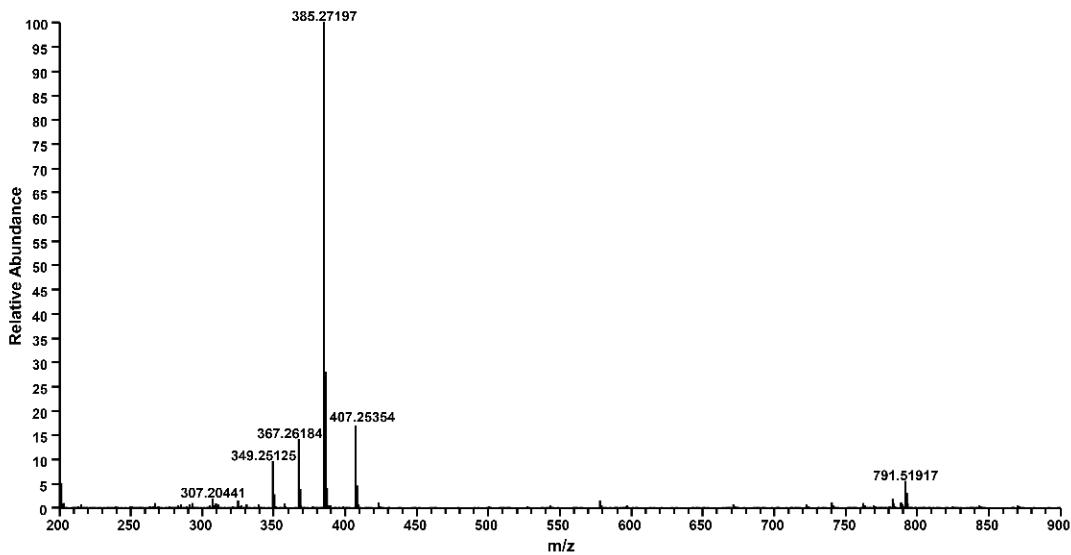


Figure S25 ESI-HRMS spectrum of compound 6

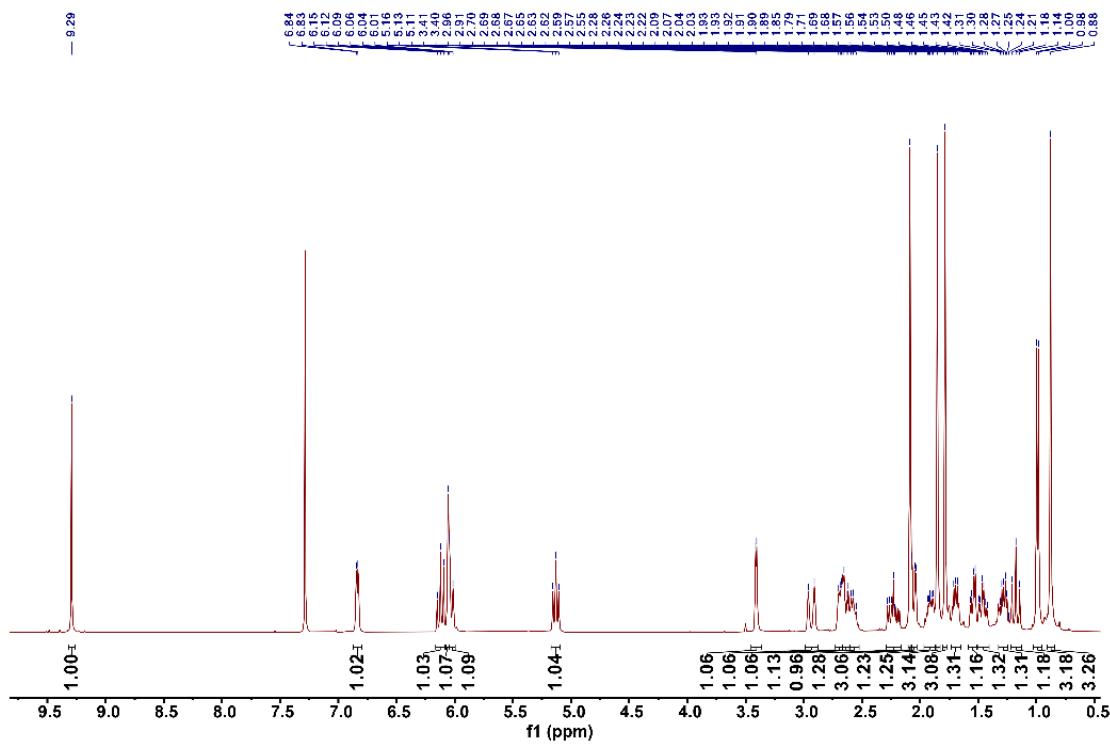


Figure S26 ^1H NMR spectrum of compound 7 (400 MHz, CDCl_3)

