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Short Synthesis of the 6,6-Spiroketal Cores of Spirofungins A and B†

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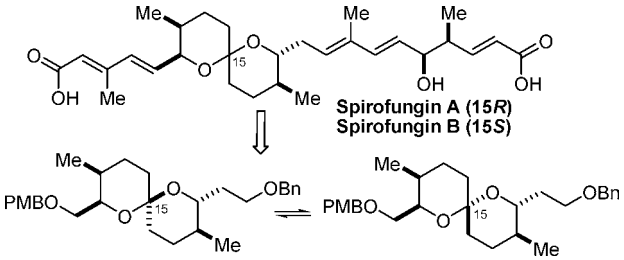
ABSTRACT



Initial efforts toward the total synthesis of the antifungal antibiotics spirofungins A and B are reported. A short and efficient synthesis of the C9–C20 6,6-spiroketal fragments of both compounds is described. This asymmetric approach uses a very efficient alkylation of a lithiated *N,N*-dimethylhydrazone followed by spiroketal formation under acidic conditions.

Spirofungins A (**1**) and B (**2**) were isolated (as a ∼4:1 mixture, respectively) from the culture filtrate and extracts of *Streptomyces violaceusniger* Tü 4113 as new polyketide-spiroketal-type antibiotics related to reveromycins, antibiotics produced by another *Streptomyces* strain (Figure 1).[1,2]

Spirofungins A and B showed high inhibition activity against yeasts such as the human pathogen *Candida albicans* and a moderate antifungal activity against filamentous fungi such as *Botrytis cinerea* and *Mucos miehei*. Their mode of antifungal action may be the same as for reveromycin, in which activity is related to the inhibition of protein synthesis in eukaryotic cells.

Spirofungins A (**1**) and B (**2**) are polyketide-spiroketals with seven stereogenic centers, two diene systems, and carboxylic acid residues at both side chain termini. The structure of spirofungins A and B (determined using the ∼4:1 mixture) was determined by NMR spectroscopic methods in combination with HR-FAB-MS data. The structures of spirofungins A and B resemble those of reveromycin A (**3**), except for the C18 position, since spirofungins lack the

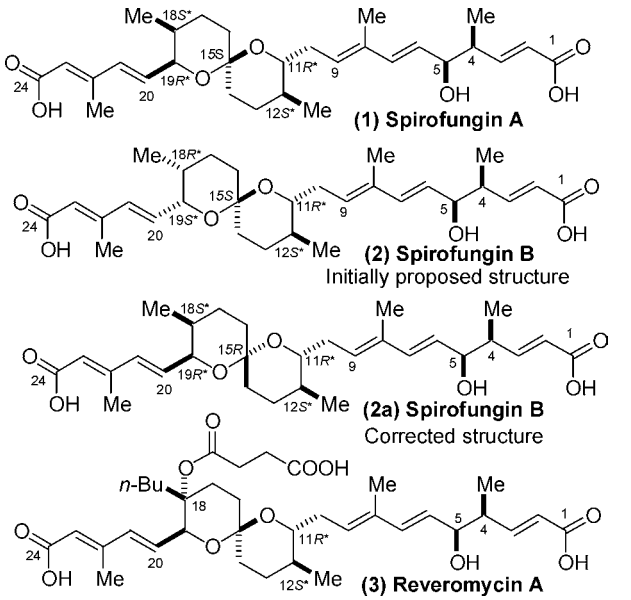


Figure 1. Spirofungins A and B and reveromycin A.

† This paper is dedicated to Prof. Edmundo Alfredo Ruveda on the occasion of his 70th birthday.

