ORGANIC LETTERS

2010
Vol. 12, No. 15
3394−3397

Total Synthesis and Assignment of the Side Chain Stereochemistry of LI-F04a: An Antimicrobial Cyclic Depsipeptide

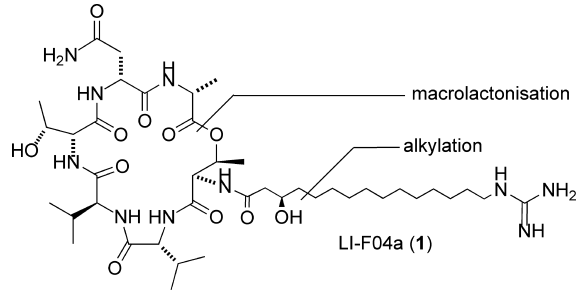
James R. Cochrane, Christopher S. P. McErlean, and Katrina A. Jolliffe*

School of Chemistry, The University of Sydney, 2006, NSW, Australia

kate.jolliffe@sydney.edu.au

Received May 31, 2010

ABSTRACT



The total synthesis of the potent antifungal and antibiotic cyclic depsipeptide LI-F04a and its side chain epimer was accomplished using macrolactonization to assemble the cyclic peptide core, followed by attachment of the 15-guanidino-3-hydroxypentadecanoyl (GHPD) side chain. The side chain was assembled by Yamaguchi−Hirao alkylation of both enantiomers of a chiral epoxide to provide a pair of enantiomeric side chains. The attachment of both these chains to the cyclic peptide allowed the absolute configuration of the side chain hydroxyl group in LI-F04a to be assigned as (*R*).

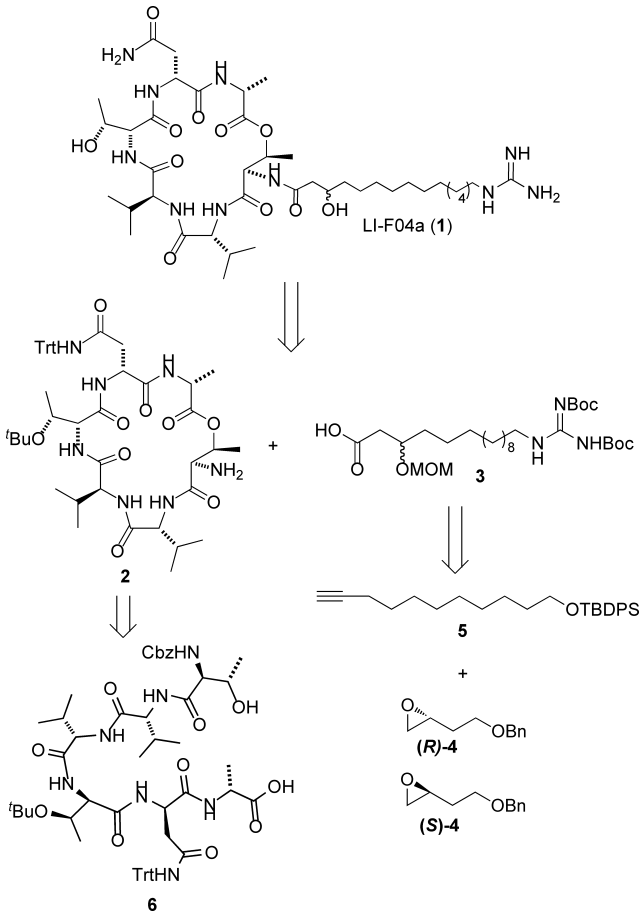
The increasing incidence of invasive fungal mycoses and the development of problematic multidrug resistant bacterial infections, such as hospital associated methicillin resistant *Staphylococcus aureus* (HA-MRSA), are emerging as serious threats to public health, and there is an increasingly urgent need for the discovery of new antimicrobial agents active against these pathogens.[1−7] LI-F04a is a cyclic depsipeptide produced as the major component of a family of closely related cyclic depsipeptides (collectively labeled as the LI-Fs) isolated from the L-1129 strain of *Paenibacillius polymyxa* (formerly *Bacillus polymyxa*).[8,9] These compounds are analogous to the fuscaricidins which were originally isolated from the KT-8 strain of *Paenibacillus polymyxa*.[10−14] The LI-F peptides have been found to exhibit antifungal activity against a wide range of fungi including the clinically relevant species *Candida albicans* and *Cryptoccoccus neoformans*.[8−14] They also exhibit activity against Gram-positive bacteria such as strains of *Staphylococcus aureus* and *Micrococcus luteus*[8,11] and have been shown to have low acute toxicity in mice.[14]