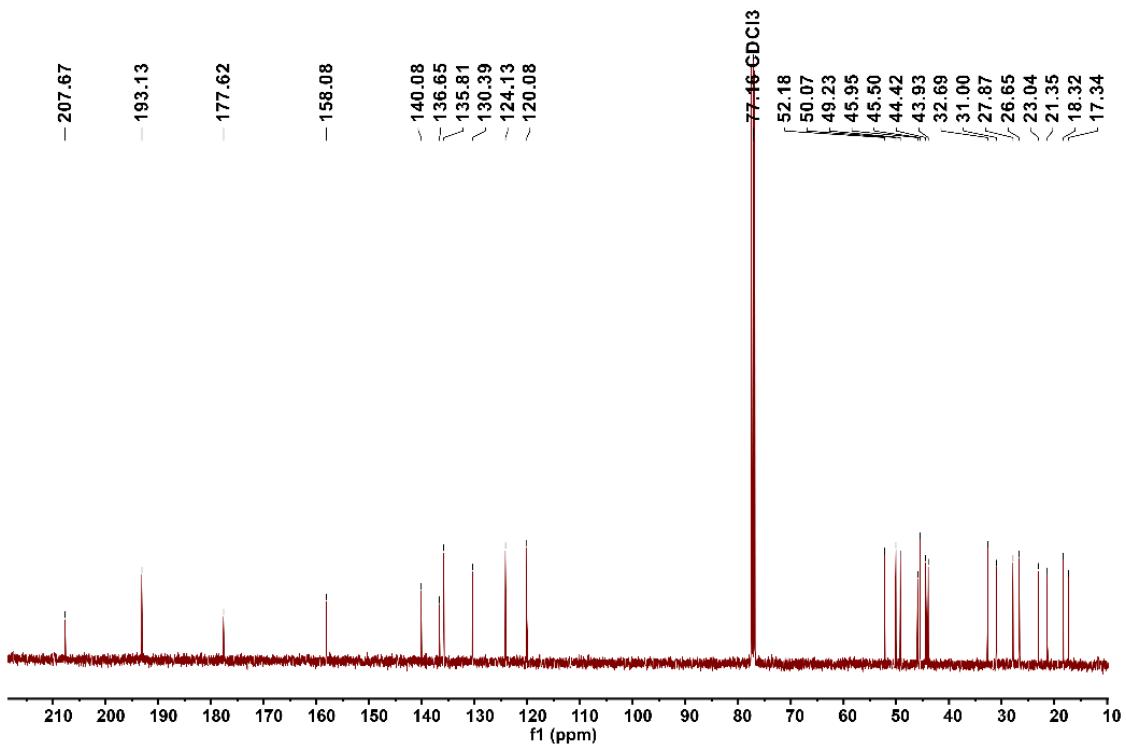


Figure S27 ^{13}C NMR spectrum of compound 7 (100 MHz, CDCl_3)

