Enzymatic spiroketal formation via oxidative rearrangement and distortion of a polycyclic polyketide backbone

*Britta Frensch[1,2], Thorsten Lechtenberg[1,2], Michel Kather[2,3], Zeynep Yunt[5], Bernd Kammerer[2,4], Michael Müller[6], Jörn Piel[7], Robin Teufel[1,2]**

[1]Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany.

[2]ZBSA, Center for Biological Systems Analysis, University of Freiburg, 79104 Freiburg, Germany.

[3]Hermann Staudinger Graduate School, University of Freiburg, Hebelstrasse 27, 79104 Freiburg, Germany

[4]University of Freiburg, BIOSS Center for Biological Signaling Studies, Freiburg, Germany.

[5]Department of Molecular Biology and Genetics, Koç University, Istanbul, 34450, Turkey.

[6]Institute of Pharmaceutical Sciences, University of Freiburg, Albertstrasse 25, 79104 Freiburg, Germany.

[7]Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zürich, Vladimir-Prelog-Weg 4, 8093 Zürich, Switzerland.

Enzyme-catalyzed redox tailoring steps often confer structural diversity and bioactivity to natural products. A prime example is the generation of the bisbenzannulated [5,6]-spiroketal pharmacophore in the bacterial rubromycin/griseorhodin family of aromatic polyketides that exhibit a wide array of bioactivities such as the inhibition of human telomerase or DNA helicase. Here we elucidate the complex flavoenzyme-driven formation of the spiroketal moiety that is markedly distinct from conventional (bio)synthetic strategies. Accordingly, a planar, polycyclic aromatic precursor undergoes extensive enzymatic oxidative rearrangement that ultimately results in a drastic distortion of the carbon skeleton. The one-pot *in vitro* reconstitution of these key tailoring steps as well as the characterization of reactive intermediates allow unravelling of the intricate underlying reactions during which several carbon-carbon bonds are broken and two C1-moieties become eliminated. This work provides detailed insight into a highly perplexing redox

tailoring processes that sets the stage for the (chemo)enzymatic production and bioengineering of bioactive spiroketal-containing polyketides.