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

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

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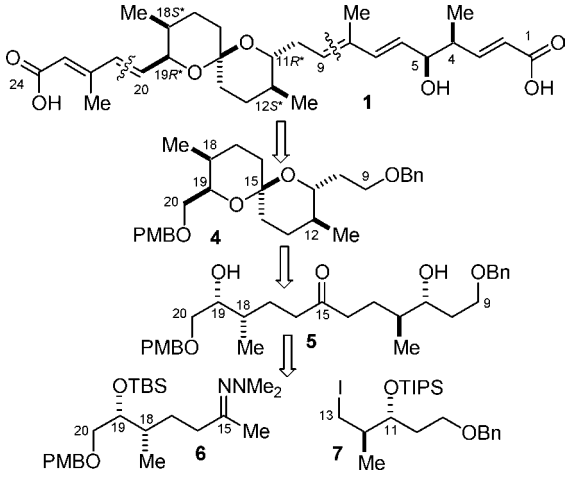
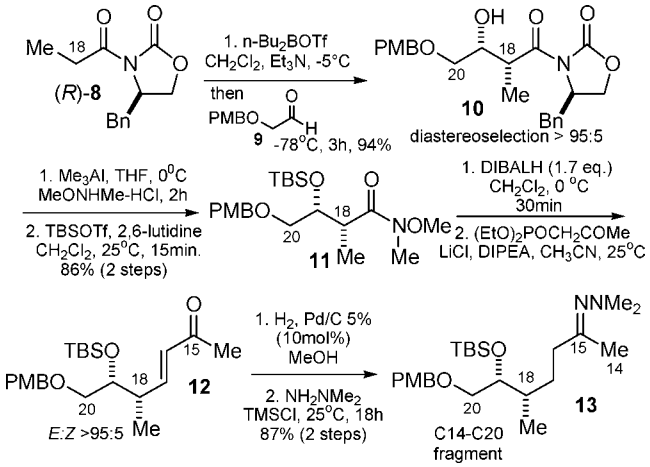
Scheme 1. Retrosynthetic Analysis



Scheme 2. Synthesis of the C14−C20 Fragment



succinate residue and have a methyl group instead of an *n*-butyl group (Figure 1).[1,2] By analogy to reveromycins, it was assumed initially that the absolute configuration of the spiroketal fragments of spirofungins A and B possessed 11*R*,12*S*,15*S*,18*S*,19*R* and 11*R*,12*S*,15*S*,18*R*,19*S* configurations, respectively. The stereogenic centers at C4 and C5 in both **1** and **2** were not determined initially but can be proposed as having 4*S*,5*S* configurations in analogy to the reveromycins.

In very elegant work, Rizzacasa et al.[3] recently proposed a reassignment of the stereochemistry for spirofungin B with the proposed corrected structure corresponding to 15-*epi*-spirofungin A (structure **2a**, 11*R*,12*S*,15*R*,18*S*,19*R*), having a spiroketal with one less anomeric stabilization and not epimeric at C18 and C19, as suggested earlier (Figure 1).[1−3] We also were very intrigued by the fact that the initially proposed structure for spirofungin B possesses different absolute configurations at C18 and C19, when compared to spirofungin A and the reveromycins. As the natural supply is extremely restricted, and attracted by their promising anticancer activity, we initiated a project directed toward the total synthesis of spirofungins A and B.[3,4]

An efficient and flexible synthesis of their spiroketal parts is essential to confirm the absolute configurations at C4, C5, C18, and C19 as well as to provide further material for more extensive biological studies, along with access to novel analogues.[5]

Our disconnection strategy summarized in Scheme 1 (for spirofungin A) involved cleavage of the C8−C9 as well as the C20−C21 double bonds to give spiroketal **4**.[6] The latter contains five of the seven stereogenic centers of the spirofungins. Our synthetic strategy for the 6,6-spiroketal system, having the functional groups for the synthesis of both **1** and **2a**, was based on the treatment of **5** under acidic conditions. Ketone **5** may be further dissected in a straightforward manner to give *N*,*N*-dimethylhydrazone **6** and primary alkyl iodide **7**. Of the available options, we speculated that the desired syn stereocenters at C18 and C19 in **6** might be established through a boron enolate-mediated aldol reaction and that the anti stereocenters at C11 and C12 in **7** might arise from a *trans*-epoxide opening reaction.[6]

Our approach began with an asymmetric aldol addition of the boron enolate derived from oxazolidinone (*R*)-**8** with aldehyde **9** to give aldol adduct **10** in 94% yield and >95:5 diastereoselectivity (Scheme 2).[7,8] Exchange of the oxazolidinone auxiliary in the *syn*-aldol **10** with *N*,*O*-dimethylhydroxylamine[9] followed by protection of the alcohol functionality as its TBS ether cleanly provided the Weinreb amide **11**, in 86% yield (over two steps). This amide was smoothly reduced to the aldehyde on treatment with diisobutylaluminum hydride at 0 °C (Scheme 2).

