Biohalogenation: Nature's Way to Synthesize Halogenated Metabolites†

Claudia Wagner, Mustafa El Omari, and Gabriele M. König*

Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, D-53115 Bonn, Germany

Received October 15, 2008

Halogenated natural products are widely distributed in nature, some of them showing potent biological activities. Incorporation of halogen atoms in drug leads is a common strategy to modify molecules in order to vary their bioactivities and specificities. Chemical halogenation, however, often requires harsh reaction conditions and results in unwanted byproduct formation. It is thus of great interest to investigate the biosynthesis of halogenated natural products and the biotechnological potential of halogenating enzymes. This review aims to give a comprehensive overview on the current knowledge concerning biological halogenations.

Introduction

Approximately 4000 compounds known to be produced by living organisms are organohalogens.[1−3] Structural classes range from simple phenolic and aliphatic compounds to complex polyketides and oligopeptides (Figures 1−5). Biological activities are equally diverse, e.g., antibiotic and antitumor activity as known for chloramphenicol (**34**) (Figure 6), glycopeptide antibiotics (**9**−**12**), and cryptophycin (**7**, Figure 3), respectively, or specific proteasome inhibition as found for salinosporamide A (**57**, Figure 11). A detailed mechanistic understanding not only will further our understanding of natural biological halogenation processes but will also give rise to alternative methods for generating halogenated analogues of biologically active metabolites.[4−7] By means of such enzymatic reactions, modified compounds may be generated with potentially improved biological properties.[8−11] Halogenating enzymes have been discovered from a broad range of organisms and mainly can be grouped into two classes: (i) highly substrate-specific halogenases requiring dioxygen for enzymatic activity and (ii) less specific haloperoxidases (HPO) utilizing hydrogen peroxide. In dioxygen-dependent halogenases either flavin ($FADH_2$-dependent halogenases) or α-ketoglutarate (non-heme Fe^{II}/α-ketoglutarate/O_2-dependent halogenases) are found to function as co-substrates. Furthermore, methyltransferases are involved in the formation of the carbon halogen bonds of CH_3Cl, CH_3Br, and CH_3I, and other enzymes requiring *S*-adenosyl-L-methionine (**53**) as catalyst have been identified to be involved in fluorination and chlorination.[12,13]

Special credit is due to the work of van Pée and Walsh for conducting pioneering research in the field of dioxygen-dependent halogenases and for providing comprehensive reviews.[4,7,11,14−20] A recently published comparison of the halide-binding sites of different halogenating enzymes also discussed the corresponding selectivity,[10] and further insights concerning the mechanisms of enzymatic halogenation were described by Dolfing,[21] Anderson and Chapman,[2] and Murphy,[5] as well as Naismith.[22] In addition, reviews focusing on haloperoxidases have recently been published by Hofrichter and Ullrich,[6] Butler and Carter-Franklin,[23] and Raugei and Carloni,[24] as well as Bortolini and Conte.[25] The present review aims to summarize current knowledge on enzymes involved in biohalogenation processes with a special emphasis on the structure of halogenated secondary metabolites of organisms.

$FADH_2$-Dependent Halogenases Involved in the Halogenation of Aromatic Moieties

$FADH_2$-dependent halogenases were first discovered when investigating pyrrolnitrin (**1**, Figure 1) and chlorotetracycline (**17**, Figure 3) biosynthesis.[26,27] The four enzymes (PrnA, B, C, D) involved in pyrrolnitrin production have been identified from a 6.2 kb genomic DNA region of *Pseudomonas fluorescens* BL915,[26] and their function was proved by construction of deletion mutants for all four genes of the cluster.[28] In each case, the appropriate gene was shown to complement the pyrrolnitrin-negative phenotype of the corresponding deletion mutant. Further demonstration that these four genes are sufficient for the production of pyrrolnitrin (**1**) was derived from a transfer of the gene cluster to *E. coli*, which subsequently resulted in the production of pyrrolnitrin (**1**).[26] Thus, PrnA (Table 1) and PrnC (Table 3) were shown to act as highly substrate-specific halogenases; that is, tryptophan-halogenase (PrnA) was not able to chlorinate the PrnC substrate monodechloraminopyrrolnitrin.[29] Both enzymes also perform the respective chlorination reaction highly regioselectively, and van Pée and coworkers demonstrated that PrnA is able to halogenate L-tryptophan in a site-specific manner to yield 7-chlorotryptophan.[30] Even though other indole derivatives were also accessible to halogenation by PrnA, they were chlorinated at different sites, i.e., at position C-2 or C-3. Thus, for regioselectivity, the exact positioning of the specific biosynthetic intermediate at the active site of the halogenating enzyme seems to be of major importance and allows chlorination at a site of the molecule not accessible to chemical halogenation.

It was further shown that the enzymes, PrnA and PrnC ascribed to the two halogenating steps of pyrrolnitrin biosynthesis, also require $FADH_2$ as cofactor,[17,31] which is produced from FAD and NADH by a flavin reductase. A direct contact between the halogenase and the flavin reductase is thereby not absolutely necessary since free diffusible $FADH_2$ can also be used. Even chemically reduced flavin, regenerated using organometallic complexes, can be utilized.[32] However, free $FADH_2$ is rapidly decomposed into FAD and H_2O_2 with molecular oxygen, which leads to a significant decrease in halogenase activity. Recent sequencing of the *Pseudomonas fluorescens* Pf-5 genome[33] has revealed that the complete gene clusters for pyrrolnitrin (**1**) as well as pyoluteorin (**19**) biosynthesis contain genes encoding for a flavin reductase. This gives rise to the assumption that a distinct flavin reductase specifically interacts with each halogenase to prevent loss of $FADH_2$ through autoxidation.[15] The mechanism for regioselective chlorination has been further characterized using a purified protein from a *P. fluorescens* BL915 PrnA overexpression system. Cocrystallization of the enzyme with tryptophan and FAD yielded yellow diamond-shaped crystals, indicating that FAD was bound to the protein.[34] From further investigations, it could be demonstrated that FAD and Cl^- are bound at the same site of the enzyme, while the tryptophan-binding module is located at a 10 Å distance.[35] $FADH_2$ reacts with O_2 and forms a peroxide-linked isoalloxazine ring, as generally known from flavin-dependent monooxygenases. Subsequently, HOCl is produced as chlorinating agent and channeled

† Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed. Tel: +49 228 733747. Fax: +49 228 733250. E-mail: g.koenig@uni-bonn.de.

10.1021/np800651m CCC: $40.75
Published on Web 02/26/2009

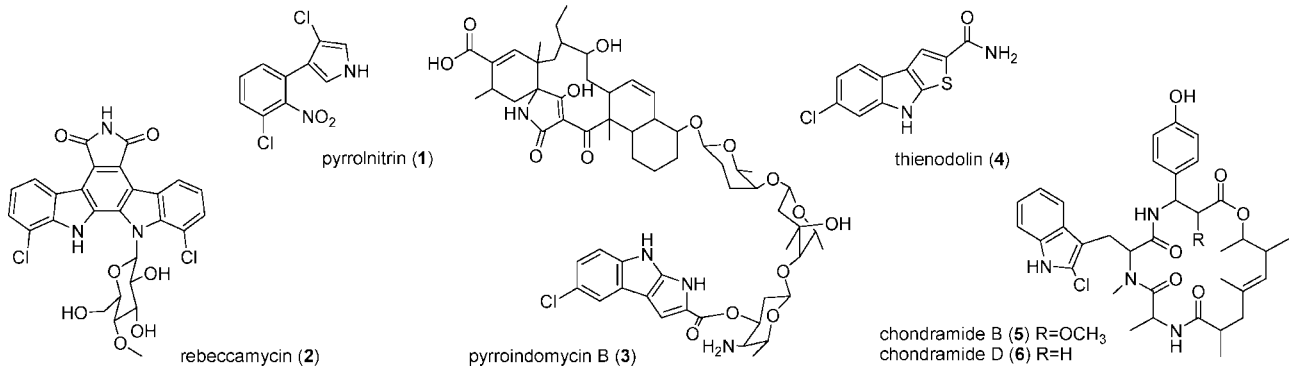

Figure 1. Compounds with chlorinated indole moieties formed from tryptophan. In pyrrolnitrin and rebeccamycin biosynthesis a tryptophan-7-halogenase, in thienodolin biosynthesis a tryptophan-6-halogenase, and in pyrroindomycin biosynthesis a tryptophan-5-halogenase was identified to catalyze the initial biosynthetic step. The tryptophan halogenation at position C-2 in chondramide B and D biosynthesis occurs during NRPS assembly.

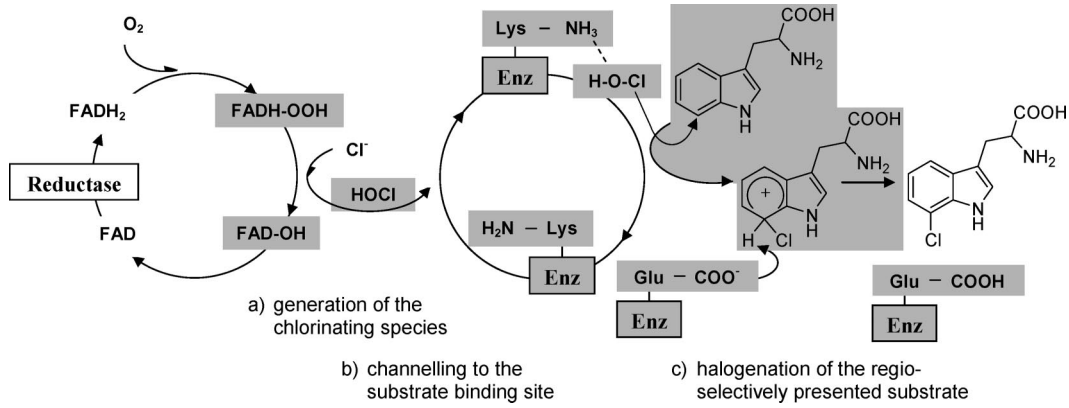

Figure 2. Mechanism of $FADH_2$-dependent halogenation. (The processes that proceed within the halogenase are shaded gray.)

through an intraenzymatic tunnel to the substrate binding pocket. However, HOCl is not released from the enzyme to act in a freely diffusible form in solution. Dong et al. postulated that chlorine may be hydrogen bound to the Lys-79 residue located at the end of the tunnel. In this manner, chlorine would be activated by increased electrophilicity, allowing it to react with tryptophan. In addition, the importance of Glu-346 to stabilize and then deprotonate the substrate intermediate during this process was confirmed by site-directed mutagenesis experiments. The basis for regioselective halogenation is thus the controlled presentation of the substrate to the bound Cl species via particular amino acid residues in the substrate binding site (Figure 2).[36]

RebH (Table 1) is another tryptophan-7-halogenase, which has been identified within the rebeccamycin (**2**, Figure 1) biosynthetic gene cluster of *Lechevalieria aerocolonigenes* ATCC39243.[37,38] RebH was shown to catalyze the initial step of rebeccamycin biosynthesis converting L-tryptophan to 7-chlorotryptophan.[39] RebF catalyzes the NADH-dependent reduction of FAD to provide $FADH_2$ and thus acts together with RebH in a robust two-component reductase/halogenase system. From spectroscopically performed kinetic analysis of RebH a detailed reaction scheme was proposed.[40] Initially, $FADH_2$ is transferred to a FAD(C4a)-oxygenated species, and from this FAD(C4a)-OOH intermediate (observed as an increase in absorbance at 390 nm) an OH^+ equivalent reacts with a chlorine ion, yielding HOCl and FAD(C4a)-OH. Finally, an increase in absorbance at 450 nm indicated the dehydration of FAD(C4a)-OH to fully oxidized FAD. The actual chlorination of tryptophan thus occurs independently after the oxidative reaction of flavin is completed. Protein dynamics leading to conformational changes are assumed to shield the oxygenated FAD(C4a) intermediates from solvent, thereby preventing their rapid breakdown and providing a site for generating the chlorinating agent. Recently, it has been demonstrated that a stable long-living enzyme-bound chlorinating species ($t_{1/2}$ of 28 h at 25 °C) is formed during the reaction of RebH with $FADH_2$, Cl^-, and O_2, which remains on the active site even after removal of FAD until a substrate becomes available for reaction.[41] This chlorinating intermediate is proposed to be a covalently bound lysine chloramine (Lys79-εNH-Cl), located ideally to deliver a Cl^+ equivalent for the electrophilic aromatic substitution of the tryptophan indole ring at C-7.

