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Absolute Configuration and Total Synthesis of a Novel Antimalarial Lipopeptide by the de Novo Preparation of Chiral Nonproteinogenic Amino Acids

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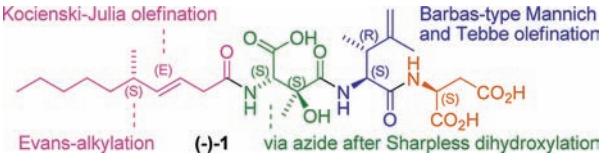
ABSTRACT



The absolute configuration (via degradation and Marfey's derivatization studies) and the total synthesis of a novel antimalarial lipid-peptide isolated from *Streptomyces* sp. (IC_{50} = 0.8 μM, *Plasmodium falciparum* 3D7) is disclosed. To this end, versatile stereocontrolled routes to nonproteinogenic amino acids (via catalytic Mannich, Sharpless methods) and enantiomeric *trans* fatty acids (via Evans alkylation, Kocienski–Julia olefination) have been developed.

Without multiple modes of attack, even our best and latest antimalarial agent will face drug resistance in the field. New lead structures with atypical bioactivities are therefore of high interest. In our efforts to understand drug resistance and synthesize lipid conjugates,[1] we became interested in elucidating the novel structure and function of a *Streptomyces*-derived lipidated peptide metabolite (**1**, Figure 1). This was originally disclosed in a 2004 patent application by the Yamanouchi Pharmaceutical Co. (now Astella Pharma) to inhibit the growth of *Plasmodium falciparum*.[2] Independent to this disclosure, MerLion Pharmaceuticals isolated the same metabolite (−)-**1** from various *Streptomyces* species and confirmed its planar structure by NMR and MS analysis. Similar to the patent,[2] they found the lipopeptide (−)-**1** to have little or no activity against a panel of mammalian, fungal, and Gram positive bacteria cell lines. This is curious since antimalarial peptides, although poorly understood, can often possess antibacterial activity via membrane disruption.[3] In view of providing primary antimalarial data and material for mode of action studies, we communicate the stereochemical determination and first total synthesis of (−)-**1**. This required the nontrivial absolute stereogenic identification and *de novo* synthesis of two nonproteinogenic amino acids (**3** and **4**) and the synthesis of both enantiomers of the methyl-branched *trans* fatty acid **5** (Figure 1).

Our studies began by determining the absolute configuration of each constituent **2**–**5** (Figure 1). A sample of the natural product (−)-**1** (5 mg) was refluxed in 6 M HCl for

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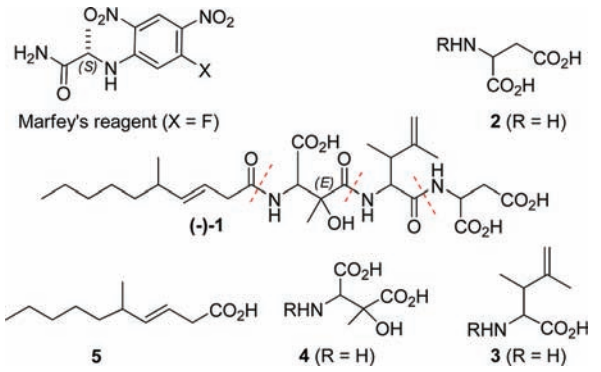


Figure 1. Structures of Marfey's reagent, amino acid, and fatty acid components of the natural product including the two nonproteinogenic amino acids **3** and **4**.

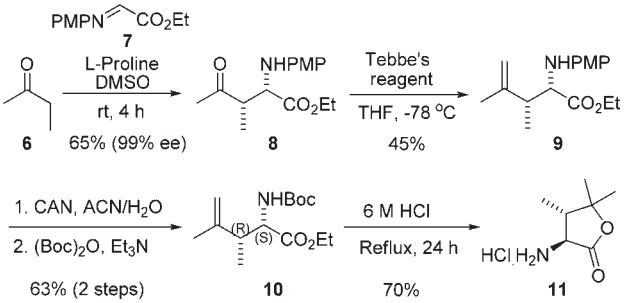
24 h, and the hydrolyzed amino acid mixture was derivatized with either UV-active CbzCl or Marfey's reagent.[4] The lipid portion **5** did not survive the 6 M HCl treatment, and attempts to hydrolyze the acid under milder conditions were unsuccessful. However, the aspartic acid (**2**) in the natural product was recovered and readily assigned as the L-isomer (*S*). This was achieved by comparison of the HPLC retention time and MS of the Marfey's derivatized hydrolysis products with authentic standards of L- and D-aspartic acid (see Supporting Information).

