The Biosynthesis of Vancomycin-Type Glycopeptide Antibiotics—The Order of the Cyclization Steps**

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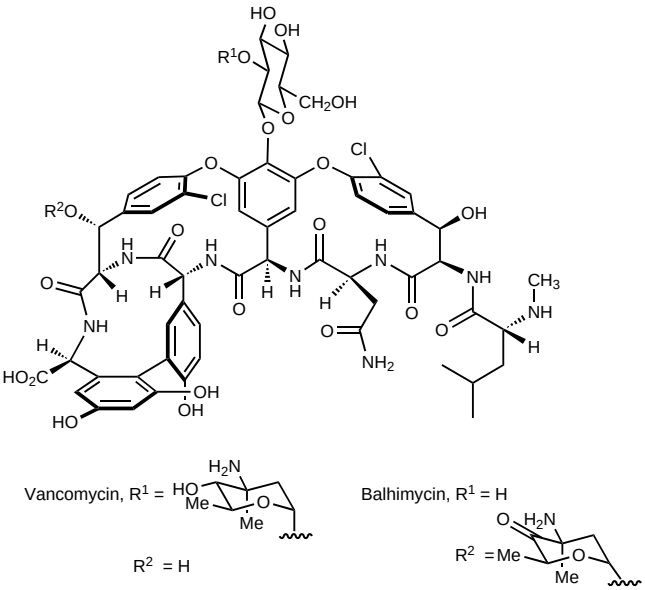
Dedicated to Professor K. Barry Sharpless on the occasion of his 60th birthday

Vancomycin (Scheme 1),[1] a glycopeptide antibiotic produced by *Amycolatopsis orientalis*, is the most prominent representative of a family comprising several hundred natural products with common structural features. A tremendous amount of work has been focused on the elucidation of its structure, mode of action, and on the total synthesis.[1, 2] The potency of vancomycin against methicillin-resistant *Staphylococcus aureus* strains (MRSA) has led to its widespread application as an antibiotic of last resort.[3] Until recently, little work has been done on the molecular biology of the glycopeptide biosynthesis, with the exception of a few biochemical studies.[4] The sequencing of the chloroeremomycin biosynthesis gene cluster of *A. orientalis* provided first insights into the genes participating in the glycopeptide biosynthesis.[5] However, the nature and sequence of many essential biosynthesis steps and the structures of biosynthesis intermediates remained unknown. The identification of the balhimycin gene cluster in *A. mediterranei*[6] and the development of a host–vector system for the balhimycin producer[7] enables the biosynthesis to be manipulated for the first time.

Our work addresses the question of how nature assembles the unique aglycon structure. This moiety confers antibiotic activity and its assembly thus represents a key step in the glycopeptide antibiotic biosynthesis. Therefore, we focused our attention on three oxygenases (OxyA, OxyB, and OxyC) in the glycopeptide biosynthesis gene cluster of the balhimycin producer *A. mediterranei DSM5908*. These are presumably involved in the three oxidation steps leading from a linear precursor peptide to the side chain cyclized aglycon.



Scheme 1. Structures of the glycopeptide antibiotics vancomycin and balhimycin.

From an integration mutant within the oxygenase region we characterized hexa- and heptapeptides, which we suggested to be linear intermediates of the aglycon.[6, 8] In a subsequent study we isolated the first monocyclic peptides from a gene replacement mutant in the *oxyA* gene, the ring closure of which possibly represents the first step in the three oxidative bridging reactions.[9] Herein, we report the first bicylic peptides. These structures allow us to deduce the sequence of the three oxidative bridging steps of the aromatic side chains leading to the formation of the aglycon.

From the culture filtrate of a gene replacement mutant SPoxyBcat (Figure 1), two compounds, SP-901 (**1**) and SP-1066 (**2**) with molecular masses of 901.3 and 1066.4 Da, respectively, were isolated. Full structural analysis showed that they were the linear dechlorinated analogues of compounds previously described (Scheme 2).[8]

From the culture filtrate of a second gene replacement mutant SPoxyCcat of *A. mediterranei* (Figure 1), three compounds SP-911 (**3**), SP-1073 (**4**), and SP-1238 (**5**) with molecular masses of 911.3, 1073.4, and 1238.4 Da, respectively, were isolated (Scheme 3). As in the case of compounds **1** and **2**, the isotopic pattern of the $[M+H]^+$ signals of compounds **3**–**5** indicated the absence of chlorine atoms. By means of chiral amino acid analysis, D-*N*-methylleucine (MeLeu), L-Asx, and D-4-hydroxyphenylglycine (Hpg) were identified as constituents for all compounds. In the case of compound **5**, L-3,5-dihydroxyphenylglycine (Dpg) was detected additionally. Edman degradation of compounds **3**–**5** gave the sequence ^{1}MeLeu-^{2}-———-^{3}Asn-^{4}-———-^{5}Hpg-^{6}-———.

