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Synthesis of the Spiroiminal Moiety of Marineosins A and B

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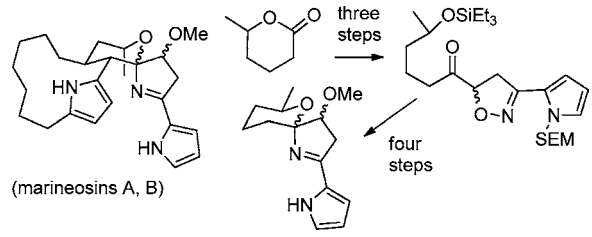
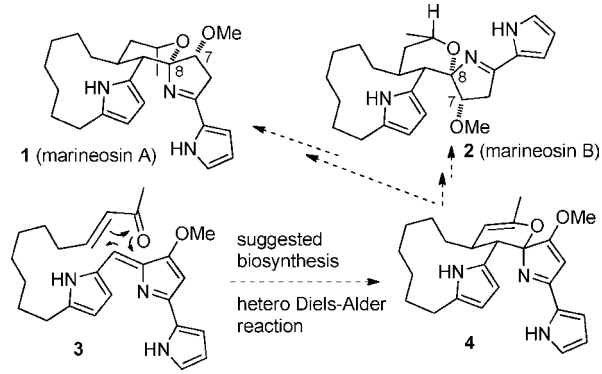
ABSTRACT



A model for the spiroiminal moiety of marineosins A and B was prepared starting from methylvalerolactone. Addition of vinylmagnesium bromide, protection of the alcohol, and reaction of the vinyl ketone with a protected pyrrole-2-carbonitrile *N*-oxide gave an isoxazoline. Hydrogenolysis of the N−O bond with Raney nickel gave a keto imine that cyclized to a hemi-iminal. O-Methylation, acid-catalyzed cleavage of the TES group and spiroiminal formation, and deprotection completed a seven-step synthesis.

Fenical and co-workers recently reported the isolation of the cytotoxic spiroiminals marineosins A (**1**) and B (**2**) from a marine-derived *Streptomyces*-related actinomycete (see Scheme 1).[1] The structures were assigned by analysis of the NMR spectra, and the stereochemistry was assigned from the NOESY spectra. These compounds are clearly members of the prodigiosin family of bacterial pigments.[2] It is noteworthy that marineosins A (**1**) and B (**2**) differ in stereochemistry at both C-7 and C-8. Analysis of models and molecular mechanics calculations suggest that the isomers of the marineosins at the spiroiminal center (C-8) are much less stable than marineosins A and B because of steric interactions between the methoxy group and the adjacent pyrrole that is part of the macrocycle in the two isomers that were not isolated. The major isomer, marineosin A (**1**), inhibits human colon carcinoma HCT-116 with an IC_{50} of 0.5 μM. Testing in the NCI 60 cell line panel showed considerable selectivity against melanoma and leukemia cell lines.

Scheme 1. Structures of Marineosins A and B and Biosynthesis Suggested by Fenical



Fenical suggested that the marineosins might be biosynthesized by an intramolecular hetero Diels−Alder reaction of **3** to give **4**, which would then be reduced to give marineosins A and B. However, this biosynthetic pathway seemed unlikely to us since it would require a six-electron oxidation of a likely precursor, undecylprodigiosin (**5**) (see

(1) Boonlarppradab, C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2008**, *10*, 5505–5508.

(2) (a) Fürstner, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 3582–3603. (b) Williamson, N. R.; Fineran, P. C.; Gristwood, T.; Chawrai, S. R.; Leeper, F. J.; Salmond, G. P. C. *Future Microbiol.* **2007**, *2*, 605–618.

