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SYNTHESIS AND EVALUATION OF A DESIGNED INHIBITOR FOR NONACTIN BIOSYNTHESIS IN *S. griseus* ETH A7796

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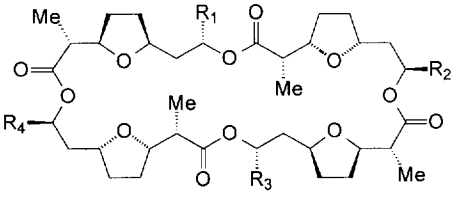
Abstract: A designed suicide inhibitor of a postulated, enzyme-catalyzed step in the biosynthesis of the macrotetrolide antibiotic nonactin (**1**) was synthesized and evaluated in vivo. The inhibitor reduced the rate of nonactin biosynthesis by over 75% while affecting neither biomass production nor the synthesis of other secondary metabolites. The role of the inhibitor in nonactin biosynthesis is discussed.

Introduction

Nonactin (**1**)[1-9] is the parent compound of a group of ionophore antibiotics produced by *S. griseus* ETH A7796 known as the macrotetrolides (Fig. 1). Nonactin has been shown to possess antitumor activity both against mammalian cell lines in vitro and against Crocker Sarcoma 180 in studies in mice.[9] Nonactin was recently shown to be a novel inhibitor of the 170-kDa-P-glycoprotein mediated efflux of 4-*O*'-tetrahydropyranyl-adriamycin in multidrug-resistant erythroleukemia K562 cells at sub-toxic concentrations.[10] The natural macrotetrolide homologues show a wide range of potency. For example, the minimum inhibitory concentration (MIC) of nonactin against *Staphylococcus aureus* and *Mycobacterium bovis* is over an order of magnitude greater than that of dinactin, a difference that is paralleled by the changes in the stability constants of their Na^+ and K^+ complexes.[1,11]



		R1	R2	R3	R4
1	Nonactin	Me	Me	Me	Me
2	Monactin	Me	Me	Et	Me
3	Dinactin	Et	Me	Et	Me
4	Trinactin	Et	Et	Et	Me
5	Tetranactin	Et	Et	Et	Et

Figure 1. The naturally occurring macrotetrolides

Non-natural analogs of the macrotetrolides can be envisaged that may extend the activity trends seen for the natural homologues. Obtaining quantities of such analogs by total synthesis, however, is a difficult undertaking.[12] Separate, enantioselective synthesis of each antipode of the precursor monomer, nonactic acid, is required,[13-18] followed by selective assembly of a tetrameric species, and finally a macrolactonization reaction. By comparison, the producing organism *S. griseus* makes over 1 gL^{-1} of nonactin even in unoptimized fermentation. Our goal is to use precursor directed biosynthesis, in a genetically altered strain of *S. griseus*, so that the native production of macrotetrolides is blocked yet the strain retains the ability to effect the difficult, stereospecific, transformation of chemically synthesized monomer analogs into new macrotetrolides. To this end we have studied the key reaction in the biosynthesis of nonactin, the stereospecific formation of the tetrahydrofuran ring of nonactic acid (**7**, X = OH) by the enzyme nonactate synthase (Fig. 2, **6** to **7**). We report here our efforts to design, synthesize, and evaluate a mechanism-based 'suicide' inhibitor of nonactate synthase which can be used either to chemically block *de novo* macrotetrolide biosynthesis or later in studies of the enzyme itself.

