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Natural Product Structures

The Structure Elucidation and Total Synthesis of β-Lipomycin**

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Abstract: *Here we describe the synthesis of β-lipomycin, a secondary metabolite isolated from the fermentation broth of Corallococcus coralloides. The synthesis relies on the structural assignment made by a statistical method, the so-called profile hidden Markov model. Using this protocol, not only the configuration of the secondary alcohol, but also of the adjacent methyl branch could be deduced. The synthesis therefore not only provides access to this natural product but also confirms the validity of this approach for configurational assignment at methyl branches of modular polyketides.*

The lipomycins were first isolated in 1972 by the group of Axel Zeeck from a strain of *Streptomyces aureofaciens*.[1] The orange–red compounds were shown to inhibit the growth of several Gram-positive bacteria (with MICs ranging from 0.3–3 $\mu g\,mL^{-1}$) and to have no effect on fungi and yeasts. The name of these natural products arose from the fact that their antibiotic activity was antagonized by naturally occurring lipids such as lecithin and other sterols. Whereas the sugar of α-lipomycin (**1**) was quickly identified as D-digitoxose, the structure elucidation of the aglycon β-lipomycin (**2**) took a further year and was accomplished by chemical degradation, mass spectrometry, and NMR spectroscopy.[2] However, apart from the (*S*)-glutamate stereocenter (C5′) within the acyltetramic acid[3] ring system, the configuration of the C12 and C13 stereocenters remained unknown (Figure 1).

Additionally, although Schabacher and Zeeck proposed the *N*1′-methyl-4′-hydroxy-$\Delta^{3'}$ pyrrolidin-2′-one form (as in **1**) for the cyclic ring system, Steyn and co-workers predicted the lipomcyins to rather exist as a tautomer with a *Z*-configured exocyclic double bond (C1–C3′ within **2**) after NMR and X-ray studies of related compounds.[4]

Structural relationships exists with the polyenoyl tetramic acids oleficin (**3**)[5] and altamycin (**4**),[6] both of which were also isolated from *Actinomycetales* and differ only by the number of the double bonds in their aliphatic tail. In contrast to almost all other naturally occurring tetramic acids, the slime mold pigment fuligorubin (**5**)[7] shows an *R* configuration at the glutamate stereocenter.

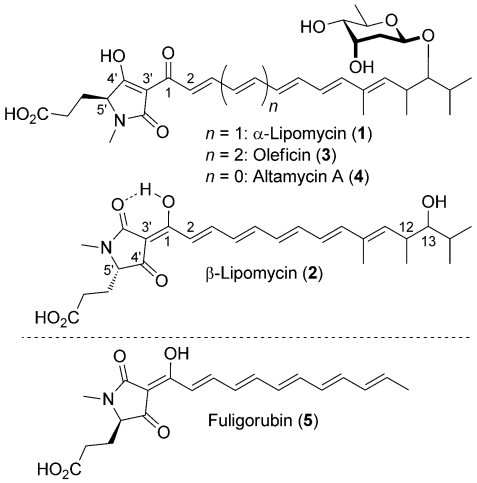

Figure 1. Structures of the lipomycins as published by Schabacher/Zeeck and Steyn (numbering according to Schabacher/Zeeck).[2]

Gene cluster analysis has contributed to the structure elucidation of many polyketides when classical methods like chemical degradation, NMR, MS, and X-ray methods were not sufficient.[8] McDaniel[9] and Caffrey[10] independently found conserved amino acid residues within the ketoreductases (KR) and were able to predict the configuration of the formed alcohols. Thus, a pivotal LDD motif at position 93 (Caffrey's numbering) leads to the formation of a D-configured alcohol, whereas a tryptophan (W) in position 141 gives an L alcohol. Leadlay et al.[11] published a gene analysis of enoylreductases (ER) that revealed the configuration of isolated methyl groups with the help of a pivotal tyrosin (Y) residue in position 52. With these two simple tools in hand, the prediction of several configurations within polyketides can be done with high accuracy even though there are rare examples for which this analysis does not provide the correct assignment. Additionally, a reliable tool to predict the configuration of methyl branches following secondary alcohols, as observed for lipomycin, is missing as well. In the course of our work on the synthesis of complex natural products, we have developed a profile hidden Markov model (HMM)[12,13] that allows the accurate assignment of secondary alcohols as well as methyl branches following L-configured alcohols (Figure 2). The advantage of using the hidden Markov model for predicting the configuration of modular polyketides is the fact that this model uses all amino acids (M) for the configurational prediction and not only selected residues. Additionally, it accounts for insert (I) and deleted (D) states which correspond to additional or missing amino acids. That increases the reliability of the prediction and makes previous sequence alignment superfluous. The term

