JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Stereochemical Determination of the Leupyrrins and Total Synthesis of Leupyrrin A_1

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Supporting Information

ABSTRACT: The stereochemical determination of the potent antifungal agents leupyrrin A_1 and B_1 and the total synthesis of leupyrrin A_1 are reported. The relative and absolute configuration was determined by a combination of high field NMR studies, molecular modeling, and chemical derivatization. The expedient total synthesis involves a one-pot sequential Zr-mediated oxidative diyne-cyclization/regioselective opening sequence for preparation of the unique dihydrofuran ring, a highly stereoselective one-pot approach to the butyrolactone, a challenging sp^2–sp^3 Suzuki coupling and a high-yielding Shiina macrolactonization.

The leupyrrins (Figure 1) are structurally unique macrodiolides from the myxobacterium *Sorangium cellulosum*.[1]

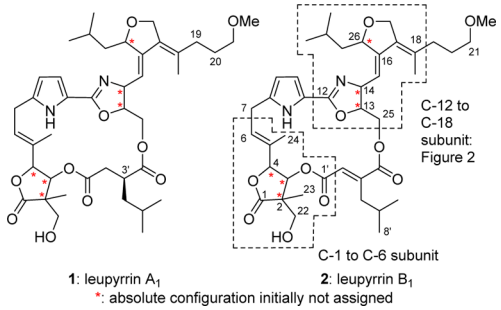


Figure 1. Planar structures of leupyrrins A_1 (**1**) and B_1 (**2**): potent antifungal agents from the myxobacterium *Sorangium cellulosum*.

They display impressive biological properties, including antiproliferative and anti-HIV activities[2] and highly potent antifungal effects in nanomolar concentrations.[1] On a molecular level they efficiently inhibit DNA, RNA, and protein syntheses. In cellular systems, no interaction with the respiratory chain, membrane disruption, or conductivity changes were observed, which suggest that an unusual and so far undefined molecular target may be involved. As shown for the most prominent representatives leupyrrins A_1 (**1**) and B_1 (**2**) their unique architectures are derived by a most diverse biosynthesis reported for myxobacteria so far, that includes a polypropionate (C-1 to C-7), a nonribosomal peptide (C-8 to C-15), a dicarboxylic acid (C-1′ to C-8′), and a polyketide segment (C-16 to C-21) and also includes highly unusual biosynthetic constructions of the γ-butyrolactone and the dihydrofuran moieties.[3] They are distinguished by an 18-membered nonsymmetric macrodiolide core incorporating an unusually substituted γ-butyrolactone ring together with a pyrrole and an oxazoline ring in combination with a side chain containing a unique dihydrofuran with two exocyclic alkylidenes.[4] The important biological properties and their natural scarcity, coupled with their intriguing molecular architectures and most unusual biosyntheses, render the leupyrrins highly attractive targets for further development.

Herein we report determination of the full stereochemistry of the leupyrrins and the first total synthesis of leupyrrin A_1 (**1**). Configurational assignment relied on application of high-field NMR studies in combination with molecular modeling and derivatization. The total synthesis was accomplished by an expedient strategy, involving innovative, highly stereoselective sequential approaches to the butyrolactone and the furan moieties, a challenging sp^2-sp^3 coupling of advanced intermediates and a high yielding Shiina macrolactonization.

The planar constitutions of the leupyrrins (Figure 1) were elucidated by Bode and Höfle on the basis of NMR data (^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC).[1] They contain up to seven stereogenic centers. Only one of them (C-3′) has been rigorously assigned by degradation. Furthermore, a *trans*-orientation of H-13 to H-14 in the oxazoline ring as well as *trans*-configurations of H-3 to both H-4 and C-23 in the butyrolactone moiety have been proposed for leupyrrin A_1 (**1**) on the basis of conformational NMR studies.

For full stereochemical determination, we focused our studies on leupyrrin B_1, due to optimal signal resolution and low degrees of conformational flexibility.[5] Optimum ^{1}H signal dispersion was obtained in CD_3OD and $CDCl_3$ at the highest available field strength (600 MHz) allowing for complete assignment of all resonances. Notably, the coupling constants and NOESY correlations suggest the macrocyclic core to be relatively rigid, while a certain degree of flexibility has to be considered for the C-6 to C-8 and the C-25 region.

