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Synthesis of the Isoxazolo[4,3,2-*de*]phenanthridinone Moiety of the Parnafungins

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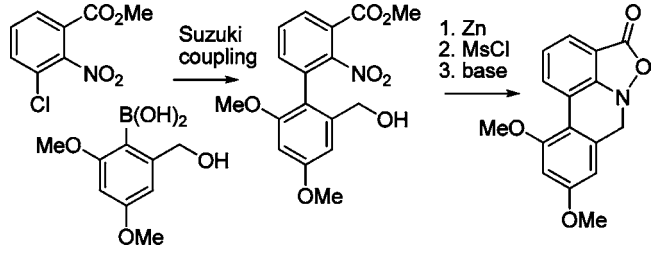
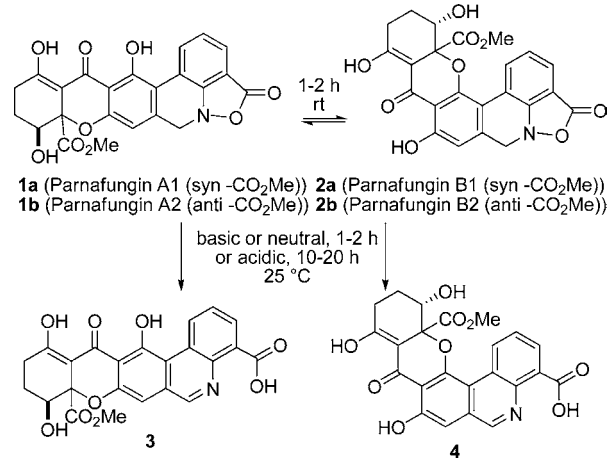
ABSTRACT



A practical route to the labile tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety of the antifungal parnafungins has been developed. Zinc reduction of a methyl 2′-hydroxymethyl-2-nitro-3-biphenylcarboxylate, which was prepared by a Suzuki coupling, afforded a benzisoxazolone that was treated with MsCl and base to generate the labile tetracyclic ring system in 37−47% yield. This compound decomposes to the phenanthridine in $CDCl_3$ and the phenanthridine *N*-oxide in aqueous base.

A Merck group recently reported the isolation of parnafungins A1 (**1a**), A2 (**1b**), B1 (**2a**), and B2 (**2b**) that interconvert readily by an elimination that opens the xanthone ring to form an enone and a phenol and then a conjugate addition from either phenol OH group to either face of the enone to reform the xanthone (see Scheme 1).[1] The parnafungins demonstrate broad spectrum antifungal activity by inhibiting fungal polyadenosine polymerase (PAP) and show in vivo efficacy against *Candida albicans* in a mouse model.[1b] Affinity selection/mass spectrometry demonstrated that the linear parnafungin A binds

Scheme 1. Parnafungin Structures and Rearrangement Products



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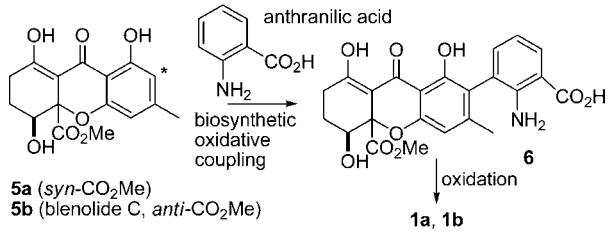
preferentially to PAP.[1c] The *O*-methylated parnafungins C and D were recently isolated.[1d]

The isoxazolone ring of **1** and **2** is very labile. Treatment of **1** and **2** at neutral or basic pH generated phenanthridines **3** and **4**, respectively, in less than 1 h. The same compounds were generated over 10−20 h at pH 3. Unfortunately, phenanthridines **3** and **4** are biologically inactive.[1a]

The parnafungins might be biosynthesized by oxidative coupling of **5** with anthranilic acid to give **6** (see Scheme 2). Further oxidation of the benzylic methyl group and aniline

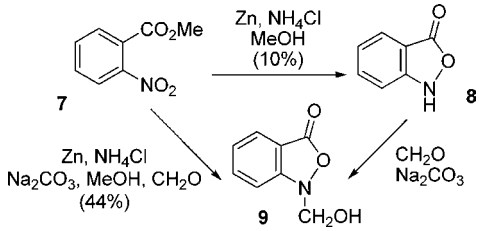
Scheme 2. Possible Biosynthesis of the Parnafungins



could give parnafungins A1 (**1a**) and A2 (**1b**). Blenolide C (**5b**) was recently isolated[2] and numerous natural products are known that are derived from blenolide C by oxidative dimerization or coupling at the carbon marked by an asterisk. Bräse[3] and Nicolaou[4] have recently reported syntheses of blenolide C (**5b**).