Further specific halogenases (Table 1) capable of chlorinating tryptophan at positions C-6 and C-5 were also found. A selective tryptophan-5-halogenase (PyrH) was shown to be involved in pyrroindomycin B (**3**, Figure 1) biosynthesis of *Streptomyces rugosporus* LL-42D005[42] as well as a tryptophan-6-halogenase (Thal) in the thienodolin (**4**, Figure 1) production by *Streptomyces albogriseolus*,[36] both ascribed to catalyze initial steps in the biosynthesis.

In contrast to the above-mentioned halogenases, the tryptophan-2-halogenase, CmdE, which is associated with the formation of chondramides B (**5**) and D (**6**) (Figure 1, Table 2) from the myxobacterium *Chondromyces crocatus Cm* c5, was demonstrated to be part of a biosynthetic assembly line.[43] Chondramides are of mixed nonribosomal peptide (NRPS)/polyketide (PKS) origin, and to date, CmdE is the only example of a tryptophan-halogenase integrated in such a modular biosynthetic pathway. Consequently, it is not surprising that the highest homologies of CmdE can be found to halogenases like those involved in cryptophycin (**7**) (CrpH), avilamycin A (**29**) (AviH), and clorobiocin (**28**) (CloHal) biosynthesis, rather than to the above-described typical tryptophan-halogenases, i.e., only 20% identity to PryH, while no significant similarity to the tryptophan-7-halogenases, PrnA and RebH, was found.[43]

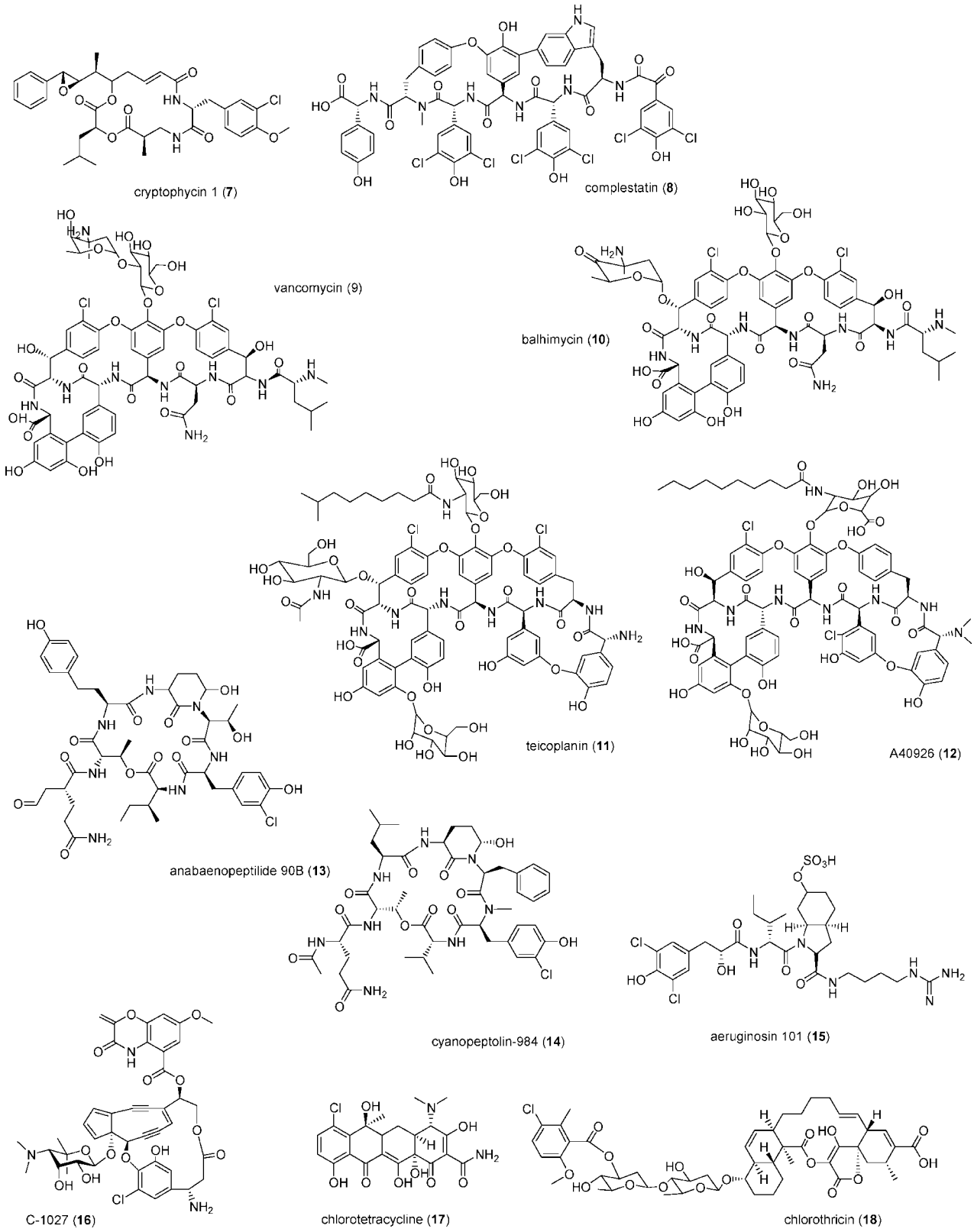

Figure 3. Compounds with chlorinated phenolic structures where $FADH_2$-dependent halogenation most likely occurs involving multienzymatic biosynthesis assembly lines (e.g., PKS/NRPS).

Further halogenases (Table 2) involved in multienzymatic NRPS assembly lines are suggested to chlorinate phenolic moieties in complestatin (**8**)[44] and glycopeptide antibiotic formation,[45] e.g., vancomycin (**9**) (Figure 3). Concerning balhimycin (**10**) biosynthesis, feeding experiments specified that chlorinated β-OH-tyrosine is not accepted for incorporation in the growing peptide chain, excluding halogenation occurring before or during β-OH-tyrosine formation.[45] Also in anabaenopeptilide 90B (**13**) biosynthesis, it is presumed that chlorination of the tyrosine moiety occurs when the growing peptide is bound to ApdB,[46] and similar biosynthetic considerations have been proposed for halogenated cyanopeptolin (**14**) and aeruginosin peptides (**15**).[47,48] In vivo studies on the formation of the enediyne C-1027 (**16**) from *Streptomyces globisporus* likewise revealed a halogenase responsible for the

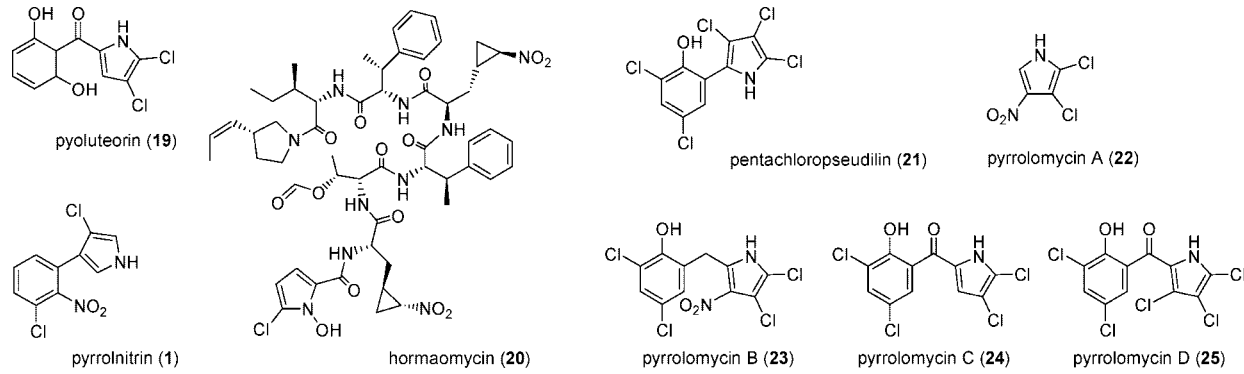

Figure 4. Compounds with chlorinated pyrrole structural elements halogenated by $FADH_2$-dependent halogenases.

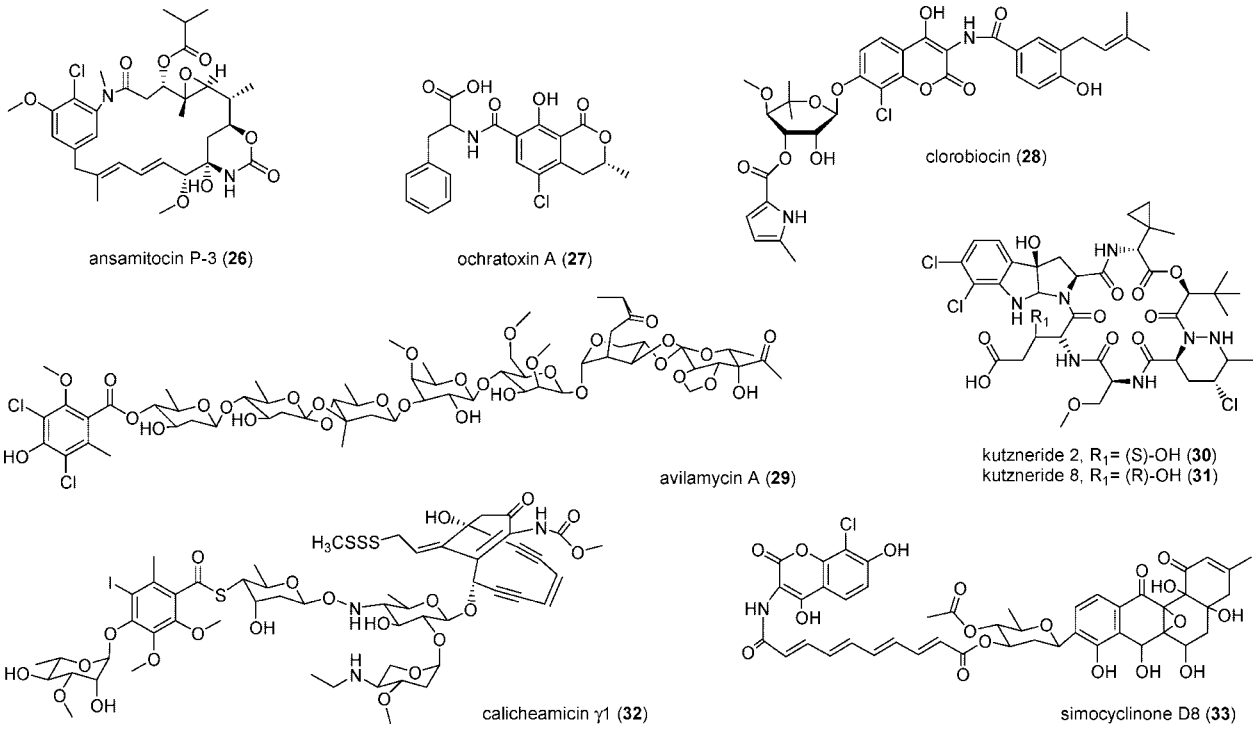

Figure 5. Compounds with halogenated phenolic substructures for which an involvement of $FADH_2$-dependent halogenases is discussed. Cyclopropyl ring formation of the kutznerides might occur via a chlorinated intermediate involving a Fe^{II}/α-ketoglutarate/O_2-dependent halogenase.

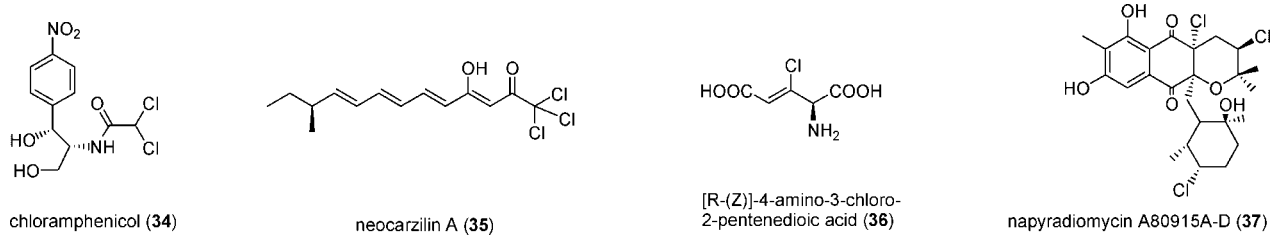

Figure 6. Compounds with chlorinated aliphatic moieties, for which an involvement of $FADH_2$-dependent halogenases is discussed.