Within the C-1 to C-6 subunit, a *trans*-, *trans*-configuration between H-3 and H-4 and between H-3 and Me-23 was readily confirmed,[6] as shown in Figure 3 for the macrocyclic core **4**, in full agreement with the proposal of Bode and Höfle for leupyrrin A_1 and a partial synthesis from our laboratory.[7] As depicted in Figure 2 for the C-12 to C-18 fragment, a homonuclear coupling

Received: February 20, 2015
Published: March 13, 2015

DOI: 10.1021/jacs.5b01894
J. Am. Chem. Soc. 2015, 137, 4086–4089

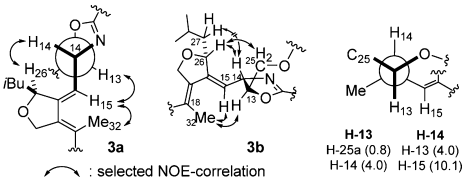


Figure 2. Conformations determined for the C12 to C18 subunit **3** of leupyrrin B_1 (**2**); coupling constants, $^3J_{H,H}$ (Hz) in parentheses.

constant of 4.0 Hz between H-13 and H-14[8] and a large coupling between H-14 and H-15 (10.1 Hz) indicated an antiperiplanar relationship between these protons. In combination with strong NOE correlations from H-13 to H-15 and from H-14 to CH_2-25, a relative *trans*-relationship between H-13 and H-14 was deduced, as shown for **3a**, again in full agreement with previous proposals.[1,7]

Stereochemical correlation of the chiral center at C-26 to the oxazoline moiety was based on a characteristic NOE-signal from H-26 to H-14. In contrast, an analogous correlation between H-13 and H-26 was much less pronounced, which defines the dihedral angle between the oxazoline and the furan moiety, as shown in Figure 3b.[9] Together with a large coupling between H-14 and H-15, the rigidity of the alkylidenesystem, and the absence of NOE correlations of CH_2-27 to either H-14 or H-25, these data suggest a relative *syn*-configuration between H-14 and H-26. Notably, the characteristic NOE signal from H-14 to H-26 had also been observed independently for a structurally related synthetic fragment, which further corroborates this assignment.[7]

For determination of the relationship between the C1 to C6 and the C12 to C18 stereoclusters, molecular modeling was applied on the two possible stereochemical permutations of a truncated analogue (**4**) of leupyrrin B_1 (Figure 3). Using

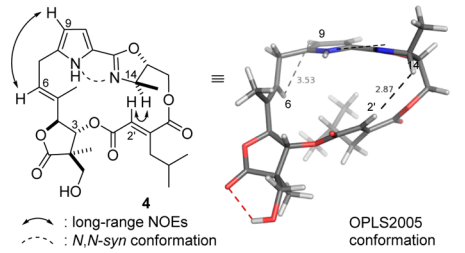
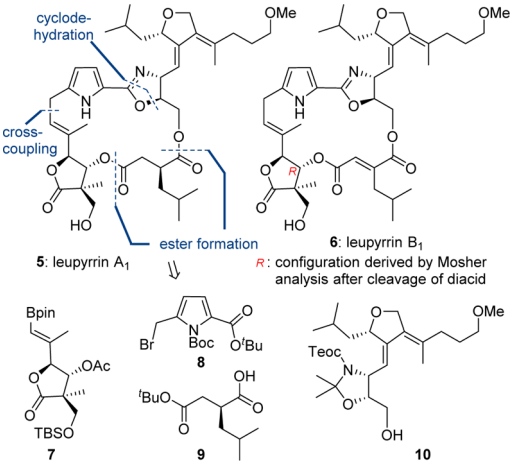


Figure 3. Lowest energy conformation (OPLS2005; GB/SA water solvent model) of the truncated analogue **4** of leupyrrin B_1.

Macromodel (Version 8.5), 20,000 step Monte Carlo searches were carried out with the OPLS2005 force field and the generalized Born/surface area (GB/SA) water solvent model and with implementation of a conformational restraint of the antiperiplanar orientation of H-4 and C-24 based on the NOE data (see above).[10] As starting geometries for each of the two stereochemical permutations, both *N,N-syn* and *N,N-anti* orientations of the nitrogen atoms of the pyrrole and the oxazoline heterocycles (cf. C11–C12 dihedral angle) were employed, resulting in four input structures.[11] The calculations revealed a series of discrete families of low energy conformations for the stereoisomers within 20.00 kcal mol^{-1} of the global minimum.[12] The calculated dihedral angles for the lowest energy conformation of **4** (see Figure 3) to the corresponding series of coupling constants, as determined by NMR, resulted in a close match, while for the other three stereochemical permutations, a much lower degree of resemblance between spectral and calculated data was obtained. Furthermore, the calculated conformation of **4** with pyrrole and oxazoline nitrogen atoms adopting an *N,N-syn* orientation accounted most accurately for several key long-range ROESY correlations (i.e., from H6 to H9 and from H2′ to H14).