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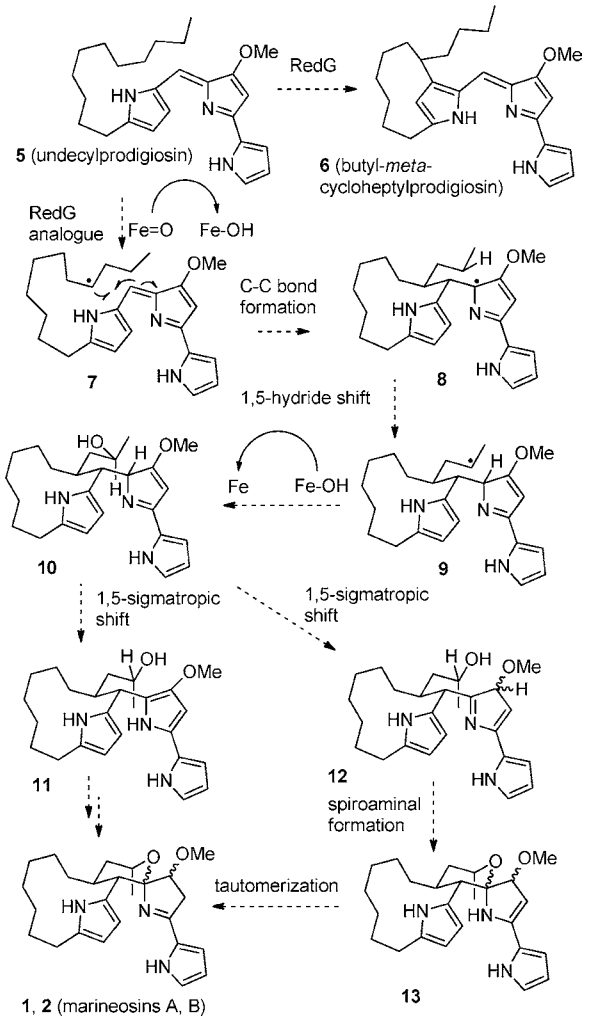
Scheme 2), to form **3**, followed by a four-electron reduction of **4** to give marineosins A and B. Lindsley recently reported that the conversion of **3** (prepared by an eight-step route) to **4** could not be achieved in the laboratory.[3]

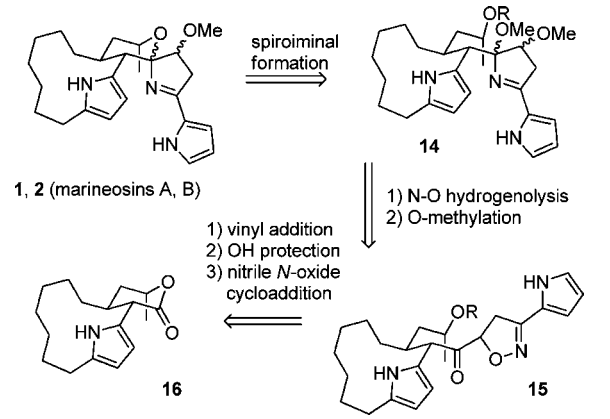
Scheme 2. Alternative Biosynthesis of the Marineosins



The gene cluster responsible for the biosynthesis of undecylprodigiosin (**5**) and butyl-*meta*-cycloheptylprodigiosin (**6**) in *Streptomyces coelicolor* has been sequenced (see Scheme 2).[4] A single enzyme RedG, which is probably a nonheme iron-dependent dioxygenase, appears to convert **5** to **6** by oxidation of the CH_2 group to a radical or cation that undergoes an intramolecular Friedel−Crafts reaction to the pyrrole to form **6**. A related enzyme could easily oxidize **5** at the adjacent carbon to give radical **7**, which could add to the exocyclic double bond to give macrocyclic radical **8**. Radical **8** could undergo a facile 1,5-hydride shift to give macrocycle **9** with the radical at the side chain carbon adjacent to the methyl group. This radical is close to the enzyme active site that generated radical **7** and could therefore be easily oxidized to form macrocyclic alcohol **10**. 2*H*-Pyrrole **10** could undergo a facile 1,5-sigmatropic hydride shift to form 1*H*-pyrrole **11**, which could cyclize to form marineosins A and B. However, cyclization of **11** to **1** and **2** would require the loss of aromaticity of pyrrole **11**. Alternatively, a 1,5-sigmatropic hydride shift from **10** could also give 3*H*-pyrrole **12**, which should rapidly cyclize to form spiroaminal **13**. Tautomerization of the enamine to an imine would give marineosins A (**1**) and B (**2**). This scheme is appealing because only a single enzyme would be needed to convert undecylprodigiosin (**5**) to the marineosins.

There are numerous examples of spiroaminals and many examples of iminals, but the spiroiminal moiety of the marineosins appears to be unprecedented. We therefore chose to start our synthetic planning for the marineosins by exploring routes to the spiroiminal moiety. We were particularly interested in routes in which the dihydropyrrole ring of the spiroiminal was formed before the tetrahydropyran because this approach is related to the biosynthesis proposed in Scheme 2. Our retrosynthesis is shown in Scheme 3. The marineosins should be available by spiroiminal formation from **14**, which should be readily available from keto isoxazoline **15** by hydrogenolysis of the N−O bond over Raney nickel with spontaneous formation of the hemi-iminal[5] and then *O*-methylation. Isoxazoline **15** will be prepared by nitrile *N*-oxide cycloaddition to the vinyl ketone prepared by addition of vinylmagnesium bromide to lactone **16** and protection of the secondary alcohol.

