

Total Synthesis of the Immunosuppressants Myriocin and 2-*epi*-Myriocin

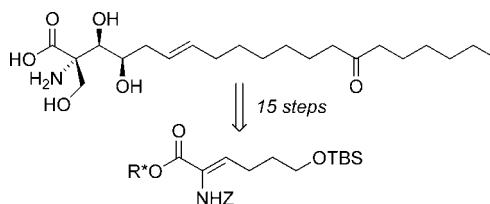
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ABSTRACT



Total syntheses of the natural immunosuppressant myriocin (1) and the equipotent unnatural analogue 2-*epi*-myriocin (in protected form) have been achieved through a common strategy. The key transformations are the efficient synthesis of a quaternary (*E*)-vinylglycine by asymmetric deconjugative alkylation of a dehydroamino acid and an unusually highly diastereoselective dihydroxylation of the vinylglycine alkene.

Myriocin (1) is a complex amino acid natural product that has been isolated independently from three fungal sources.^{1–3} Initially of interest owing to its antifungal properties,^{1,2} considerable interest was engendered by the finding that myriocin is a potent immunosuppressant.³ Myriocin has been shown to be an inhibitor of serine palmitoyl transferase,⁴ a key enzyme in the biosynthesis of sphingolipids. On the basis of this activity, myriocin has been examined as a potential antiatherosclerotic agent.⁵

The juxtaposition of the quaternary α -amino acid and the neighboring dihydroxylated alkyl chain within myriocin represents a challenging motif which, combined with the interesting biological activity, has made myriocin an attrac-

tive target for total synthesis.⁶ The first successful total synthetic approach was achieved in 15 linear steps from D-fructose, but the quaternary stereocenter was installed by a Strecker reaction in which the desired diastereoisomer was the minor component in a 7:2 mixture.⁷ Subsequent total and formal synthetic studies have employed a variety of methods for the stereoselective introduction of the quaternary stereocenter, including epoxide opening with internal^{8,9} or external^{10,11} nitrogen nucleophiles, aldol reactions on chiral bislactim ether¹² or oxazoline templates,¹³ Overman re-

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arrangement,¹⁴ and photolytic hydroxymethylation of α -carboxoximes.¹⁵

We recently reported a convenient method for the asymmetric synthesis of quaternary (*E*)-vinylglycines based upon the deconjugative alkylation of chiral dehydroamino acid derivatives.^{16,17} We reasoned that a quaternary vinylglycine such as **2** would be an attractive precursor to myriocin, provided that a diastereoselective dihydroxylation of the alkene could be achieved under substrate, reagent, or catalyst control (Figure 1). We report herein the successful application of

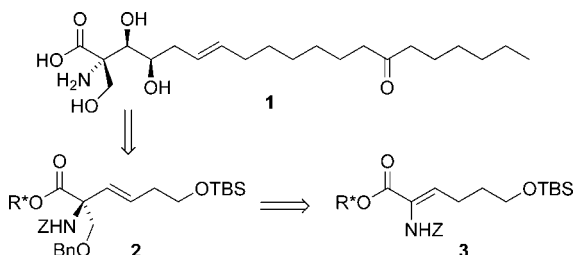
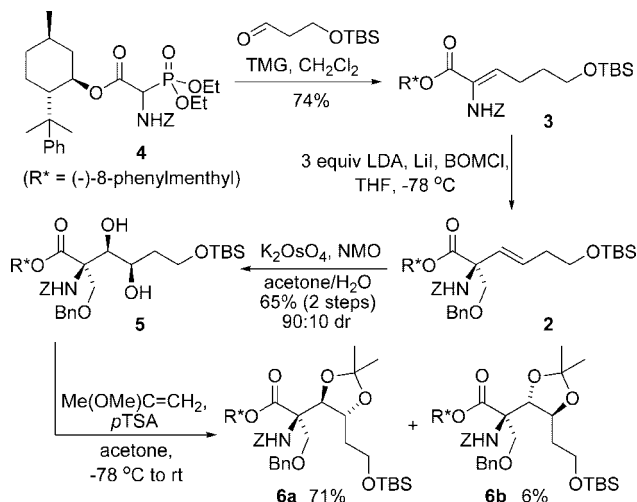


Figure 1. Schematic retrosynthetic analysis of myriocin.

this strategy to the syntheses of both myriocin and a protected form of the equipotent non-natural isomer 2-*epi*-myriocin.