Figure 7. General amino acid (aa) sequence assembly of $FADH_2$-dependent halogenases, showing the conserved motifs in all $FADH_2$-dependent halogenases.

chlorination of a peptidyl carrier protein tethered β-tyrosine within the corresponding biosynthetic machinery.[49]

Further examples of enzyme-bound precursors to be halogenated are found for $FADH_2$-dependent halogenases involved in the chlorination of pyrrole moieties (Table 3, Figure 4), e.g., PltA in pyoluteorin (**19**) biosynthesis.[50] In this case, proline is bound as a thioester to the carrier protein PltL and enzymatically desaturated to a pyrrolyl-*S*-PltL intermediate. This is then chlorinated regiospe-

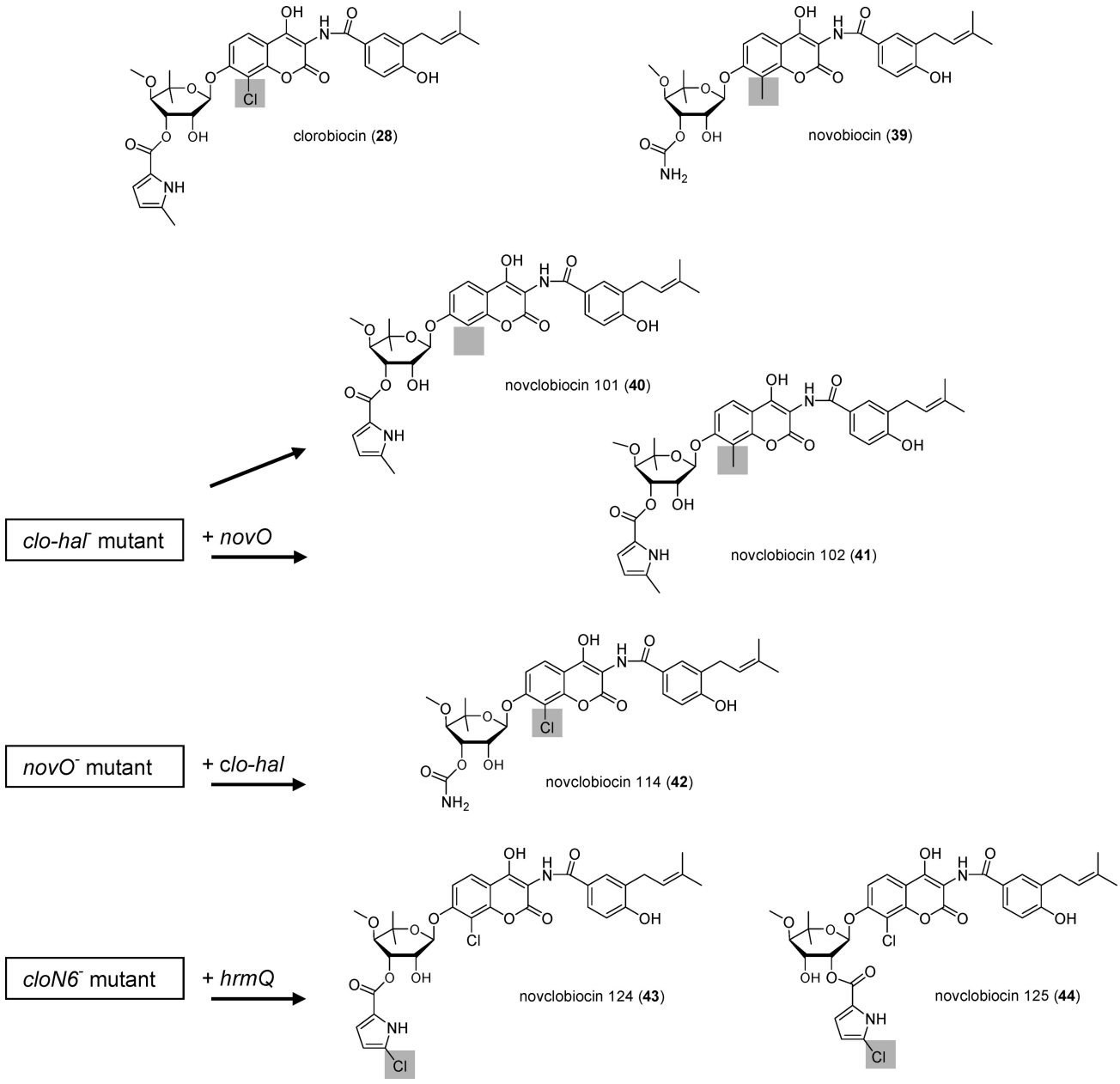

Figure 8. Novel clorobiocin derivatives derived by combinatorial biosynthesis.

cifically at position C-5 and subsequently at position C-4 of the heteroaromatic ring, as observed through temporal product formation. Both halogenations are catalyzed by the same enzyme, i.e., PltA. During this process, chlorination is specific for pyrrolyl-*S*-PltL, since prolyl-*S*-PltL or free pyrrole-2-carboxylate was not accepted as substrate for halogenation by PltA. Recently, the identified HrmQ from the hormaomycin (**20**) biosynthetic gene cluster of *Streptomyces griseoflavus* W-384 was similarly hypothesized to act on a peptidyl carrier bound proline.[51] In contrast to this, halogenation of the nonbound pyrrole-substrate monodechloroaminopyrrolnitrin by PrnC has been demonstrated in pyrrolnitrin (**1**) biosynthesis.[26]

Yet another group of halogenases involved in the chlorination of phenolic moieties, but not explicitly described to function as an integral part of enzymatic assembly lines was recognized (Table 4, Figure 5). Asm12 in ansamitocin P-3 (**26**) biosynthesis halogenates proansamitocin in a post-PKS step.[52,53] In this case, it was demonstrated that halogenation is the first tailoring reaction. Interestingly, 19-deschloro-*N*-demethylansamitocin P-3, formed as a late-stage intermediate by an *asm12*-mutant, is also accessible to the enzymatic chlorination.[54,55] Post-PKS halogenation was also postulated for the mellein intermediate of ochratoxin A (**27**) biosynthesis,[56] and halogenation of clorobiocin (**28**) using Clo-hal could be ascribed to be a tailoring reaction of the aminocoumarin ring.[9,57,58] In avilamycin A (**29**) production, a dichloroisoeverninic acid moiety is attached to the preformed heptasaccharide; however, the direct substrate (free or bound orsellinic acid) for halogenation by AviH is not yet clearly identified. In the biosynthesis of kutzneride 1-9 (**30/31**), two further $FADH_2$-dependent halogenases have been identified.[59] However, their specific involvement in the formation of the dichlorinated indole moiety or that of γ-chloropiperazate remains to be proven.

$FADH_2$-Dependent Halogenases Involved in the Halogenation of Aliphatic Moieties

Besides the $FADH_2$-dependent halogenases involved in halogenation of aromatic compounds, recognizing tryptophan/indole or phenol/pyrrole moieties, enzymes involved in the halogenation of aliphatic compounds represent a third group of $FADH_2$-dependent halogenases (Table 5, Figure 6). For chloramphenicol (**34**) biosyn-

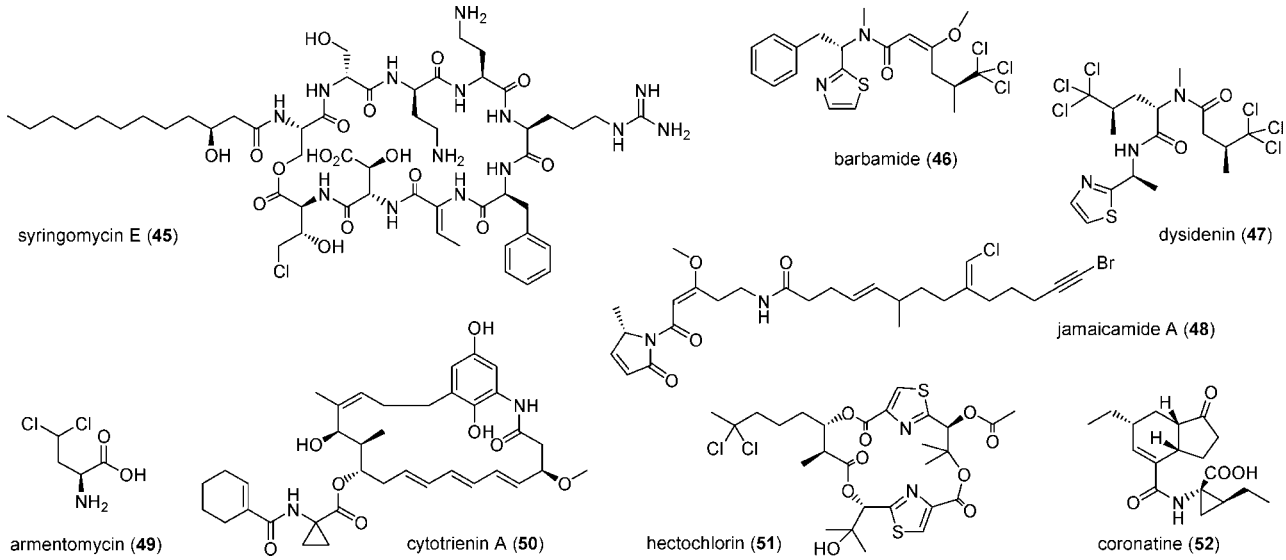

Figure 9. Natural products to be halogenated by Fe^{II}/α-ketoglutarate/O_2-dependent halogenases. Cyclopropyl ring formation of coronatine is executed via a γ-chlorination of L-*allo*-isoleucine followed by intramolecular cyclization.

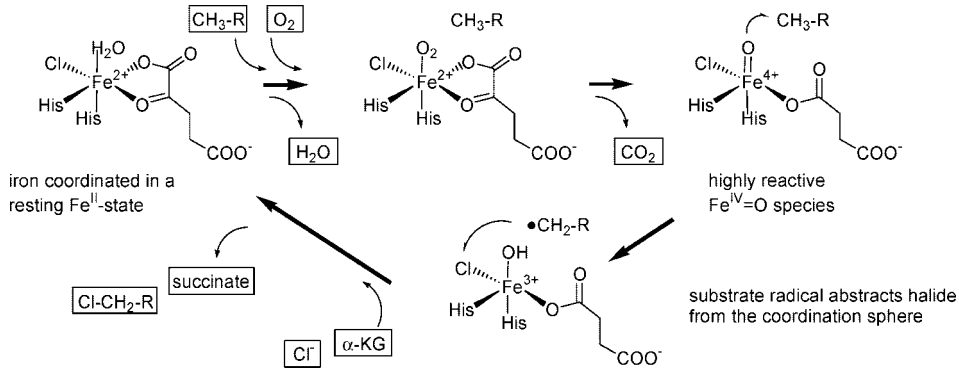

Figure 10. Proposed mechanism of the catalytic cycle of Fe^{II}/α-ketoglutarate/O_2-dependent halogenases. R-CH_3 is the methyl group of the threonyl-*S*-protein substrate (Anderson and Chapman, 2006).

thesis in *Streptomyces venezuelae* ISP5230, the function of CmlS as a halogenating enzyme has been proved by gene disruption experiments, whereby corynecins were produced in place of chloramphenicol (**34**).[60] CmlS shows highest homology to the $FADH_2$-dependent halogenase PltA (Table 3). It has been suggested that a substrate derived from glucose is halogenated and that dichloroacetyl-CoA is then attached to the amino group of the side chain of an enzyme-tethered *p*-aminophenylalanine.[61] The antitumor polyenone neocarzilin (**35**), produced by *Streptomyces carinostaticus* var. F-41, is formed via a type I PKS assembly line and is chlorinated in a tailoring step.[62] ORF3 of the biosynthetic gene cluster shows similarity to the $FADH_2$-dependent halogenase HalB (Table 3); however its disruption completely abolished neocarzilin production. Thus, the authors concluded that the expression of the PKS and the halogenase would be coordinated by an as yet uncharacterized regulatory mechanism. For [*R*-(*Z*)]-4-amino-3-chloro-2-pentenedioic acid (**36**) biosynthesis in *Streptomyces viridogenes* ATCC39387, a halogenation mechanism via a pyrrole intermediate has been postulated, by analogy with the formation of the dichloropyrrole moiety of pyoluteorin.[63] This is consistent with the finding that glutamic acid is the starting point in this biosynthesis, as confirmed by isotopic (^{15}N)-labeling.

