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Total Synthesis of the Proposed Structure for Spirofungin B: A Reassignment of the Stereochemistry

Shannon D. Zanatta, Jonathan M. White, and Mark A. Rizzacasa*

School of Chemistry, The University of Melbourne, Victoria 3010, Australia

masr@unimelb.edu.au

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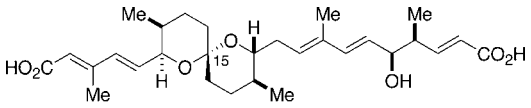
ABSTRACT

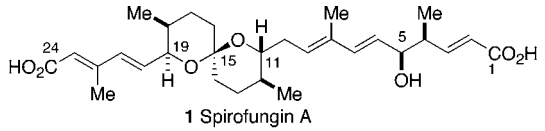


Corrected structure for spirofungin B

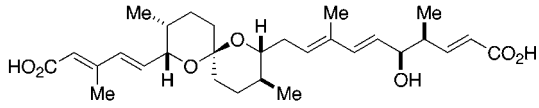
The total synthesis of the proposed structure for spirofungin B (2) is described. The data for the synthetic material did not compare with that for the natural product leading to the conclusion that the structure 2 assigned for spirofungin B is incorrect. Analysis of the NMR data reported for spirofungins A and B as well as related spiroketals allowed for the reassignment of the stereochemistry of spirofungin B to be that corresponding to 15-*epi*-spirofungin A (27).

The spirofungins A (**1**) and B (**2**) were isolated from a *Streptomyces* strain Tü 4113 collected in the Otway National Park, Australia.[1] Compounds **1** and **2** possess a similar 6,6-spiroketal core to reveromycin A (**3**)[2] and have the same C1−C10 triene acid and C20−24 diene acid segments; however, there is no succinate half ester at C18 and the C18 substituent is a methyl group. The spirofungins show high inhibition activity against yeast and moderate activity against some fungi with a MIC of 15 mg mL^{-1} against *Candida albicans*.[1]

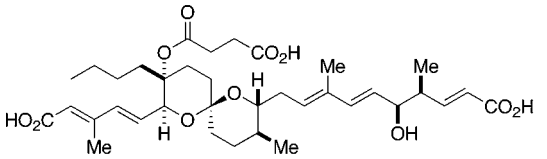
Compounds (**1**) and (**2**) were isolated as a ∼4:1 mixture, respectively. Although they possessed slightly different retention times on reverse phase HPLC, they were not separated and the structural elucidation was conducted on this mixture. The structure of the major isomer spirofungin A (**1**) was assigned using various NMR techniques with the minor component spirofungin B (**2**) identified as a diastereoisomer of **1**, epimeric at both C18 and C19. The absolute configurations of **1** and **2** are suggested by analogy to the reveromycins.



1 Spirofungin A



2 Spirofungin B



3 Reveromycin A

It was proposed[1] that the spiroketal core of **1** possessed the conformation **A** as depicted in Figure 1, with the oxygens (maximum anomeric stabilization) and the C19 diene acid side chain in axial orientations while the C18 methyl group is equatorial. In compound **2**, the C19 side chain is now equatorial while the C18 methyl group is oriented axial as shown in proposed conformer **B**. This was supported by the vicinal coupling constant measured between H19 and H18, the C18 change in chemical shift, and the ROE observed between H19 and H11.

(1) Holtzel, A.; Kempter, C.; Metzger, J. W.; Jung, G.; Groth, I.; Fritz, T.; Fiedler, H.-P. *J. Antibiot.* **1998**, *51*, 699.

(2) Takahashi, H.; Osada, H.; Koshino, H.; Kudo, T.; Amano, S.; Shimizu, S.; Yoshihama, M.; Isono, K. *J. Antibiot.* **1992**, *45*, 1409. Takahashi, T.; Osada, H.; Koshino, H.; Sasaki, M.; Onose, R.; Nakakoshi, M.; Yoshihama, M.; Isono, K. *J. Antibiot.* **1992**, *45*, 1414.