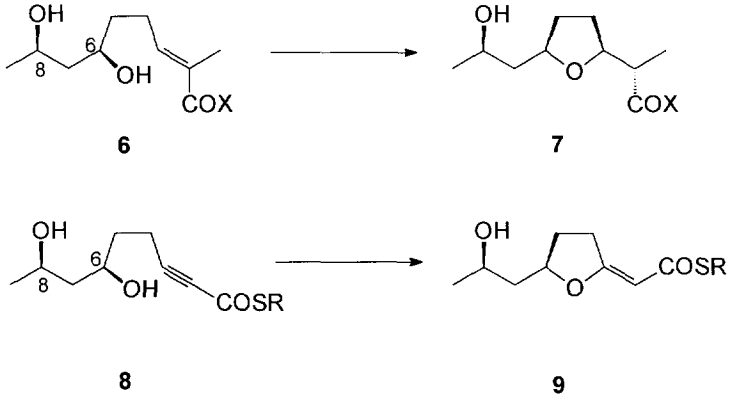
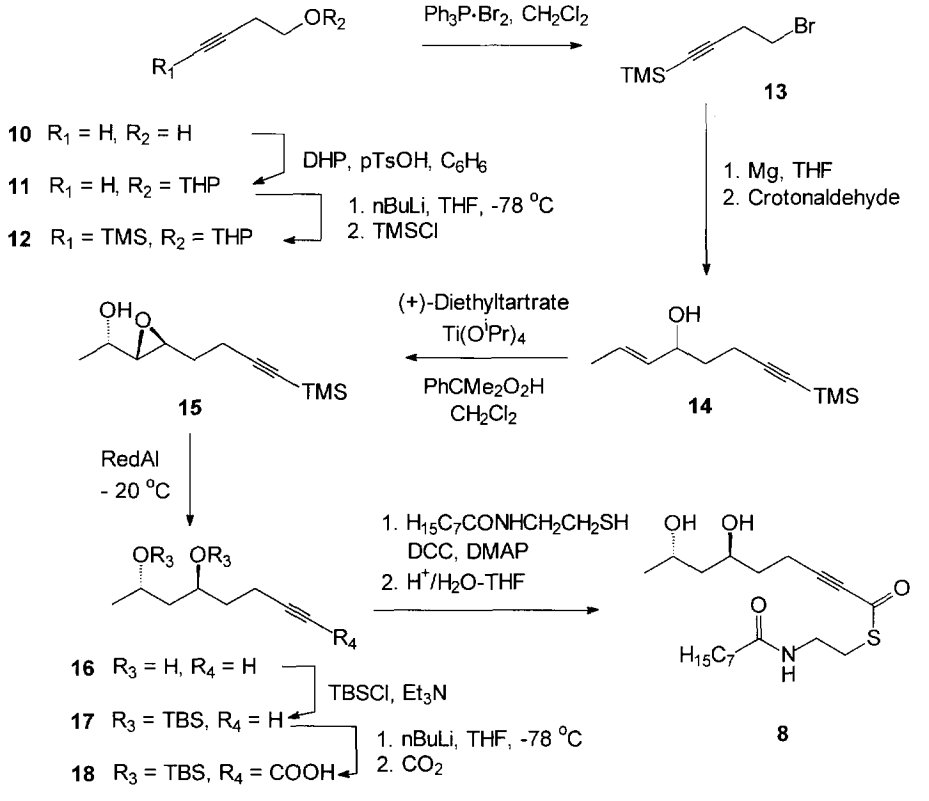


Figure 2. Reaction catalyzed by the nonactate synthase (6 → 7) and its possible inhibition by a 2-alkynoyl analog, 8

Results

The thioester-activated alkynoyl analog **8** (Scheme 1) was chosen as a possible suicide inhibitor of the nonactate synthase based upon the expectation that nucleophilic 1,4-addition of the 6-OH of **8** to the alkynoyl thioester (Fig. 2), in a manner similar to the natural reaction, would produce a reactive intermediate **9** capable of covalently attaching at the active site of the enzyme. The inhibitor was synthesized as outlined in Scheme 1. After protection of the available 4-butyn-1-ol **10** as a THP-ether and silylation of the terminal alkyne to give **12**, the key bromide **13** was obtained by reaction of **12** with Ph_3PBr_2 in 70–80% overall yield from **8**.

Production of the bromide by treatment of a precursor alcohol with CBr_4/PPh_3, as well as other routes to the bromide, were found to give inferior overall yields. Reaction of the Grignard reagent derived from **13** with freshly distilled crotonaldehyde gave the racemic allylic alcohol **14**. Sharpless epoxidation[19,20] of **14**, with subsequent Payne rearrangement of the epoxide upon workup, gave epoxyalcohol **15** in 80% overall yield based on only 50% of the starting material being available for reaction in the kinetic resolution. The enantiomeric purity of **15** was determined by ^1H NMR analysis of a mixture of **15** with the chiral solvating agent (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol and from ^1H NMR analysis of its diastereomeric (2*R*)-2-acetoxy-2-phenylacetate derivative. No signals assigned to the minor diastereoisomer were observed, indicating that **15** had been obtained in greater than 98% ee. Regioselective opening of the epoxide using Red-Al® with concurrent removal of the silyl protecting group gave **16**, which was protected to give alkyne **17** in about 80% yield from **15**. Formation of the carboxylic acid **18**, thioesterification, and deprotection gave the desired inhibitor **8** in approximately 60% yield from **14** (*ca.* 18% yield overall from **10**).



