A genetic and biochemical map for the biosynthesis of occidiofungin, an antifungal produced by *Burkholderia contaminans* strain MS14

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Supplementary Table 1

Table S1. Primers used for this study

Primer	**Sequence (5'-3')**
ORF10F	5'-GAGCGTCTGCAGGTTCGGATAGG
ORF10R	5'-TCTCGGCCTGGATTCGCTGGT
occEF	5'-CTTCCCGGCGCACTTCACAG
occER	5'-ATCGTCGCCGGCCGCAATCA
ORF12F	5'-GGAACAGATGGGCCTGATTGAAG
ORF12R	5'-AGCCTTCTGCGCGGATAACG
ORF13F	5'-CCGCATCACGGCTTCATTGAC
ORF13R	5'-CTCCTTCCCGCGGCTGTTTAC
ORF14F	5'-GCACGATGAAGTTGGACACG
ORF14R	5'-CGCCTGCTACGACGAAGG
ORF15F	5'-GCCATCGTTCGCATTTCGTTTC
ORF15R	5'-CAGGCTGGCGGTGGACATCA
ORF16F	5'-CGAGACCGGCTGGCATGTTCA
ORF16R	5'-CTGCGGGAAGTCGGGGCGTAT
MoccEF	5'-GTCCGGGGCAAACACGAAGTC
MoccER	5'-CTCCTTGGATTACGGGGCAGAC
EoccEF2*	5'-CCCAAGCTTATGCTTCCCGATACA
EoccER2*	5'-CCCAAGCTTGCTTCTTGTAGTCAG

* Underlined letters represent restriction endonuclease sites.

Supplementary Table 2

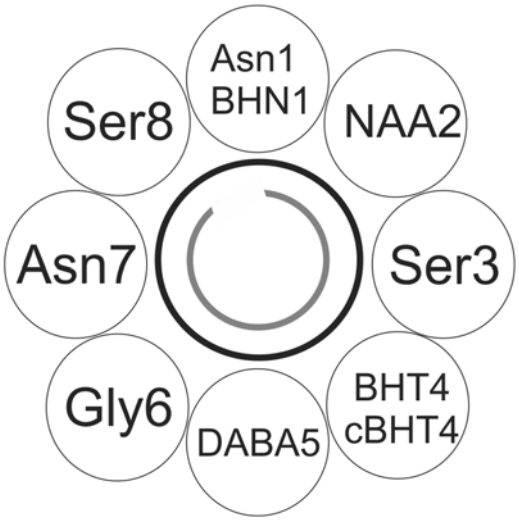
Chemical Shifts[a]

Amino Acid	H^N	H^α	H^β	Other proton
Asn 1	8.08	4.49[50.39]	2.52, 2.32 [37.42]	γ-NH2: 7.23, 6.84
	7.97	4.51[50.19]	2.52, 2.32 [37.42]	γ-NH2: 7.23, 6.84
	7.89	4.51[50.27]	2.59, 2.41 [37.42]	γ-NH2: 7.23, 6.84
	7.76	4.56[50.27]	2.59, 2.41 [37.42]	γ-NH2: 7.23, 6.84
BHN 1	7.89	4.60[55.95]	3.98[72.41]	γ-NH2: 7.20, 6.77, β-OH: 5.68
	7.81	4.63[55.95]	4.03[72.41]	γ-NH2: 7.20, 6.77, β-OH: 5.63
NAA 2	7.53			C2:CH2- 2.34[41.32], C3:CH- 4.12[44.85], C4:CH2- 1.74, 1.37[39.07], C5:CH- 3.43[67.70], C5:OH- 4.15, C6:CH- 3.07[75.06], C6:OH- 4.08, C7:CH- 3.73[77.70], C8:CH2- 1.52[30.48], C9-C17:CH2- 1.25[25.30], C18:CH3- 0.83[14.36]
	7.49			C2:CH2- 2.35[41.32], C3:CH- 4.13[44.85], C4:CH2- 1.72, 1.37[39.07], C5:CH- 3.43[67.70], C5:OH- 4.15, C6:CH- 3.07[75.06], C6:OH- 4.08, C7:CH- 3.73[77.70], C8:CH2- 1.52[30.48], C9-C17:CH2- 1.25[25.30], C18:CH3- 0.83[14.36]
	7.34			C2:CH2- 2.38, 2.29[41.32], C3:CH- 4.19[44.85], C4:CH2- 1.74, 1.33[39.07], C5:CH- 3.43[67.70], C5:OH – 4.15, C6:CH- 3.07[75.06], C6:OH- 4.08, C7:CH- 3.73[77.70], C8:CH2- 1.52[30.48], C9-C17:CH2- 1.25[25.30], C18:CH3- 0.83[14.36]
	7.31			C2:CH2- 2.38, 2.29[41.32], C3:CH- 4.16[44.85], C4:CH2- 1.74, 1.3[39.07], C5:CH- 3.43[67.70], C5:OH- 4.15, C6:CH- 3.07[75.06], C6:OH- 4.08, C7:CH- 3.73[77.70], C8:CH2- 1.52[30.48], C9-C17:CH2- 1.25[25.30], C18:CH3- 0.83[14.36]
Ser 3	8.14	4.11[56.15]	3.42, 3.27 [61.75]	β-OH: 4.95
	8.11	4.04[56.15]	3.42, 3.27 [61.75]	β-OH: 4.97
	8.06	4.21[56.15]	3.38 [61.75]	β-OH: 4.97
	8.00	4.17[56.15]	3.43, 3.36 [61.75]	β-OH: 4.95
Chloro-BHY4	8.13	4.20[60.55]	5.08[70.77]	β-OH: - 5.86, OH – 10.03, C2:CH – 7.33[127.9], C5:CH – 6.87[116.59], C6:CH – 7.09[126.19]
	7.99	4.18[60.55]	5.08[70.77]	β-OH: - 5.86, OH – 10.03, C2:CH – 7.33[127.9],