Scheme 3. Retrosynthesis of the Marineosins

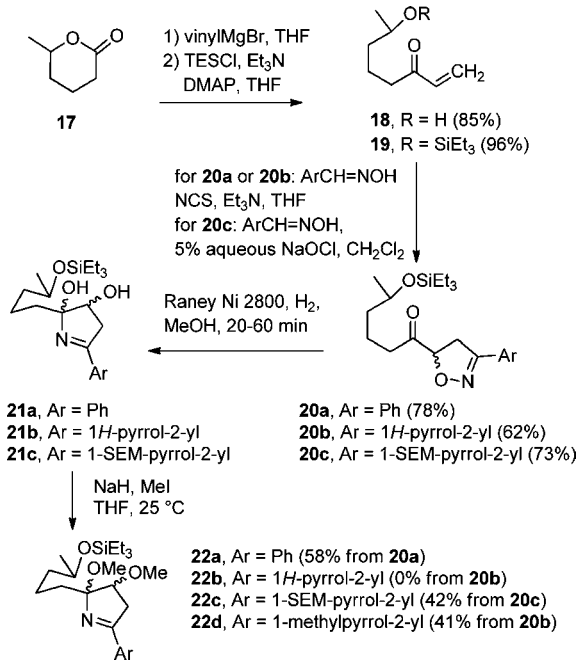


We chose to investigate this sequence with the readily available model lactone **17**, which lacks the macrocyclic ring of **16** (see Scheme 4). Addition of vinylmagnesium bromide to lactone **17** afforded the known hydroxy ketone **18** in 85% yield,[6] which was protected as its triethylsilyl ether to give enone **19** in 96% yield. The pyrrole ring is relatively unstable[7] and susceptible to methylation, so we initially

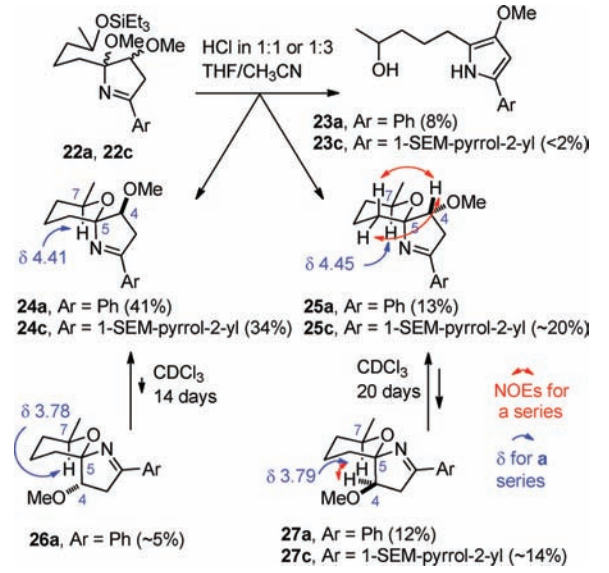
(3) Aldrich, L. N.; Dawson, E. W.; Lindsley, C. W. *Org. Lett.* **2010**, *12*, 1048–1051.

(4) (a) Cerdenõ, A. M.; Bibb, M. J.; Challis, G. L. *Chem. Biol.* **2001**, *8*, 817–829. (b) Williamson, N. R.; Fineran, P. C.; Leeper, F. J.; Salmond, G. P. C. *Nat. Rev. Microbiol.* **2006**, *4*, 887–899. (c) Mo, S.; Sydor, P. K.; Corre, C.; Alhamadsheh, M. M.; Stanley, A. E.; Haynes, S. W.; Song, L.; Reynolds, K. A.; Challis, G. L. *Chem. Biol.* **2008**, *15*, 137–148.

Scheme 4. Preparation of Iminal **22**



Scheme 5. Preparation of Spiroiminals **24a**,**c**, **25a**,**c**, and **27a**,**c**



investigated the reaction of **19** with benzonitrile *N*-oxide. Reaction of **19**, benzaldehyde oxime, NCS, and Et_3N in THF at −78 to +25 °C provided isoxazoline **20a** in 78% yield. Hydrogenolysis (1 atm) over Raney nickel 2800 in MeOH for 45 min afforded hemi-iminal **21a** as a mixture of isomers. Methylation with NaH and MeI in THF at 25 °C gave methyl ether iminal **22a** in 58% yield from **20a**.