Sequence Specificities and Enzymatic Activity of $FADH_2$-Dependent Halogenases

To date, the enzymatic activity of halogenases in vitro has been investigated only in a very few cases, and the function of many biosynthetic genes as halogenases was assigned only putatively. Nevertheless, halogenases are known to have an approximate size of 550 amino acids and, thus, to be encoded by a corresponding DNA consisting of 1500−1600 bp (Figure 7). At the amino terminal end, the flavin binding site is located and includes a GxGxxG motif, which is extremely conserved within this enzyme group.[7] Another conserved region, i.e., WxWxIP, is found in the middle of the sequence.[7] The tryptophan residues of the latter motif are assumed to prevent the enzyme from catalyzing monooxygenase-type reactions by sterically hampering substrate binding next to the isoalloxazine ring of $FADH_2$.[36] The only different amino acids lining the active site in the tryptophan-halogenases PrnA and Thal (Table 1), I56/I82/T348 (PrnA) and V56/V86/S364 (Thal), are potentially involved in the directed substrate presentation for regioselective halogenation.[36]

Early studies investigating the incorporation of bromine instead of chlorine in chloramphenicol (**34**), chlorotetracycline (**17**), and pyrrolnitrin (**1**) resulted in the formation of the corresponding bromo analogues, giving the first indication that the involved enzymes are not exclusively specific for chloride.[64−66] Thus, the ability of $FADH_2$-dependent halogenases to alternatively incorporate bromide instead of chloride has been subjected to further detailed investigations. For PyrH (Table 1), it could be shown that, in the presence of bromide, 5-bromotryptophan was obtained, although chlorination is favored. In any case, only the regioselective monohalogenation of tryptophan at the C-5 position occurred.[42] Similarly, Thal and RebH (Table 1) were demonstrated to catalyze monochlorination

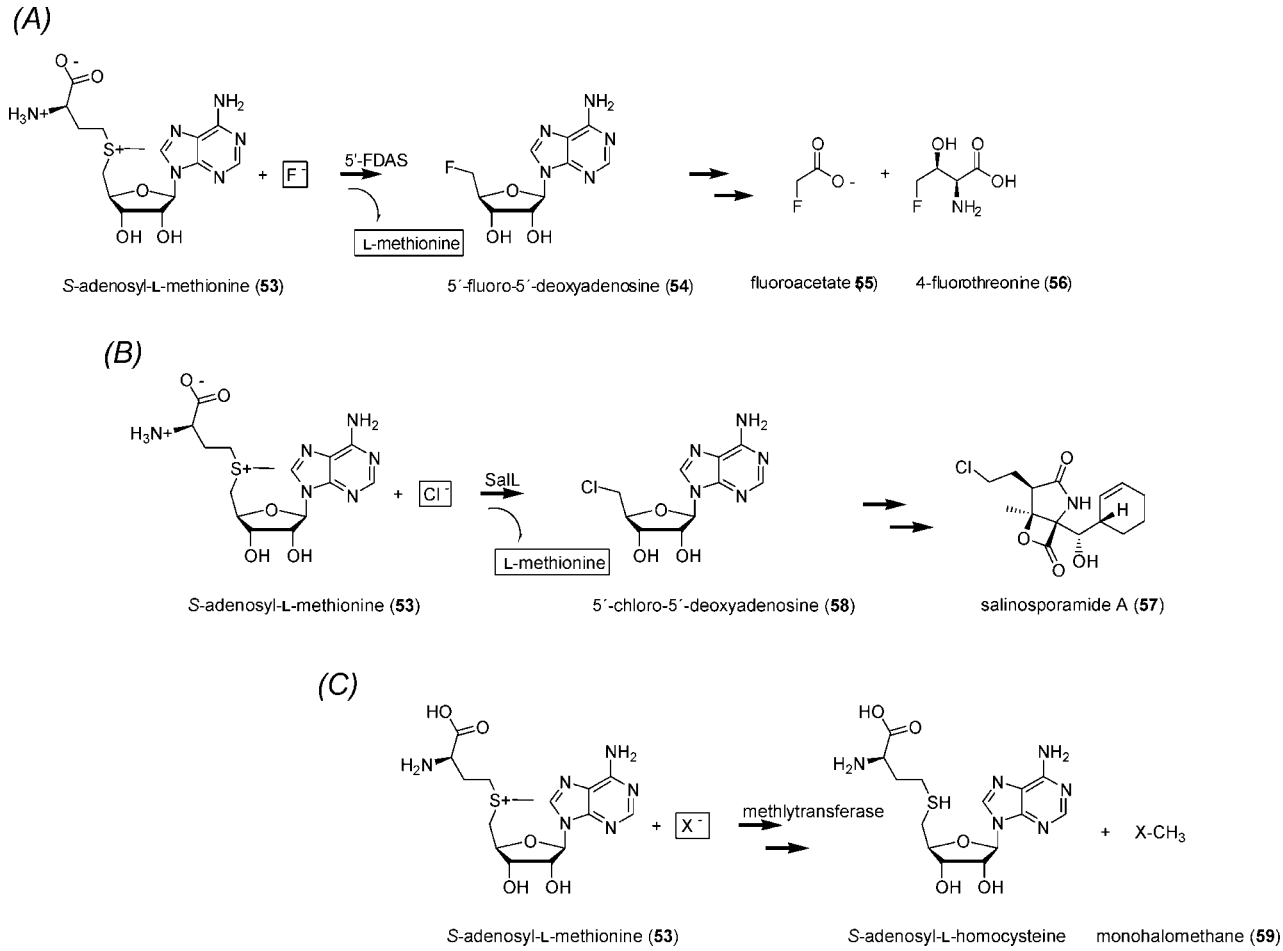

Figure 11. SAM in biohalogenation processes. (A) Enzymatic conversion of inorganic fluoride to organic fluoride by 5′-fluoro-5′-deoxyadenosine synthetase. (B) The chlorinated moiety of salinosporamide A could be shown to derive from tetrose, and SalL was identified as a 5′-FDAS analogue halogenating enzyme. (C) Methyltransferases mediate the transfer of a methyl group from SAM to a halide (X: halide ion).

Table 1. $FADH_2$-Dependent Halogenases that Halogenate Tryptophan in the Initial Step of the Respective Natural Product Biosynthesis

halogenase	natural product *organism*	halogenated structural element	GenBank accession number (reference)
PrnA	pyrrolnitrin (**1**) *Pseudomonas fluorescens* BL915	tryptophan at position C-7	PFU74493[26]
RebH	rebeccamycin (**2**) *Lechevalieria aerocolonigenes* ATCC 39243	tryptophan at position C-7	AJ414559[37]
PyrH	pyrroindomycin B (**3**) *Streptomyces rugosporus* LL-42D005	tryptophan at position C-5	AY623051[42]
Thal	thienodolin (**4**) *Streptomyces albogriseolus*	tryptophan at position C-6	EF095207[36]

regioselectively, as well as monobromination of tryptophan in vitro, resulting in 6- and 7-halotryptophan, respectively.[36,39] Bromide ions were also able to compete with and replace chloride in the PltA (Table 3) reaction on pyrrolyl-*S*-PltL; however, the sterically larger bromide slowed the halogenation reaction to 10−20% conversion in 1 h, as opposed to >90% when chloride was the only halide present.[50]

In an attempt to investigate the role of halogen substitutions in relation to the activity of glycopeptide antibiotics, total substitution of chloride by the other halide ions, fluoride, bromide, and iodide, in the culture medium of *Amycolatopsis balhimycina* was carried out. While fluoride and iodide supplementation proved to be toxic to *A. balhimycina* cultures, fermentation with bromide salts yielded bromobalhimycin.[67] Incubation with equimolar amounts of bromide and chloride resulted in the formation of chlorobromobalhimycin without any preference for either halide. Therefore, it was concluded that halogenases involved in glycopeptide biosynthesis can use bromide or chloride equally well and that the natural occurrence of chloride-modified glycopeptides is due to the predominance of chloride over bromide in common fermentation media. In another study dealing with the influence of bromide, iodide, and fluoride on ochratoxin A production by *Aspergillus ochraceus*, it was again shown that iodide and fluoride inhibited the growth of the fungus, whereas with potassium bromide a respective bromo analogue, bromo-ochratoxin B, was formed.[68] However, *A. ochraceus* was less versatile in the production of bromo analogues and the organism utilized bromide only when present at relatively high concentrations, indicating that in this case chloride would be the preferred halogen for incorporation into ochratoxin-type molecules. From in vivo investigations concerning cryptophycin (**7**) (Table 2, Figure 3) biosynthesis in a *Nostoc* species, evidence was obtained that this cyanobacterial halogenase can also incorporate bromide and even iodide. Addition of calcium bromide or calcium iodide to culture media resulted in the formation of brominated and iodinated analogues.[69]

Table 2. $FADH_2$-Dependent Halogenases That Halogenate Phenolic and Indole Moieties as an Integrated Step in Multienzymatic Biosynthesis

halogenases	natural product *organism*	halogenated structural element	GenBank accession number (reference)
CmdE	chondramides B/D (**5**/**6**) *Chondromyces crocatus Cm* c5	tryptophan at position C-2	AM179409[43]
CrpH	cryptophycin 1 (**7**) *Nostoc* sp. GSV224	tyrosine	EF159954[69]
ComH	complestatin (**8**) *Steptomyces lavendulae*	hydroxyphenylglycine	AF386507[44]
"halogenase-gene"	vancomycin (**9**) *Amycolatopsis orientalis*	n.s.[a]	AF486630[126]
BhaA	balhimycin (**10**) *Amycolatopsis balhimycina* DMS5908	β-hydroxytyrosine	Y16952[45,127]
Tcp21	teicoplanin (**11**) *Actinoplanes teichomyceticus* ATCC31131	tyrosine and β-hydroxytyrosine	AJ605139[128]
ORF10 (=*dbv10*)	A40926 (**12**) *Nonomuraea sp.* ATCC39727	dihydroxyphenylglycine and β-hydroxytyrosine	AJ561198[129]
ApdC	anabaenopeptilide 90B (**13**) *Anabaena circinalis* 90	tyrosine	AJ269505[46]
McnD[b]	cyanopeptolin-984 (**14**) *Microcystis* sp. NIVA-CYA 172/5	tyrosine	DQ075244[47]
AerJ[c]	aeruginosin peptides (**15**) *Microcystis* sp. PCC 9812	3-(4-hydroxyphenyl)lactic acid	AM773664[48]
SgcC2	C-1027 (**16**) *Streptomyces globisporus*	β-tyrosine[d]	n.d.[e] [130]
CTC-chl (=*cts4*)	chlorotetracycline (**17**) *Streptomyces aureofaciens* NRRL3203	4-ketoanhydrotetracycline[f]	D38214[19,27]
ChlB4	chlorothricin (**18**) *Streptomyces antibioticus* DSM 40725	6-methylsalicylic acid	DQ116941[131]

[a] Not specified. [b] Also identified from other *Microcystis* strains (AM773679, AM773678, AM773677, AM773676, AM773675, AM773674).[48] [c] Also identified from other *Microcystis* strains (AM773663, AM773662, AM773661, AM773660, AM773659, AM773658, AM773657, AM773656, AM773655, AM773654).[48] [d] In vitro characterized to halogenate β-tyrosine tethered to a peptidyl carrier protein. [e] No sequence data available in GenBank. [f] Indicates that halogenation occurs while the elongating acyl chain is still tethered to carrier proteins during the PKS assembly line.

Table 3. $FADH_2$-Dependent Halogenases Involved in the Halogenation of Pyrrole Moieties

halogenase	natural product *organism*	halogenated structural element	GenBank accession number (reference)
PltA	pyoluteorin (**19**) *Pseudomonas fluorescens* Pf-5	proline[a]	AF081920[50]
HrmQ	hormaomycin (**20**) *Streptomyces griseoflavus* W-384	pyrrole[b]	n.d.[c] [51]
PrnC	pyrrolnitrin (**1**) *Pseudomonas fluorescens* BL915	monodechloramino-pyrrolnitrin	PFU74493[26]
HalB	pentachloropseudilin (**21**) *Actinoplanes* sp. ATCC33002	pyrrole	AF450451[132]
Pyr29[d]	pyrrolomycins A, B, C, D (**22**−**25**) *Actinosporangium vitaminophilum* ATCC31673	proline	EF140901[133]

[a] Proline halogenation while bound to a peptidyl carrier protein. [b] Most likely halogenation via a peptidyl carrier protein-bound proline. [c] No sequence data available in GenBank. [d] 65% similarity to PltA.

Biotechnological Potential of $FADH_2$-Dependent Halogenases

The considerable progress made in understanding biological halogenation mediated through $FADH_2$-dependent halogenases gave rise to probing the biotechnological potential of these enzymes. The aim of such investigations was to allow halogenations at specific sites of substrates not accessible to direct chemical halogenation, as well as to avoid the formation of unwanted byproduct.

