Penitrem A is one of the most elaborated members of the fungal indole diterpenes. Two separate penitrem gene clusters were identified using genomic and RNA sequencing data, and 13 out of 17 transformations in the penitrem biosynthesis were elucidated by heterologous reconstitution of the relevant genes. These reactions involve 1) a prenylation-initiated cationic cyclization to install the bicyclo[3.2.0]heptane skeleton (PtmE), 2) a two-step P450-catalyzed oxidative processes forming the unique tricyclic penitrem skeleton (PtmK and PtmU), and 3) five sequential oxidative transformations (PtmKULNJ). Importantly, without conventional gene disruption, reconstitution of the biosynthetic machinery provided sufficient data to determine the pathway. It was thus demonstrated that the Aspergillus oryzae reconstitution system is a powerful method for studying the biosynthesis of complex natural products.