Our initial analysis of the LC-MS data of the hydrolysis mixture failed to identifiy derivatives of **3**. However, NMR and ESI-MS analysis of a Cbz *N*-protected isolate indicated that the carboxylic acid **3** had cyclized to a γ-lactone during hydrolysis. The lactone **11** was thus isolated after basifying the acidic aqueous phase and extracting with $CHCl_3$. This conveniently allowed a *trans* relative stereochemistry to be assigned to the isolated lactone **11** by ^{1}H NMR and NOE experiments ($^1J_{H\alpha/\beta}$ 12 Hz).[5] A Marfey's derivative was then formed from naturally isolated **11**.

To determine the absolute stereochemistry of **11** and provide further evidence for the cyclization process, an expedient synthetic route was designed (Scheme 1). Thus, the L-proline catalyzed Mannich reaction[6] between butan-2-one (**6**) and the imine **7** generated the *syn*-adduct **8** in 99% ee. Although Wittig-type reactions failed, presumably due to the sterics of α-methylation, standard Tebbe treatment[7] of the keto-ester **8** produced the olefin **9** chemoselectively in 45% yield.

Next, deprotection of the amino *p*-methoxyphenyl (PMP) group was found problematic, but reverse addition[8] of compound **9** to a ceric ammonium nitrate (CAN)

Scheme 1. Organocatalytic Synthesis of α-Amino-γ-lactone **11**



solution and Boc *N*-protection cleanly afforded the desired amino ester **10**. Refluxing **10** in 6 M HCl for 24 h then produced the expected γ-lactone **11**. Further derivatization with Marfey's reagent, and analysis with naturally derived material, indicated **3** to be (2*S*,3*R*).

Due to the practical limitations and inefficiencies of reported routes to selected diastereomers of the amino acid **4**,[9] we designed a general strategy to provide all four diastereomers of **4** via a stereoselective dihydroxylation of a *cis* or *trans* olefin (cf. **15**).[10] The specific methodology developed to obtain the naturally occurring isomer in **1** is shown in Scheme 2. Thus the propargylic PMB ether **12** was activated with methyl chloroformate **13** to generate **14**[11] for a carefully controlled Michael addition of Me_2-CuLi to afford the *Z*-olefin **15**[12] exclusively. Sharpless asymmetric dihydroxylation of **15** then gave the diol **16** in 82% ee.[13] Cyclic sulfate formation to **17** (using $SOCl_2$ and then $RuCl_3-NaIO_4$ oxidation)[14] followed by regioselective azide opening and acidic hydrolysis afforded the azido alcohol **18**.

After extensive studies to convert **18** to **20**, we found that the tertiary alcohol of **18** first needed TES protection before oxidation of the PMB-protected alcohol. However, the PMB deprotection step also needed care and DDQ oxidative removal to **19** required buffered pH 7.5.[15] In both cases, undesired lactone formation could be circumvented, and the best oxidation sequence for **19** was achieved by using TEMPO combined with $PhI(OAc)_2$[16]

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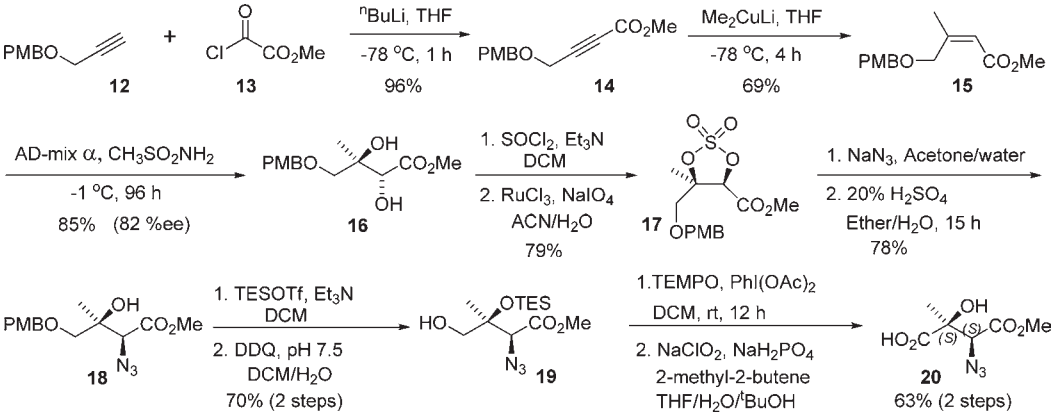
Scheme 2. Stereocontrolled Synthesis of Monomethyl Ester of Amino Acid **4**