The unpurified aldehyde was directly subjected to a Horner−Wadsworth−Emmons homologation with the required phosphonate reagent to give the α,β-unsaturated ketone **12** in 90% isolated yield (*E*:*Z* > 95:05) over the two-step sequence.[10] Selective hydrogenation[11] of the double bond proceeded smoothly, leaving the PMB group intact to give the corresponding methyl ketone, which, after treatment with *N*,*N*-dimethyl hydrazine in the presence of TMSCl as a dehydrating agent, gave the corresponding hydrazone **13** (18*S*,19*R*) in 87% yield for the two-step sequence.[12] Starting from oxazolidinone (*S*)-**8**, we were able to prepare hydrazone

(3) Zanatta, S. D.; White, J. M.; Rizzacasa, M. A. *Org. Lett.* **2004**, *6*, 1041.

(4) Approaches to the spiroketal parts of spirofungins A and B: (a) Shimizu, Y.; Kiyota, H.; Oritani, T. *Tetrahedron Lett.* **2000**, *41*, 3141. (b) Shimizu, T.; Kusaka, J.; Ishiyama, H.; Nakata, T. *Tetrahedron Lett.* **2003**, *44*, 4965.

(5) For a review on spiroketals, see: Vaillancourt, V.; Pratt, N. E.; Perron, F; Albizati, K. F. In *The Total Synthesis of Natural Products*; ApSimon, J., ed.; John Wiley & Sons: New York, 1992; Vol. 8, pp 533−691.

(6) Numbering of **1**, **2**, and **2a** as well as of each intermediate follows that suggested in ref 1.

(7) Gage, J. R.; Evans, D. A. *Org. Synth.* **1990**, *68*, 83.

(8) (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. (b) Evans, D. A.; Taber, T. R. *Tetrahedron Lett.* **1980**, *21*, 4675.

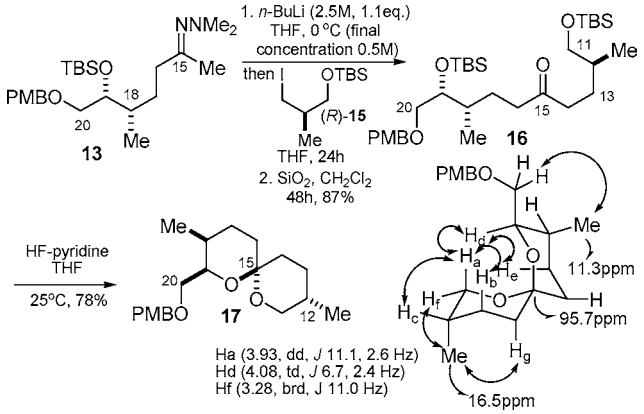
(9) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.

(10) Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863.

(11) Dias, L. C.; Campano, P. L. *J. Braz. Chem. Soc.* **1998**, *9*, 97.

(12) (a) Evans, D. A.; Bender, S. L.; Morrisy, J. *J. Am. Chem. Soc.* **1988**, *110*, 2506. (b) Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Org. Chem.* **2001**, *66*, 7001. (c) Panek, J. S.; Jain, N. F. *J. Am. Chem. Soc.* **1988**, *110*, 2747.

