Biomimetic Total Synthesis of Enterocin

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In memory of Klaus Hafner

Abstract: The first chemical total synthesis of the highly oxygenated polyketide enterocin has been accomplished. The key step of the synthesis was a late-stage biomimetic reaction cascade involving two intramolecular aldol reactions in which each step proceeded in 52% yield (averaged) and which established four of the seven stereogenic centers. The pivotal precursor for the cascade reaction was assembled from three readily available building blocks. A chiral dithioacetal with two stereogenic centers originating from L-arabinose represented the core fragment to both ends of which the other building blocks were attached by aldol reactions. The remaining stereogenic center was installed by Davis oxygenation immediately prior to the key step.

Enterocin (1, Scheme 1) is a polyketide natural product, the structure of which was first described in independent reports by the groups of Miyairi[1] and Seto.[2] The former group isolated the compound from Streptomyces candidus var. enterostaticus WS-8096 and from variant M-127 of Streptomyces viridochromogenes. The latter group, who originally named the natural product vulgamycin, reported its production by Streptomyces hygroscopicus No A-5294. Subsequently, the metabolite was isolated from other biological sources, including several marine-derived Streptomyces strains.[3] While the biological activity of enterocin remains to be comprehensively studied,[2,3a,e] the biosynthesis of enterocin has been successfully unraveled. Moore and co-workers cloned and sequenced the gene cluster that is responsible for the production of not only enterocin, but also related natural products, including the wailupemycins.[5] The biosynthesis is a Favorskii-type rearrangement, which proceeds via cyclopropanone 4 to precursor 5 of the natural product: Two aldol reactions establish the key bonds C3-C4 and C8-C9 of the tricyclic skeleton.

Supported by MALDI-TOF mass spectrometry, the relative stereochemistry of the core fragment was established by X-ray crystallography. The pivotal precursor was synthesized from L-arabinose, which undergoes the rearrangement via intermediate cyclopropanone 5. The latter is trapped by the oxygen atom of the C6 alcohol to a δ-lactone with subsequent formation of two C=C single bonds (C5-C4 and C8-C9) by aldol reactions and of the α-pyrene ring by lactonization. Methylation of the free pyrene hydroxy group in intermediate 5 generates 5-deoxy-enterocin, which has been isolated as a minor metabolite from typical producers of enterocin.[3a,3b,4] The biosynthesis is concluded by selective oxygenation at C5 mediated by a cytochrome P-450 monooxygenase (EncK).

Despite the elegance and brevity of its biosynthesis, enterocin has not yet surrendered to chemical total synthesis.[11] The highly oxygenated tricyclic core structure poses an extraordinary challenge regarding the selective, sequential introduction of functional groups and their compatibility. A few synthetic studies exist: Flores-Parra and Khuong-Huu showed that an early precursor with the 2-oxabicyclo-
synthetic disconnection and key features of the pivotal aldol reaction. In this precursor, we additionally intended to install a protected hydroxy group at C5 while retaining the acetonide protecting group and installing the desired alcohol at C4. Although its configuration was required after the final step of the sequence. Initial attempts to retain the acetonide protecting group and install the oxygenated lactone moiety of compound 6 were not successful. Instead, we decided to cleave the acetonide under mild conditions, which furnished 1,2-diols 11. The primary alcohol was temporarily TBS-protected and the secondary alcohol 12 was converted into its MEM ether 13. Selective deprotection of the primary silyl ether was achieved by treatment with pyridinium para-toluenesulfonate (PPTS) in ethanol. Careful product monitoring was required to avoid two-fold deprotection to the undesired 1,3-diol. Oxidation of primary alcohol 14 to aldehyde 15 was performed under Parikh–Doering conditions, thus avoiding any undesired oxidation of the dithioacetal.

The aldol-type addition of deprotonated methyl pyrone 7 to aldehyde 15 proceeded with remarkable diastereoselectivity, delivering the desired alcohol 16 essentially as a single diastereoisomer (diastereomeric ratio = d.r. = 95/5) at the newly generated stereogenic center C4. Although its configuration was inconsequential for the further course of the synthesis of enterocin commenced with the preparation of dithioacetal 8 from L-arabinose (10, Scheme 3). The reported procedure[18] was optimized so that only a single chromatographic purifica-

Although Nature beautifully masters the combination of lactonization to the δ-lactone ring with the pivotal aldol reactions, we hypothesized that this process might be too challenging entropically and we rather focused on the synthesis of a precursor with an intact δ-lactone ring. In this precursor, we additionally intended to install a protected hydroxy group at position C5 with the correct relative configuration, reasoning that, by adopting an equatorial position, this would favor the necessary pre-orientation in the six-membered transition state required to form the C8-C9 bond. Compound 6 evolved from these considerations as an ideal precursor for the aldol cascade (Scheme 2).