Treatment of **22a** with 2 M aqueous hydrochloric acid in 1:1 THF/CH_3CN hydrolyzed the triethylsilyl ether and effected loss of methanol to give the desired spiroiminals **24a** (41%), **25a** (13%), and **27a** (12%) and the undesired pyrrole **23a** (8%) (see Scheme 5). Solutions of either pure **25a** or **27a** equilibrated to an identical 3:1 mixture of **25a** and **27a** in $CDCl_3$ (containing adventitious HCl) over 2−3 weeks, establishing that these two compounds have the identical relative stereochemistry at C-4 and C-7 and differ only at the iminal center C-5. The major isomer **24a** equilibrated in 2 weeks to give a 19:1 mixture of **24a** and **26a**, establishing that these two compounds have the identical relative stereochemistry at C-4 and C-7. We were unable to convert methoxypyrrole **23a** to the desired spiroiminals **24a**−**27a** under a variety of acidic conditions, which resulted in either no reaction or extensive decomposition. This suggests that methoxypyrrole **11** may not be an intermediate in the biosynthesis of the marineosins (see Scheme 2).

The presence of the imine double bond makes the formation of spiroiminals from **22** quite different from that of spiroketals and spiroaminals. Protonation of the methoxy group and loss of MeOH would give a cumulene (C=N^+=C) in a five-membered ring. Spiroiminal formation may proceed by tautomerization to an enamine analogous to **13**, which can easily lose MeOH. Other pathways are presented in the Supporting Information. Equilibration of the spiroiminals can also occur by formation of enamines or by protonation on the imine nitrogen and ring-opening of the dihydropyrrole ring to give a six-membered oxocarbenium ion. Remarkably, very little pyrrole **23a** was formed from **22a** or during the equilibration of the spiroiminals.

The structure of **27a** was established by an NOE between proton H-4 adjacent to the methoxy group and proton H-7 adjacent to the methyl group, as shown. These protons are too far apart in the other three isomers for an NOE to be observed. This NOE also allowed us to assign the structure of **25a**, which differs from **27a** only in the stereochemistry at the spiroiminal center. The structure of **25a** was confirmed by NOEs between proton H-4 adjacent to the methoxy group and the adjacent CH_2 group on the tetrahydropyran group as shown. The axial nitrogen deshields proton H-7 adjacent to the methyl group in **24a** (δ 4.41) and **25a** (δ 4.45), which absorbs much further downfield than H-7 in **26a** (δ 3.78) and **27a** (δ 3.79) with an axial carbon.[8] As expected, the major isomer **24a** has no NOEs between the protons on the tetrahydropyran ring and those on the dihydropyrrole ring.

Having developed a route to phenyl-substituted spiroiminals, we turned our attention to the problem of a pyrrole substituent. Treatment of **19** with the oxime of pyrrole-2-carboxaldehyde in THF with NCS and Et_3N at −78 °C afforded isoxazoline **20b** in 62% yield. Hydrogenolysis of

(5) Hydrogenolysis of a keto isoxaoline afforded a compound whose spectral data are in agreement with those of a hemi-iminal. See compound **12** in: Andersen, S. H.; Sharma, K. K.; Torssell, K. B. G. *Tetrahedron* **1983**, *39*, 2241–2245.

(6) Cohen, N.; Banner, B. L.; Blount, J. F.; Weber, G.; Tsai, M.; Saucy, G. *J. Org. Chem.* **1974**, *39*, 1824–1833.

(7) Cycloadditions with the nitrile *N*-oxide derived from benzaldehyde oxime can be carried out at room temperature, whereas those with the nitrile *N*-oxide derived from the oxime of pyrrole-2-carboxaldehyde must be carried out at −70 °C. See: Ghabrial, S. S.; Thomsen, I.; Torssell, K. B. G. *Acta Chem. Scand. B* **1987**, *B41*, 426–434.