Finally, for assigning the absolute configuration of leupyrrin B_1, selective cleavage of the dicarboxylic acid was effectuated by basic hydrolysis using K_2CO_3/MeOH to give a corresponding triol (see SI). Mosher ester analysis then allowed designation of the C3-configuration, as shown (Scheme 1). Structure **6** summarizes the assignment of the full relative and absolute configuration for leupyrrin B_1. On the basis of a common biogenesis and close similarity of the spectral data, the stereochemistry of leupyrrin A_1 was assigned as **5**, and the structure of the other leupyrrins can be assigned accordingly.[13]

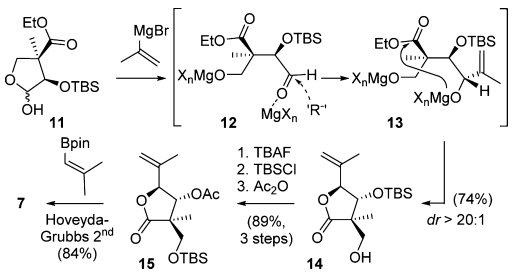
Scheme 1. Stereostructures for Leupyrrins A_1 and B_1 and Retrosynthetic Analysis of Leupyrrin A_1



Efforts were then directed to a validation of our assignment by a total synthesis of leupyrrin A_1. As outlined retrosynthetically in Scheme 1, our synthetic approach relies on the assembly of four building blocks, i.e., **7**, **8**, **9**, and **10**. The oxazoline moiety was planned to arise from a cyclodehydration of **8** with the vicinal aminoalcohol derived from **10**, while a cross-coupling of boronate **7** with benzylic bromide **8** should deliver the sp^2–sp^3 fusion between C-6 and C-7. In principle, either methodology could be employed to close the macrocyclic ring, as an alternative to more conventional macrolactonizations with either end of **9**, thus offering considerable flexibility in the synthesis.

For a synthesis of the butyrolactone **7**, an innovative 3-step one-pot process was designed. As shown in Scheme 2, this involves a hemiacetal opening of readily available lactol **11**,[14] nucleophilic addition to the resulting aldehyde **12** with *iso*-

Scheme 2. One-Pot Synthesis of the Butyrolactone Fragment

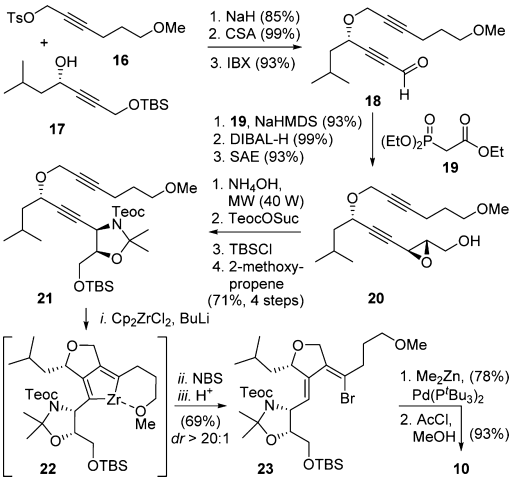


DOI: 10.1021/jacs.5b01894
J. Am. Chem. Soc. 2015, 137, 4086–4089

propenylmagnesium bromide and an intramolecular transesterification to **14**. This process proceeded with excellent selectivity giving the desired hydroxy ester **14** as a single diastereomer.[15] Notably, this route is highly concise and compares favorably to a previous preparation of the butyrolactone from our group.[7] After installment of suitable protective groups, the derived terminal alkene **15** was elaborated to vinylic boronic ester **7** by a cross metathesis with sterically demanding methyl-propenylboronic acid pinacol ester in the presence of Hoveyda–Grubbs II catalyst, which proceeded in good yields and *E*/*Z*-selectivities (9:1).[16,17]

For synthesis of bis-alkylidene-substituted dihydrofuran **10**, a further advancement and application of our Zr-mediated cyclization strategy, as previously developed in or group, was evaluated.[7,18] In detail, the elaboration toward a densely functionalized diyne, i.e., **21** was envisioned (Scheme 3). The