The LI-F peptides contain a cyclic depsihexapeptide core with a unique 15-guanidino-3-hydroxypentadecanoyl (GHPD) side chain attached to the nitrogen atom of an L-threonine residue (Scheme 1). Three amino acids, L-Thr, D-*allo*-Thr, and D-Ala,

(1) Brickner, S. J.; Mobashery, S. *Curr. Opin. Microbiol.* **2007**, *10*, 425.
(2) Lipsitch, M. *Trends Microbiol.* **2004**, *8*, 515.
(3) Barrett, J. F. *Expert Opin. Ther. Targets* **2005**, *9*, 253.
(4) Lamagni, T. L.; Evans, B. G.; Shigematsu, M.; Johnson, E. M. *Epidemiol. Infect.* **2001**, *126*, 397.
(5) Pfaller, M. A. *Clin. Infect. Dis.* **1998**, *27*, 11481150.13.
(6) Marr, K. A.; Carter, R. A.; Crippa, F.; Wald, A.; Corey, L. *Clin. Infect. Dis.* **2002**, *34*, 909.
(7) Norby, S. R.; Nord, C. E.; Finch, R. *Lancet Infect. Dis.* **2005**, *5*, 115.
(8) Kaneda, M.; Kajimura, Y. *Yakugaku Zasshi* **2002**, *122*, 651.
(9) Kuroda, J.; Fukai, T.; Konishi, M.; Uno, J.; Kurusu, K.; Nomura, T. *Heterocycles* **2000**, *53*, 1533.
(10) Choi, S. K.; Park, S. Y.; Kim, R.; Lee, C. H.; Kim, J. F.; Park, S. H. *Biochem. Biophys. Res. Commun.* **2008**, *365*, 89.
(11) Kajimura, Y.; Kaneda, M. *J. Antibiot.* **1996**, *49*, 129.
(12) Kajimura, Y.; Kaneda, M. *J. Antibiot.* **1997**, *50*, 220.
(13) Kajimura, Y.; Sugiyama, M.; Kaneda, M. *J. Antibiot.* **1995**, *48*, 1095.
(14) Kurusu, K.; Ohba, K.; Arai, T.; Fukushima, K. *J. Antibiot.* **1987**, *40*, 1506.

10.1021/ol101254m
Published on Web 07/07/2010

Scheme 1. Retrosynthesis



are conserved throughout the LI-F series, while there are slight variations in the other three amino acids present. In LI-F04a, these are D-Asn, L-Val, and D-Val. The GHPD side chain is conserved among the LI-F series of antifungal cyclic peptides, but to the best of our knowledge, the absolute stereochemistry of the 3-hydroxyl group has not previously been confirmed.[9,11] This, together with the biological activity and limited availability of isolated LI-F04a, makes this compound an attractive target for total synthesis. The ability to synthesize individual members of the LI-F peptide family will allow the structural basis for the biological activity of these cyclic peptides to be determined in the future.

The synthesis of a simplified analogue of LI-F04a, in which the side chain 3-hydroxy group was omitted, has previously been reported.[15] We report here a total synthesis of both LI-F04a (**1**) and its GHPD side chain epimer. The synthesis of both compounds allowed the unambiguous assignment of the absolute stereochemistry of the alcohol in the natural product as the (*R*)-isomer.