We therefore decided to start our synthetic studies by developing a route to the tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety **10** that could then be extended to the synthesis of the parnafungins by starting with blenolide C or an analogue. We were guided by the studies of Wierenga[5] that were based on earlier work of Bamberger[6] and Cohen.[7] Wierenga reported that reduction of methyl *o*-nitrobenzoate (**7**) with zinc, NH_4Cl, and Na_2CO_3 in MeOH containing formaldehyde afforded hydroxymethylbenzisoxazolone (**9**) in 44% yield (see Scheme 3). A similar reduction

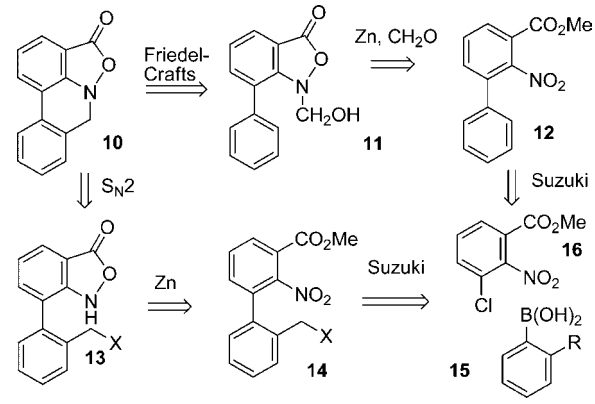
Scheme 3. Wierenga Benzisoxazolone Synthesis



without formaldehyde gave **8** in low yield, which could be converted to **9** by reaction with basic formaldehyde.

Our retrosynthesis of **10** is shown in Scheme 4. An intramolecular Friedel−Crafts alkylation of **11** could give **10**. Hydroxymethylbenzisoxazolone **11** should be accessible

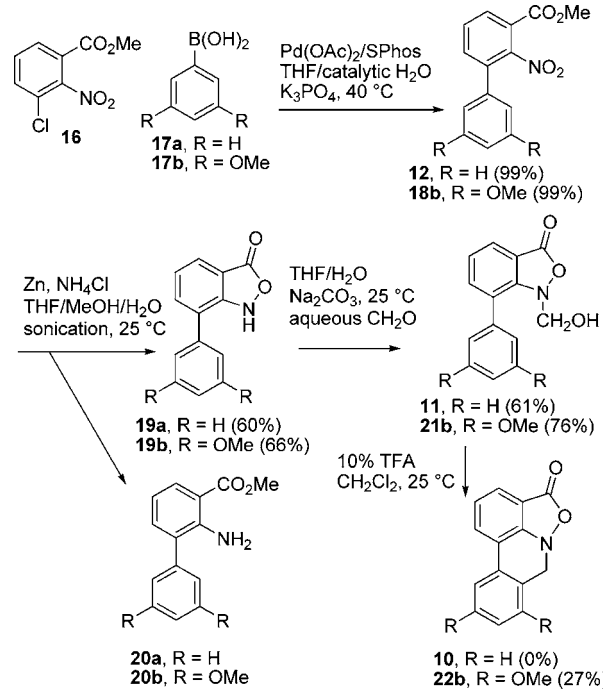
Scheme 4. Retrosynthesis of Tetracycle **10**



by Wierenga's procedure from biphenylcarboxylate **12**, which has been prepared by Liu from methyl 3-chloro-2-nitrobenzoate (**16**) by Suzuki coupling with phenylboronic acid (**15**, R = H).[8] However, the Friedel−Crafts reaction may not work well with an unactivated aromatic ring, and this route differs from the proposed biosynthesis in which the ring is closed by C−N rather than C−C bond formation. We therefore considered an alternate approach in which the C−N bond of **10** is formed from **13** by an intramolecular S_N2 reaction. Suzuki coupling of **16** with the appropriate boronic acid **15**, R = CH_2OH, should provide **14**, which should form **13** by zinc reduction without formaldehyde.

Suzuki coupling of **16** and phenylboronic acid (**17a**) as described by Liu afforded **12** in 65% yield (see Scheme 5).