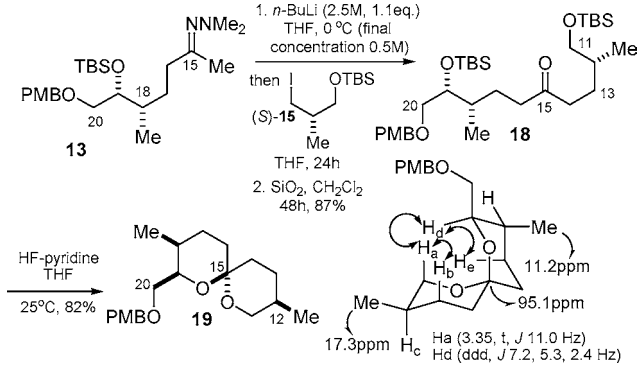
Scheme 3. Synthesis of Spiroketal **17**



14 (18*R*,19*S*), with the intention of preparing the initially proposed spiroketal of spirofungin B (see Scheme 6).

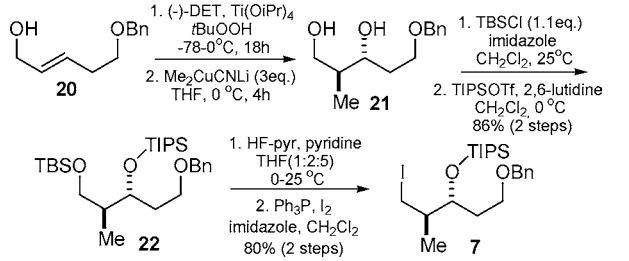
To check the best conditions for carrying out the required hydrazone coupling as well as the spiroketalization reactions leading to analogues of spirofungins lacking the stereocenter at C11, we first prepared the two enantiomeric primary alkyl iodides (*R*)-**15** and (*S*)-**15** from commercially available methyl (*R*)- and (*S*)-3-hydroxy-2-methylpropionate esters.[13] The lithiated *N*,*N*-dimethylhydrazone derived from hydrazone **13** was used for a very efficient subunit coupling with alkyl iodides (*R*)-**15** and (*S*)-**15** (Schemes 3 and 4).[11] After extensive experimental work, we found that the best conditions for this coupling involved treatment of hydrazone **13** with *n*-BuLi (1.1 equiv) in THF at −78 °C, to give a lithiated hydrazone that participated in a smooth alkylation reaction with primary iodide (*R*)-**15**. This reaction led to a coupled product that was directly subjected to hydrazone hydrolysis using silica gel in CH_2Cl_2 for 48 h to provide ketone **16** in 87% overall yield.

Scheme 4. Synthesis of Spiroketal **19**



Removal of the TBS protecting groups with HF-pyridine followed by spirocyclization gave the desired spiroketal **17** as the only isolated product, in 78% yield.[14] The relative stereochemistry for spiroketal **17** was confirmed by the illustrated NOESY interactions as well as by NMR analysis

Scheme 5. Preparation of Primary Iodide **7**



(Scheme 3). The ^{13}C chemical shift of the spiro carbon C15 (95.7 ppm) was typical for a bis-axial orientation with two anomeric stabilizations. In addition, the ^{13}C chemical shift of the methyl group at C18 (11.3 ppm) was appropriate for an axial position, although the ^{13}C chemical shift of the methyl group at C12 (16.5 ppm) was more deshielded than expected for a methyl group in an axial position. The axial orientation for the methyl group was confirmed by NOESY interactions and the observed coupling constants for Ha (3.93, dd, *J* 11.1, 2.6 Hz) and Hf (3.28, brd, *J* 11.0 Hz).