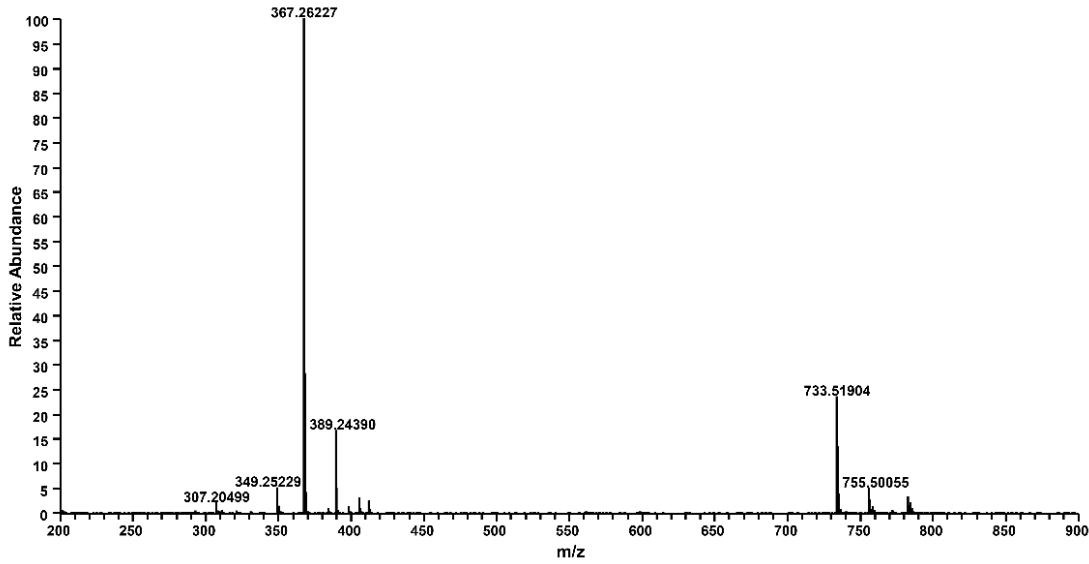


Figure S28 ESI-HRMS spectrum of compound 7

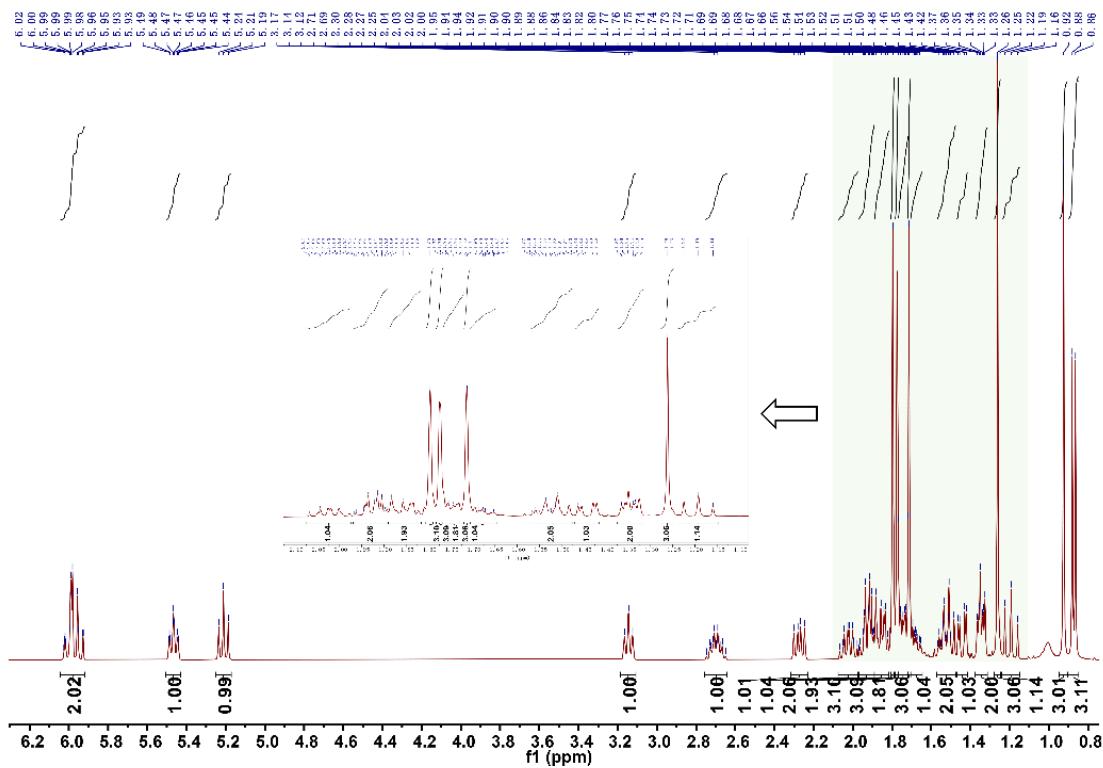


Figure S29 ^1H NMR spectrum of compound **10** (400 MHz, CDCl_3)