Our first target was the substrate for the deconjugative asymmetric alkylation, namely, dehydroamino acid **3**. This was readily prepared by Horner–Wadsworth–Emmons condensation of the previously reported¹⁶ phosphonate reagent **4** (available in two steps from commercial *Z*-phosphonoglycinate) with 3-*tert*-butyldimethylsilyloxybutanal (Scheme 1). Alkylation of **3** was achieved by double

Scheme 1. Synthesis and Diastereoselective Dihydroxylation of Quaternary Vinylglycine **2**



deprotonation with lithium diisopropylamide in the presence of lithium iodide, followed by addition of benzyloxymethyl

chloride. The alkylated product **2** was formed apparently as a single diastereoisomer by ¹H NMR.¹⁸ It should be noted that the expected¹⁶ absolute stereochemistry of the amino acid center arising from alkylation of **3** (incorporating the more readily available (–)-enantiomer of the 8-phenylmenthol auxiliary) is opposite to that required for myriocin. Our initial intention was to work in this antipodal series to optimize the synthetic route and to address the “correct” stereochemical series later.

Compound **2** could not easily be purified to homogeneity and so was used directly in the subsequent dihydroxylation reaction. Reaction of **2** with potassium osmate under slightly modified Upjohn conditions gave a highly diastereoselective reaction, furnishing diol **5** in 65% yield (over two steps) as an inseparable 90:10 mixture of diastereoisomers. Protection of **5** as an acetonide facilitated separation of the two diastereoisomers **6a** (71%) and **6b** (6%) by careful chromatography. At this stage, the identity of the major diastereoisomer was confirmed by X-ray crystallographic studies of a solid derivative.¹⁹ This confirmed both the stereochemical outcome of the alkylation reaction and that dihydroxylation had occurred selectively *syn* to the protected amine function.

The *syn*-1,2-aminoalcohol relationship in **6a** is diastereomeric to that in myriocin. However, both the relative and absolute stereochemistries in **6a** match those in the non-natural analogue 2-*epi*-myriocin **7**, which has previously been prepared by Yoshikawa and shown to be equipotent to myriocin in the suppression of the mouse allogenic mixed lymphocyte reaction (IC₅₀ = 9 nM).²⁰ We therefore elected to progress **6a** to 2-*epi*-myriocin, providing an alternative approach to this valuable biochemical tool as well as allowing us to scope end-game chemistry for a later assault on myriocin itself.

To construct the C6–C7 *E*-olefin in **7**, we elected to use a Julia–Kocienski olefination.^{21,22} Thus, removal of the TBDMS protecting group of **6a** was followed by two-step conversion to the phenyltetrazolyl sulfone **8** according to the

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(18) **2** was inferred to be of 95:5 dr, by reductive cyclization of the ester and comparison (90% ee) to racemic oxazolidinone. See ref 16.

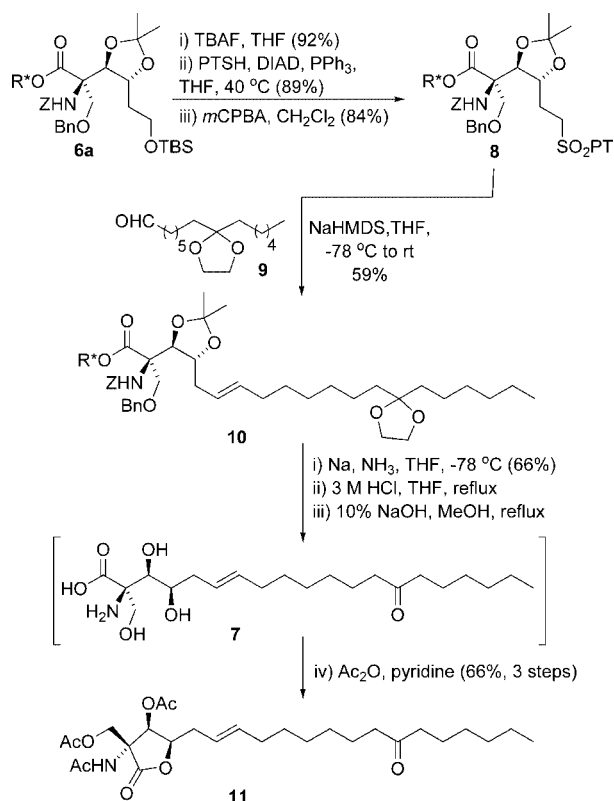
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Scheme 2. Synthesis of 2-*epi*-Myriocin **7**