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[**] This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323) and by a grant of the EU (MEGATOP, QLK3-1999-00650). R. D. S. gratefully acknowledges the support of a Feodor-Lynen Fellowship granted by the Alexander-von-Humboldt Stiftung. We thank Corina Bihlmaier and Volker Pfeifer for help with transformation and Southern hybridization, J. A. Moss (La Jolla (USA)) for critical comments on the manuscript and Prof. Dr. M. E. Maier and Prof. Dr. H.-P. Fiedler (Tübingen) for generous support.

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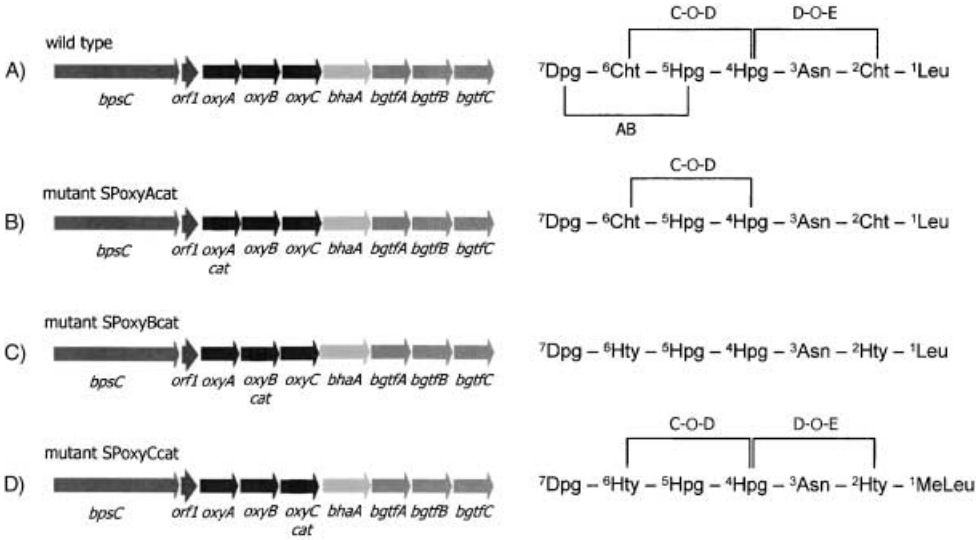
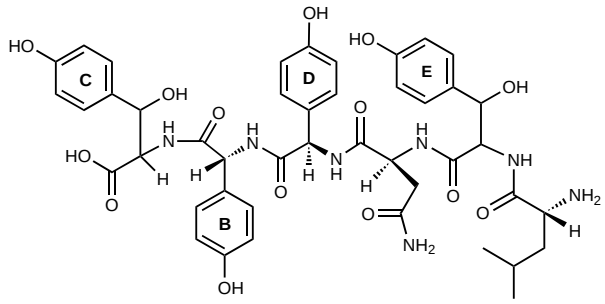


Figure 1. Gene organization of *A. mediterranei* wild type strains and of the corresponding mutants. The degree of cyclization is shown schematically for the example of the characterized heptapeptides. A) Gene organization of a part of the balhimycin biosynthetic gene cluster from *A. mediterranei* wild type. B) *oxyA* gene replacement mutant SPoxyAcat. C) *oxyB* gene replacement mutant SPoxyBcat. D) *oxyC* gene replacement mutant SPoxyCcat. *bpsC*: peptide synthetase gene; *orf1*: Orf with unknown function; *oxyA – C*: oxygenase genes; *bhaA*: halogenase gene; *bgtfA – C*: glycosyltransferase genes. *cat*: chloramphenicol resistance gene; Hty = β-hydroxytyrosine).