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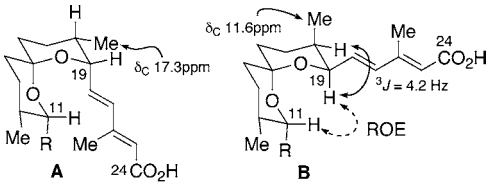
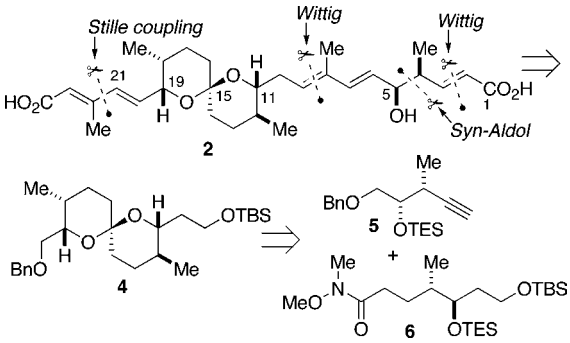


Figure 1.

The natural occurrence of spirofungin B (**2**) is curious since it possesses a different absolute configuration at C19 to that from both **1** and the related reveromycins. Several groups have reported approaches[3,4] to the spiroketal systems of spirofungins A (**1**) and B (**2**) as well as reveromycin A (**3**)[5,6,7] which has also succumbed to total synthesis.[8,9] In this paper, we report the total synthesis of the structure **2** proposed for the minor component spirofungin B which led to the reassignment of the stereochemistry of this natural product.

A brief retrosynthetic analysis of **2** is shown in Scheme 1. The key bond disconnections are similar to those utilized

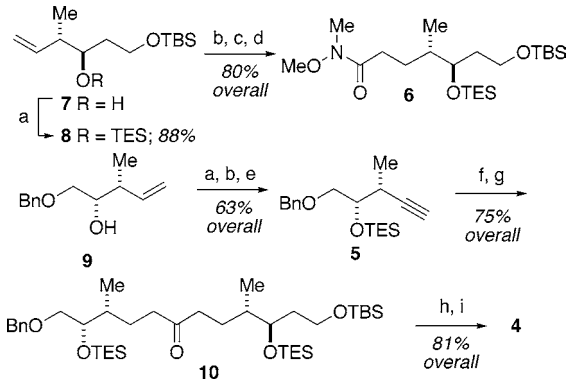
Scheme 1



for our total synthesis of reveromycin B.[10] The C2−C3 and C8−C9 bonds could be formed via stabilized Wittig reactions while the C4−C5 bond might be formed by a reagent-controlled asymmetric *syn*-aldol reaction.[11] Finally, the C21−C22 bond could be installed using a Stille cross-coupling reaction.[12] This leads to the key spiroketal intermediate **4** which could be constructed from a coupling[13] between the anion derived from alkyne **5** and Weinreb amide **6** followed by alkyne saturation and acid induced deprotection and spiroketal formation.[14]

The route to the nominal structure for spirofungin B began with the known optically pure alcohol **7**[10] (Scheme 2). Sily-

Scheme 2 [a]



[a] Reagents and conditions: (a) TESCl, imidazole, DMF, rt; (b) cat. OSO_4, H_2O/THF, then $NaIO_4$; (c) $Ph_3P{=}CHCON(OMe)Me$, CH_2Cl_2; (d) H_2, Pd/C; (e) Ph_3P, CBr_4, CH_2Cl_2, then *n*-BuLi (2 equiv), THF, −78 °C; (f) *n*-BuLi, THF, −78 to 0 °C, cool to −78 °C then add **6** in THF; (g) Lindlar catalyst, H_2, EtOAc, rt; (h) PPTS, MeOH/CH_2Cl_2; (i) TBSCl, imidazole, DMF, rt.

lation of **7** gave ether **8** which was smoothly converted into Weinreb amide **6** by oxidative alkene cleavage, Wittig extension, and hydrogenation.