Combinatorial biosynthesis yielded new derivatives of the aminocoumarin antibiotics novobiocin (**39**) and clorobiocin (**28**) (Figure 8). Inactivation of the *clo-hal* gene in the clorobiocin biosynthetic gene cluster of *Streptomyces roseochromogenes* resulted in the formation of the hydrogen analogue, novclobiocin 101 (**40**), and co-expression of the putative methlytransferase *novO* led to the production of novclobiocin 102 (**41**), which is methylated at C-8 of the aminocoumarin ring.[9] More interestingly, co-expression of *clo-hal* in a *novO*$^-$ mutant resulted in the chlorinated novobiocin derivative novclobiocin 114 (**42**).[70] An attempt to functionally replace *clo-hal* by the balhimycin halogenase (Table 2) *bhaA,* in both the *clo-hal*$^-$ and *novO*$^-$ mutant, did not yield the respective chlorinated aminocoumarin derivatives (clorobiocin or novclobiocin 114), thus underlining the different substrate specificity of *clo-hal* and *bhaA*.[70] In a study investigating the halogenating potential of the hormaomycin (**20**) halogenase HrmQ (Table 3) in combinatorial biosynthesis, co-expression experiments of *hrmQ* in a *cloN6*$^-$ clorobiocin mutant strain were conducted.[51] The results confirmed that HrmQ specifically chlorinates pyrrole moieties at the C-5 position and thus yielded the new clorobiocin derivatives novclobiocin 124 (**43**) and 125 (**44**).

As a result of their potential therapeutic applications as antitumor and neuroprotective agents,[71,72] great efforts have been made to generate derivatives of indolocarbazole alkaloids. Novel halogenated compounds were obtained by recombining genes found within the biosynthetic gene clusters of rebeccamycin (**2**) and staurosporine with the halogenases RebH, PyrH, and Thal (Table 1). In this way, Salas and co-workers were able to generate "hybrid" indolocarbazoles by modifying the staurosporine aglycon using rebeccamycin chlorination, glycosylation, and sugar methylation activities. Besides nonhalogenated products, this approach yielded chloro- and dichlorostaurosporine derivatives.[72] The major bis-indole products obtained

Table 4. $FADH_2$-Dependent Halogenases Involved in the Halogenation of Phenolic Moieties

halogenase	natural product *organism*	halogenated structural element	GenBank accession number (reference)
Pyr16/17	pyrrolomycins A, B, C, D (**22**−**25**) *Actinosporangium vitaminophilum* ATCC31673	phenol[a]	EF140901[133]
Asm12	ansamitocin P-3 (**26**) *Actinosynnema pretiosum* ATCC31565	proansamitocin[b]	AF453501[52,55]
n.d.[c]	ochratoxin A (**27**) *Penicillium nordicum*	mellein intermediate[d]	n.d.[c] [56]
Clo-Hal	clorobiocin (**28**) *Strepromyces roseochromogenes*	aminocoumarin	AF329398[9,57]
AviH	avilamycin A (**29**) *Stepromyces viridochromogenes* Tü57	orsellinic acid[e]	AF333038[134]
KtzQ and KtzR	kutznerides (**30/31**) *Kutzneria* sp. 744	indole and piperazate	EU074211[59]
CalO3	calicheamicin γ1 (**32**) *Micromonospora echinospora* ssp. *calichensis*	orsellinic acid	AF497482[135]
SimD4	simocyclinone D8 (**33**) *Streptomyces antibioticus* Tü6040	aminocoumarin	AF324838[136]

[a] Phenol moiety acetate derived. [b] Halogenation of proansamitocin as first PKS-tailoring reaction. [c] No sequence data available in GenBank. [d] Post-PKS. [e] Dichloroisoeverninic acid attached to the preformed heptasaccharide.

Table 5. $FADH_2$-Dependent Halogenases Involved in the Chlorination of Aliphatic Structural Elements

halogenase	natural product *organism*	halogenated structural element	GenBank accession number (reference)
CmlS	chloramphenicol (**34**) *Streptomyces venezuelae* ISP5230	aliphatic moiety[a]	AY026946[60]
ORF3[b]	neocarzilin A (**35**) *Steptomyces carinostaticus*	aliphatic moiety	AB097904[62]
n.d.[c]	(*R*-(*Z*))-4-amino-3-chloro-2-pentenedioic acid (**36**) *Streptomyces viridogenes* ATCC39387	aliphatic moiety[d]	DQ782376[63]
NapH2	napyradiomycin A80915A-D (**37**) *Streptomyces* sp. CNQ-525	aliphatic moiety[e]	EF397638[120]

[a] Likely in an enzyme-bound assembly line (similarity to PltA). [b] Similarity to HalB. [c] Only partial gene sequence. [d] Halogenation via a pyrrole intermediate postulated. [e] Also Va-CPOs (NapH1,3,4) identified within the gene cluster.

when co-expressing PyrH and Thal (Table 1) had a chloride substitution at positions C-5 and C-6 of the indole moiety, respectively.

In another approach, Thal was investigated in a recombinant pyrrolnitrin producer strain.[36] Overexpesstion of *thal* yielded the new phenylpyrrole derivative 3-(2′-amino-4′-chlorophenyl)pyrrole, but no pyrrolnitrin (**1**) or aminopyrrolnitrin was detected. The authors concluded that the overproduction of Thal resulted in the chlorination of all the available tryptophan to 6-chlorotryptophan, which was then accepted by PrnB, the second enzyme in pyrrolnitrin biosynthesis catalyzing the subsequent ring rearrangement. However, the phenylpyrrole compound produced was not a substrate to PrnC for further halogenation reaction.

The use of tryptophan-5-halogenases (PyrH) (Table 1) in producing halogenated serotonin precursors has been the focus of further research.[73] The aim of these efforts was to obtain 5-bromotryptophan, which is regarded as a suitable intermediate for a subsequent nucleophilic hydroxylation to yield 5-hydroxytryptophan (oxitriptan). The latter is a component of many antidepressant drugs and, to date, commonly obtained by extraction of the seeds of the African plant *Griffonia simplicifolia*. The main limitation at this point is the low activity of the purified enzyme as well as the unsatisfactory production of the recombinant enzyme. Optimization of the reaction conditions, however, has already led to a nearly 20-fold improvement of the 5-bromotryptophan yield, and ongoing efforts aim to enhance the recombinant enzyme production.[73]

Non-heme Fe^{II}/α-Ketoglutarate/O_2-Dependent Halogenases

In the past few years, evidence has accumulated that another class of halogenating enzymes carries out the chlorination of inactivated carbon centers. Using α-ketoglutarate (α-KG) as co-substrate, these halogenases are involved in the chlorination of terminal methyl groups of amino acids linked to peptidyl carrier proteins (Table 6, Figure 9).

Initial insight into their catalytic mechanism is derived from investigations on SyrB2, which chlorinates the γ-methyl group of L-threonine in syringomycin E (**45**) biosynthesis in *Pseudomonas syringae*.[74,75] Halogenation of L-threonine occurs only when bound to the peptidyl carrier protein SyrB1, while free L-threonine is not accepted as a substrate. Reactions performed with an excess of sodium bromide demonstrated that SyrB2 was also capable of incorporating bromine into the threonyl-*S*-protein.[76] From crystallographic data,[77] it was confirmed that Fe^{II} of the active site is bound by two histidine residues. A halide ligand (chloride or bromide) together with α-KG and water coordinates the iron in the resting Fe^{II} state. Dioxygen attack results in decarboxylation of α-KG and a highly reactive iron-oxo-species (Fe^{IV}═O) is formed as a general key feature of catalysis by these α-oxo-acid-dependent dioxygenases.[78] The Fe^{IV}═O abstracts a hydrogen radical from an aliphatic carbon center of the actual substrate, which, in turn, abstracts the halide from the coordination sphere (Figure 10). It has been shown that in vitro SyrB2 was rapidly deactivated during catalysis and no more than about seven turnovers of the enzyme were possible.[78]

For further natural products that are halogenated at an unactivated carbon center (Figure 9), analogous halogenating enzymes were suggested to be involved in their biosynthesis, e.g., barbamide (**46**), dysidenin (**47**), dysideathiazole, and jamaicamides (**48**).[76,79] Indeed, in the barbamide gene cluster of *Lyngbya majuscula*, two genes, i.e., *barB1* and *barB2*, homologous to SyrB2 could be identified. The initial biosynthetic step is the chlorination of L-leucine to a trichloroleucine derivative, followed by a subsequent conversion to a unique trichloroisovaleryl starter unit in a mixed PKS/NRPS pathway.[80,81] Regarding dysidenin (**47**) production in dictyoceratid sponges, similar genes (*dysB1/dysB2*) were found and could be ascribed to the cyanobacterial symbiont *Oscillatoria spongeliae*.[82]

Table 6. Fe^{II}/α-Ketoglutarate/O_2-Dependent Halogenases

halogenase	natural product *organism*	halogenated structural element	GenBank accession number (reference)
SyrB2	syringomycin E (**45**) *Pseudomonas syringae*	L-threonine[a]	AAD50521[137,138]
BarB1/BarB2	barbamide (**46**) *Lyngbya majuscula*	L-leucine[b]	AF516145[80,81,139]
DysB1/DysB2	dysidenin (**47**) *Oscillatoria spongeliae* 35P1[c]	n.s.[d]	AY628171 AY628172[82]
CytC3	armentomycin (**48**) *Streptomyces* spp	L-aminobutyrate[e]	n.d.[f 83]
HctB	hectochlorin (**50**) *Lyngbya majuscula*	n.s.[d]	AY974560[85]
CmaB[g]	coronatine (**51**) *Pseudomonas syringae*	L-*allo*-isoleucine	U14657[86,140]
KtzD[h]	kutznerides (**30/31**) *Kutzneria* sp. 744	n.s.[d]	EU074211[59]
JamE[i]	jamaicamides *Lyngbya majuscule* JHB	n.s.	AAS98776[79]

[a] Only when bound to the peptidyl carrier protein SyrB1. [b] The triple chlorination is catalyzed by a tandem action of the two enzymes. [c] *Oscillatoria spongeliae* 39P1: AY628173, AY628174;[82] *Oscillatoria spongeliae* AY648943.[141] [d] Not specified. [e] Bound to the peptidyl carrier protein CytC2. [f] No sequence data available in GenBank. [g] Mediates the formation of the cyclopropane ring. [h] Hypothesized to be involved in the formation of the cyclopropane ring. [i] JamE is a PKS module of the jamaicamides NRPS/PKS assembly line, harboring a region with weak homology to Fe^{II}/α-ketoglutarate/O_2-dependent halogenases.

A Fe^{II}/α-ketoglutarate/O_2-dependent halogenase homologous enzyme, CytC3, involved in the formation of the γ,γ-dichloroaminobutyrate (armentomycin (**49**), Figure 9) was identified from cytotrienin A (**50**)-producing *Streptomyces* spp.[83] The γ,γ-dichloroaminobutyryl moiety is postulated to be further cyclized and to act as a precursor in cytotrienin A formation. CytC3 could be demonstrated to act on L-aminobutyrate bound to the peptidyl carrier protein, CytC2. Spectroscopic monitoring of the enzymatic reaction confirmed that the chlorination proceeds through an Fe^{IV} intermediate that cleaves the C−H bond of the amino acid substrate,[84] as described above. Also, in the hectochlorin (**51**) biosynthetic gene cluster, a region with high homology to Bar1, Bar2, and SyrB2 was found and is presumed to be involved in the formation of the *gem*-dichloro group.[85] Interestingly, γ-chlorination of an L-*allo*-isoleucine by the Fe^{II}/α-ketoglutarate/O_2-dependent halogenase CmaB mediates the formation of the cyclopropane ring in coronatine (**52**, Figure 9), which is produced by several *Pseudomonas syringae* strains.[86] The intermediate γ-chloroaminoacyl β-thioester is then converted to a γ-carbanion, which, in turn, is accessible to intramolecular cyclization. By analogy, methylcyclopropylglycine formation is postulated as the initial reaction of the NRPS assembly line in kutzneride (**30/31**, Figure 5) biosynthesis, since KtzD was identified as being homologous to Fe^{II}/α-ketoglutarate/O_2-dependent halogenases.[59]

Halogenating Enzymes Utilizing *S*-Adenosyl-L-methionine

Fluoride is the most nucleophilic halogen and does not allow any electrophilic or radical biological chemistry, as is known for Cl^-, Br^-, and I^-. Even though, thermodynamically, the formation of carbon−fluoride bonds would be favorable, due to the extremely high desolvation energy required to activate F^- in aqueous solution, biological reactions with fluoride are rather rare.[22] Unique enzymes are needed to overcome this kinetic barrier, and the first to be characterized was 5′-fluoro-5′-deoxyadenosine synthetase (5′-FDAS). This enzyme was isolated from *Streptomyces cattleya* and utilizes *S*-adenosyl-L-methionine (SAM) (**53**) as co-substrate.[12] Fluoride is thereby thought to be placed in a hydrophobic pocket where desolvation is achieved as protein residues coordinate to water. In turn, a S_N2 substitution of methionine at SAM (**53**) takes place.[87] In this way 5′-fluoro-5′-deoxyadenosine (**54**) is formed as an intermediate and subsequently converted to fluoroacetate (**55**) and 4-fluorothreonine (**56**) (Figure 11A).