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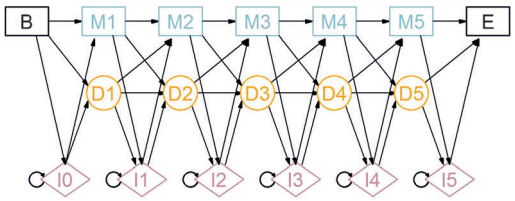


Figure 2. Architecture of the profile HMM with five match states. Blue squares indicate match states, pink diamonds insert states, and orange circles delete states. B and E refer to the beginning and end of an amino acid sequence, respectively.

"profile" refers to different emission and transition propabilities of individual positions. Moreover, the absolute value of the ScoreDiff value derived from the HMM provides a measure of reliability of the predicted configuration.

When the lipomycin biosynthetic gene cluster was published in 2006,[14] Bechthold et al. found W141 and the absence of the LDD motif within the KR to be responsible for the formation of the C12 and C13 stereocenters. Therefore, they predicted the C13 alcohol to be L configured, but they were unable to assign the C12 methyl configuration.

Using our profile HMM approach all amino acids of the specific subset of each ketoreductase were taken into account and they were allocated to one of the two families of secondary alcohols. Additionally, we used Viterby scores[15] to quantify the reliability of our predictions. In the case of the C13 alcohol of the lipomycins we found a negative ScoreDiff value (−53.96) that is consistent with the L configuration and therefore supports the configurational assignment made by the Bechthold group. For the C12 methyl group we found a ScoreDiff of −39.37 and thust identified the stereocenter to be L configured as well. This led to the structure of β-lipomycin (**2**) as depicted in Figure 3.

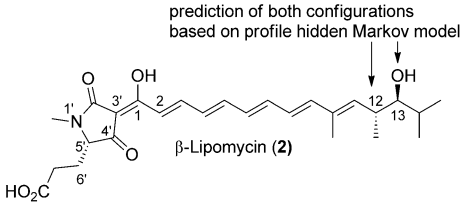
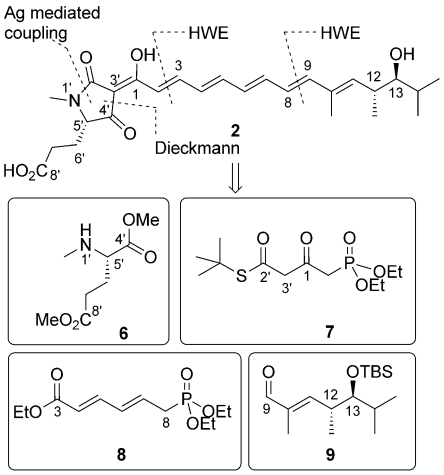


Figure 3. The proposed absolute configuration of β-lipomycin (**2**).

Here we report on the total synthesis of β-lipomycin to provide support for the correct structural assignment made by the profile hidden Markov model. In a retrosynthetic analysis β-lipomycin (**2**) can be divided into the four segments **6**, **7**, **8**, and **9** (Scheme 1).

Although the lipomycins were reported to be more stable than other polyenes[1] to light and air, we planned to build up the pentane system in the endgame of the synthesis. Our plan was to join the glutamate-derived fragment **6** and the bifunctional fragment **7**[16] through a silver(I)-promoted coupling reaction[17] to give the corresponding amide. This amide should be a suitable substrate for a Dieckmann

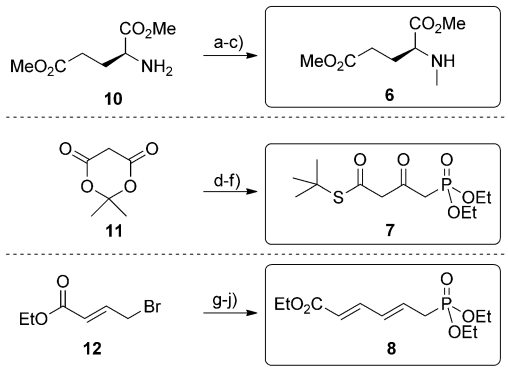


Scheme 1. Retrosynthetic analysis of β-lipomycin (**2**).