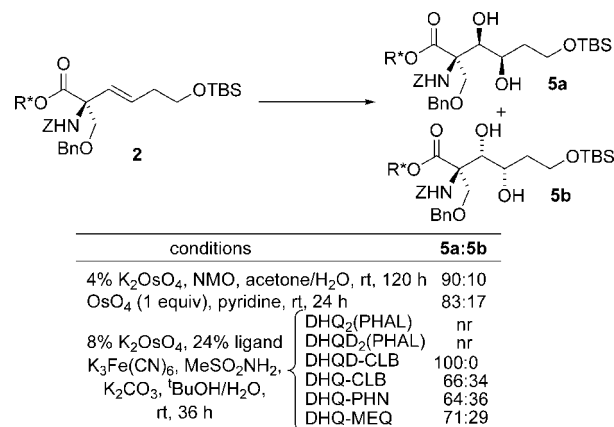
procedure of Kocienski²¹ (Scheme 2). Condensation of the sodium anion of **8** with aldehyde **9**²³ gave the target olefin **10** as a single *E*-isomer in 59% yield, highlighting the functional group tolerance as well as the high levels of stereoselectivity attainable with this reaction.

After extensive studies, deprotection of **10** was achieved by stepwise removal of the benzyl ether and carbamate by Birch reduction, acidic hydrolytic cleavage of the acetonide, and basic hydrolysis of the ester. Since 2-*epi*-myriocin and myriocin are almost indistinguishable by their ¹H NMR spectra and no ¹³C NMR data are reported for the former,²⁰ we elected to isolate the product as the peracetyl lactone **11**. This facilitated the efficient isolation of the product in 66% yield over three steps. Compound **11** exhibits different NMR spectral data from the known^{14b} peracetyl lactone of myriocin (vide infra). Additionally, we were able to confirm the relative stereochemistry of the lactone core as corresponding to 2-*epi*-myriocin by NOE studies.¹⁹

Having established the efficacy of the Julia–Kocienski olefination as a route to the 2-*epi*-myriocin structure, we returned to our approach to myriocin itself and specifically an examination of the diastereoselective dihydroxylation of **2**. A search of the literature revealed scattered examples of the dihydroxylation of acyclic alkenes substituted with amine-bearing quaternary asymmetric centers.^{24,25} The diastereoselectivities observed range from 50:50 to 100:0, and

additionally, examples of preferential dihydroxylation both *syn* and *anti* to the amine are known. In the absence of any precedent for the origins of the observed stereoselectivity and how one might overturn it, we therefore embarked on a screen of different dihydroxylation conditions (Scheme 3).

Scheme 3. Studies on the Diastereoselective Dihydroxylation of **2**



We first excluded the possibility of amplification of stereoselectivity through a “second cycle”-type system²⁶ since the use of stoichiometric osmium tetroxide returned a ratio of diastereoisomers (in favor of **5a**) similar to the catalytic conditions. We next attempted to use catalyst control to engineer the desired selectivity. The highly hindered substrate **2** proved resistant to dihydroxylation under standard Sharpless AD conditions using either pseudoenantiomeric catalyst system, even at high catalyst loadings. On moving to the less sterically demanding monomeric “first-generation” ligands,²⁷ the reactions were still sluggish but gave sufficient conversion for the diastereoselectivity to be examined. As expected, pseudoenantiomeric ligand pairs exhibited matched/mismatched behavior with the chiral substrate: the dihydroquinidine-derived ligand DHQD-CLB gave exclusively the undesired *syn*-diastereomer **5a**, in accord with the expected facial preference of this catalyst system.²⁷ The dihydroquinine-derived variant DHQ-CLB, however, gave a 66:34 mixture of **5a:5b**, and this ratio could not be substantially improved upon with other dihydroquinine ligands. It appears that the powerful intrinsic bias of the substrate cannot be overridden by ligand effects.

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We therefore elected to complete our synthesis of myriocin by performing a formal inversion of the quaternary asymmetric center, wherein the benzyloxymethyl substituent of **6a** ultimately forms the carboxylic acid of myriocin and the carboxylate becomes the hydroxymethyl side chain. This approach has the additional advantage that **6a**, derived from the readily available (–)-8-phenylmenthol auxiliary, will furnish the natural enantiomer of myriocin.