For the biosynthesis of the potent proteasome inhibitor salinosporamide A (**57**, Figure 11B), produced by *Salinispora tropica*, the deschloro analogue, salinosporamide B, was initially presumed to be the direct precursor, and thus the halogenation of the unactivated C-13 methyl group was suggested to be catalyzed by a non-heme iron halogenase. However, from feeding experiments a different origin of the four-carbon moiety was demonstrated for these two molecules.[88] Thus, for salinosporamide B, a butyrate-derived ethylmalonyl-CoA was identified as a building block, whereas in salinosporamide A (**57**), an unprecedented PKS extender unit was shown to originate from a sugar precursor. The enzyme involved in the chlorination step (SalL) has been characterized as a 5′-FDAS similar enzyme, which converts SAM (**53**) to 5′-chloro-5′-deoxyadenosine (**58**).[13] This 5′-halo-5′-deoxyadenosine synthetase was shown to also be capable of utilizing bromide as well as iodide, but not fluoride.

SAM (**53**) is also involved in the formation of monohalomethanes (**59**), albeit in a different way, namely, as a methyl donor (Figure 11C). At an early stage, Harper predicted that most likely enzymes other than haloperoxidases (discribed below) would be involved in the formation of halomethanes,[89] and Wuosmaa and Hager were the first to detect and partially purify a corresponding methytransferase responsible for the methyl chloride production in the rot fungus *Phellinus promaceus*.[90] Several studies dealing with the emission of gaseous iodine and chlorine from the biosphere to the atmosphere resulted in the identification of respective enzymes in marine as well as terrestrial bacteria, macro- and microalgae, wood-rotting fungi, and terrestrial higher plants.[91−93]

Haloperoxidases

Until the mid 1990s, the understanding of biological halogenation reactions was restricted to haloperoxidases (HPO). These enzymes generate hypohalous acid (HOX) or related halogenating intermediates, such as OX^-, X_3^-, and X^+, by the reaction of a metal-bound hydroperoxy species with a halide ion (Figure 12). In most cases, the halogenating species is then released from the enzyme to act on organic substrates that are susceptible to an attack by electrophiles. The enzymes are found to contain either heme or vanadate as cofactor in their active site.[6,23,94]

In heme-containing HPOs, the formation of hypohalous acid is driven by a redox mechanism, whereas vanadate-containing HPOs do not change their oxidation state during the enzymatic cycle but rather function as Lewis acids. HPOs are further classified according to the halide ion that they are predominantly able to utilize for halogenation.[95] Thus, the nomenclature is based on the most electronegative halide oxidized by the enzyme. Chloroperoxidases

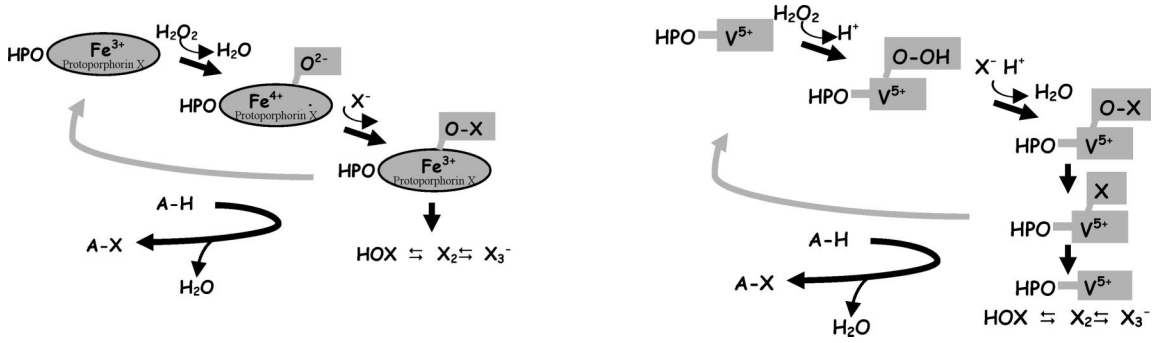

Figure 12. Both heme- and vanadium-dependent HPOs are thought to generate bound hypohalite intermediates and hypohalous acid, which reacts as X^+ equivalents with electron-rich substrates (X: halide, A: organic substrate).

(CPOs) catalyze the oxidation of chloride, bromide, and iodide, while bromoperoxidases (BPOs) catalyze only the oxidation of bromide and iodide, and iodoperoxidases (IPOs) are specific for iodide oxidation.[96] However, hydrogen peroxide does not have the driving force to oxidize fluoride.[23]

The first halogenating enzyme was described from the caldariomycin-producing fungus *Caldariomyces fumago* as a heme-containing HPO.[97] Hager and co-workers developed a spectrophotometric assay, using monochlorodimedone as a mimic of the natural precursor of caldariomycin, to monitor HPO activity. On the basis of this assay, several heme-HPOs were subsequently discovered from diverse fungi, algae, and microorganisms.[6,94,98] Furthermore, other heme-proteins were found to have halogenating activity, i.e., an unusual lignin peroxidase from *Agrocybe aegerita*,[99] mammalian myeloperoxidase and eosinophil peroxidase,[100] the catalase-peroxidase KatG of *Synechocystis* PCC 6803,[101] and a nitrate reductase from *Thioalkalivibrio nitratireducens*.[102] The detected HPOs were shown to have a broad substrate specifity, accepting organic compounds, which are generally susceptible to electrophilic attack. Thus, regioselectivity was the same as that seen in chemical halogenations.

In addition to halogenating activity, ferric heme-HPOs have been shown to share catalytic properties with at least three further classes of heme-containing oxidoreductases, namely, classical peroxidases, cytochrome P450 monooxygenases, and catalases.[6] Intensive studies on structural and functional aspects of the heme-HPO have focused mainly on the initially described CPO from *C. fumago*. Under investigation was the chlorination activity of this enzyme, as well as its epoxidation and sulfoxidation activity in the absence of halide.[6,103−107] A recent study on the chlorination mechanism confirmed that the final chlorine transfer to various substrates occurs outside the active site via a free diffusible species without any special mode of substrate recognition.[108] In contrast, epoxidation and sulfoxidation are performed regioselectively, providing *C. fumago* CPO as a useful tool in synthetic applications. Introduction of an oxygen atom into organic substrates such as sulfides, olefins, and aromatic rings, was thereby obtained enantioselectively and with high yields.[105,109] A rigid architecture of the active site as recognized from crystal structures of *C. fumago* CPO complexed with various ligands possibly explains the observed enantioselective reactions.[107] However, a potential industrial use is hampered by the limited stability of the enzyme due to inactivation through hydrogen peroxide and by the low water solubility of many organic substrates of synthetic interest.[104] Further investigations were conducted toward the dehalogenation of trihalophenols and *p*-halophenols.[110] *C. fumago* CPO proved to be capable of catalyzing such degradation processes and was significantly more robust than other peroxidases. Thus, this CPO might be applicable in biodegradation of polychlorinated phenols and further noxious haloaromatic compounds, under reaction conditions often too harsh for other biocatalysts.

Vanadate ion containing bromoperoxidases (Va-BPOs) have been isolated mainly from marine algae. This group of enzymes most likely seems to play a role in the formation of marine natural products, e.g., the halogenation and cyclization of terpenes.[111] Va-HPOs bind hydrogen peroxide and halides, leading to a putative enzyme-bound or active-site-trapped brominating moiety.[112] X-ray crystal structures of the Va-BPO from *Curvularia inaequalis*,[113] *Ascophyllum nodosum*,[114] and *Corallina officinalis*[115] displayed a channel leading to the vanadium binding site, which is proposed to influence substrate specificity.[116] Competitive kinetic studies, comparing the bromination of indole substrates by Va-HPO with enzyme-free preparations under aqueous conditions, showed that these reactions were not congruent, since Va-HPO preferred brominated indole derivatives even in the presence of other substrates, such as monochlorodimedone or phenol red.[23,117] In the absence of a suitable substrate however, HOBr is released from the enzyme and carries out unselective bromination as observed for aqueous bromine. The ratio of formed diastereomers was also found to differ between purely chemical and enzymatic reactions. Accordingly, asymmetric bromination and cyclization of the terpenoid precursor (*E*)-(+)-nerolidol could be demonstrated for Va-BPO, producing single diastereomers of the marine natural products, α- and β-snyderol, as isolated from *Laurencia obtusa*.[116]

The first Va-BPO to be characterized was from the brown seaweed *Ascophyllum nodosum.*[118] However, with the isolation of a Va-BPO from the lichen *Xanthoria parietina*, it became obvious that this group of enzymes not only occurs in the marine environment but can also be found in terrestrial eukaryotic organisms.[119] Va-CPOs on the other hand have been mainly found in fungi, even though, to date, halogenated natural products have not been identified from the corresponding strains. In *Streptomyces* strains the first indication of a potential involvement of Va-CPOs has recently been discussed regarding the chlorination and cyclization of napyradiomycin (**37**, Figure 6).[120]

In contrast to the iron heme haloperoxidases, Va-HPOs do not catalyze the direct disproportion of hydrogen peroxide and, therefore, have no catalase activity.[121] However, their structural similarity to acid phosphatases is noticeable, particularly in the domains providing the vanadate and phosphate binding site. Therefore, a common evolutionary origin is proposed as being likely.[112] From different approaches toward substituting the vanadate group with phosphate and vice versa a functional relationship could be confirmed.[122] However, at least for the phosphatases from *Shigella flexneri* and *Salmonella enterica*, it was shown that the turnover frequencies were low, clearly demonstrating that the active site of acid phosphatases is not optimized for haloperoxidase activity.[123]

Hypohalorous acid is a bactericidal agent, and many halogenated compounds have antimicrobial activity, illustrating the possible involvement of HPO systems in natural defense mechanisms. This might prevent biofouling by microorganisms on the surface of

marine algae, as well as act as an antifeeding system. Experiments with the marine alga *Laminaria digitata* demonstrated that natural HPO systems are also capable of mediating the deactivation of acylated homoserine lactones, which are important as cell-to-cell signaling molecules for Biofilm formation.[124] This suggests that oxidized halogens may control biofouling not only via a bactericidal mechanism but also by possibly interfering with bacterial cell signaling systems. As described above, an involvement of HPOs in the biosynthesis of natural products has been suggested only in a very few cases.[111,116,120] The physiological function of CPOs is mainly proposed as being important for the degradation of plant cell walls. The hypohalorous acid produced thus oxidizes lignocellulose and facilitates penetration of the fungal hyphae into a host.[112] Furthermore, Va-IPOs seem to play a role in iodine accumulation of brown algae as a main vector of the iodine biogeochemical cycle.[125] Species like *Laminaria digitata* are thereby able to uptake an average content of 1% of their dry weight, representing approximately a 30 000-fold accumulation of iodine from seawater. Iodine efflux and the production of volatile halocarbons, then again, are proposed to be part of an early defense response to various biotic and abiotic stresses by these organisms.

Concluding Remarks

In the last years, our understanding of biohalogenation processes has been extended tremendously. Several groups of halogenating enzymes have been discovered and investigated on the biochemical and genetic level. Each group of these enzymes performs halogenation reactions on chemically distinct and different substructures, i.e., indole, phenol, pyrole, and aliphatic moieties, using a specific reaction mechanism. Investigations concerning the biotechnological potential of halogenating enzymes, however, are still in their infancy. Results obtained in the latter area so far prove that several halogenases can be produced recombinantly and subsequently applied to directly halogenate suitable substrates (Table 1). Other halogenases function only in a multienzymatic biosynthetic environment, and combinatorial biosynthetic strategies need to be implemented to use their enzymatic capabilities.