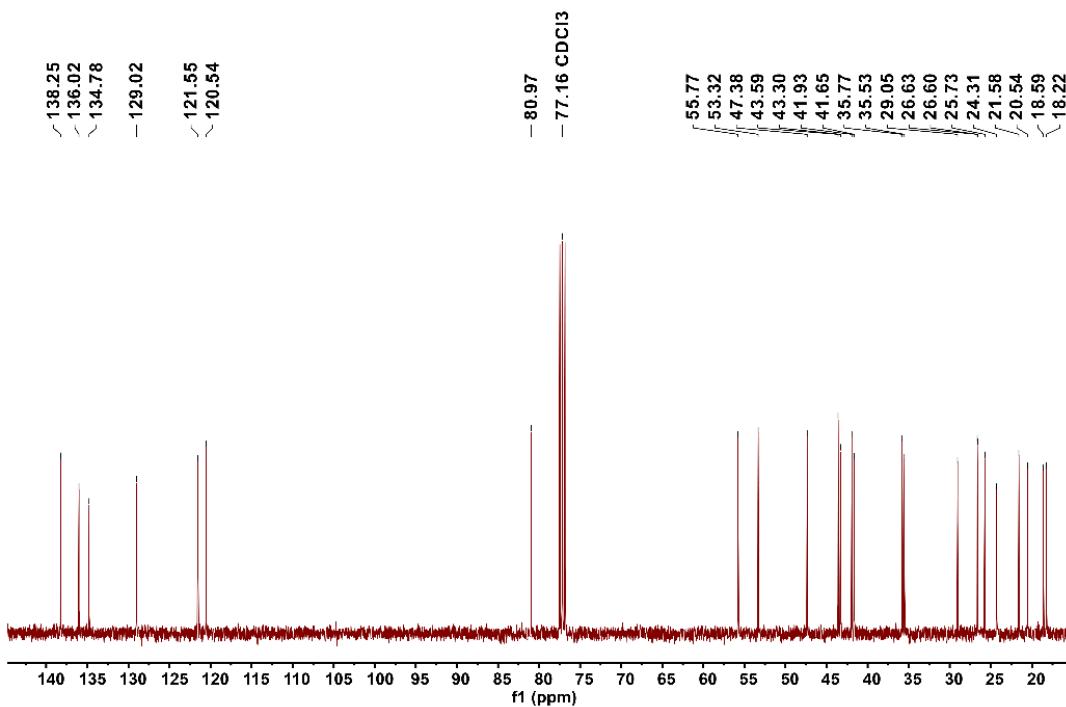


Figure S30 ^{13}C NMR spectrum of compound **10** (100 MHz, CDCl_3)