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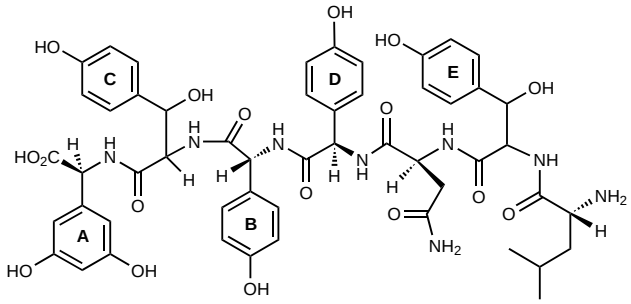

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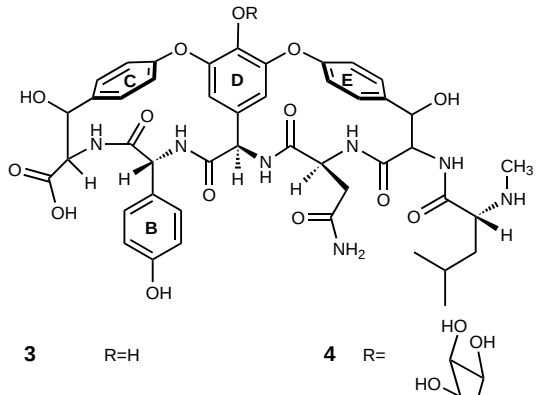
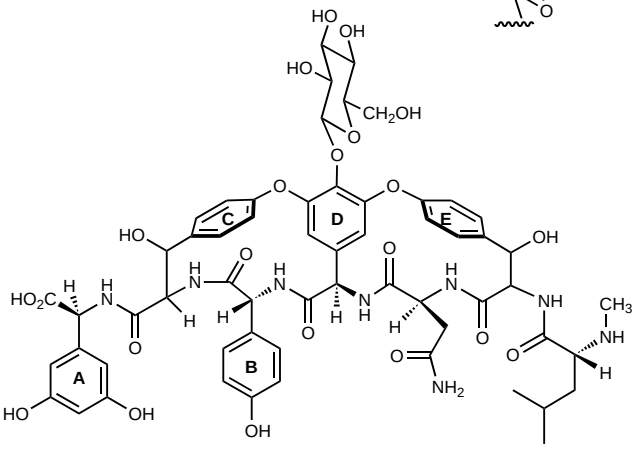
Scheme 2. Structures of SP-901 (**1**) and SP-1066 (**2**).

Curiously, Dpg did not appear in cycle 7 of Edman degradation of compound **5**. In accordance with previous data obtained by Edman degradation of SP-1134 (linear) and SP-1132 (monocyclic),[8, 9] cyclization of the C-O-D and the D-O-E ring systems was found for **3** – **5** (Table 1). Sugar analysis by GC-MS revealed D-glucose for compounds **4** and **5**. 2D-NMR experiments (COSY, TOCSY, NOESY, HSQC, HMBC) on **5** (Table 2) confirmed these results and revealed the structure of **5**[10] with β-glycosylation at ^{4}Hpg as shown in Scheme 3.



3 R=H **4** R=



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Scheme 3. Structures of SP-911 (**3**), SP-1073 (**4**), and SP-1238 (**5**).

Compounds **1** – **5** as well as those characterized in previous studies[8, 9] all contain β-hydroxylated tyrosine groups at positions 2 and 6. This confirms our former suggestion that

Table 1. Results of Edman degradation of intermediates isolated from different *A. mediterranei* mutants.

Peptide	Degradation cycle[a]						
	1	2	3	4	5	6	7
SP-1134[b]	Leu	Cht	Asn	Hpg	Hpg	Cht	Dpg
SP-1132[c]	Leu	Cht	Asn	–	Hpg	–	Dpg
SP-1238[d]	MeLeu	–	Asn	–	Hpg	–	n.d.
Aglycon	MeLeu	–	Asn	–	–	–	–

[a] – = no amino acid identified; n.d. = assignment ambiguous. [b] Linear heptapeptide SP-1134.[8] [c] Monocyclic heptapeptide SP-1132.[9] [d] Newly identified bicyclic heptapeptide SP-1238.

β-hydroxylation is already present at the linear precursor-peptide stage. In contrast to the linear and the monocyclic peptide intermediates, the bicyclic metabolites **3**–**5** isolated from culture filtrates of SPoxyCcat are N-methylated at ^{1}Leu. These data also show that the early formed bicylic C-O-D-O-E ring intermediate is subject to N-methylation. The results are in accordance with the enzyme assays of the overexpressed *N*-methyltransferase of *A. orientalis*, which showed that the demethylated aglycon and demethylated glycopeptides are clearly favored over linear peptides for the N-methylation.[11] Similarly, the O-glycosylation of ^{4}Hpg in **4** and **5** (β-D-Glc), as found in balhimycin, shows that the bicyclic C-O-D-O-E system is already recognized by a glycosyltransferase of the balhimycin biosynthesis gene cluster. In contrast to the chlorinated peptides from the SPoxyAcat mutant, the unexpected absence of chlorine in peptides **1**–**5**, which were isolated from SPoxyBcat and SPoxyCcat, cannot be explained at the current stage of our investigations. It is conceivable that gene replacement of *oxyB* and *oxyC* also affected expression of *bhaA* which codes for the respective halogenase[12] and which is located directly downstream. Furthermore, it is still unknown why hexapeptides can be isolated, as already shown for the postulated linear biosynthesis precursors.[8]