Scheme 1. Synthesis of the acetylenic suicide inhibitor

The effects of the inhibitor on nonactin production in a fermentative culture of *S. griseus* were determined. A fermentative culture of *S. griseus* ETH A7796 was prepared from a standard vegetative liquid culture, split into two equal volumes, and grown on under standard conditions.[21] Small samples of the broth were removed and frozen every 24 h. At 96 h after inoculation the inhibitor (33 mg, in 1 mL EtOH, 450 μM final concentration) was added to the assay culture and a blank sample (1 mL EtOH) added to the control culture. Daily sampling of the cultures was continued for a total of 7 days: the macrotetrolide concentration in the samples was determined by the procedure of Suzuki et al.[22] The control culture developed as expected to a peak of 1 gL^{-1} in nonactin production at 144 h. The nonactin production curve in the inhibited culture substantially lagged behind the control culture with initial rates of nonactin biosynthesis being less than 25% of that observed in the control. The cell mass produced in both the control and inhibited cultures was almost identical. Further, in a zone-diffusion assay on solid culture media, only a marginal inhibition zone was observed for the inhibitor, and then only when milligram quantities of the inhibitor were used. Other secondary metabolites, responsible for the distinctive green color of *S. griseus* extracts, were produced in apparently equal quantities in both the control and assay culture.

Discussion

Spavold and Robinson were able to show that a labeled form of **6** (Fig. 2), activated as its *N*-caprylcysteamine thioester, was efficiently incorporated into nonactin in vivo, indicating that **6** was a probable intermediate in nonactin biosynthesis.[23] We have demonstrated that inhibitor **8**, which is a close analog of biosynthesis intermediate **6**, has a significant effect on nonactin production, lowering initial rates of synthesis by over 75% when present at only 450 μM. The blockade of nonactin production is not due to nonspecific toxicity since **8** shows only marginal effects on cell growth in zone-diffusion assays when used at substantially higher concentrations than those in the fermentative culture. Further, cell mass production and other secondary metabolite synthesis are almost identical in both the control and assay culture. We conclude that the effects of the inhibitor are specific for nonactin biosynthesis. Our result gives further evidence that **6** is a valid intermediate in nonactin biosynthesis.

Although **8** is a close structural analog of **6**, it is conceivable that two other reactions of the postulated nonactin biosynthesis pathway[24,25] may be inhibited by the compound. First, a late β-ketodehydrogenase activity (Fig. 3: **19** to **20**) of the nonactin PKS may be blocked by **8**: 2-alkynoyl thioesters are known mechanism-based inhibitors of acyl-CoA dehdrogenases.[26,27] This would imply that reduction of the 6,8-diketo moiety would occur earlier in the pathway so that the 6,8-diol (**19b**) was present in this substrate.

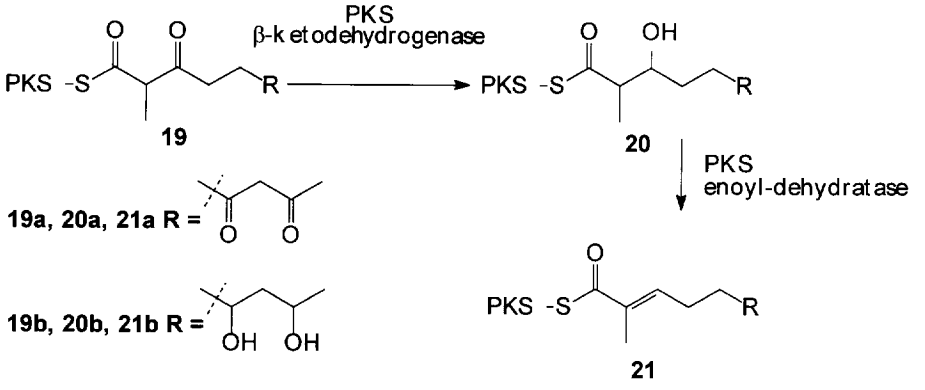


Figure 3. Alternate sites for inhibitor action

Second, a late enoyl-dehydratase activity of the nonactin polyketide synthase (PKS) may be inhibited (Fig. 3: **20** to **21**): conversion of **6** into **7** is highly analogous to enoyl-CoA hydratase catalyzed reactions. Inhibition of the PKS enoyl-dehydratase would imply that the substrate in that dehydratase reaction was the 6,8-diol (**20a**), not the 6,8-diketo-compound (**20b**).