				C5:CH – 6.87[116.59], C6:CH – 7.09[126.19]
BHY4				
	8.09	4.15[60.55]	5.05[70.77]	β-OH: - 5.73, OH – 9.29, C2&C6:CH – 7.15[127.66], C3&C5:CH – 6.68[115.11]
	8.04	4.07[60.88]	4.94[71.39]	β-OH: - 5.79, OH – 9.29, C2&C6:CH – 7.15[127.66], C3&C5:CH – 6.68[115.11]
	7.95	4.13[60.55]	5.05[70.77]	β-OH: -5.73 , OH – 9.29, C2&C6:CH – 7.15[127.66], C3&C5:CH – 6.68[115.11]
	7.83	4.02[61.05]	4.94[71.39]	β-OH: - 5.65, OH – 9.29, C2&C6:CH – 7.15[127.66], C3&C5:CH – 6.68[115.11]
DABA 5	7.67	4.39[50.84]	2.08, 1.88 [30.09]	γ-H: 2.88[36.53], NH2: 7.74
Gly 6	8.04	3.76, 3.67[42.32]		
	8.00	3.76, 3.67[42.32]		
	7.91	3.79, 3.67[42.32]		
	7.86	3.80, 3.65[42.32]		
	7.71	3.83, 3.53[42.32]		
	7.68	3.83, 3.53[42.32]		
Asn 7	8.41	4.56[50.34]	2.56, 2.38 [37.42]	γ-NH2: 7.38,6.92
	8.36	4.48[50.34]	2.56, 2.38 [37.42]	γ-NH2: 7.38,6.92
	8.33	4.53[50.34]	2.56, 2.38 [37.42]	γ-NH2: 7.38,6.92
	8.28	4.44[50.34]	2.60, 2.38 [37.42]	γ-NH2: 7.38,6.92
Ser 8	7.80	4.30[55.77]	3.58[62.09]	β-OH: 4.80
	7.76	4.19[55.77]	3.58[62.09]	β-OH: 4.80
Xylose				C1:CH - 4.16 [102.65], C2:CH - 2.96 [73.55], C2:OH – 4.94, C3:CH - 3.09 [76.93], C3:OH – 4.94, C4:CH - 3.30 [61.76], C4:OH – 4.93, C5:CH2 - 3.68, 3.06 [66.18]