Finally, the inactivation of oxygenase genes (*oxyA*, *oxyB*, *oxyC*) through replacement mutants reveals peptides with different degrees of cyclization (Figure 1). According to these results, that is the characterization of compounds **1**–**5**, and those described in our previous contribution,[9] we postulate the order of the cyclization steps as follows: 1. Formation of the C-O-D ring system; 2. Formation of the D-O-E ring system; 3. Formation of the biaryl AB system. Thus, the oxygenases act in a stepwise fashion in the sequence OxyB $\rightarrow$OxyA $\rightarrow$OxyC.

The N-methylation of Leu as well as the glycosylation at ^{4}Hpg can partially occur already at the stage of the bicylic intermediate as indicated by the occurrence of compounds **3**–**5** and **4**, **5**, respectively. Since glycosylation in position 6 is not observed, glycosylation at ^{4}Hpg by a glycosyltransferase is considered to be the first step in the glycosylation sequence. These results indicate a relaxed substrate specificity of the participant enzymes and are in agreement with the known biochemical action of the enzymes methyltransferase (Mtf)[11] and glycosyltransferase (Gtf)[13] in the chloroeremomycin and vancomycin gene cluster. We assume that the sequence of the aglycon assembly is representative for type-I and type-II glycopeptide antibiotics.

Experimental Section

The mutants SPoxyBcat and SPoxyCcat of *Amycolatopsis mediterranei DSM5908* were grown in liquid culture. All peptides were purified (yields SPoxyBcat: 8.6 mg L^{-1} (**1**), 10.6 mg L^{-1} (**2**); yields SPoxyCcat: 1.2 mg L^{-1} (**3**), 0.8 mg L$^-$ (**4**), and 0.5 mg L^{-1} (**5**)) and analyzed according to procedures described previously.[8, 9]

Received: July 12, 2001 [Z17490]

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Table 2. ^{1}H and ^{13}C NMR shifts of the peptide SP-1238 (**5**) ([D6]DMSO, 298 K).

		NH, CO	α	β	Others		
^{1}Leu	$\delta(^1$H)	8.93	4.00	1.68/1.57	1.64 (γ), 0.86/0.90 (δ), 2.63 (NCH$_3$)		
	$\delta(^{13}$C)	167.6	59.2	38.7	23.6 (γ), 22.5/22.2 (δ), 31.0 (NCH$_3$)		
^{2}Hty	$\delta(^1$H)	8.62	4.90	5.23	5.86 (OH$^\gamma$), 7.60 (2), 7.22 (3), 7.06 (5), 7.25 (6)		
	$\delta(^{13}$C)	–[a]	58.8	71.2	136.8 (1), 127.5 (2), 154.7 (4), 122.2 (3), 121.2 (5), 128.1 (6)		
^{3}Asn	$\delta(^1$H)	–[a]	4.34	2.58/2.19	7.53/7.07 (δ)		
	$\delta(^{13}$C)	169.7	50.3	36.0	171.5 (γ)		
^{4}Hpg	$\delta(^1$H)	8.00	5.21	–	5.49 (2), 5.78 (6)		
	$\delta(^{13}$C)	166.8	54.7	–	106.3 (2), 132.1 (1), 152.9 (3), 156.4 (4), –[a] (5), 106.6 (6)		
^{5}Hpg	$\delta(^1$H)	8.80	5.18	–	7.11 (2,6), 6.68 (3,5), 9.44 (OH^4)		
	$\delta(^{13}$C)	168.4	55.6	–	128.1 (2,6), 114.8 (3,5), 158.4 (4), –[a] (1)		
^{6}Hty	$\delta(^1$H)	–[a]	4.75	5.44	5.65 (OH$^\gamma$), 7.37 (2), 7.06 (3), 7.16 (5), 7.55 (6)		
	$\delta(^{13}$C)	167.8	58.8	71.0	138.2 (1), 127.6 (2), 121.2 (3), 153.4 (4), 121.9 (5), 126.4 (6)		
^{7}Dpg	$\delta(^1$H)	8.73	5.15	–	6.28 (2,6), 9.35 (OH^{3,5}), 6.15 (4)		
	$\delta(^{13}$C)	171.7	55.9	–	105.2 (2,6), 158.1 (3,5), 101.9 (4), –[a] (1)		
Glc		1′	2′	3′	4′	5′	6′
	$\delta(^1$H)	5.18	3.34	3.26	3.16	3.18	3.72/3.46
	$\delta(^{13}$C)	102.6	73.7	76.3	69.8	77.1	60.8
OH	$\delta(^1$H)	–	–[a]	–[a]	–[a]	–	–[a]

[a] Assignment not determined. Hty = β-hydroxytyrosine.

