Table S1. Benzomalvin Products and Intermediates

Name	Exact Mass	Molecular Formula	Compound
benzomalvin A/D	381.1477	$C_{24}H_{19}N_3O_2$	1
macrocyclic intermediate	399.1583	$C_{24}H_{21}N_3O_3$	2
linear tripeptide	417.1689	$C_{24}H_{23}N_3O_4$	3
desmethyl benzomalvin A/D	367.1321	$C_{23}H_{17}N_3O_2$	4

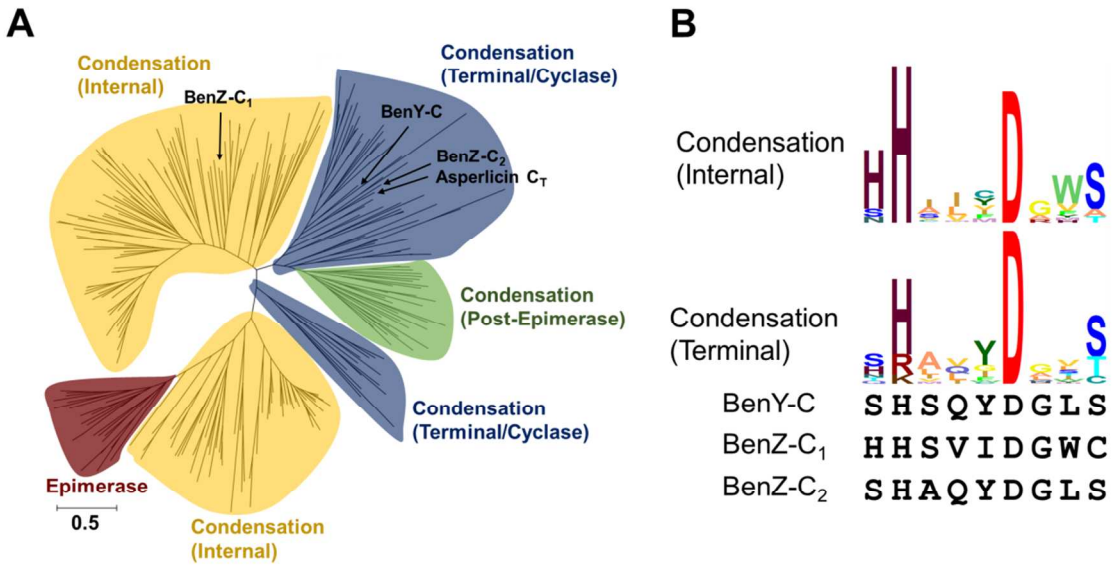

Figure S1. Bioinformatic analysis of benzomalvin C. A) Phylogenetic tree of fungal C-domains. While BenZ-C_1 clearly groups with internal C-domains, BenY-C and BenZ-C_2 both group as terminal C-domains, such as the C_T domain involved in asperlicin biosynthesis. B) Sequence alignment benzomalvin C domains and comparison to consensus sequences for internal and terminal C domains. BenY-C and BenZ-C_2

cannot be readily clearly assigned as either terminal or internal based on sequence analysis.