Scheme 5. Synthesis of Tetracycle **22b**

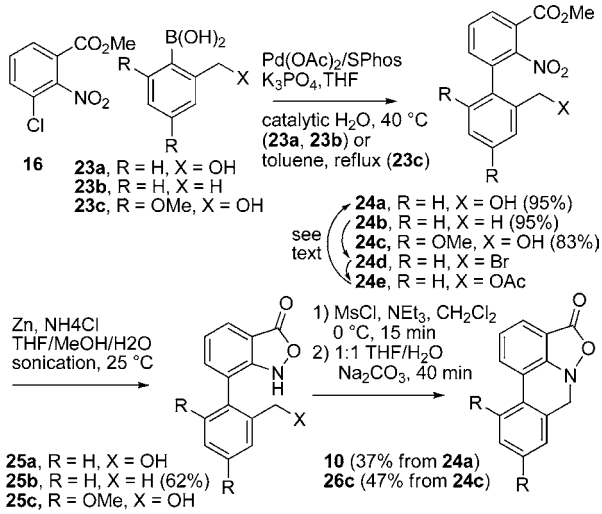


The yield was improved to 99% using SPhos, $Pd(OAc)_2$, and K_3PO_4 in wet THF.[9] Reduction of **12** with Zn and NH_4Cl in THF/MeOH/H_2O with sonication for 30 min afforded a 3:1 mixture of the desired benzisoxazolone **19a** and amino ester **20a**. Benzisoxazolone **19a** decomposed on chromatography but could be obtained in 60% yield by washing the mixture with 9:1 hexanes/Et_2O to remove **20a**. Reaction of **19a** with formaldehyde and Na_2CO_3 in aqueous THF afforded the desired hydroxymethylbenzisoxazolone **11** in 61% yield. This two-step sequence gave pure **11**; impure **11** was obtained in one step by reduction of **12** with Zn and formaldehyde. Unfortunately, all attempts to form **10** by intramolecular Friedel−Crafts alkylation on the phenyl ring of **11** were unsuccessful.

We then turned to a more electron-rich aromatic ring to see if the Friedel−Crafts alkylation would work with an optimized substrate. The analogous Suzuki coupling of **16** with 3,5-dimethoxyphenylboronic acid (**17b**) gave **18b** in 99% yield. Zinc reduction gave **19b** in 66% yield, which was treated with formaldehyde to give **21b** in 76% yield. We were delighted to find that treatment of **21b** with 10% TFA in CH_2Cl_2 generated an iminium cation that added to the aromatic ring to give **22b** in 27% yield. Although this route to **22b** provided the first synthesis of an isoxazolo[4,3,2-*de*]phenanthridinone, it is not applicable to the synthesis of the parnafungins because the Friedel−Crafts reaction would have to occur meta to the oxygen substituents and para to the carbonyl group. We therefore turned our attention to the alternate route using C−N bond formation.

The analogous Suzuki coupling of **16** with 2-hydroxymethylphenylboronic acid (**23a**) afforded biphenyl **24a** in 95% yield (see Scheme 6). Zinc reduction afforded a mixture of

Scheme 6. Synthesis of Tetracycles **10** and **26c**



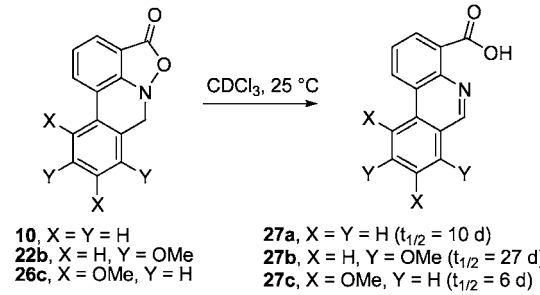
benzisoxazolone **25a** and the amino ester analogous to **20**. Treatment of this mixture with MsCl and Et_3N in CH_2Cl_2 for 15 min at 0 °C afforded the mesylate of **25a**, which was treated with Na_2CO_3 in 1:1 THF/H_2O for 40 min to give the desired tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety **10** in 37% yield from **24a**.

Application of this route to a coupling product of blenolide C (**5b**) would require functionalization of a benzylic methyl group. We therefore prepared **24b** in 95% yield by Suzuki coupling of **16** with *o*-tolylboronic acid (**23b**). Bromination of **24b** with NBS and $(BzO)_2$ in CCl_4 at reflux gave **24d**, R = H, X = Br. Reaction of **24d** with KOAc in DMF afforded **24e**, R = H, X = OAc, which was hydrolyzed with K_2CO_3 in MeOH to give **24a** in 79% overall yield from **24b**. Other approaches were unsuccessful. Reduction of **24d** with Zn gave **25b** resulting from reduction of both the nitro and bromomethyl groups. Functionalization of the methyl group must be carried out before formation of the benzisoxazolone ring. Zn reduction of **24b** gave **25b** in 62% yield, which decomposed on treatment with NBS.