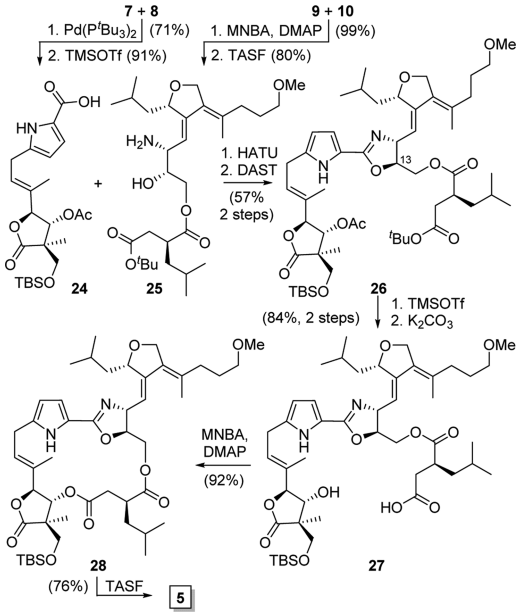
Scheme 3. Tandem Synthesis of the Dihydrofuran Moiety



required diyne was obtained from readily available propargylic alcohol **17**[19] involving an etherification with **16**,[7,18] an HWE homologation of a derived aldehyde **18**, a Sharpless epoxidation, subsequent regioselective opening of the epoxide **20** with ammonia at the propargylic center,[20] and introduction of suitable protective groups.[21,22] Following our previously developed procedure, the crucial 3-step sequential process was initiated by a zirconocene-mediated cyclization. After regioselective opening of the derived zirconacyclopentadiene **22** with NBS *in situ*, final protonation of **22** gave rise to the desired furan **23**. This process proceeded with high yields, considering the complexity of the substrate, and excellent selectivities (>20:1), even on gram scale. This outcome is presumably based on a remote coordination of the metal center to the terminal OMe-ether, in agreement with our mechanistic and protective group concept.[18] To the best of our knowledge, this presents the most advanced application of Zr-mediated oxidative cyclizations in complex target synthesis. Finally, the required methyl group was installed using slightly modified Negishi coupling conditions, and the terminal hydroxyl was liberated with *in situ* generated HCl in high yields.[23,24]

In a rationale to allow for high degrees of convergence, our strategy for fragment union relied on first building up the Western and Eastern fragments, i.e., **7** with **8**[25] and **9**[26] with **10**, respectively (Scheme 4). After extensive evaluation of reaction parameters (catalyst, base, and solvent), it was found that the challenging sp^2–sp^3 Suzuki cross-coupling between **7** and **8**

Scheme 4. Completion of the Total Synthesis of Leupyrrin A_1



could be accomplished in good yields using catalytic amounts of $(^tBu_3P)_2Pd$ and cesium carbonate in a THF/water mixture. To the best of our knowledge this presents the first example of a Suzuki-reaction at the benzylic position of a pyrrole.[27] Simultaneous removal of the Boc- and *tert*-butyl ester group was achieved under Lewis acidic conditions to give **24**.[28]

For esterification of **9** with **10**, the Shiina protocol proceeded smoothly and in high yields.[29] Mild reaction conditions (TASF) then had to be applied for the liberation of the vicinal aminoalcohol **25** to minimize partial migration of the ester group to C-13. For coupling of **24** with **25**, a protocol of Wipf and co-workers[30] was applied, involving an HATU mediated amide formation and cyclodehydration with DAST, giving the desired oxazoline **26** in good yields and with inversion of the hydroxyl-bearing stereogenic center at C-13, as expected.[30]

After liberation of *seco*-acid **27** by cleavage of the *tert*-butyl ester with TMSOTf and mild saponification of the acetate using potassium carbonate in a methanol/THF/water mixture, the desired macrolactonization to **28** could be effectuated using the Shiina protocol under high dilution conditions (0.001 M) in excellent yield (92%). Ultimately, removal of the remaining primary TBS-group proceeded smoothly with TASF in acetonitrile to reveal the natural product **5** in 76% yield. The spectroscopic data (^1H NMR, ^{13}C NMR) and specific rotation (synthetic, $[\alpha]_D^{20}$ = +11.0, *c* = 0.31, MeOH; natural, $[\alpha]_D^{20}$ = +12.0, *c* = 4.06, MeOH) of our synthetic material were in agreement with those published for an isolated sample of leupyrrin A_1, thus allowing confident assignment of the relative and absolute configuration of leupyrrin A_1 and validating our earlier proposal.[1]

In summary, a full stereochemical assignment for the leupyrrin class of natural products was suggested based on the results of high field NMR studies, molecular modeling, and chemical derivatization. This assignment was ultimately validated by a stereocontrolled total synthesis of leupyrrin A_1 (**5**), which proceeds in 21 steps (longest linear sequence, starting from known compounds **16** and **17**) with a 6.3% overall yield.[31] This represents the first synthesis of a member of this family. Salient features of our strategy were an advantageous sp^2–sp^3 Suzuki