Our synthetic strategy was based upon the retrosynthetic analysis presented in Scheme 1. The late-stage coupling of the cyclic peptide **2** with the GHPD side chain **3** would allow ready access to both side chain epimers of **1**. It was envisaged that the chiral alcohol functionality at C3 of **3** could be introduced by a Yamaguchi−Hirao alkylation[16] of the known chiral epoxides (*S*)-**4** and (*R*)-**4** with alkyne **5**, thereby providing both side chain enantiomers for attachment to the cyclic peptide core **2**.[17,18] While there are a number of possible macrocyclization sites possible for the synthesis of **2**, we anticipated that Yamaguchi macrolactonization[19,20] of linear peptide **6** would yield the required cyclic depsipeptide and enable rapid access to libraries of LI-F04a analogues (including the other peptides in the LI-F family) for future biological studies, by standard Fmoc solid phase peptide synthesis of the linear precursors.

Synthesis of the (*R*)-enantiomer of the GHPD side chain, (*R*)-**3**, began with the enantiomerically enriched epoxide (*S*)-**4** which was obtained in 32% yield (>99% ee) by hydrolytic kinetic resolution (HKR) of (±)-**4** in the presence of Jacobsen's (*S*,*S*)-(salen)Co(III) catalyst.[17] Nucleophilic opening of epoxide (*S*)-**4** with the lithioacetylide of alkyne **5**[21] via a $BF_3{\cdot}OEt_2$-promoted alkylation[16] at −78 °C gave the alcohol (*R*)-**7** in 69% yield (Scheme 2). Protection of the secondary alcohol as a methoxy methyl ether, followed by desilylation (TBAF), gave the primary alcohol (*R*)-**8** in 77% yield over 2 steps. Reaction of (*R*)-**8** with di(*tert*-butoxycarbonyl)guanidine under Mitsunobu conditions proceeded smoothly to give (*R*)-**9** in 90% yield.[22] After optimization of both the catalyst and solvent, debenzylation and concomitant reduction of the internal alkyne were achieved upon treatment with H_2 in the presence of $Pd(OH)_2/C$ to give (*R*)-**10** in 79% yield. The use of the basic catalyst and mild conditions was necessary as the guanidine Boc-protecting groups were very acid labile and prone to cleavage under more forcing conditions. Finally, alcohol (*R*)-**10** was subjected to a ruthenium tetroxide catalyzed oxidation[23−25] to give the protected GHPD fragment (*R*)-**3** in 63% yield. The synthesis of this fragment was thus achieved in 6 steps and 23% overall yield from (*S*)-**4**. The (*S*)-GHPD enantiomer (*S*)-**3** was prepared in an identical manner starting from the chiral epoxide (*R*)-**4**, which was obtained in 40% yield (>99% ee) upon HKR of (±)-**4** in the presence of Jacobsen's (*R*,*R*)-(salen)Co(III) catalyst (Scheme 3).

With both enantiomers of the GHPD side chain in hand, our attention turned to the synthesis of the cyclic peptide core of LI-F04a. Thus, linear peptide precursor **6** was

(15) Stawikowski, M.; Cudic, P. *Tetrahedron Lett.* **2006**, *47*, 8587.

(16) Yamaguchi, M.; Hirao, I. *Tetrahedron Lett.* **1983**, *24*, 391.

(17) Schaus, S. E.; Brandes, B. D.; Larrow, J. F.; Tokunaga, M.; Hansen, K. B.; Gould, A. E.; Furrow, M. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2002**, *124*, 1307.

(18) Liang, Q.; Sun, Y.; Yu, B.; She, X.; Pan, X. *J. Org. Chem.* **2007**, *72*, 9846.

(19) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

(20) Sarabia, F.; Chammaa, S.; Lopez-Herrera, F. J. *Tetrahedron Lett.* **2002**, *43*, 2961.