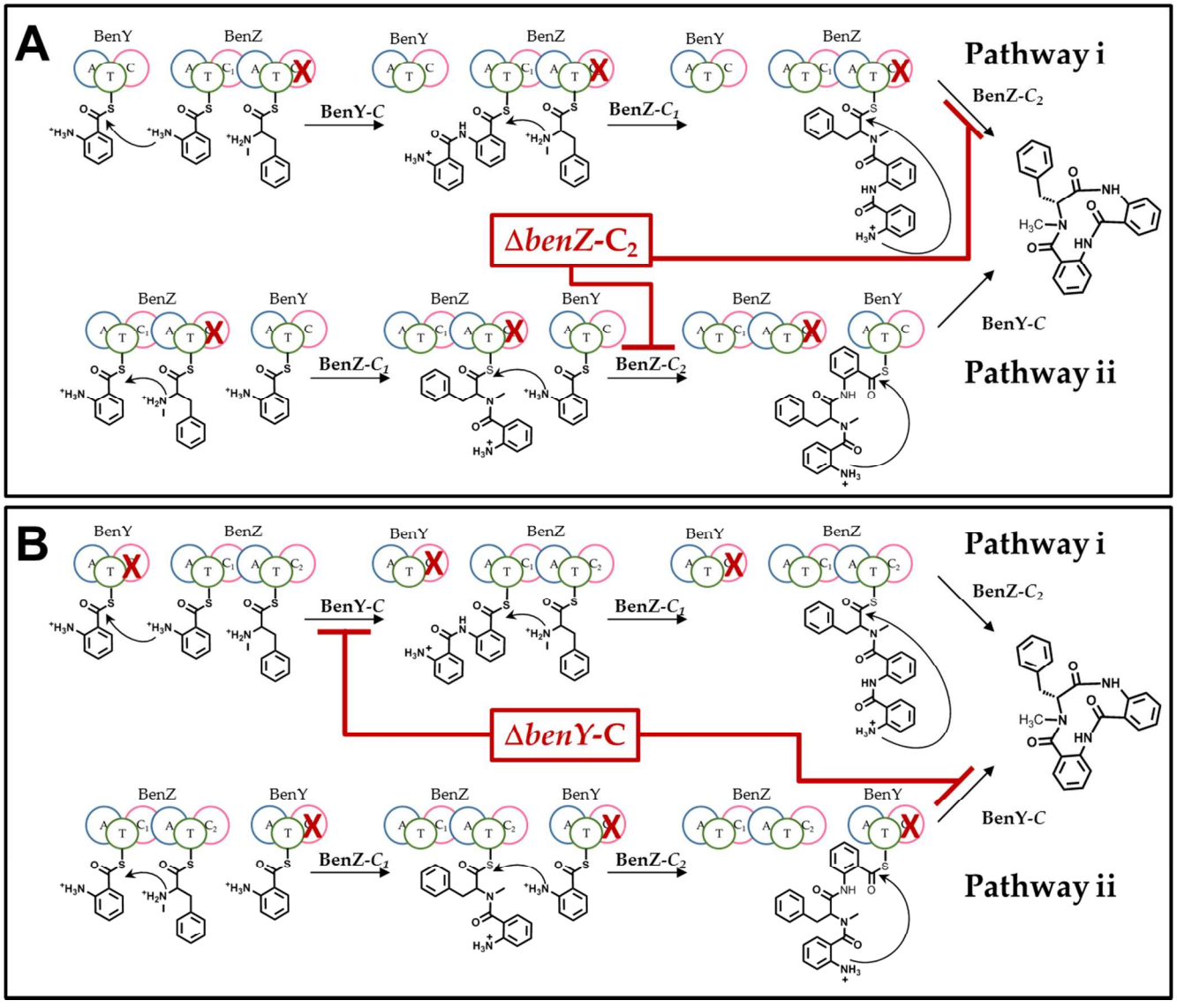

Figure S2. BenY-C is the terminal condensation domain. (A) The effect of deletion of *benZ*-C_2 (elimination of all detectable production of benzomalvin A/D or its precursors) is consistent with either i or ii. (B) The effect of deletions of *benY*-C (reduction but not elimination of benzomalvin A/D production) is inconsistent with i,

since BenY-C would be needed to facilitate protein-protein interactions between BenZ and BenY, and uncatalyzed formation of the BenZ-dipeptidyl precursor would be highly unlikely. The effect of *benY*-C deletion is however consistent with ii, since the local concentration of the terminal amino group is likely sufficiently high enough for intramolecular nucleophilic attack on the thioester bond without catalytic support from BenY-C.

Table S2

ΔAtFAC9J20BenY-C-domain	
At20BenYCkan-F	GTGAGCTCTGTCTGTCGCATCTTCGGGAACAGACATCGTTGCTGGACTCTcgacctgcagcctgttgacaa
At20BenYCkan-R	GAACGCGACCCCATACGATCAATCAAAGCACCCTTCCAATCACCATCTCAgtcgaggctgatcagcga
ΔAtFAC9J20BenZ-C_2-domain	
At20BenZCkan-F	ATGATACATTCTGGGTGGTGGATGTACCCTCCTCCGGAAGAGATAGATCAcgacctgcagcctgttgacaa
At20BenZCkan-R	TTGACGGTGAGCTCCGTGCAGTGGCAACGCGCGAGCTAGTCAGCCGCGGCgtcgaggctgatcagcga