DOI: 10.1021/jacs.5b01894
J. Am. Chem. Soc. 2015, 137, 4086–4089

cross-coupling for C6/C7 bond formation and a Zr-mediated diyne-cyclization of a highly functionalized substrate for the regioselective dihydrofuran synthesis. This route will be readily amenable for preparing significant amounts of leupyrrin A_1 as well as designed analogues to explore the biological potential and highly unusual biosynthesis of this unique class of metabolites.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and full characterization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the DFG (ME 2756/7-1; GK 850: scholarship to S.D.) for generous funding, Andreas J. Schneider for HPLC-support, and Dr. Frank Rominger (Univ. Heidelberg) for X-ray analysis.

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(4) Four more leupyrrins have been reported. Leupyrrins A_2 and B_2 bear an additional double bond between C-19 and C-20. Leupyrrins C and D are formal derivatives of leupyrrins A_1 and B_1 with a terminal OH-group in the side chain instead of the OMe-ether.

(5) Higher degrees of signal overlap were observed for the other leupyrrins. In addition, the double bond in the dicarboxylic part makes this compound less flexible, which was critical for correlation of remote stereogenic centers. The observed coupling constants and NOE data in CD_3OD and $CDCl_3$ were very similar for the leupyrrins, which suggest that they adopt similar conformations in these solvents.

(6) In detail, characteristic NOE correlations were observed from H-3 to H-22 and H-24 as well as from H-4 to H-6 and H-23. In combination with an antiperiplanar relationship of H-3 and H-4, as deduced from a large vicinal coupling constant between these protons (9.2 Hz), these data suggest an *anti*-, *anti*-configuration between H-2 and H-3 and between H-3 and Me-23: see SI for details.

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(8) These values are in full agreement with usual data, see e.g.: (a) Morris, L. A.; Jantina Kettenes van den Bosch, J.; Versluis, K.; Thompson, G. S.; Jaspars, M. *Tetrahedron* **2000**, *56*, 8345. (b) Toske, S. G.; Fenical, W. *Tetrahedron Lett.* **1995**, *36*, 8355.

(9) Furthermore, H-15 shows a strong NOE contact both to H-13 and to Me-32, which confirms the configuration of the exocyclic double bonds between C-15 and C-16, as well as between C-17 and C-18.

(10) Conformational searches without constraints resulted in structures with an *anti*-periplanar orientation of Me-24 to C-3, which can be excluded due strong NOE correlation from H-3 to H-24 and the absence of a correlation between H-3 and H-6.

(11) The chemical shift of the pyrrole NH proton in $CDCl_3$ (9.0 ppm) indicates an *N,N-syn*-orientation between the pyrrole and the oxazoline: Afonin, A. V.; Ushakov, I. A.; Pavlov, D. V.; Ivanov, A. V.; Mikhaleva, A. I. *Magn. Reson. Chem.* **2010**, *48*, 685.

(12) For analysis of the conformers, all output structures were superimposed and identical conformations of the macrocycle were clustered, resulting in two to five families of macrocyclic conformations.

(13) This assignment is in agreement with biosynthetic considerations suggesting C-14 to be (*R*)-configured based on the origin from threonine.

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(15) The high degrees of asymmetric induction may possibly be derived via a cyclic Cram or a Felkin-Ahn transition state.

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(17) The minor isomer could be removed by column chromatography; little or no conversion was observed with vinylboronic or 1-propenylboronic acid pinacol ester under a variety of conditions.

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(21) A TEOC protective group was chosen to enable high selectivities in the zirconacyclopentadiene opening, based on our model.[18]

(22) The absolute configuration was confirmed by Mosher ester analysis of an epoxide-reduction product of **20**: see SI. The relative conformation was confirmed by NOE experiments on **21**.

(23) This method proved more reliable as compared to a previously evaluated procedure using BuLi and Me_2SO_4, see refs. 7 and 18.

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(26) Dicarboxylic acid **9** was synthesized in 3 steps (57%) following a reported procedure involving an Evans-type asymmetric alkylation: Hoekstra, M. S.; Sobieray, D. M.; Schwindt, M. A.; Mulhern, T. A.; Grote, T. M.; Huckabee, B. K.; Hendrickson, V. S.; Franklin, L. C.; Granger, E. J.; Karrick, G. L. *Org. Process Res. Dev.* **1997**, *1*, 26.

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(31) The stereochemical assignment for leupyrrin B_1 was independently proven by X-ray crystallography (see SI for details).

DOI: 10.1021/jacs.5b01894
J. Am. Chem. Soc. 2015, 137, 4086−4089