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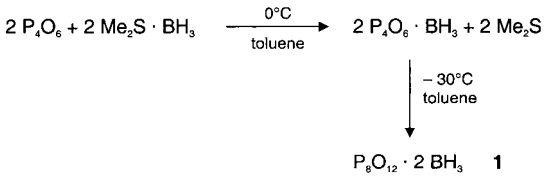
Dimerization of Molecular Phosphorus Oxides

Akbar Tellenbach und Martin Jansen*

Highly symmetric, spatially closed molecular compounds are frequently much less willing to undergo reactions. Such inhibition of a reaction based on its kinetics may have considerable effects in the most diverse fields in chemistry. For instance, in chemical transport reactions involving SiF_4 as a component of the gas phase, the thermodynamically calculated transport rates are not reproduced experimentally, because the reaction back to the solid is strongly inhibited.[1] In addition, SF_6 or CCl_4 are stable towards water at room temperature, although in view of the thermodynamics they should undergo vigorous hydrolysis.[2]

In this respect P_4O_6 appears particularly ambivalent, on the one hand it adds smoothly as donor ligand or reacts rapidly with water giving phosphonic acid, on the other hand it is remarkably resistant to directed ring-opening reactions. Among the few controlled and selective reactions on the adamantane-like P_4O_6 cage are the nitrene insertion[3] and several alcoholyses.[4] In view of this, the observation reported herein of a spontaneous dimerization of the monoborane adduct of P_4O_6 without the need for special activation was unexpected.

In the attempt to crystallize $P_4O_6 \cdot BH_3$,[5, 6] which forms in the stoichiometric reaction of P_4O_6 with $Me_2S \cdot BH_3$, at $-30\,^\circ C$ in a concentrated toluene solution, we obtained instead the dimer $P_8O_{12} \cdot 2BH_3$ (**1**) as crystalline material (Scheme 1). After three months single crystals had formed which were suitable for a crystal structure analysis.[7]



Scheme 1. Synthesis of **1**.

The principal building block of **1** is a novel P_8O_{12} framework, which evidently originates from the fusion of two P_4O_6 cages after cleavage of one P–O bond in each cage. As shown in Figure 1, two $P_4O_6 \cdot BH_3$ units were connected through two common oxygen atoms (O1, O7) in such a manner that the borane groups point in opposite directions (head – tail linkage). The dimer is folded along the two bridging oxygen atoms, resulting in dihedral angles of 151° (P1-O1-O7-P5) and 164° (P4-O7-O1-P8), respectively. The different dihedral

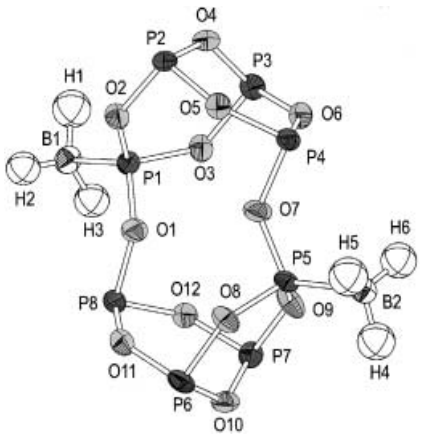


Figure 1. Molecular structure of **1** (ellipsoids for 50% probability). Selected bond lengths [pm] and angles [°]: P1-B1 187.2(2), P1-O1 158.0(2), P1-O2 158.9(2), P1-O3 158.5(2), P2-O2 166.5(2), P2-O4 163.7(2), P2-O5 162.7(2), P3-O3 166.0(2), P3-O4 163.9(2), P3-O6 162.4(2), P4-O5 162.5(2), P4-O6 163.2(2), P4-O7 165.6(2), B1-H1 113(4), B1-H2 101(3), B1-H3 119(3); O1-P1-O2 102.1(1), O1-P1-O3 103.2(1), O2-P1-O3 104.6(1), P1-O1-P8 126.8(1), P1-O2-P2 131.5(1), P1-O3-P3 135.4(1), P2-O4-P3 126.6(1), H1-B1-H2 111(3), H1-B1-H3 117(2), H2-B1-H3 118(2).

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  1433-7851/01/4024-4691 $ 17.50+.50/0