(21) Carpita, A.; Mannocci, L.; Rossi, R. *Eur. J. Org. Chem.* **2005**, 1859.

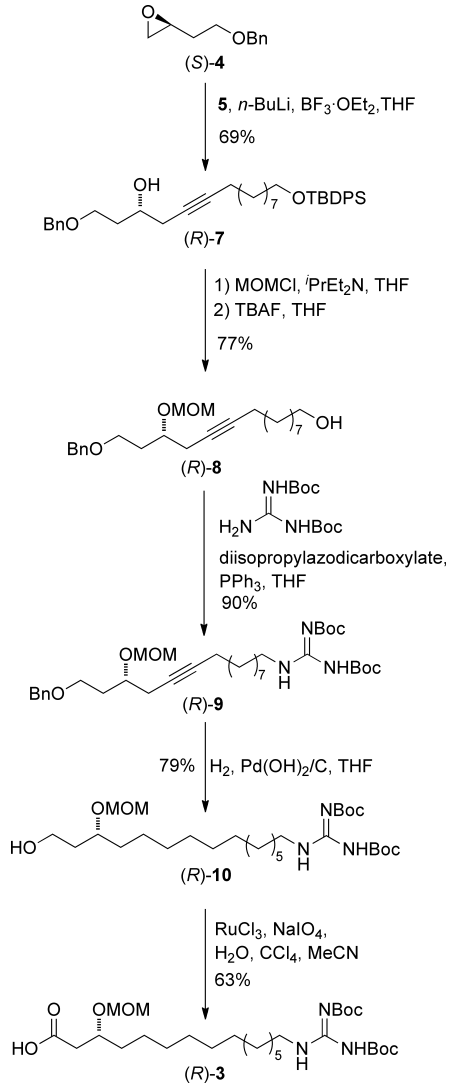
(22) Dodd, D. S.; Kozikowski, A. P. *Tetrahedron Lett.* **1994**, *35*, 977.

(23) Djerassi, C.; Engle, R. R. *J. Am. Chem. Soc.* **1953**, *75*, 3838.

(24) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.

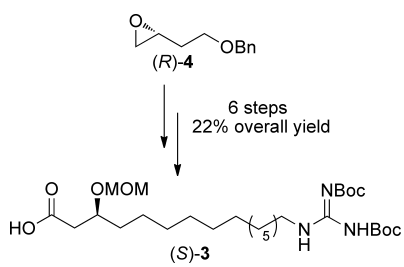
(25) Joo, J. E.; Pham, V. T.; Tian, Y. S.; Chung, Y. S.; Oh, C. Y.; Lee, K. Y.; Ham, W. H. *Org. Biomol. Chem.* **2008**, *6*, 1498.