We next investigated the coupling of hydrazone **13** with alkyl iodide (*S*)-**15** (Scheme 4). Treatment of hydrazone **13** with *n*-BuLi (1.1 equiv) in THF at −78 °C, followed by addition of the primary iodide (*S*)-**15** and hydrazone hydrolysis provided ketone **18** in 87% overall yield (Scheme 4).[11] Removal of the silicon protecting groups with HF-pyridine followed by spiroketalization gave the desired spiroketal **19** as the only isolated product, in 82% yield. The relative stereochemistry for spiroketal **19** was confirmed by the illustrated NOESY interactions as well as by NMR analysis (Scheme 4). The ^{13}C chemical shift of the spiro carbon C15 (95.1 ppm) was typical for a bis-axial orientation with two anomeric stabilizations. The ^{13}C chemical shifts of the methyl groups at C12 (17.3 ppm) and C18 (11.2 ppm) were appropriate for equatorial and axial positions, respectively. The equatorial orientation of the methyl group at C12 was confirmed by the illustrated NOESY interactions and the observed coupling constants for Ha (t, $J = 11.0$ Hz). We have also observed that hydrogens Ha (3.93 ppm) and Hb (3.80 ppm) in spiroketal **17** are more deshielded than the same hydrogens, Ha (3.80 ppm) and Hb (3.35 ppm), in spiroketal **19**, which further supports the assignment of the methyl group in an axial position at C12 in spiroketal **17**.

Having established the best conditions for hydrazone coupling and spiroketal formation, we next moved to the preparation of alkyl iodide **7** (Scheme 5).

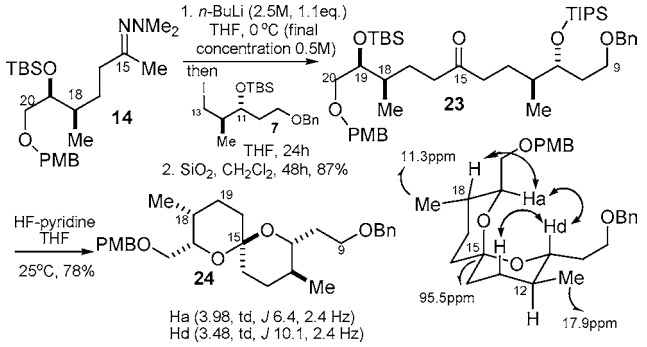
This was accomplished by epoxidation of allylic alcohol **20** using the Sharpless asymmetric procedure[15] with D-(−)-DET, $Ti(O^iPr)_4$ and ^{t}BuOOH to produce the epoxy alcohol.[16] Epoxide opening proceeded smoothly with high regioselec-

(13) Protection of the OH function, DIBALH reduction to the alcohol, followed by treatment with PPh_3 and I_2 to give primary iodides **15** in 80% yield for the three-step sequence.

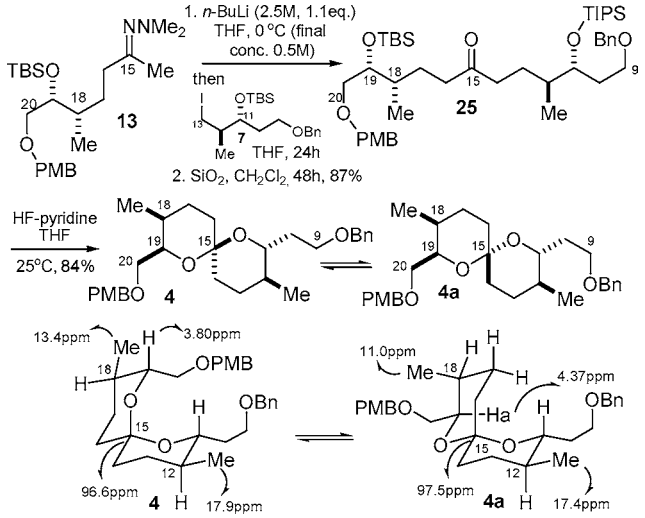
(14) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639. (b) Bloch, R.; Brillet, C. *Synlett* **1991**, 829.

(15) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

Scheme 6. Synthesis of Spiroketal **24**



Scheme 7. Equilibrium between Spiroketals **4** and **4a**



tivity after treatment of the epoxy alcohol with $Me_2CuCNLi_2$ to give diol **21** with good yield and selectivity.[17] Selective protection of the primary OH function as its TBS ether followed by protection of the secondary alcohol functionality as the TIPS ether gave **22** in 86% overall yield (two steps).