References and Notes

(1) Gribble, G. W. *Chemosphere* **2003**, *52*, 289–297.
(2) Anderson, J. L. R.; Chapman, S. K. *Mol. BioSyst.* **2006**, *2*, 350–357.
(3) Vetter, W. *Rev. Environ. Contam. Toxicol.* **2006**, *188*, 1–57.
(4) van Pée, K. H. *Annu. Rev. Microbiol.* **1996**, *50*, 375–399.
(5) Murphy, C. D. *Nat. Prod. Rep.* **2006**, *23*, 147–152.
(6) Hofrichter, M.; Ullrich, R. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 276–288.
(7) Kling, E.; Schmid, C.; Unversucht, S.; Wage, T.; Zehner, S.; van Pée, K. H. *Ernst Schering Res. Found. Workshop* **2005**, 165–194.
(8) Reed, K. A.; Manam, R. R.; Mitchell, S. S.; Xu, J. L.; Teisan, S.; Chao, T. H.; Deyanat-Yazdi, G.; Neuteboom, S. T. C.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2007**, *70*, 269–276.
(9) Eustáquio, A. S.; Gust, B.; Luft, T.; Li, S. M.; Chater, K. F.; Heide, L. *Chem. Biol.* **2003**, *10*, 279–288.
(10) Blasiak, L. C.; Drennan, C. L. *Acc. Chem. Res.* **2008**, *42*, 147−155.
(11) Neumann, C. S.; Fujimori, D. G.; Walsh, C. T. *Chem. Biol.* **2008**, *15*, 99–109.
(12) Dong, C. J.; Deng, H.; Dorward, M.; Schaffrath, C.; O'Hagan, D.; Naismith, J. H. *Acta Crystallogr. D: Biol. Crystallogr.* **2003**, *59*, 2292–2293.
(13) Eustáquio, A. S.; Pojer, F.; Noel, J. P.; Moore, B. S. *Nat. Chem. Biol.* **2008**, *4*, 69–74.
(14) Chen, X.; van Pée, K. H. *Acta Biochim. Biophys. Sin. (Shanghai)* **2008**, *40*, 183–193.
(15) van Pée, K. H.; Dong, C. J.; Flecks, S.; Naismith, J.; Patallo, E. P.; Wage, T. *Adv. Appl. Microbiol.* **2006**, *59*, 127–157.
(16) van Pée, K. H.; Patallo, E. P. *Appl. Microbiol. Biotechnol.* **2006**, *70*, 631–641.
(17) van Pée, K. H. *Arch. Microbiol.* **2001**, *175*, 250–258.
(18) van Pée, K. H.; Hölzer, M. *Adv. Exp. Med. Biol.* **1999**, *467*, 603–609.
(19) Vaillancourt, F. H.; Yeh, E.; Vosburg, D. A.; Garneau-Tsodikova, S.; Walsh, C. T. *Chem. Rev.* **2006**, *106*, 3364–3378.
(20) Fujimori, D. G.; Walsh, C. T. *Curr. Opin. Chem. Biol.* **2007**, *11*, 553–560.
(21) Dolfing, J. *FEMS Microbiol. Lett.* **1998**, *167*, 271–274.
(22) Naismith, J. H. *Chem. Soc. Rev.* **2006**, *35*, 763–770.
(23) Butler, A.; Carter-Franklin, J. N. *Nat. Prod. Rep.* **2004**, *21*, 180–188.
(24) Raugei, S.; Carloni, P. *J. Phys. Chem. B* **2006**, *110*, 3747–3758.
(25) Bortolini, O.; Conte, V. *J. Inorg. Biochem.* **2005**, *99*, 1549–1557.
(26) Hammer, P. E.; Hill, D. S.; Lam, S. T.; van Pée, K. H.; Ligon, J. M. *Appl. Environ. Microbiol.* **1997**, *63*, 2147–2154.
(27) Dairi, T.; Nakano, T.; Aisaka, K.; Katsumata, R.; Hasegawa, M. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 1099–1106.
(28) Kirner, S.; Hammer, P. E.; Hill, D. S.; Altmann, A.; Fischer, I.; Weislo, L. J.; Lanahan, M.; van Pée, K. H.; Ligon, J. M. *J. Bacteriol.* **1998**, *180*, 1939–1943.
(29) Hohaus, K.; Altmann, A.; Burd, W.; Fischer, I.; Hammer, P. E.; Hill, D. S.; Ligon, J. M.; van Pée, K. H. *Angew. Chem.* **1997**, *109*, 2102–2104.
(30) Hölzer, M.; Burd, W.; Reissig, H.-U.; van Pée, K. H. *Adv. Synth. Catal.* **2001**, *343*, 591–595.
(31) Keller, S.; Wage, T.; Hohaus, K.; Hölzer, M.; Eichhorn, E.; van Pée, K. H. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2300–2302.
(32) Unversucht, S.; Hollmann, F.; Schmid, A.; van Pée, K. H. *Adv. Synth. Catal.* **2005**, *347*, 1163–1167.
(33) Paulsen, I. T.; Press, C. M.; Ravel, J.; Kobayashi, D. Y.; Myers, G. S. A.; Mavrodi, D. V.; Deboy, R. T.; Seshadri, R.; Ren, Q. H.; Madupu, R.; Dodson, R. J.; Durkin, A. S.; Brinkac, L. M.; Daugherty, S. C.; Sullivan, S. A.; Rosovitz, M. J.; Gwinn, M. L.; Zhou, L. W.; Schneider, D. J.; Cartinhour, S. W.; Nelson, W. C.; Weidman, J.; Watkins, K.; Tran, K.; Khouri, H.; Pierson, E. A.; Pierson, L. S.; Thomashow, L. S.; Loper, J. E. *Nat. Biotechnol.* **2005**, *23*, 873–878.
(34) Dong, C.; Kotzsch, A.; Dorward, M.; van Pée, K. H.; Naismith, J. H. *Acta Crystallogr. D: Biol. Crystallogr.* **2004**, *60*, 1438–1440.
(35) Dong, C. J.; Flecks, S.; Unversucht, S.; Haupt, C.; van Pée, K. H.; Naismith, J. H. *Science* **2005**, *309*, 2216–2219.
(36) Seibold, C.; Schnerr, H.; Rumpf, J.; Kunzendorf, A.; Hatscher, C.; Wage, T.; Ernyei, A. J.; Dong, C. J.; Naismith, J. H.; van Pée, K. H. *Biocatal. Biotransform.* **2006**, *24*, 401–408.
(37) Sánchez, C.; Butovich, I. A.; Braña, A. F.; Rohr, J.; Méndez, C.; Salas, J. A. *Chem. Biol.* **2002**, *9*, 519–531.
(38) Onaka, H.; Taniguchi, S.; Igarashi, Y.; Furumai, T. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 127–138.
(39) Yeh, E.; Garneau, S.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3960–3965.
(40) Yeh, E.; Cole, L. J.; Barr, E. W.; Bollinger, J. M.; Ballou, D. P.; Walsh, C. T. *Biochemistry* **2006**, *45*, 7904–7912.
(41) Yeh, E.; Blasiak, L. C.; Koglin, A.; Drennan, C. L.; Walsh, C. T. *Biochemistry* **2007**, *46*, 1284–1292.
(42) Zehner, S.; Kotzsch, A.; Bister, B.; Süssmuth, R. D.; Méndez, C.; Salas, J. A.; van Pée, K. H. *Chem. Biol.* **2005**, *12*, 445–452.
(43) Rachid, S.; Krug, D.; Kunze, B.; Kochems, I.; Scharfe, M.; Zabriskie, T. M.; Blöcker, H.; Müller, R. *Chem. Biol.* **2006**, *13*, 667–681.
(44) Chiu, H. T.; Hubbard, B. K.; Shah, A. N.; Eide, J.; Fredenburg, R. A.; Walsh, C. T.; Khosla, C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8548–8553.
(45) Puk, O.; Bischoff, D.; Kittel, C.; Pelzer, S.; Weist, S.; Stegmann, E.; Süssmuth, R. D.; Wohlleben, W. *J. Bacteriol.* **2004**, *186*, 6093–6100.
(46) Rouhiainen, L.; Paulin, L.; Suomalainen, S.; Hyytiainen, H.; Buikema, W.; Haselkorn, R.; Sivonen, K. *Mol. Microbiol.* **2000**, *37*, 156–167.
(47) Tooming-Klunderud, A.; Rohrlack, T.; Shalchian-Tabrizi, K.; Kristensen, T.; Jakobsen, K. S. *Microbiology* **2007**, *153*, 1382–1393.
(48) Cadel-Six, S.; Dauga, C.; Castets, A. M.; Rippka, R.; Bouchier, C.; de Marsac, N. T.; Welker, M. *Mol. Biol. Evol.* **2008**, *25*, 2031–2041.
(49) Lin, S.; Van Lanen, S. G.; Shen, B. *J. Am. Chem. Soc.* **2007**, *129*, 12432–12438.
(50) Dorrestein, P. C.; Yeh, E.; Garneau-Tsodikova, S.; Kelleher, N. L.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13843–13848.
(51) Heide, L.; Westrich, L.; Anderle, C.; Gust, B.; Kammerer, B.; Piel, J. *ChemBioChem* **2008**, *9*, 1992–1999.
(52) Yu, T. W.; Bai, L. Q.; Clade, D.; Hoffmann, D.; Toelzer, S.; Trinh, K. Q.; Xu, J.; Moss, S. J.; Leistner, E.; Floss, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7968–7973.
(53) Cassady, J. M.; Chan, K. K.; Floss, H. G.; Leistner, E. *Chem. Pharm. Bull.* **2004**, *52*, 1–26.
(54) Floss, H. G. *J. Nat. Prod.* **2006**, *69*, 158–169.
(55) Spiteller, P.; Bai, L. Q.; Shang, G. D.; Carroll, B. J.; Yu, T. W.; Floss, H. G. *J. Am. Chem. Soc.* **2003**, *125*, 14236–14237.
(56) Färber, P.; Geisen, R. *Eur. J. Plant Pathol.* **2004**, *110*, 661–669.
(57) Li, S. M.; Heide, L. *Planta Med.* **2006**, *72*, 1093–1099.
(58) Pacholec, M.; Hillson, N. J.; Walsh, C. T. *Biochemistry* **2005**, *44*, 12819–12826.
(59) Fujimori, D. G.; Hrvatin, S.; Neumann, C. S.; Strieker, M.; Marahiel, M. A.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16498–16503.