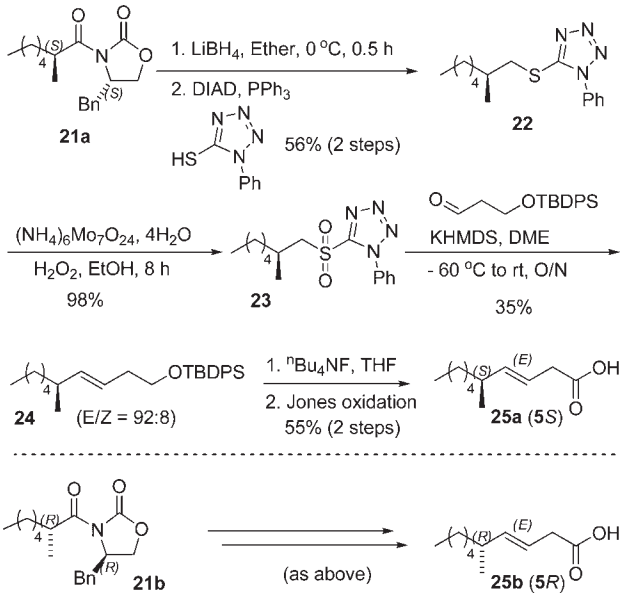
followed by Pinnick oxidation[17] to afford the monoester **20** in 63% yield over two steps.

To confirm the absolute and relative stereochemistry, the azide ester **20** was reduced and hydrolyzed with 6 M HCl to form the amino acid **4** (see Supporting Information). Marfey's derivatization again matched synthetic material data with those from the natural product by HPLC and LCMS, confirming the (2*S*,3*S*) stereochemistry for **4**.

After determining the absolute configuration of each amino acid **2**–**4**, we focused on synthesizing the fatty acid **5** (Scheme 3). Having no isolated natural derivative of **5** to hand, a total synthesis of diastereomeric **1** with both enantiomers was undertaken. To this end, the Evans-based oxazolidinones **21a/b** were prepared[18] and converted to the thiotetrazoles **22** after reduction and Mitsunobu reaction. Oxidation to the sulfone **23** and Kocienski–Julia olefination to **24** gave high selectivity (E/Z = 92:8, by GCMS) with the use of KHMDS.[19] Deprotection of TBDPS followed by Jones oxidation then gave the fatty acids **25a** (**5***S*) and **25b** (**5***R*).

With all synthetic fragments available, the Boc-*N*-protected amino ester **10** was hydrolyzed with LiOH in THF/MeOH to give the acid **26**, which was coupled with the (*S*)-aspartate methyl ester **27** under HATU conditions to produce the dipeptide **28** (Scheme 4). After Boc deprotection, the TFA salt from **28** was coupled with the azido methyl ester **20** to afford the azido tripeptide **29**. Although other phospines failed, the azide functionality was successfully reduced with Me_3P[20] and the amine formed coupled with either lipid **25a** or **25b**. This provided the trimethyl ester stereoisomers **30** and **31** of the targeted lipopeptide with $[\alpha]^{25}_D$ values of +18.8 (c = 0.25) and −23.1 (c = 0.22), respectively.

Scheme 3. Synthesis of Chiral *Trans* Fatty Acids **25a** and **25b**



Although MeOD revealed minor NMR differences between (+)-**30** and (−)-**31**, comparison in $CDCl_3$ revealed significant differences in the C-2 and C-3 positions (proximal to the C5 chiral center). The NMR data and optical rotation of (+)-**30** correlated closely to the trimethylated natural product, $[\alpha]^{25}_D$ = +31.6 (c = 0.18), as prepared by reacting (−)-**1** with $TMSCHN_2$.[21] This indicated a C-5 *S* configuration for the fatty acid **5**. Furthermore, data from hydrolysis of (+)-**30** to its triacid matched the NMR and optical rotation data of the natural product: synthetic (−)-**1** $[\alpha]^{25}_D$ = −20.0 (c = 0.1), natural (−)-**1** $[\alpha]^{25}_D$ = −23.7 (c = 0.3) (see Supporting Information).