Coupling fragment **5** was synthesized from known alcohol **9**[15] by protection, alkene cleavage, and Corey−Fuchs[16] alkyne formation. Treatment of the alkyne **5** with *n*-BuLi followed by addition of the amide **6** and subsequent complete saturation using H_2 and Lindlar catalyst gave the corresponding ketone **10** in excellent yield without any benzyl group removal. Compound **10** was subjected to acid deprotection and spiroketalization by treatment with PPTS in MeOH to give **4** as the only spiroisomer. Some of the primary TBS group was cleaved in this sequence but this was easily reinstalled by subjection of the crude product to resilylation. With the spiroketal fragment **4** in hand we next appended the C3−C10 side chain as outlined in Scheme 3.

Debenzylation of **4** followed by oxidation[17] and homologation using the Bestmann protocol[18] gave alkyne **11**. TBAF induced desilylation then afforded the alcohol **12** which fortunately was crystalline and an X-ray crystal structure[19]

(3) Shimizu, Y.; Kiyota, H.; Oritani, T. *Tetrahedron Lett.* **2000**, *41*, 3141.
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(13) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.
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(15) Nicolaou, K. C.; Patron, A. P.; Ajito, K.; Richter, P. K.; Khatuya, H.; Bertinato, P.; Miller, R. A.; Tomaszewski, M. J. *Chem. Eur. J.* **1996**, *2*, 847.
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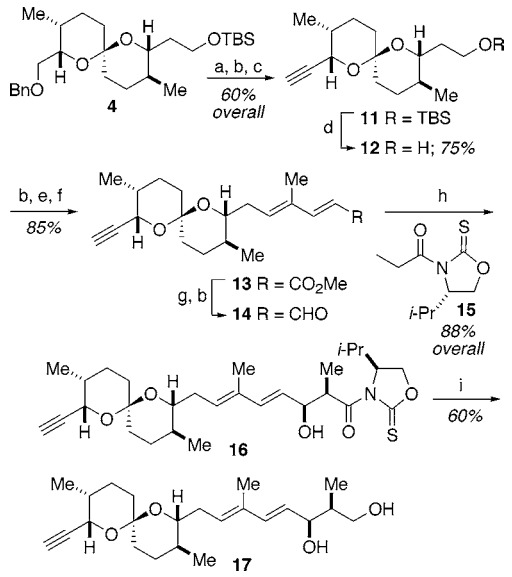
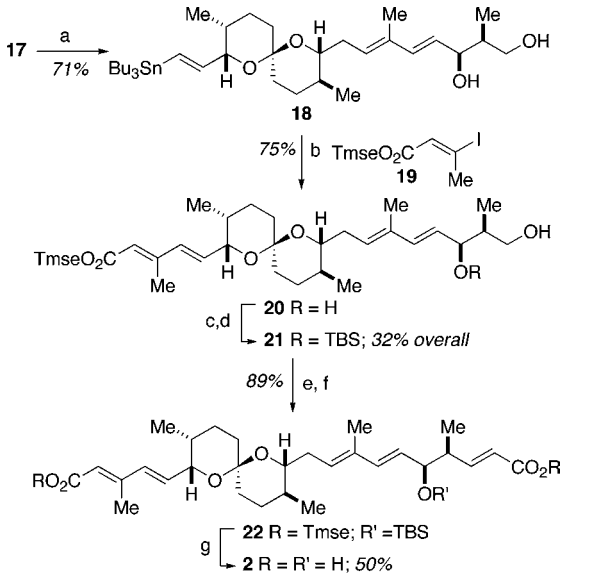
(17) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
(18) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, J. *Synlett* **1996**, 521.
(19) Crystallographic data have been deposited with the Cambridge Crystallographic Centre as supplementary publication CCDC-224793.

Scheme 3 [a]



[a] Reagents and conditions: (a) H_2, Pd/C, EtOAc; (b) Dess−Martin periodinane, CH_2Cl_2, rt; (c) MeOH, K_2CO_3, $(MeO)_2P(O)C(N_2)$-C(O)Me; (d) TBAF, THF, rt; (e) $Ph_3P{=}C(Me)CHO$, toulene, reflux; (f) $Ph_3P{=}CHCO_2Me$, toulene, reflux; (g) DiBALH, CH_2Cl_2, −78 °C; (h) Oxazolidine-2-thione **15**, $Sn(OTf)_2$, *N*-ethylpiperidine, −40 °C, CH_2Cl_2, then add **14**, −78 °C; (i) $NaBH_4$, THF/H_2O.