Suzuki coupling of **16** with boronic acid **23c**[10] provided biphenyl **24c** (83%) with the oxygen substituents on the same carbons as in the parnafungins. Zinc reduction gave benzisoxazolone **25c**, which was treated with MsCl and Et_3N to give the mesylate. Treatment of the mesylate with Na_2CO_3 in 1:1 THF/H_2O provided **26c** in 47% overall yield from **24c**. This sequence provides a very short and high yield route to the labile tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety of the parnafungins that should be applicable to the synthesis of the natural product.

The Merck group suggested that the rearrangement of parnafungins **1** and **2** to **3** and **4**, respectively, could occur by either an E2 reaction or by hydrolysis of the isoxazolone followed by loss of water. We explored the stability of **10**, **22b**, and **26c** in $CDCl_3$. All three compounds rearranged cleanly to the respective phenanthridine **27a**,[11] **27b**, and **27c** without any evidence for the formation of an intermediate (see Scheme 7). This indicates either that the reaction

Scheme 7. Rearrangement of **10**, **22b**, and **26c** in $CDCl_3$



proceeds by an E2 elimination or that loss of water is much faster than hydrolysis of the isoxazolone. Tetracycle **26c** with

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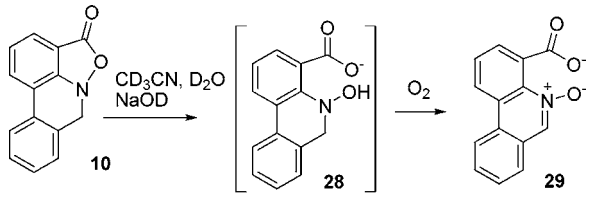
the oxygen substituents in the same position as the parnafungins rearranges fastest with a half-life of 6 days. Tetracycle **10** rearranges with a half-life of 10 days and tetracycle **22b** rearranges slowest with a half-life of 27 days. The C-methyl group of *m*-methoxytoluene is deprotonated twice as fast as that of toluene by lithium amide bases, whereas the C-methyl group of *o*-methoxytoluene is deprotonated 10 times slower than that of toluene.[12] Deprotonation occurs in the rate-determining step of an E2 elimination even if protonation of the carbonyl group by adventitious HCl is the initial step. Therefore, the two methoxy groups meta to the methylene group of **26c** should accelerate deprotonation and E2 elimination, whereas the methoxy groups ortho and para to the methylene group of **22b** should retard deprotonation and elimination. The parnafungins have oxygen substituents meta to the methylene group as in **26c** and a carbonyl group para to the methylene group, which should further increase its acidity and accelerate the E2 elimination.

We also explored the decomposition of **10** under basic condition with NaOD in CD_3CN/D_2O. Under these conditions, we detected an intermediate with the CH_2 group shifted upfield to δ 4.32 from δ 4.72 in **10**. We tentatively assigned structure **28** to this intermediate (see Scheme 8). To our surprise, the final product is not **27a** but a 1:10 mixture of **27a** and the *N*-oxide **29** resulting from oxidation of **28**, presumably by air. The autoxidation of hydroxylamine in aqueous base has been extensively studied[13] and the oxidation of alkylhydroxylamines to oximes in MeOH has been reported.[14] The formation of **29** by this decomposition pathway is noteworthy because we were unable to prepare **29** directly by oxidation of **27a** using procedures that work well on phenanthridine iteself. Both electron-withdrawing groups and peri substituents are known to retard the *N*-oxidation of heterocycles.[15]

Scheme 8. Decomposition of **10** in Base



In conclusion, a practical route to the labile tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety (**10** and **26c**) of the antifungal parnafungins has been developed. Zinc reduction of methyl 2′-hydroxymethyl-2-nitro-3-biphenylcarboxylates **24a** and **24c**, which were prepared by Suzuki couplings, afforded benzisoxazolones **25a** and **25c** that were treated with MsCl and then base to generate the labile tetracyclic ring systems **10** (37%) and **26c** (47%). These compounds rearrange to phenanthridines **27a** and **27c** in $CDCl_3$ and **10** decomposes to phenanthridine *N*-oxide **29** in aqueous base.

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Supporting Information Available: Complete experimental procedures, copies of ^1H 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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