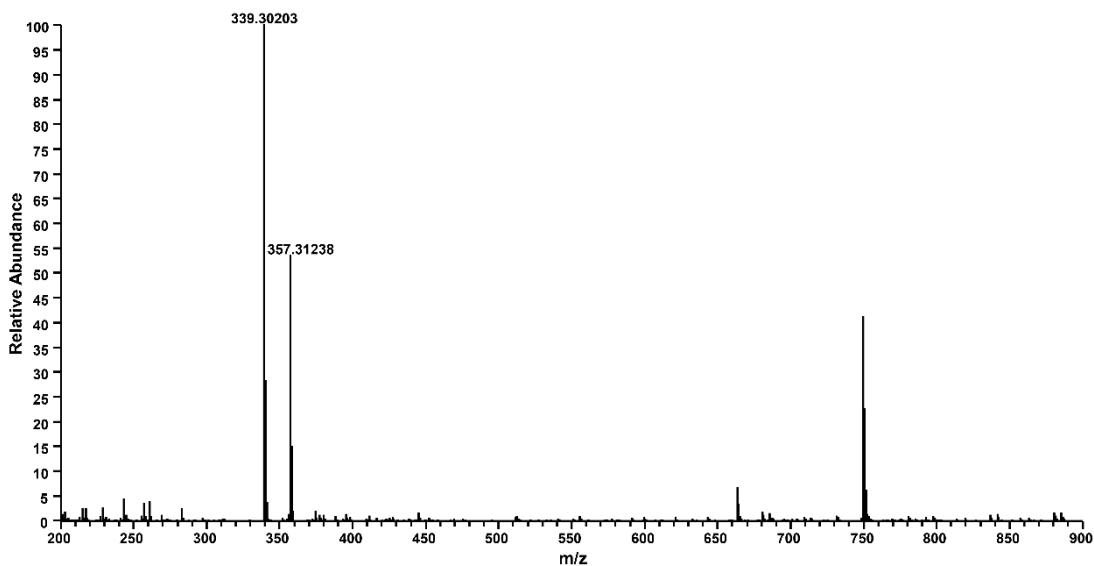


Figure S31 ESI-HRMS spectrum of compound **10**

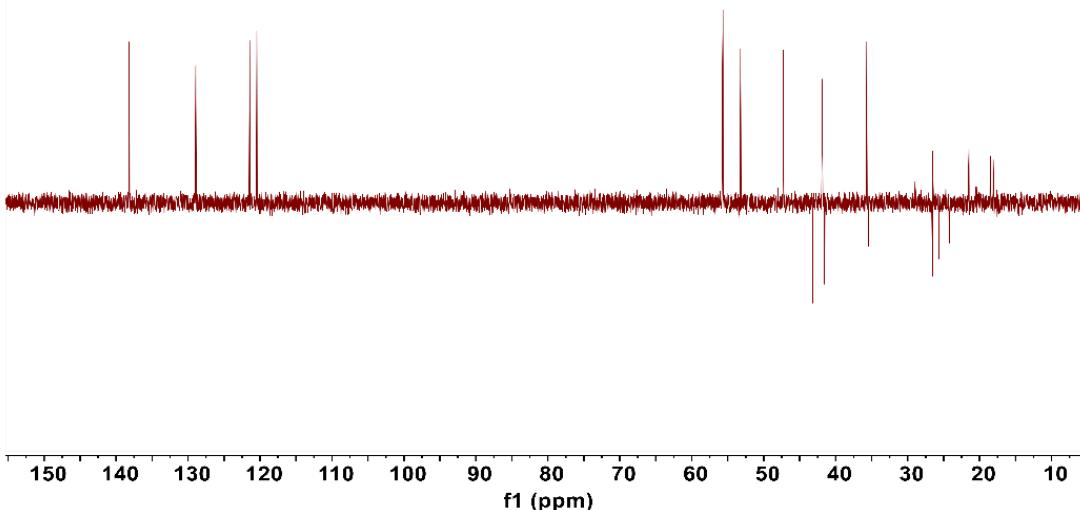


Figure S32 DEPT135 of compound **10** (100 MHz, CDCl_3)