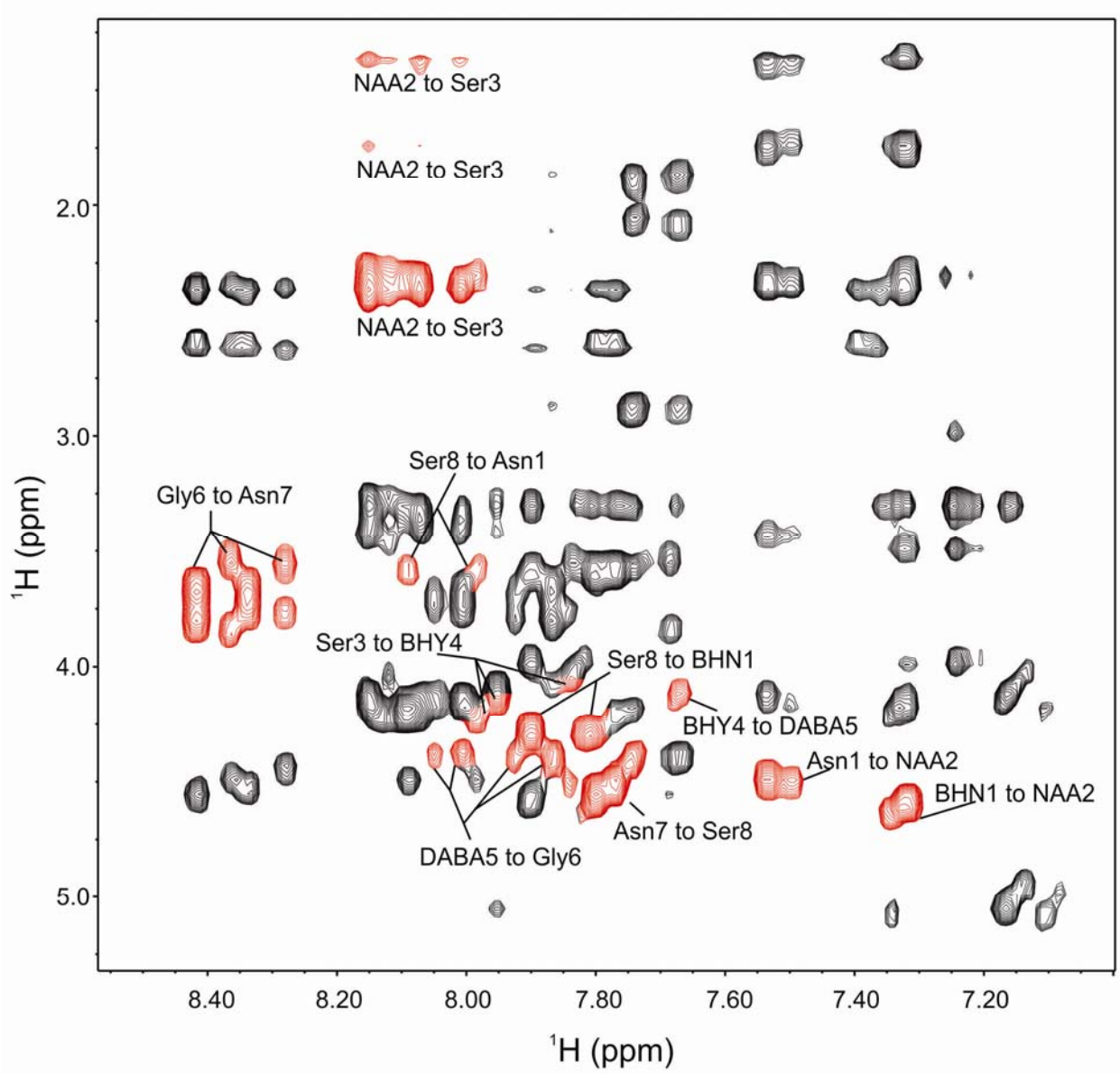
[a] Proton chemical shift values are from a TOCSY and NOESY experiments. Chemical shifts in brackets are ^{13}C values from the HSQC experiment.