The 2-methoxyethoxymethyl (MEM) group was chosen as a protecting group at the C5 alcohol, which was to be removed after the projected aldol reactions. Since the highly oxygenated lactone fragment had previously been found to be capricious,[17] we planned to install the hydroxy group at C2 in a late stage of the synthesis before establishing the required ketone functionality at C4. Further retrosynthetic analysis led to three key precursors 7–9, which would combine the 22 carbon atoms of enterocin in a convergent fashion. The core fragment 8 is a known compound[19] which is readily accessible from L-arabinose (vide infra) and displays the stereo- genic centers at carbon atoms C5 and C6 with the required relative configuration. Similarly, the other two building blocks 7[19] and 9[20] are also known and readily prepared in a few steps.

Following the plan outlined above, the synthesis of enterocin commenced with the preparation of dithioacetal 8 from L-arabinose (10, Scheme 3). The reported procedure[18] was optimized so that only a single chromatographic purifi-

\[ \text{Scheme 3. Synthesis of the left-hand fragment 17 of enterocin from L-arabinose (10): An aldol addition of methoxypyrene 7 to aldehyde 15 establishes the C-C bond at positions C4 and C9} \]

\[ \text{[TFA = trifluoroacetic acid, PG = protecting group, im = imidazole, PPTS = pyridinium para-toluenesulfonate, py = pyridine, LHĐMS = lithium hexamethyldisila-}
\]

\[ \text{zide, DMAP = 4-(N,N-dimethylamino)pyridine}. \]

\[ \text{[20] are also known and readily prepared in a few steps.}\]
Iodine-mediated oxidative cleavage\cite{18c} transpired to liberate an aldehyde group at the future carbon atom C8. Mosher analysis.\cite{25} The facial diastereoselectivity is thus governed by a Felkin–Anh- or Cornforth-controlled addition of the carbon nucleophile to the carbonyl carbon atom.\cite{26} The synthesis, the alcohol was shown to be (S)-configured by Mosher analysis.\cite{22} The facial diastereoselectivity is thus governed by a Felkin–Anh- or Cornforth-controlled addition of the carbon nucleophile to the carbonyl carbon atom.\cite{24} The steric hindrance around the newly formed secondary alcohol was reflected in the relatively harsh conditions required for its isolation. Strategically, the presence of two TBS protecting groups was considered ideal because in the diol resulting from their simultaneous removal, only the C6 hydroxy group would be competent to form the desired \( \delta \)-lactone; we would thus eschew a separate deprotection step for the C4 alcohol.

Having withstood a remarkable array of reactions, the dithioacetal group in compound 17 was to be removed to liberate an aldehyde group at the future carbon atom C8. Iodine-mediated oxidative cleavage\cite{16} transpired to be a fortuitous choice, delivering the unstable aldehyde 18 in high yield (Scheme 4). The ensuing aldol addition to aldehyde 18 involved ester 9 as the nucleophile, which was preferably deprotonated with lithium diisopropylamide (LDA) but not with LHMDS. In the former case, the reaction proceeded in near quantitative yield while in the latter case an unidentified side product precluded the isolation of aldol product 19 in pure form. Expectedly, there was no significant facial or simple diastereoselectivity, which is why all four diastereomers of product 19 were formed (d.r. = 38/36/14/12). Since both stereogenic centers are irrelevant for the further course of the synthesis, no attempts were made to investigate the selectivity more closely. From compound 19, oxidative cleavage of the terminal double bond was performed under Lemieux-Johnson conditions\cite{27} and the alcohol at position C8 was oxidized to ketone 20 with Dess–Martin periodinane.\cite{28}

To our surprise, deprotection of the two silyl ethers at C4 and C6 did not lead to the desired \( \delta \)-lactone but rather to hemiacetal 21. Closer inspection of this compound revealed that acetol formation had occurred by O–C bond formation between the oxygen atom at C4 and the C8 carbonyl carbon atom. The facile ring closure corroborated our expectation that a substituent adjacent to carbon atom C4 can cyclize to a six-membered ring by attack at the carbonyl group (vide supra).