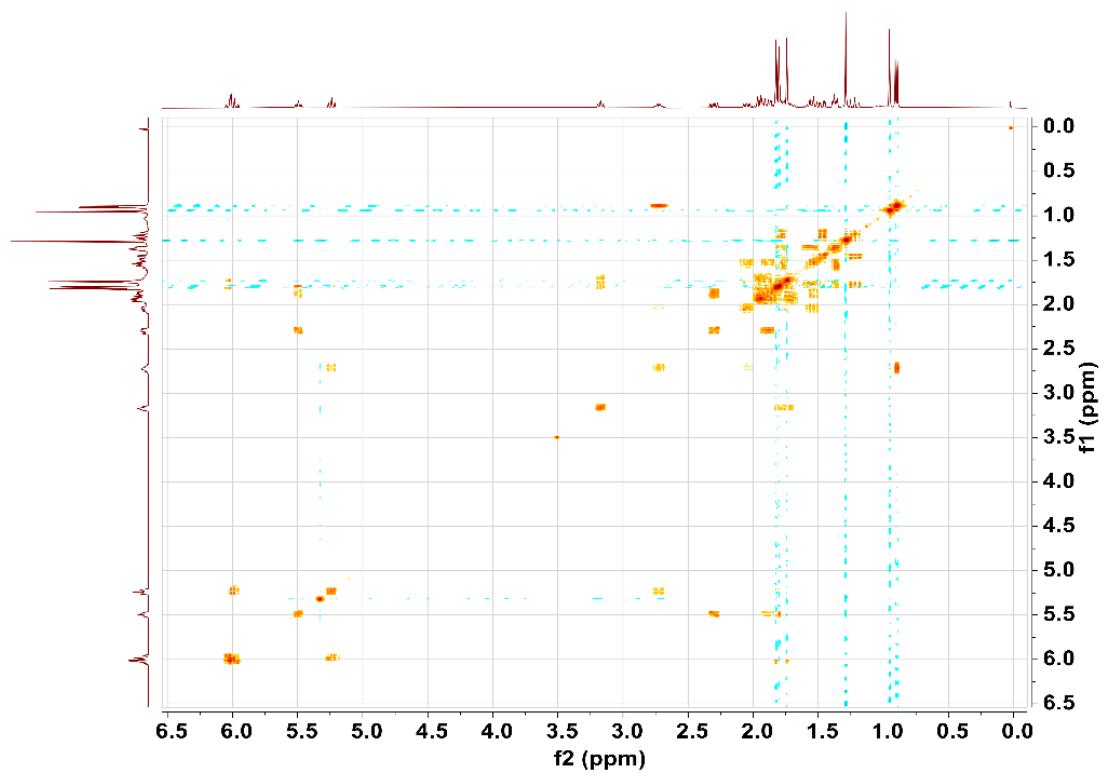


Figure S33 ¹H-¹H COSY spectrum of compound 10 (400 MHz, CDCl₃)

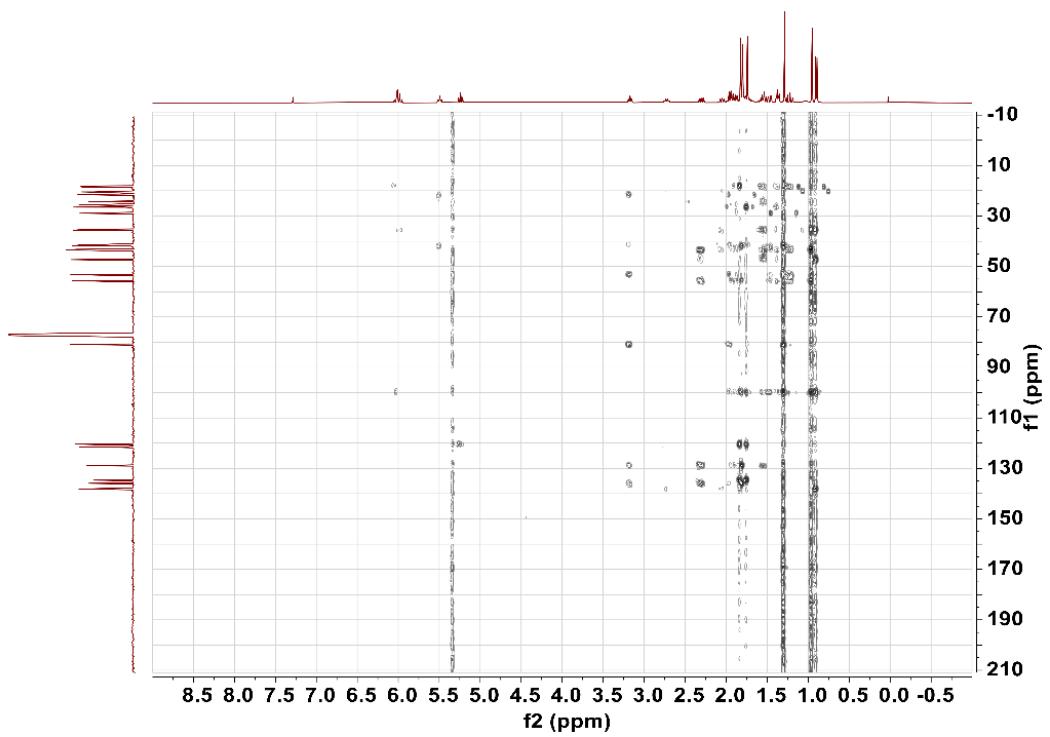


Figure S34 HMBC spectrum of compound 10 (400 MHz, CDCl₃)