Scheme 2. Synthesis of (*R*)-**3**

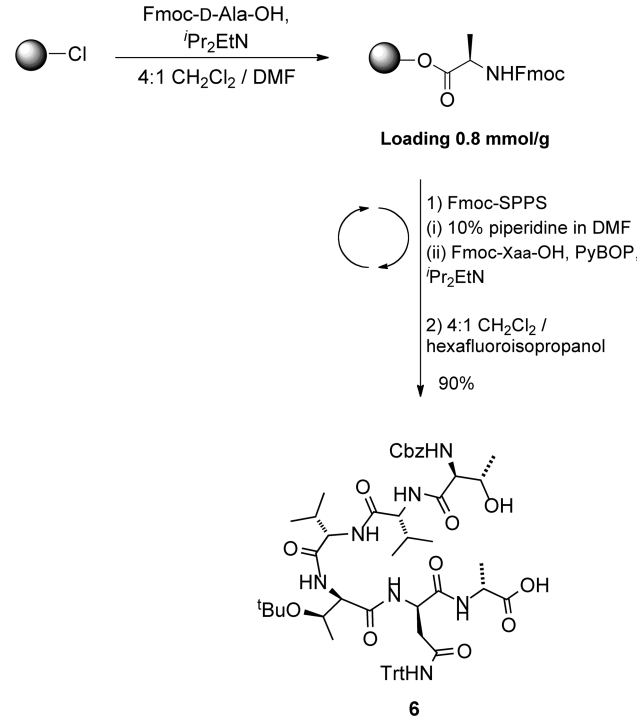


prepared by standard Fmoc solid phase peptide protocols using PyBOP/Hünig's base as the activation reagent and the 2-chlorotritylchloride resin as the solid support. Cbz-L-Thr-OH was added as the N-terminal amino acid, and then the peptide was cleaved from the resin upon treatment with hexafluoroisopropanol,[26] leaving the N-terminal and side chain protecting groups intact (Scheme 4).

Scheme 3. Synthesis of (*S*)-**3**



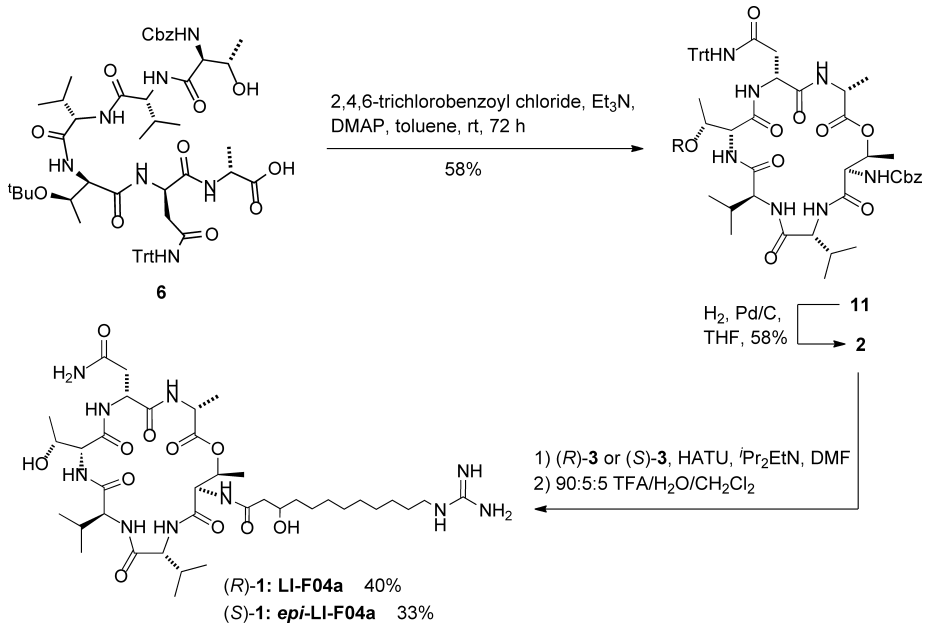
Scheme 4. Peptide Synthesis



The key macrolactonization step was then attempted under standard Yamaguchi macrolactonization conditions.[19,20] Initial experiments provided cyclized peptide in good yield (>60%). However, analysis of this material by HPLC indicated that it was a 3:1 mixture of diastereoisomers, presumably as a result of epimerization of the C-terminal D-Ala residue. Epimerization of the C-terminal amino acid prior to macrocyclization is frequently observed in the synthesis of small cyclic peptides.[27,28] Fortunately, use of the modified Yonemitsu conditions,[29] which involved slow addition of the acid to a solution of DMAP, 2,4,6-trichlorobenzoyl chloride, and triethylamine in toluene at room temperature, provided the required cyclic peptide **11** in 58% yield, without significant epimerization (<5% epimer observed by HPLC) (Scheme 5). Hydrogenolysis of the Cbz protecting group on **11** was unexpectedly problematic. Numerous conditions were employed; however, in all cases the reactions were sluggish, and mixtures of starting material and over-reduced side products were frequently obtained, despite reports that trityl protecting groups on amides are stable to hydrogenolysis.[30]

(26) Bollhagen, R.; Schiedberger, M.; Barlos, K.; Grell, E. *J. Chem. Soc., Chem. Commun.* **1994**, 2559.
(27) Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345.
(28) Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831.
(29) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.
(30) Isidro-Llobet, A.; Álvarez, M.; Albericio, F. *Chem. Rev.* **2009**, *109*, 2455.