The undesired hemiacetal formation was smoothly corrected by saponification of the methyl ester at carbon atom C1. Upon acetylation of the solution to pH 4, ring closure to the desired \( \delta \)-lactone 22 occurred. The compound exists as a mixture of the depicted enol form and the respective ketone in CDCl3 solution. In [D\(_6\)]DMSO at 300 K, only the enol form was detected by NMR spectroscopy.

Until this stage, the isolation of all compounds had been unproblematic but with the increasing density of oxygen functionalities, handling the products required greater care and optimization. Equally, any ensuing oxidative transformations were required to be compatible with the existing functional groups. Attempts to introduce the desired hydroxy group at position C2 of \( \delta \)-lactone 22 commenced with the standard Davis protocol\cite{29} employing a base and 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine as the oxygen donor. Yields remained mediocre (ca. 50%) even when the more reactive 2-para-nitrophenyl (PNP) derivative\cite{30} was used. Eventually, it turned out that the reaction was most efficient in the absence of base, delivering the desired product 23 in high yield with perfect diastereoselectivity (Scheme 5). The configuration of the newly generated stereogenic center at C2 was corroborated by NOESY studies ([D\(_6\)]DMSO, 300 K). The compound exists in a boat conformation\cite{17b} and there is an NOE contact between the hydroxy proton at C2 and the proton at C6. The preferred conformation of base, delivering the desired product

**Scheme 4.** Assembly of the lactone precursor 21 of enterocin by an aldol reaction of ester 9 to aldehyde 18 and subsequent functional group manipulations (DMP = Dess–Martin periodinane). Formation of hemiacetal 21 was a first indication that a substituent at carbon atom C4 can intramolecularly attack the C8 carbonyl group.

**Scheme 5.** Conclusion of the total synthesis of enterocin (1). Hydrox-ylation at C2 occurs presumably via the enol of \( \delta \)-lactone 22. After oxidation of alcohol 23, the formation of product 25 was observed when storing a solution of ketone 6 at room temperature. The reaction was accelerated by treatment of ketone 6 with a mild base. For further details, see the narrative.
subsequent oxidation of the alcohol at C4 to the desired cyclization precursor proved to be even more challenging than the oxygenation. Only Dess–Martin conditions[29] enabled oxidation to the ketone; careful monitoring of the reaction conditions led us to use a slight excess of oxidant and to terminate the reaction after 2.5 hours. Product 6 initially escaped isolation due to its lability under even slightly acidic conditions, for example, on silica gel, but eventually purification by semi-preparative, reversed-phase HPLC proved suitable. Ketone 6 was thus obtained in 45% yield, together with 5% of re-isolated substrate 23.

The limited quantities obtained of ketone 6 have not yet allowed to screen a wide range of conditions for the projected cyclization. Gratifyingly, though, the desired cascade reaction was found to occur in acetonitrile as the solvent and was accelerated by a mild base. The best result thus far was achieved using potassium carbonate as the base, delivering the two-fold aldol addition product 25 in 27% yield. This seemingly low yield must be put into perspective, given that (a) the two sequential reactions are highly complex and (b) on average, each C–C bond formation occurs with a yield of 52%. It is likely that the base facilitates formation of anion 24 which initiates the first cyclization followed by the second aldol reaction. An orange-colored side product was isolated in 52%. It is likely that the base facilitates formation of anion

In summary, we have achieved the first chemical total synthesis of enterocin (1) was shown by HPLC and NMR analysis to be, in all scalar properties, fully identical with the natural product.[30]

In summary, we have achieved the first chemical total synthesis of enterocin. The longest linear sequence starting from L-arabinose (10) comprises a total number of 22 steps (0.4% overall yield). The final three steps of the synthesis require further optimization, which should lead to an improved overall yield. Due to its modular synthetic scheme, substituent modification around the enterocin core should be readily possible and will be employed as a tool to study the biological relevance of this intriguing molecule in more detail.

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Conflict of Interest

The authors declare no conflict of interest.

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[31] The limited quantities did not allow us to reliably determine the specific rotation of the synthetic material, which was exacerbated by the fact that the reported value \( ([\alpha]_D^{20} = -10.5) \) is relatively low. Based on biosynthetic studies\(^{[19]}\) and on the crystal structure of neoenterocin A\(^{[23]}\), it is very likely that the absolute configuration of the natural product is identical to synthetic enterocin.