(60) Piraee, M.; White, R. L.; Vining, L. C. *Microbiology* **2004**, *150*, 85–94.
(61) Pacholec, M.; Sello, J. K.; Walsh, C. T.; Thomas, M. G. *Org. Biomol. Chem.* **2007**, *5*, 1692–1694.
(62) Otsuka, M.; Ichinose, K.; Fujii, I.; Ebizuka, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 3468–3476.
(63) Hutchinson, R. I.; Grant, R. J.; Murphy, C. D. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 3046–3049.
(64) van Pée, K. H.; Salcher, O.; Fischer, P.; Bokel, M.; Lingens, F. *J. Antibiot.* **1983**, *36*, 1735–1742.
(65) Smith, C. G. *J. Bacteriol.* **1958**, *75*, 577–583.
(66) Doerschuk, A. P.; Mccormick, J. R. D.; Goodman, J. J.; Szumski, S. A.; Growich, J. A.; Miller, P. A.; Bitler, B. A.; Jensen, E. R.; Matrishin, M.; Petty, M. A.; Phelps, A. S. *J. Am. Chem. Soc.* **1959**, *81*, 3069–3075.
(67) Bister, B.; Bischoff, D.; Nicholson, G. J.; Stockert, S.; Wink, J.; Brunati, C.; Donadio, S.; Pelzer, S.; Wohlleben, W.; Sussmuth, R. D. *ChemBioChem* **2003**, *4*, 658–662.
(68) Stander, M. A.; Steyn, P. S.; Lübben, A.; Miljkovic, A.; Mantle, P. G.; Marais, G. J. *J. Agric. Food Chem.* **2000**, *48*, 1865–1871.
(69) Magarvey, N. A.; Beck, Z. Q.; Golakoti, T.; Ding, Y. S.; Huber, U.; Hemscheidt, T. K.; Abelson, D.; Moore, R. E.; Sherman, D. H. *ACS Chem. Biol.* **2006**, *1*, 766–779.
(70) Eustáquio, A. S.; Gust, B.; Li, S. M.; Pelzer, S.; Wohlleben, W.; Chater, K. F.; Heide, L. *Chem. Biol.* **2004**, *11*, 1561–1572.
(71) Akinaga, S.; Sugiyama, K.; Akiyama, T. *Anti-Cancer Drug Des.* **2000**, *15*, 43–52.
(72) Sánchez, C.; Zhu, L. L.; Braña, A. F.; Salas, A. P.; Rohr, J.; Méndez, C.; Salas, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 461–466.
(73) Muffler, K.; Retzlaff, M.; van Pée, K. H.; Ulber, R. *J. Biotechnol.* **2007**, *127*, 425–433.
(74) Krebs, C.; Galonic, F. D.; Walsh, C. T.; Bollinger, J. M., Jr. *Acc. Chem. Res.* **2007**, *40*, 484–492.
(75) Vaillancourt, F. H.; Yin, J.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10111–10116.
(76) Vaillancourt, F. H.; Vosburg, D. A.; Walsh, C. T. *ChemBioChem* **2006**, *7*, 748–752.
(77) Blasiak, L. C.; Vaillancourt, F. H.; Walsh, C. T.; Drennan, C. L. *Nature* **2006**, *440*, 368–371.
(78) Straganz, G. D.; Nidetzky, B. *ChemBioChem* **2006**, *7*, 1536–1548.
(79) Edwards, D. J.; Marquez, B. L.; Nogle, L. M.; McPhail, K.; Goeger, D. E.; Roberts, M. A.; Gerwick, W. H. *Chem. Biol.* **2004**, *11*, 817–833.
(80) Flatt, P. M.; O'Connell, S. J.; McPhail, K. L.; Zeller, G.; Willis, C. L.; Sherman, D. H.; Gerwick, W. H. *J. Nat. Prod.* **2006**, *69*, 938–944.
(81) Chang, Z.; Flatt, P.; Gerwick, W. H.; Nguyen, V. A.; Willis, C. L.; Sherman, D. H. *Gene* **2002**, *296*, 235–247.
(82) Ridley, C. P.; Bergquist, P. R.; Harper, M. K.; Faulkner, D. J.; Hooper, J. N. A.; Haygood, M. G. *Chem. Biol.* **2005**, *12*, 397–406.
(83) Ueki, M.; Galonic, D. P.; Vaillancourt, F. H.; Garneau-Tsodikova, S.; Yeh, E.; Vosburg, D. A.; Schroeder, F. C.; Osada, H.; Walsh, C. T. *Chem. Biol.* **2006**, *13*, 1183–1191.
(84) Galonic, D. P.; Barr, E. W.; Walsh, C. T.; Bollinger, J. M., Jr.; Krebs, C. *Nat. Chem. Biol.* **2007**, *3*, 113–116.
(85) Ramaswamy, A. V.; Sorrels, C. M.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 1977–1986.
(86) Vaillancourt, F. H.; Yeh, E.; Vosburg, D. A.; O'Connor, S. E.; Walsh, C. T. *Nature* **2005**, *436*, 1191–1194.
(87) Dong, C. J.; Huang, F. L.; Deng, H.; Schaffrath, C.; Spencer, J. B.; O'Hagan, D.; Naismith, J. H. *Nature* **2004**, *427*, 561–565.
(88) Beer, L. L.; Moore, B. S. *Org. Lett.* **2007**, *9*, 845–848.
(89) Harper, D. B. *Nature* **1985**, *315*, 55–57.
(90) Wuosmaa, A. M.; Hager, L. P. *Science* **1990**, *249*, 160–162.
(91) Ohsawa, N.; Tsujita, M.; Morikawa, S.; Itoh, N. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2397–2404.
(92) Amachi, S.; Kamagata, Y.; Kanagawa, T.; Muramatsu, Y. *Appl. Environ. Microbiol.* **2001**, *67*, 2718–2722.
(93) Saxena, D.; Aouad, S.; Attieh, J.; Saini, H. S. *Appl. Environ. Microbiol.* **1998**, *64*, 2831–2835.
(94) van Pée, K. H. *Biotechnol. Adv.* **1990**, *8*, 185–205.
(95) Ballschmiter, K. *Chemosphere* **2003**, *52*, 313–324.
(96) Colin, C.; Leblanc, C.; Wagner, E.; Delage, L.; Leize-Wagner, E.; Van Dorsselaer, A.; Kloareg, B.; Potin, P. *J. Biol. Chem.* **2003**, *278*, 23545–23552.
(97) Morris, D. R.; Hager, L. P. *J. Biol. Chem.* **1966**, *241*, 1763–1768.
(98) Butler, A.; Walker, J. V. *Chem. Rev.* **1993**, *93*, 1937–1944.
(99) Ullrich, R.; Nuske, J.; Scheibner, K.; Spantzel, J.; Hofrichter, M. *Appl. Environ. Microbiol.* **2004**, *70*, 4575–4581.
(100) Henderson, J. P.; Heineche, J. W. In *Natural Production of Organohalogen Compounds. The Handbook of Environmental Chemistry*; Hutzinger, O., Gribble, G. W., Eds.; Springer: Berlin, 2003; Vol. 3,Part P.
(101) Jakopitsch, C.; Regelsberger, G.; Furtmuller, P. G.; Ruker, F.; Peschek, G. A.; Obinger, C. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 682–687.
(102) Antipov, A. N.; Sorokin, D. Y.; L'Vov, N. P.; Kuenen, J. G. *Biochem. J.* **2003**, *369*, 185–189.
(103) Sanfilippo, C.; D'Antona, N.; Nicolosi, G. *Biotechnol. Lett.* **2004**, *26*, 1815–1819.
(104) Spreti, N.; Germani, R.; Incani, A.; Savelli, G. *Biotechnol. Prog.* **2004**, *20*, 96–101.
(105) Yi, X. W.; Conesa, A.; Punt, P. J.; Hager, L. P. *J. Biol. Chem.* **2003**, *278*, 13855–13859.
(106) Baciocchi, E.; Fabbrini, M.; Lanzalunga, O.; Manduchi, L.; Pochetti, G. *Eur. J. Biochem.* **2001**, *268*, 665–672.
(107) Sundaramoorthy, M.; Terner, J.; Poulos, T. L. *Chem. Biol.* **1998**, *5*, 461–473.
(108) Manoj, K. M. *Biochim. Biophys. Acta, Proteins Proteomics* **2006**, *1764*, 1325–1339.
(109) Littlechild, J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 28–34.
(110) Osborne, R. L.; Raner, G. M.; Hager, L. P.; Dawson, J. H. *J. Am. Chem. Soc.* **2006**, *128*, 1036–1037.
(111) Carter-Franklin, J. N.; Parrish, J. D.; Tschirret-Guth, R. A.; Little, R. D.; Butler, A. *J. Am. Chem. Soc.* **2003**, *125*, 3688–3689.
(112) Wever, R.; Hemrika, W. In *Handbook of Metalloproteins*; Messerschmidt, A., Huber, R., Wieghardt, K., Poulos, T., Eds.; Wiley & Sons (England) Ltd.: Chichester, 2001; Vol. 1.
(113) Messerschmidt, A.; Wever, R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 392–396.
(114) Weyand, M.; Hecht, H. J.; Kiess, M.; Liaud, M. F.; Vilter, H.; Schomburg, D. *J. Mol. Biol.* **1999**, *293*, 595–611.
(115) Isupov, M. N.; Dalby, A. R.; Brindley, A. A.; Izumi, Y.; Tanabe, T.; Murshudov, G. N.; Littlechild, J. A. *J. Mol. Biol.* **2000**, *299*, 1035–1049.
(116) Carter-Franklin, J. N.; Butler, A. *J. Am. Chem. Soc.* **2004**, *126*, 15060–15066.
(117) Martinez, J. S.; Carroll, G. L.; Tschirret-Guth, R. A.; Altenhoff, G.; Little, R. D.; Butler, A. *J. Am. Chem. Soc.* **2001**, *123*, 3289–3294.
(118) Vilter, H. *Phytochemistry* **1984**, *23*, 1387–1390.
(119) Plat, H.; Krenn, B. E.; Wever, R. *Biochem. J.* **1987**, *248*, 277–279.
(120) Winter, J. M.; Moffitt, M. C.; Zazopoulos, E.; McAlpine, J. B.; Dorrestein, P. C.; Moore, B. S. *J. Biol. Chem.* **2007**, *282*, 16362–16368.
(121) Soedjak, H. S.; Butler, A. *Biochemistry* **1990**, *29*, 7974–7981.
(122) Littlechild, J.; Garcia-Rodriguez, E.; Dalby, A.; Isupov, M. *J. Mol. Recognit.* **2002**, *15*, 291–296.
(123) Tanaka, N.; Dumay, V.; Liao, Q. N.; Lange, A. J.; Wever, R. *Eur. J. Biochem.* **2002**, *269*, 2162–2167.
(124) Borchardt, S. A.; Allain, E. J.; Michels, J. J.; Stearns, G. W.; Kelly, R. F.; McCoy, W. F. *Appl. Environ. Microbiol.* **2001**, *67*, 3174–3179.
(125) Leblanc, C.; Colin, C.; Cosse, A.; Delage, L.; La Barre, S.; Morin, P.; Fievet, B.; Voiseux, C.; Ambroise, Y.; Verhaeghe, E.; Amouroux, D.; Donard, O.; Tessier, E.; Potin, P. *Biochimie* **2006**, *88*, 1773–1785.
(126) Zerbe, K.; Pylypenko, O.; Vitali, F.; Zhang, W.; Rouset, S.; Heck, M.; Vrijbloed, J. W.; Bischoff, D.; Bister, B.; Süssmuth, R. D.; Pelzer, S.; Wohlleben, W.; Robinson, J. A.; Schlichting, I. *J. Biol. Chem.* **2002**, *277*, 47476–47485.
(127) Pelzer, S.; Süssmuth, R.; Heckmann, D.; Recktenwald, J.; Huber, P.; Jung, G.; Wohlleben, W. *Antimicrob. Agents Chemother.* **1999**, *43*, 1565–1573.
(128) Sosio, M.; Kloosterman, H.; Bianchi, A.; de Vreugd, P.; Dijkhuizen, L.; Donadio, S. *Microbiology* **2004**, *150*, 95–102.
(129) Sosio, M.; Stinchi, S.; Beltrametti, F.; Lazzarini, A.; Donadio, S. *Chem. Biol.* **2003**, *10*, 541–549.
(130) Lin, S.; Van Lanen, S. G.; Shen, B. *J. Am. Chem. Soc.* **2007**, *129*, 12432–12438.
(131) Jia, X. Y.; Tian, Z. H.; Shao, L.; Qu, X. D.; Zhao, Q. F.; Tang, J.; Tang, G. L.; Liu, W. *Chem. Biol.* **2006**, *13*, 575–585.
(132) Wynands, I.; van Pée, K. H. *FEMS Microbiol. Lett.* **2004**, *237*, 363–367.
(133) Zhang, X.; Parry, R. J. *Antimicrob. Agents Chemother.* **2007**, *51*, 946–957.
(134) Weitnauer, G.; Mühlenweg, A.; Trefzer, A.; Hoffmeister, D.; Süssmuth, R. D.; Jung, G.; Welzel, K.; Vente, A.; Girreser, U.; Bechthold, A. *Chem. Biol.* **2001**, *8*, 569–581.
(135) Ahlert, J.; Shepard, E.; Lomovskaya, N.; Zazopoulos, E.; Staffa, A.; Bachmann, B. O.; Huang, K. X.; Fonstein, L.; Czisny, A.; Whitwam, R. E.; Farnet, C. M.; Thorson, J. S. *Science* **2002**, *297*, 1173–1176.
(136) Trefzer, A.; Pelzer, S.; Schimana, J.; Stockert, S.; Bihlmaier, C.; Fiedler, H. P.; Welzel, K.; Vente, A.; Bechthold, A. *Antimicrob. Agents Chemother.* **2002**, *46*, 1174–1182.

(137) Vaillancourt, F. H.; Yin, J.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10111–10116.
(138) Guenzi, E.; Galli, G.; Grgurina, I.; Gross, D. C.; Grandi, G. *J. Biol. Chem.* **1998**, *273*, 32857–32863.
(139) Galonic, D. P.; Vaillancourt, F. H.; Walsh, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 3900–3901.
(140) Ullrich, M.; Guenzi, A. C.; Mitchell, R. E.; Bender, C. L. *Appl. Environ. Microbiol.* **1994**, *60*, 2890–2897.
(141) Flatt, P.; Gautschi, J.; Thacker, R.; Musafija-Girt, M.; Crews, P.; Gerwick, W. *Mar. Biol.* **2005**, *147*, 761–774.

NP800651M