Scheme 5. Total Synthesis of LI-F04a and *epi*-LI-F04a



The optimal conditions were found to be H_2, Pd/C in THF, providing the free amine **2** in 58% yield.

The final key step in the synthesis of LI-F04a involved coupling of the cyclic peptide **12** to both enantiomers of the GHPD side chain **3**. This was achieved using HATU/Hünig's base as the coupling reagent. The coupled products were immediately subjected to global deprotection upon treatment with TFA/CH_2Cl_2/H_2O (90/5/5 v/v/v), providing both side chain epimers of LI-F04a in unoptimized yields of 33−40% over 2 steps.

With both compounds available with known stereochemistry at the side chain hydroxy group, we were able to compare their spectroscopic and physical properties with those of natural LI-F04a to establish the absolute configuration of the side chain hydroxy group. First, comparison of the optical rotations of the synthetic material with the (*R*)-configured side chain (+20°) and that of the (*S*)-configured side chain (−10°) with those reported for the natural product (+24°,[9] +12.8°[11]) suggested that the natural product may bear a side chain with (*R*)-stereochemistry at C3. Second, the ^{1}H and ^{13}C spectra of both synthetic epimers of **1** were fully assigned using a combination of 1- and 2-D NMR experiments and compared with the data reported for natural LI-F04a. While both side chain epimers had similar ^{1}H and ^{13}C NMR spectra, there were some subtle differences. Notably, both the chemical shift and coupling constant for one of the methylene protons on the carbon (C2) adjacent to the side chain hydroxy substituent differ significantly in the two epimers. In (*S*)-**1**, the signal attributable to this proton appears at 2.27 ppm and couples to the proton on C3 with a coupling constant of 3.7 Hz, while the signal for the same proton in (*R*)-**1** is observed at 2.36 ppm with a coupling constant of 6.6 Hz, which is identical to the data reported for the natural product. A comparison of chemical shifts for all protons (excluding the NH and OH signals) indicated that the spectrum of (*R*)-**1** showed a closer correlation with those of the natural product (maximum $\Delta\delta$ of 0.02 ppm) than that of (*S*)-**1** (maximum $\Delta\delta$ of 0.14 ppm). Similarly, while the ^{13}C NMR spectra of (*R*)-**1** and (*S*)-**1** were similar, a comparison of chemical shifts for all signals indicated that the spectrum of (*R*)-**1** matched that of the natural product more closely than that of (*S*)-**1**. Taken together, the excellent correlation of the ^{1}H and ^{13}C spectra of (*R*)-**1** with those of the natural product and the similar optical rotations of the natural material and synthetic (*R*)-**1** enabled us to assign the stereogenic center at C3 of the side chain of naturally occurring LI-F04a as (*R*)-configured.

In summary, we have completed a total synthesis of LI-F04a as well as its side chain epimer. This has allowed the complete stereochemical assignment of the natural product. The synthetic route we have developed is general, is efficient, and will enable the rapid synthesis of other members of the LI-F family. It will also be applicable to the synthesis of the related circulocin family of cyclic peptides, which have a similar cyclic peptide core bearing ω-guanidino-3-hydroxy side chains of varying length.[31]

Acknowledgment. We thank Dr. Ian Luck (USyd) and Dr. Kelvin Picker (USyd) for assistance with NMR and HPLC, respectively. We thank the University of Sydney for financial support and the award of a postgraduate scholarship to JRC.

Supporting Information Available: Experimental procedures and spectroscopic data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL101254M

(31) He, H. Y.; Shen, B.; Korshalla, J.; Carter, G. T. *Tetrahedron* **2001**, *57*, 1189.