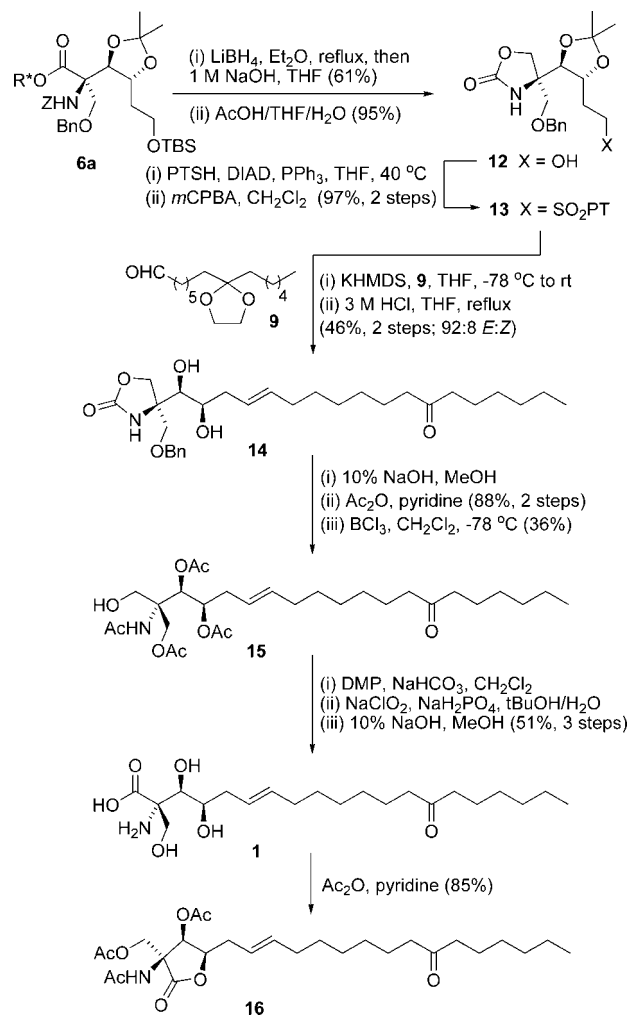
Reduction of the ester in **6a** with lithium borohydride followed by base-induced cyclization of the resulting primary alcohol generates a stable oxazolidinone. Removal of the TBDMS group under mild acidic conditions gave alcohol **12** in high yield, which was converted to the phenyltetrazolyl sulfone **13** as before. Reaction of **13** with aldehyde **9** under the previously utilized conditions (NaHMDS, THF) gave a poorly selective olefination (86:14 *E:Z*). A screen of base counteranion, solvent, and reaction conditions (premetalation or Barbier-type conditions)²¹ allowed us to identify the use of KHMDS in THF as optimal. Following removal of the acetone, olefin **14** was isolated as a 92:8 mixture of isomers. This could be improved to 97:3 by recrystallization.

All that remained was to convert the benzyloxymethyl group to the desired carboxylic acid. It was found necessary to have all of the other functionality protected as acetyl esters/amides during the oxidation: other protecting group regimes gave only product decomposition under a range of oxidative conditions. Thus, hydrolytic cleavage of the oxazolidinone was followed by peracetylation and chemoselective deprotection of the benzyl ether using boron trichloride. We were unable to force the latter reaction to completion, using even large excesses of reagent, but the desired alcohol **15** was obtained in 36% yield (82% based on 56% recovery of starting material). Stepwise oxidation of the primary alcohol (Dess–Martin periodinane followed by Pinnick oxidation) gave the desired acid. Finally, hydrolysis of the three acetate esters and acetamide allowed the isolation of myriocin (**1**) (Scheme 4). The material had spectroscopic data,^{14b} melting point,^{12,13} and optical rotation^{3,14b} in accord with those reported. As an additional confirmation of structure, we converted **1** to the known peracetyl lactone **16**. Again, the spectroscopic data^{14b} and optical rotation^{2a,9} matched the known values and were distinct from those of the 2-*epi*-myriocin peracetyl lactone **11**.

In summary, we have developed a common synthetic strategy for the synthesis of the natural immunosuppressant myriocin (**1**) and a protected form of the equipotent unnatural variant 2-*epi*-myriocin (**7**). The overall efficiencies compare well with the published routes.²⁸ The key transformations

(28) **1**: 16 linear steps from **4**, compared with the shortest previous stereoselective route of 16 steps from (Z)-but-2-ene-1,4-diol (ref 12). **7**: 11 linear steps to **7** (isolated as **11**) from **4**, compared with 16 steps to **7** from 2-deoxy-D-glucose (ref 20).

Scheme 4. Total Synthesis of Myriocin **1**



are the concise synthesis of quaternary vinylglycine **2** using our deconjugative asymmetric alkylation methodology and the highly diastereoselective substrate-controlled dihydroxylation reaction of this olefin to establish the aminodiol function.

Acknowledgment. We thank EPSRC (DTA studentship to M.C.J.) and Colin Kilner (Leeds) for the X-ray structure.

Supporting Information Available: Experimental protocols, spectroscopic data, and ¹H/¹³C NMR spectra for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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