In summary, we applied the parallel use of structure elucidation methods with total synthesis to establish the absolute stereochemistry of each amino acid fragment **3**, **4**,

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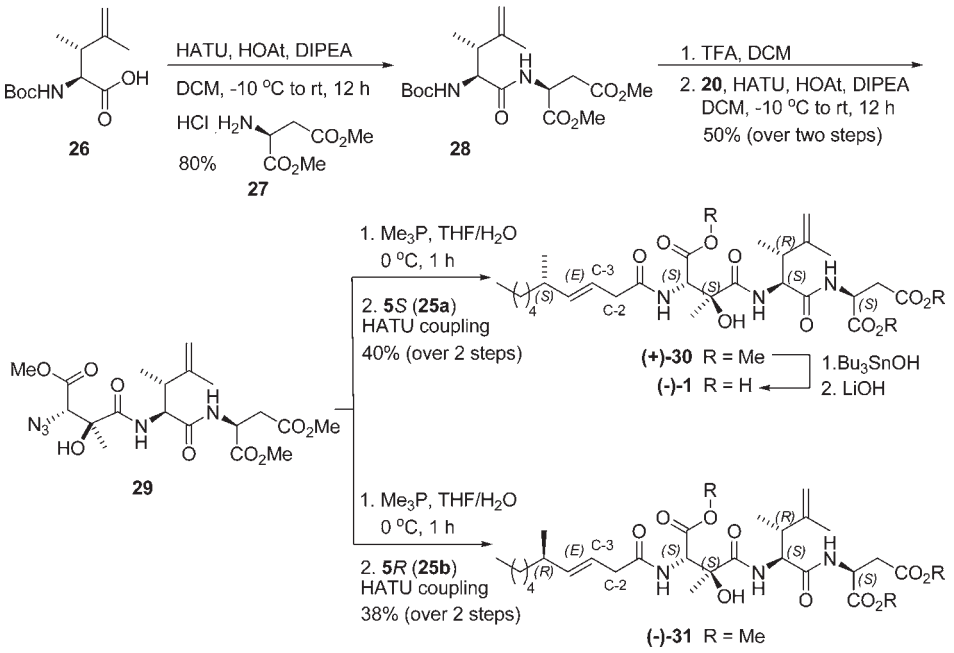
Scheme 4. Total Synthesis of Lipopeptide (−)-**1** by Peptide Coupling and Lipid Conjugation



and **5**. Assignment of the final, lipid component **5** was then confirmed via total synthesis of **1**. Noteworthy steps include the following: (1) the L-proline organocatalytic formation of a *syn*-Mannich product (**10**) in 99% ee; (2) the stereospecific formation of a *cis*-olefin (**15**) by cuprate conjugate addition onto an alkynoate; (3) the highly selective Kocienski–Blakemore Julia formation of a *trans* α-methylated olefin (**24**); (4) the Sharpless-based synthesis of an amino acid precursor (**20**) bearing a quaternary center and an azido group for a reductive-lipidation sequence to the natural product (−)-**1**.

Interestingly, it is likely that both the carboxylic acid motifs and the olefins of **1** play a key role for bioactivity. For example, the trimethyl ester derivative (+)-**30** and tetrahydrogenated version of the lipopeptide are devoid of antimalarial activity. In contrast, (−)-**1** exhibits significant in vitro activity against *P. falciparum* (IC_{50} = 0.8 μM against 3D7 strain). Moreover, primary whole cell screenings against HeLa, *B. subtilis*, *S. aureus*, *C. albicans*, *S. cerervisae*, and *A. niger*, show (−)-**1** to be inactive (< 50 μM). Mode of action studies of this rather compact, novel lipoprotein lead structure are thus anticipated to provide interesting insights and differences in microbial and mammalian cell biology.[3]

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Supporting Information Available. Experimental procedures, compound characterization, NMR spectra, and LCMS. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.