Scheme 4 [a]



[a] Reagents and conditions: (a) Bu_3SnH, $Pd(Ph_3P)_2Cl_2$, CH_2Cl_2, O °C; (b) iodide **19**, $Pd_2(dba)_3$, TFP, NMP, 55 °C, 5 h; (c) TBSCl, imidazole, DMF; (d) HF·pyridine/pyrdine, THF, 0 °C; (e) Dess−Martin periodinane, CH_2Cl_2, rt; (f) $Ph_3P{=}CHCO_2Tmse$, CH_2Cl_2, rt. (g) TBAF, DMF, rt, 24h.

was obtained for this intermediate confirming the stereochemistry (Figure 2). Oxidation of alcohol **12** and dual Wittig

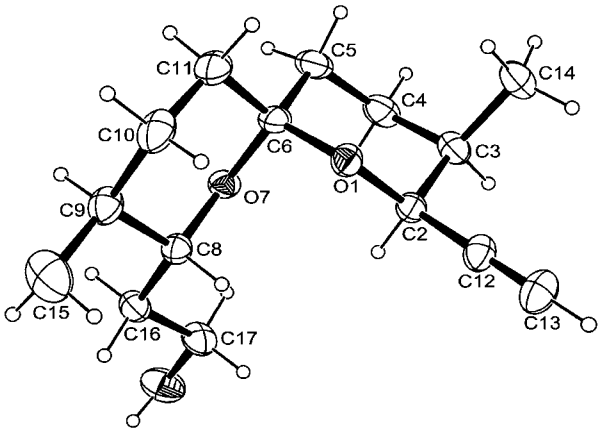


Figure 2. X-ray structure of spiroketal **12**.

extensions gave the ester **13** in good yield. Adjustment of the oxidation level in **13** gave aldehyde **14** which underwent an asymmetric aldol reaction with the tin enolate derived from oxazolidine-2-thione **15**.[10,11] This sequence of reactions which provided *syn*-aldol adduct **16** was high yielding and easily conducted on large scale. Auxiliary removal by treatment of **16** with $NaBH_4$ in wet THF gave diol **17**.

The final steps to compound **2** are outlined in Scheme 4. Conversion of alkyne **17** into vinyl stannane **18** using Pd catalysis[20] proceeded in good yield. The stannane was then cross-coupled[21] with the known iodide **19**[10] (Tmse = trimethylsilylethyl) to cleanly provide ester **20** with the C20−C24 side chain fully installed.

Protective group manipulation gave **21** which upon oxidation and Wittig reaction provided the protected precursor **22**. Exposure of **22** to excess TBAF in DMF caused global deprotection to give the nominal structure for spirofungin B (**2**) which was purified by reverse phase HPLC. Unfortunately, the physical data obtained for synthetic **2** did not compare with that reported for spirofungin B.[1] Furthermore, the ^1H NMR spectrum of an authentic spirofungin mixture measured on the same instrument did not match our synthesized material. In particular, the chemical shift for H19 in the natural product (4.76 ppm) was vastly different to that found for compound **2** (4.29 ppm). In addition to other shift differences in the ^1H NMR spectrum, the chemical shift for C15 in the ^{13}C NMR spectrum of **2** was also measurably different to the literature value (Natural spirofungin B: 97.7 ppm. Synthetic **2**: 96.2 ppm.) These differences lead to the obvious conclusion that the structure proposed for spirofungin B (**2**) is incorrect.

An investigation of the literature revealed a possible alternative for the structure of spirofungin B. In their synthesis of the spiroketal fragment of spirofungin A, Shimizu and co-workers[4] obtained a mixture of spiroketals **23** and **24** (ratio 1.53:1) isomeric at the spiro center via a thermodynamic approach similar to that utilized above (Figure 3).

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(21) Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991**, *113*, 9585.