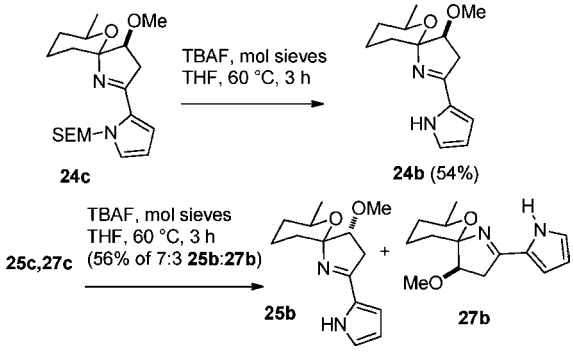
(8) For a similar effect by an axial oxygen in spiroketals, see: Doubský, J.; Šaman, D.; Zedník, J.; Vašíčková, S.; Koutek, B. *Tetrahedron Lett.* **2005**, *46*, 7923–7926.

the N−O bond over Raney Ni proceeded uneventfully to give **21b**, but methylation with NaH and MeI methylated the pyrrole NH in addition to the two hydroxy groups to give **22d** in 41% yield from **20b**. Therefore, the pyrrole NH needed to be protected.

A SEM-protected pyrrole appeared to be compatible with the reagents in this sequence.[9] *N*-SEM-Pyrrole-2-carboxaldehyde[10] was converted to the oxime in 84% yield with NH_2OH·HCl and NaOAc in aqueous MeOH. However, treatment of this oxime with NCS and enone **19** in THF at −78 °C gave **20c** in <30% yield. Fortunately, reaction of this oxime in CH_2Cl_2 at 25 °C with 5% aqueous NaOCl[11] generated the nitrile *N*-oxide, which added cleanly to enone **19** to give **20c** in 73% yield. Both hydrogenolysis over Raney Ni and methylation now proceeded uneventfully to give **22c** in 42% yield from **20c**. Treatment of **22c** with 2 M aqueous hydrochloric acid in 1:3 THF/CH_3CN hydrolyzed the triethylsilyl ether and effected loss of methanol to give the protected spiroiminal **24c** (34%), an inseparable equilibrium 3:2 mixture of spiroiminals **25c** and **27c** (34%), and the undesired pyrrole **23c** (<2%). The stereochemistry of these spiroiminals was assigned by analogy to **24a** to **27a**, which have virtually identical ^{1}H and ^{13}C NMR spectra in the aliphatic region.

Deprotection of **24c** with TBAF and molecular sieves in THF at 60 °C for 3 h provided spiroiminal **24b** in 54% yield (see Scheme 6). A similar sequence on the 3:2 mixture of **25c** and **27c** afforded a 7:3 mixture of **25b** and **27b** in 56% yield. The stereochemistry of **24b** to **27b** was again assigned by analogy to **24a** to **27a**.

Scheme 6. Preparation of Spiroiminals **24b**, **25b**, and **27b**



Comparison of the structures of model spiroiminals **24**−**27** with those of marineosins A (**1**) and B (**2**) is complicated by the trans-fused macrocyclic ring of the marineosins that forces the methyl group into an axial conformation, whereas it is equatorial in the models lacking the fused macrocyclic ring. Spiroiminal **25b** has the same relative stereochemistry as marineosin B (**2**). Although **27b** is only slightly less stable than **25b**, examination of models and molecular mechanics calculations suggest that steric interactions between the methoxy group and macrocyclic ring will make the stereoisomer of marineosin that corresponds to **27b** much less stable than marineosin B (**2**). Marineosin A (**1**) has the same relative stereochemistry as **26a**, which was only observed as a minor isomer in equilibrium with **24a**. However, steric interactions with the axial methyl group should strongly favor the spiroiminal isomer with an axial nitrogen (marineosin A) over that with the stereochemistry corresponding to **24a**, which must exist in the conformation with a large axial carbon and also has steric interactions between the methoxy group and the macrocyclic ring. Therefore, treatment of **14** with acid should afford mainly marineosins A (**1**) and B (**2**) rather than the other two, presumably less stable, isomers.

In conclusion, we have developed a seven-step route to spiroiminals **24b**, **25b**, and **27b** from lactone **17** that should be amenable to the conversion of lactone **16** to marineosins A (**1**) and B (**2**). A plausible biosynthesis from undecylprodigiosin (**5**) to the marineosins is proposed that only requires a single two-electron oxidation.

Acknowledgment. We are grateful to the National Institutes of Health (GM-50151) for support of this work.

Supporting Information Available: Complete experimental procedures, spectral assignment of **24a**−**27a**, discussion of spiroiminal formation and equilibration, and copies of ^{1}H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Jolicoeur, B.; Chapman, E. E.; Thompson, A.; Lubell, W. D. *Tetrahedron* **2006**, *62*, 11531–11563.
(10) Muchowski, J. M.; Solas, D. R. *J. Org. Chem.* **1984**, *49*, 203–205.
(11) Lee, G. A. *Synthesis* **1982**, 508–509.