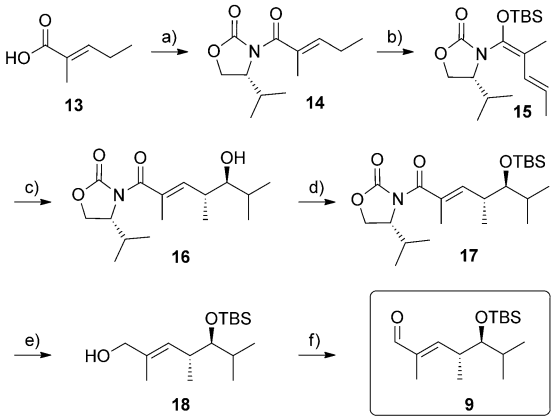
condensation, as it bears substituents on the C5′ and on the N1′ positions, both of which are important for high yields in these type of reactions.[18] The Dieckmann process was supposed to build up the five-membered tetramic acid ring system in analogy to the related step in the synthesis of fuligorubin (**5**) reported by Ley and co-workers[19] (Figure 1). The C8′-carboxylic acid was to be protected as the methyl ester in order to circumvent difficulties that might arise when with more hindered esters such as *tert*-butyl.[20] The desired cyclization giving the C4′-methyl ester rather than the C8′-methyl ester was to be controlled by the ring size (five-membered ring vs. seven-membered ring). Connections between fragments **7** and **8**,[21] as well as between **8** and **9** were achieved by HWE reactions as Ley had reported low yields and poor *E*/*Z* selectivities during corresponding Wittig transformations.[22] Compound **9** on the other hand should be easily accessible by means of a Kobayashi vinyloguos Mukaiyama aldol reaction (VMAR).[23]

In the synthetic direction, the western fragment **6** is easily accessible from commercially available H-Glu(OMe)-OMe (**10**) (Scheme 2). Boc protection, *N*-methylation, and subsequent Boc deprotection quickly furnished the desired precursor **6** in high yields. Compounds **7** and **8** were synthesized from Meldrum's acid (**11**) and ethyl 4-bromocrotonate (**12**), respectively, according to literature procedures.[16,21]

The synthesis of eastern fragment **9** started from commercially available 2-methyl-pent-2-enoic acid (**13**) (Scheme 3). Introduction of the D-valine-derived (*R*)-Evans auxiliary via the pivaloic anhydride and subsequent conversion to the TBS ketene acetal **15** utilizing sodium hexamethyldisilylamide (NaHMDS) and TBS chloride was achieved in good yields. The following Kobayashi VMAR reaction was the key step in the synthesis of **9**. With one equivalent of titanium tetrachloride and freshly distilled isobutyraldehyde the desired product **16** was obtained as a single diastereomer in 76% yield after reaction at −40°C for 16 h. TBS protection, followed by reductive cleavage of the auxiliary and allylic oxidation with activated manganese dioxide furnished α,β-unsaturated aldehyde **7** in a good yield of 70% over three steps.

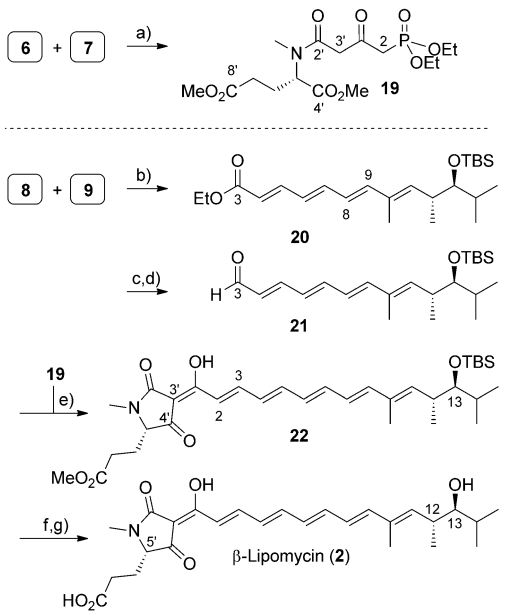


Scheme 2. Synthesis of fragments **6**, **7**, and **8**. a) $NaHCO_3$, Boc_2O, 100%; b) NaH, MeI, 75%; c) TFA, 100%; d) bromoacetyl bromide, pyridine; e) *tert*-butyl thiol, 41% over 2 steps; f) Na, diethyl phosphite, 55%; g) DiBAl-H, 80%, h) MnO_2, 77%, i) $Ph_3P{=}CHCO_2Et$, toluene, 35%; j) $P(OEt)_3$, neat, 75%. Boc = *tert*-butyloxycarbonyl; TFA = trifluoroacetic acid; DiBAl-H = diisobutylaluminum hydride.