Supplementary Figure 1



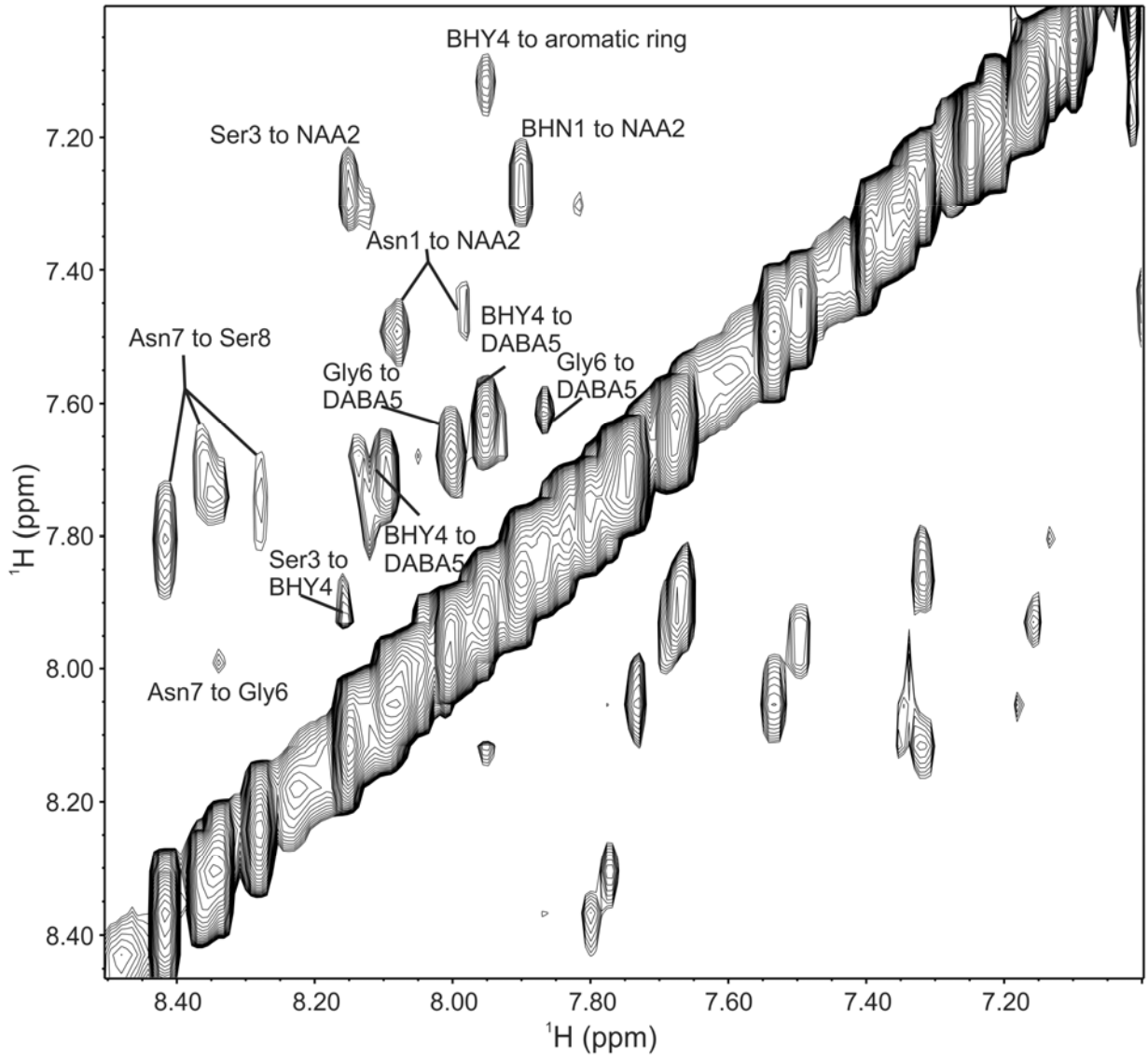
Supplementary Fig. 1 Representative diagram of the data from the NOESY NMR spectra of occidiofungin. The black circle shows the amide to alpha, and amide to side chain inter-residue interactions, while the grey circle shows amide to amide inter-residue interactions. NOEs provided a complete sequential walk along the backbone protons for each amino acid.