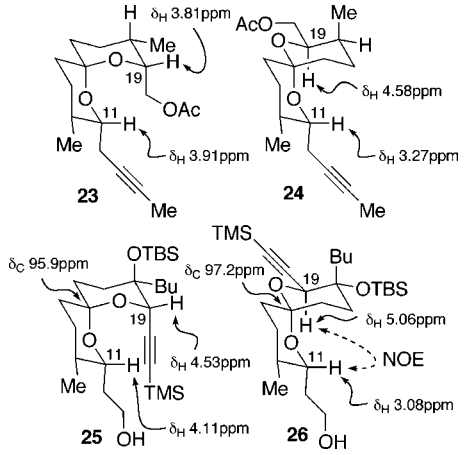

Figure 3. Related spiroketals (spirofungin numbering).

This clearly demonstrates that isomerization of the spirofungin A spiroketal is possible under acidic conditions (due to the axial orientation of the C19 substituent) to afford a spiroketal with one less anomeric stabilization.[22] The chemical shift for the H19 in the preferred isomer is upfield from the corresponding shift in the minor isomer where the axial H19 is deshielded by the axial oxygen[23] and the opposite change in chemical shift is evident for H11 (Figure 3). Comparable chemical shift differences are also observed for H11 and H19 in spirofungins A and B themselves (see **A** and **B′**, respectively, in Figure 4). Another example can be found in the synthesis of the spiroketal fragment of reveromycin A (**3**). Theodorakis and co-workers[6] also obtained a mixture of spiroketal isomers **25** and **26** in a ratio of 1.5:1 respectively by acid induced spiroketalization. The chemical shift differences (Figure 3) for the H11 and H19 protons for each isomer are similar to those cited above. In addition, the C15 chemical shifts for each isomer differ in a manner similar to that for spirofungin A (95.7 ppm) verses B (97.7 ppm). This is consistent with the trend of a slight downfield shift of the spiro carbon in going from ax-ax to ax-eq oxygen orientations.[24] An NOE was also observed between H19 and H11 which was also seen in spirofungin B (Figure 1).

(22) Deslongchamps, P. Rowan, D. D.; Potheir, N.; Sauvé, G.; Sanders, J. K. *Can. J. Chem.* **1981**, *59*, 1105.

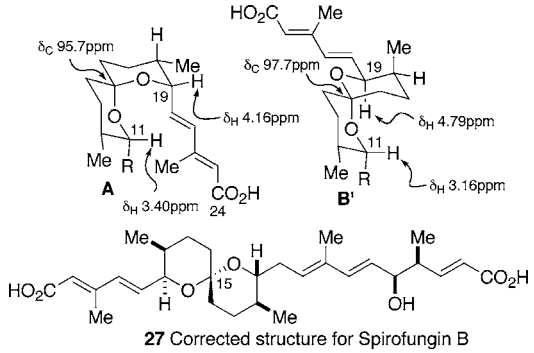

Figure 4.

The results cited above strongly suggest that the structure for spirofungin B is in fact 15-*epi*-spirofungin A (**27**) (Figure 4). The absolute configurations at C18 (*S*) and C19 (*R*) in spirofungin B are identical to that for spirofungin A (**1**) while the spiro carbon is epimeric (15*R*). Spirofungin B therefore has the conformation **B′** as shown which is consistent with the NMR data presented. It is not unreasonable to suggest that biosynthetically, spirofungin A (**1**) could give rise to **27** by simple spiroketal isomerization in analogy to the spiroisomers of the pectenotoxins.[25] Efforts toward the synthesis of spirofungin A (**1**) and its C15 epimer **27** are in progress and will be reported in due course.

Acknowledgment. We are indebted to Professors Hans-Peter Fiedler and Günther Jung (Tübingen University) for providing NMR spectra and an authentic sample of spirofungins A and B. This work was funded by the Melbourne Research Grants Scheme.

Supporting Information Available: Characterization data for compounds **2**, **4**−**6**, **10**, **12**, **13**, **16**, **17**, and **20**−**22** as well as NMR spectra of **2** and authentic spirofungins A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

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