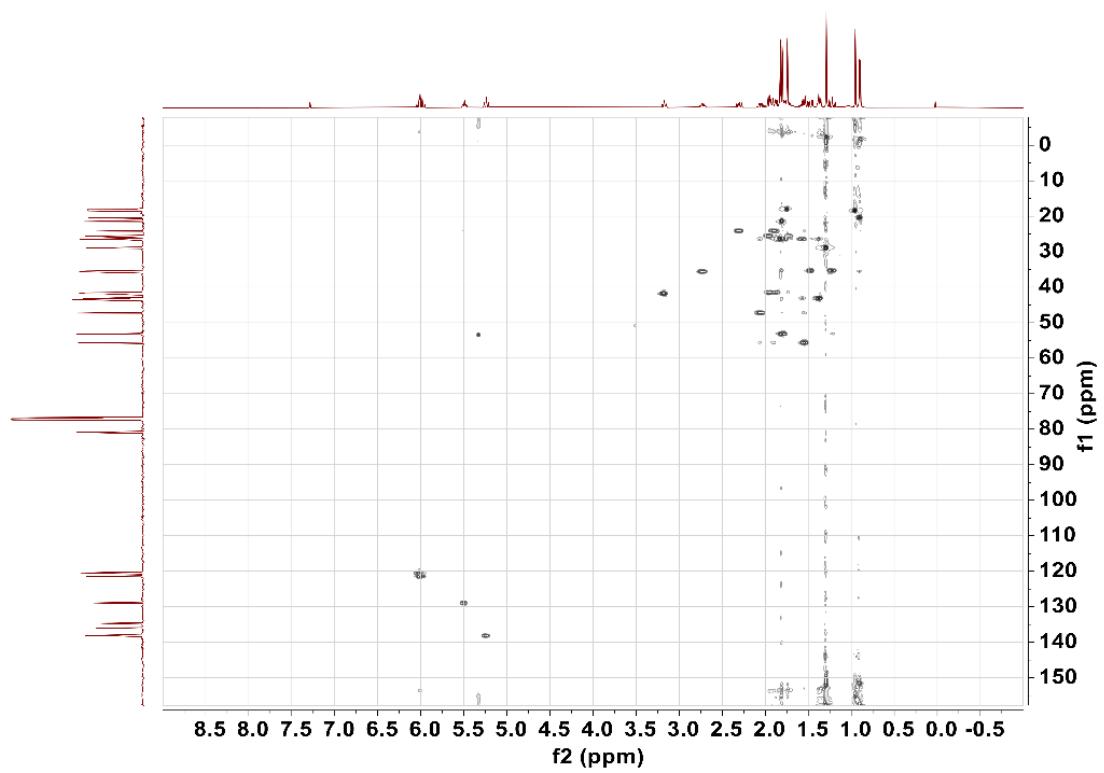


Figure S35 HSQC spectrum of compound 10 (400 MHz, CDCl₃)

Supplementary references

1. Wei, H., Itoh, T., Kinoshita, M., Nakai, Y., Kurotaki, M., & Kobayashi, M. Cytotoxic sesterterpenes, 6-epi-ophiobolin G and 6-epi-ophiobolin N, from marine derived fungus *Emericella variecolor* GF10. *Tetrahedron*. **2004**, 60, (28), 6015-6019.
2. Chiba, R., Minami, A., Gomi, K., & Oikawa, H. Identification of ophiobolin F synthase by a genome mining approach: a sesterterpene synthase from *Aspergillus clavatus*. *Org. Lett.* **2012**, 15, (3), 594-597.
3. Zhu, T., Lu, Z., Fan, J., Wang, L., Zhu, G., Wang, Y., Li, X., Hong, K., Piyachaturawat, P., Chairoungdua, A., & Zhu, W. Ophiobolins from the mangrove fungus *Aspergillus ustus*. *J. Nat. Prod.* **2018**, 81, (1), 2-9.
4. Chai, H., Yin, R., Liu, Y., Meng, H., Zhou, X., Zhou, G., Bi, X., Yang, X., Zhu, T., Zhu, W., Deng, Z., & Hong, K. Sesterterpene ophiobolin biosynthesis involving multiple gene clusters in *Aspergillus ustus*. *Sci. Rep.* **2016**, 6, 27181-27191.
5. Narita, K., Chiba, R., Minami, A., Kodama, M., Fujii, I., Gomi, K., & Oikawa, H. Multiple oxidative modifications in the ophiobolin biosynthesis: P450 oxidations found in genome mining. *Org. Lett.* **2016**, 18, (9), 1980-1983.