Supplementary Figure 2



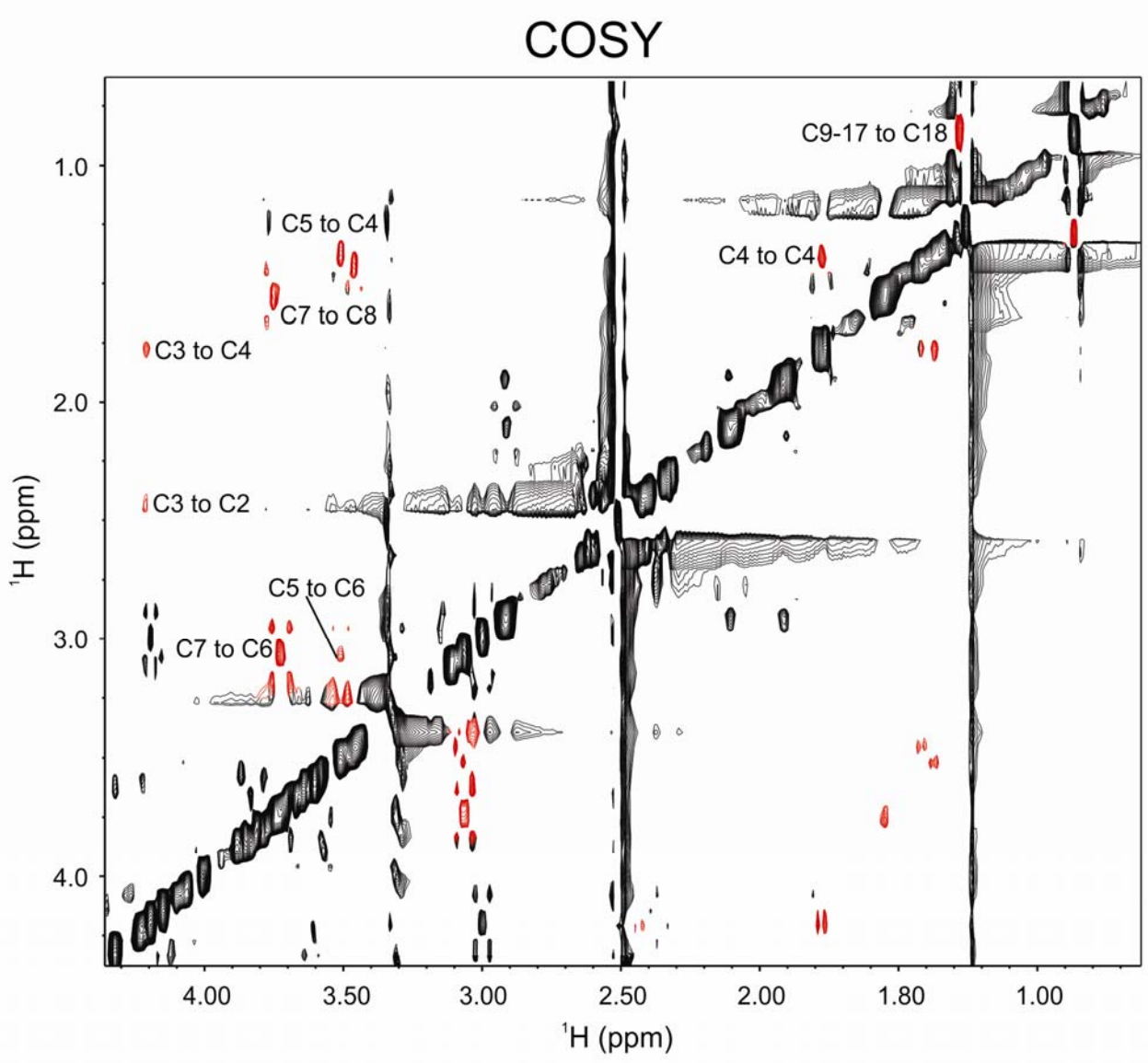
Supplementary Fig. 2: NOESY NMR spectra of occidiofungin. The expansion shows the amide to alpha, and amide to side chain interactions. The respective amino acid interactions are labeled next to their inter-residue NOE's shown in red.

Supplementary Figure 3



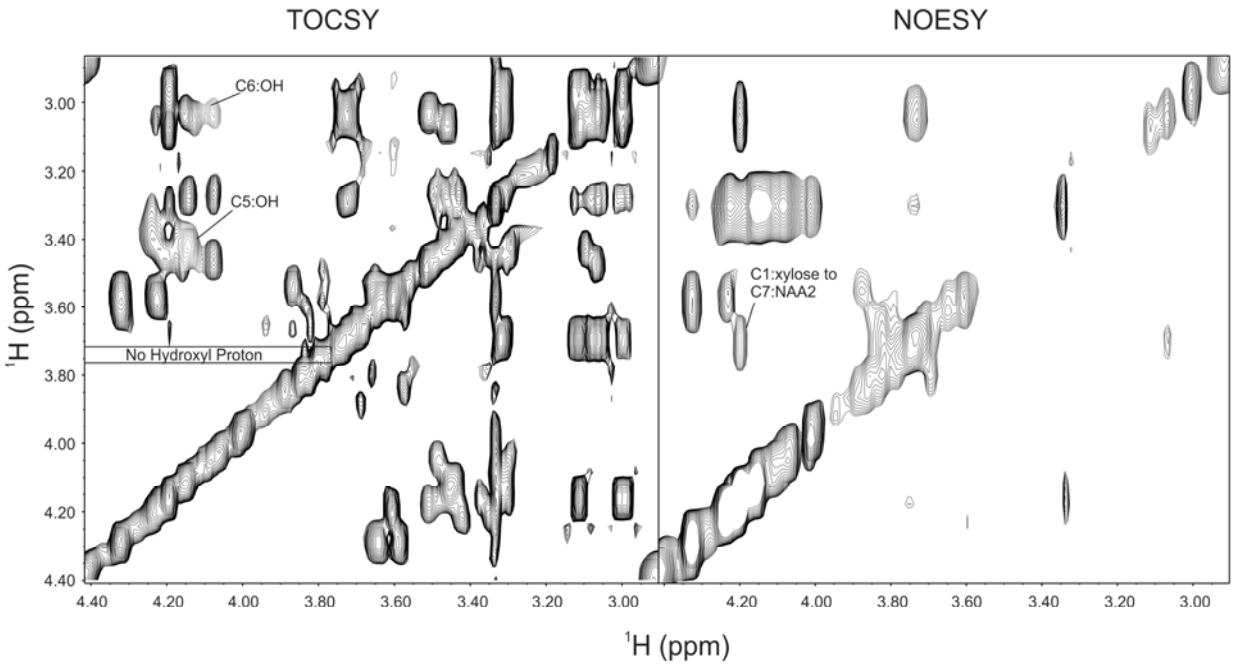
Supplementary Fig. 3: NOESY NMR spectra of occidiofungin. The expansion shows the amide to amide interactions. The respective amino acid interactions are labeled next to their inter-residue NOE's.