Scheme 3. Synthesis of aldehyde **9**. a) (*R*)-Evans auxiliary, PivCl, NEt_3, LiCl, 84%; b) NaHMDS, TBSCl, 96%; c) $TiCl_4$, isobutyraldehyde, 76%, d.r. > 95:5; d) TBSOTf, 2,6-lutidine, 95%; e) $LiBH_4$, MeOH, 77%; f) MnO_2, 95%. (*R*)-Evans auxiliary = (*R*)-4-isopropyloxazolidin-2-one; PivCl = trimethylacetyl chloride, HMDS = hexamethyldisilylamide; TBS = *tert*-butyldimethylsilyl; OTf = trifluoromethylsulfonyl.

With all the fragments in hand, we coupled glutamate-derived **6** and fragment **7** under mild conditions using silver trifluoroacetate at room temperature in 71% yield (Scheme 4). We had intended to cyclize the resulting product **19** in a Dieckmann process, but under several sets of conditions we were unable to isolate the C3′–C4′ cyclized product, probably due to high water solubility even under acidic conditions. Gratifyingly, without isolation of such an intermediate, we could obtain the Dieckmann-cyclized and HWE-coupled product **22** (66%, $E/Z > 95{:}5$ as determined by ^{1}H NMR analysis) in a one-pot process when aldehyde **21** was added.[24] The required aldehyde **21** was obtained by HWE reaction between phosphonate **8** and VMAR-derived aldehyde **9** using LiHMDS as a base to yield tetraene **20** ($E/Z >$ 95:5). We found this compound to be relatively stable, even on silica gel. DiBAl-H reduction yielded the corresponding allylic alcohol and subsequent reoxidation with manganese



Scheme 4. Completion of the total synthesis of β-lipomycin (**2**). a) Ag-(O_2CCF_3), NEt_3, 71%; b) LiHMDS, 82%; c) DiBAl-H, 99%; d) MnO_2, 95%, e) NaH, **19**, then **21**, 66%; f) LiOH, MeOH, H_2O, 92%; g) 25% aq HF, CH_3CN, 85%.

dioxide gave the α,β-unsaturated aldehyde **21**. In contrast to **20**, compound **21** and its corresponding alcohol were found to be significantly more unstable.

Subsequent to the Dieckmann/HWE one-pot protocol (vide supra) the tetramic acid moiety including pentaene **22** (if the enol double bond is not regarded as an olefin) was subjected to a smoothly proceeding saponification using aqueous lithium hydroxide. Removal of the remaining TBS group at C13 in the resulting acid was the remaining transformation before completion of the total synthesis. However, this step proved to be troublesome and needed intensive optimization. First attempts with HF-pyridine with or without additional pyridine[25] and with tris(dimethylamino)−sulfonium difluorotrimethylsilicate) (TAS-F, with or without additional water) did not lead to useful conversion. With TBAF, although deprotections in similar systems were reported at room temperature,[26] only little conversion was observed even at elevated temperatures[27] (60 °C). As we were concerned about racemization at the C5′ stereocenter under these conditions we switched to aqueous hydrogen fluoride in acetonitrile.[28] Gratifyingly, these conditions cleanly led to formation of the desired natural product β-lipomycin (**2**) in good yield (85%).

The spectroscopic data of the synthetic material match that of both nature-identical (obtained by acidic hydrolysis of α-lipomycin) and synthetic material provided by Hofferberth and Brückner, who confirmed the configuration of the lipomycins by an independent synthesis (see the preceding Communication).[29] Moreover, the fact that the optical rotation is close to the value reported by Zeeck and co-workers[1] (synthetic $[\alpha]_D^{20} = -165.0$, $c = 0.02$, MeOH; authentic $[\alpha]_D^{20} = -176$, $c = 0.09$, MeOH) indicates that no racemization at the C5′ position had occurred.

Synthetic access to the polyenoyl tetramic acid β-lipomycin (**2**) was achieved in a longest linear sequence of 12 steps starting from commercially available substances with a yield of 17.0%. This synthesis and its preceding structural assignment solely rely on the statistical analysis of the pivotal ketoreductase and confirm the validity and practicability of this analysis.

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