Selective removal of the primary TBS group with HF-pyr in pyridine followed by treatment with PPh_3 and I_2 gave primary alkyl iodide **7** in 80% yield for the two-step sequence.

With fragments C11−C13 (**7**) and C14−C20 (**13** and **14**) in hand, their coupling was undertaken (Schemes 6 and 7). To prepare the initially proposed structure of spirofungin B, treatment of hydrazone **14** with *n*-BuLi (1.1 equiv) in THF at −78 °C, followed by addition of primary alkyl iodide **7** and hydrolysis gave ketone **23** in 87% overall yield (Scheme 6).[11] Compound **23** was subjected to acid deprotection and spiroketal formation by treatment with HF-pyr in THF to give **24** as the only spiroisomer in 78% yield.

We next moved to the preparation of the desired spiroketals of spirofungins A and B (Scheme 7). Treatment of hydrazone **13** with *n*-BuLi (1.1 equiv) in THF at −78 °C, followed by addition of primary alkyl iodide **7**, gave an intermediate hydrazone that was directly subjected to hydrolysis without further purification, providing the ketone **25** in 87% overall yield (Scheme 7).[11] At this point, all that remained was to carry out the necessary spiroketalization. Compound **25** was subjected to acid deprotection and spiroketal formation by treatment with HF-pyr in THF to give a 30:70 mixture of spiroketals **4** and **4a**, respectively, in 84% combined yield.

It is noteworthy that under these conditions, spiroketal **4a**, with only one anomeric stabilization, was isolated as the major isomer. Although these spiroketals were readily separated by flash column chromatography, we observed that each isolated pure spiroketal led to the same 30:70 equilibrium mixture of **4**:**4a** during attempts to obtain the NMR spectra in $CDCl_3$. The relative stereochemistries for spiroketals **4** and **4a** were confirmed by NMR analysis (Scheme 7). For spiroketal **4** (needed for the synthesis of spirofungin A), the ^{13}C chemical shift of the spiro carbon C15 (96.6 ppm) was typical for a bis-axial orientation with two anomeric stabilizations. In addition, the ^{13}C chemical shifts of the methyl groups at C12 (13.4 ppm) and C18 (17.9 ppm) were typical for equatorial positions.

For the major spiroketal **4a** (needed for the synthesis of the corrected structure of spirofungin B), the ^{13}C chemical shift of the spiro carbon C15 (97.5 ppm) was typical for an orientation with only one anomeric stabilization. The ^{13}C chemical shift of the methyl group at C12 (17.4 ppm) was that expected for an equatorial position, and the ^{13}C chemical shift of the methyl group at C18 (11.0 ppm) was typical for an axial position. This equilibrium between **4** and **4a** is in perfect agreement with the fact that spirofungins A and B were not separated and the structure elucidation was conducted on the 4:1 mixture. On the basis of these results, we support the assignment of the Rizzacasa group[3] that the spiroketal cores of spirofungins A and B have the same absolute configurations at C18 and C19 and that spirofungin B is epimeric at the C15 spiro carbon, being a spiroketal with one less anomeric stabilization.

Our synthesis requires nine steps from (*R*)-**8** (longest linear sequence) and produced the 6,6-spiroketal **4** and **4a** in good yields. This approach is, in principle, readily applicable for the preparation of spirofungins A and B as well as to additional analogues. Extension of this work to the synthesis of spirofungins A and B is underway, and the results will be described in due course.

Acknowledgment. We are grateful to FAEP-UNICAMP, FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support. We thank also Prof. Carol H. Collins for helpful suggestions about English grammar and style.

Supporting Information Available: Spectral data for compounds **4**, **4a**, **7**, **10**−**13**, and **16**−**25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Díez-Martin, D.; Kotecha, N. R.; Ley, S. V.; Mnategani, S.; Menéndez, H. M.; White, A. D.; Banks, B. J. *Tetrahedron* **1992**, *48*, 7899.

(17) Relative stereochemistry for diol **21** was determined by conversion to the PMB acetal followed by analysis of coupling constants and NOESY interactions in the corresponding ^1H NMR spectra.