Supplementary Figure 4



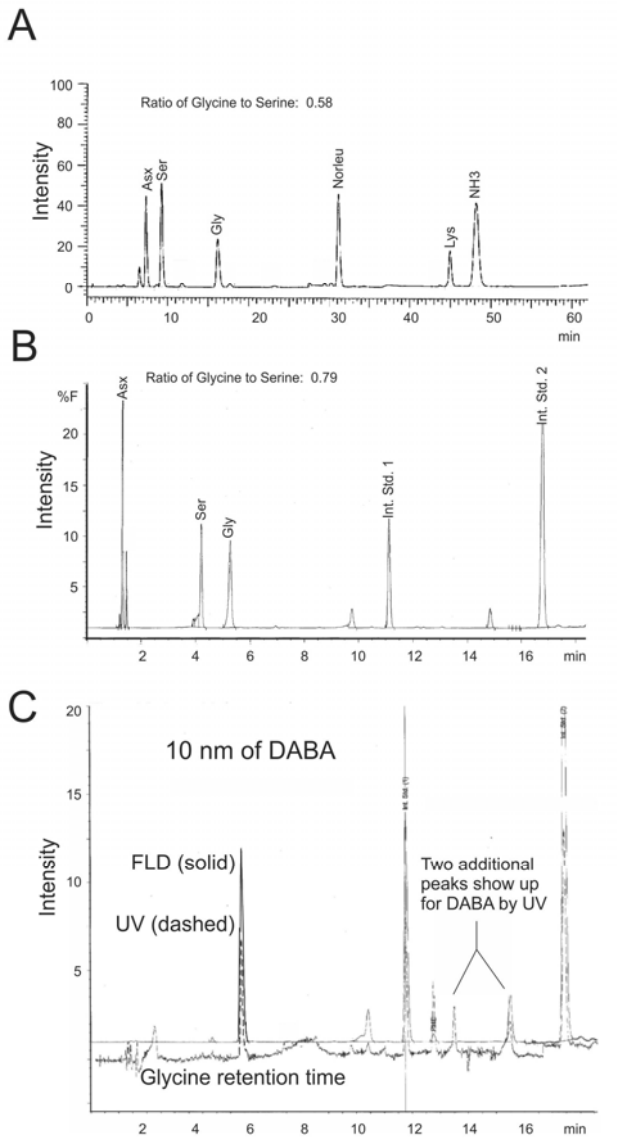
Supplementary Fig. 4: Expansion of the COSY 2D NMR Spectra showing the proton couplings within the fatty acid chain of NAA2. The couplings are shown in red and the representative carbon for each coupled proton is shown.

Supplementary Figure 5



Supplementary Fig. 5 TOCSY and NOESY NMR spectra of occidiofungin. Expansion of the TOCSY 2D NMR spectra shows the proton coupling between the proton on C5 and C6 of fatty acid chain of NAA2 to their corresponding hydroxyl proton. C7 proton has no such coupling, suggesting that the oxygen is involved in an ether linkage to the xylose sugar. Expansion of the NOESY 2D NMR spectra shows an NOE spanning the ether linkage between the proton on C7 and the proton on C1 of the xylose sugar.

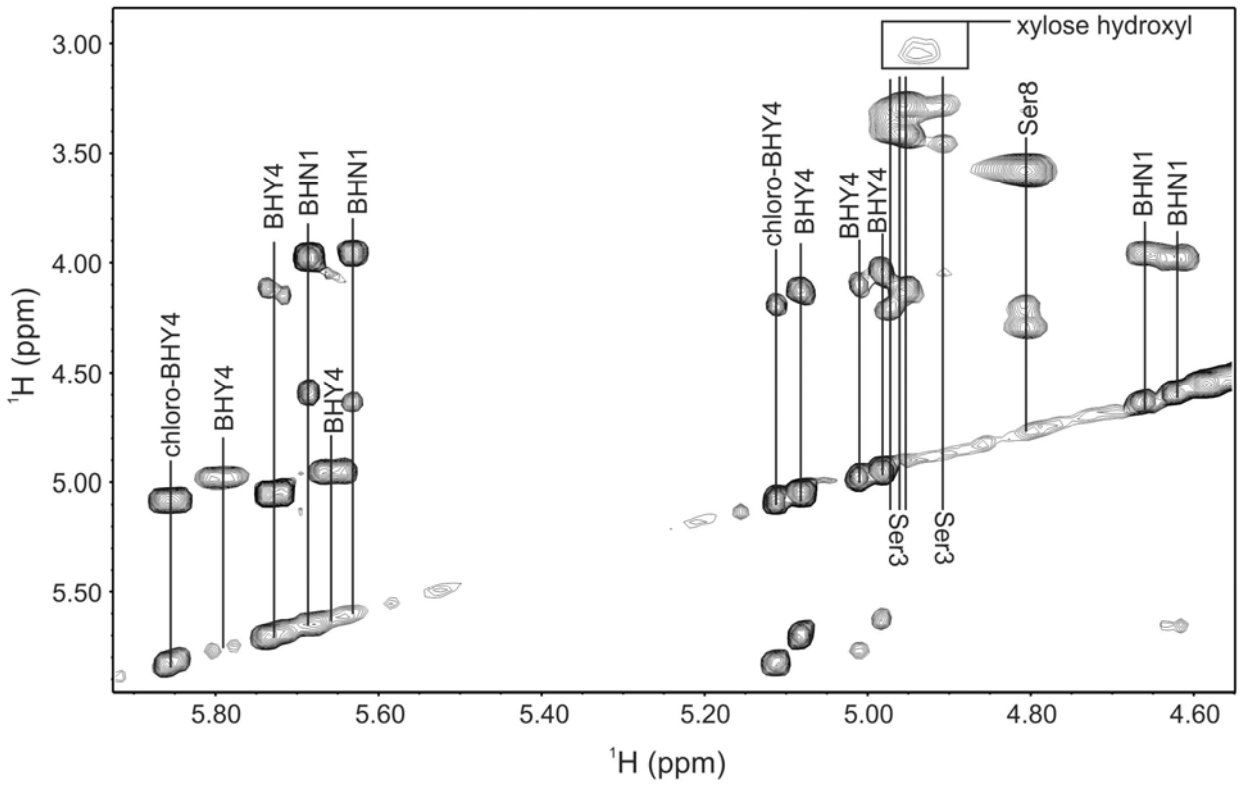
Supplementary Figure 6



Supplementary Fig. 6: Amino acid analyses. A. Data collected at the Molecular Structure Facility, UC Davis in which a "post-ion-exchange column" ninhydrin reaction detection system is used. This analysis shows the presence of lysine. B. Data collected at Texas A&M Protein Chemistry Lab using a pre-column derivatization method followed by post-column fluorescent detection. There was an increase in the ratio of glycine to serine using this method compare to the method used by Molecular Structure Facility, UC Davis. C. Using the pre-column

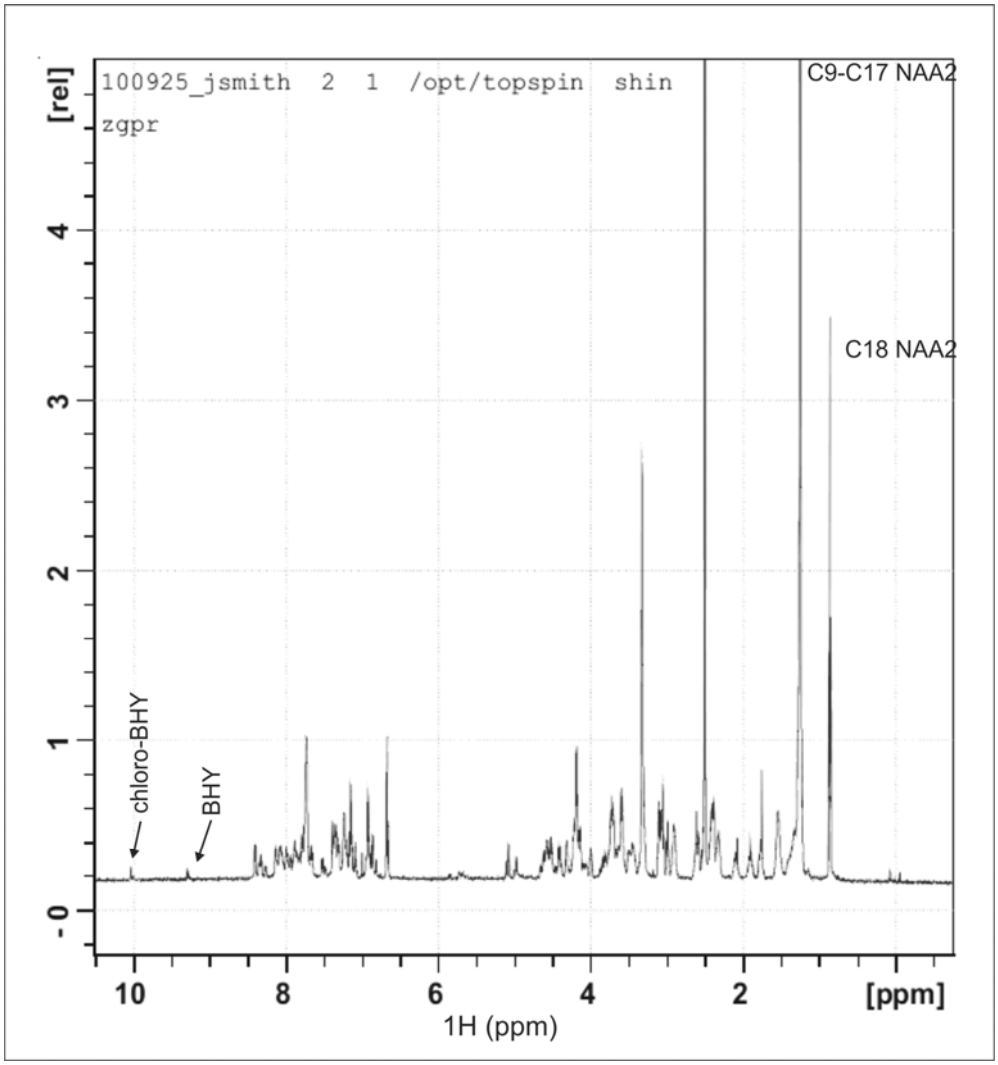
derivatization method, 10 nm of 2,4-diaminobutyric acid was prepped and is shown to co-elutes with glycine using UV and FLD detection.

Supplementary Figure 7



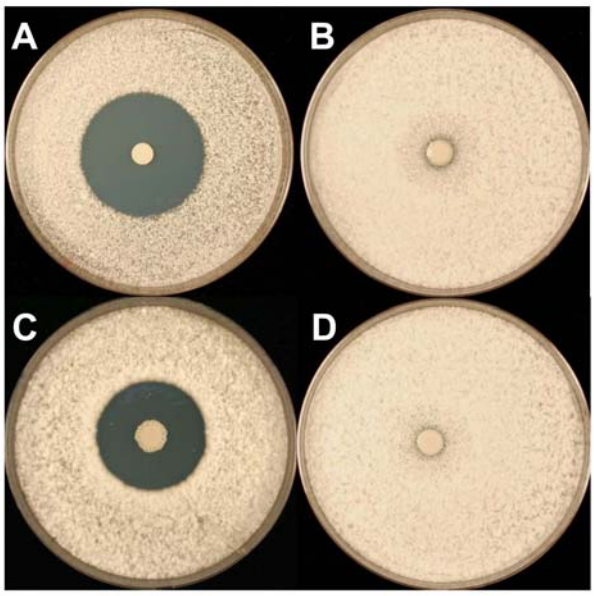
Supplementary Fig. 7 Expansion of the TOCSY 2D NMR spectra shows the hydroxyl proton chemical shifts spin systems for BHN1, Ser3, chloro-BHY4, BHY4, and Ser8.

Supplementary Figure 8



Supplementary Fig. 8: 1D Proton NMR spectra of Occidiofungin

Supplementary Figure 9



Supplementary Fig. 9 Plate bioassays of antifungal activities of *Burkholderia contaminans* strain MS14 with its mutants. Potato dextrose agar plates were inoculated with each of the strains and incubated for 3 days at 28^{o}C. The plates were oversprayed with the indicator fungus *Geotrichum candidum* and further incubated overnight. A: The wild-type strain MS14; B: MS14GG78(*ocfJ*::*nptII*); C: MS14GG78 (pGG24); D: MS14MT24(*ocfH*::Tn*5*).