In conclusion, it is most likely that **8** acts as an inhibitor of the nonactate synthase, although exact proof of the site of action of **8** needs to be sought once the nonactate synthase or the nonactin PKS have been obtained in homogeneous form.

References

1. Keller-Schlierlein, W.; Gerlach, H. *Fortschritte d. Chem. Org. Naturstoffe* **1968**, *26*, 161.
2. Corbaz, R.; Ettinger, L.; Gäumann, E.; Keller-Schlierlein, W.; Kradolfer, F.; Kyburz, E.; Neipp, L.; Prelog, V.; Zähner, H. *Helv. Chim. Acta* **1955**, *38*, 1445.
3. Menshikov, G. P.; Rubinsthein, M. M. *J. Gen. Chem. U. S. S. R.* **1956**, *26*, 2267.
4. Dutcher, J. D. *Antimicr. Agents Chemother.* **1961**, 173.
5. Gerlach, H.; Hutter, R.; Keller-Schlierlein, W.; Seibl, J.; Zähner, H. *Helv. Chim. Acta* **1967**, *50*, 1782.
6. Ando, K.; Oishi, H.; Hirano, S.; Okutomi, T.; Suzuki, K.; Okazaki, H.; Sawada, M.; Sagawa, T. *J. Antibiot.* **1971**, *24*, 347.
7. Dobler, M. *Helv. Chim. Acta* **1972**, *55*, 1371.
8. Kilbourne, R. T.; Dunitz, J. D.; Pioda, L. A. R.; Simon, W. *J. Mol. Biol.* **1967**, *30*, 559.
9. Bennett, R. E.; Brindle, S. A.; Giuffre, N. A.; Jackson, P. W.; Kowald, J.; Pansy, F. E.; Perlman, D.; Trejo, W. H. *Antimicr. Agents Chemother.* **1961**, 169.

10. Borrel, M. N.; Pereira, E.; Fiallo, M.; Garnier-Suillerot, A. *Eur. J. Biochem.* **1994**, *223*, 125.
11. Meyers, E.; Pansy, F. E.; Perlman, D.; Smith, D. A.; Weisenborn, F. E. *J. Antibiot.* **1965**, *18*, 128.
12. Lee, J. Y.; B. H. Kim, B. H. *Tetrahedron Lett.* **1995**, *36*, 3361.
13. Bartlett, P. A.; Jernstedt, K. K. *Tetrahedron Lett.* **1980**, *21*, 1607.
14. Kim, B.; Lee, J. Y. *Tetrahedron Lett.* **1992**, *33*, 2557.
15. Kim, B. H.; Lee, J. Y. *Tetrahedron Lett.* **1993**, *34*, 1609.
16. Fleming, I.; Ghosh, S. K., *J. Chem. Soc. Chem. Commun.* **1994**, 2285.
17. Ahmar, M.; Duyck, C.; Fleming, I. *Pure App. Chem.* **1994**, *66*, 2049.
18. Solladie, G.; Domiguez, C. J. *J. Org. Chem.* **1994**, 59, 3898.
19. Katsuki, T; Martin, V. S. *Org. Reactions* **1996**, 48, 1.
20. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
21. Priestley, N. D. Ph.D. Thesis, University of Southampton, 1991.
22. Suzuki, K; Nawata, Y.; Ando K. *J. Antibiot.* **1971**, *24*, 675.
23. Spavold, Z. M.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 4.
24. Ashworth, D. M.; Robinson, J. A.; Turner, D. L. *J. Chem. Soc., Perkin Trans. I* **1988**, 1719.
25. Ashworth, D. M.; Clark, C. A.; Robinson, J. A. *J. Chem. Soc., Perkin Trans. I* **1989**, 1461.
26. Freund, K; Mizzer, W. D.; Thorpe, C. *Biochem.* **1985**, *24*, 5996.
27. Powell, P; Thorpe, C. *Biochem.* **1988**, *27*, 8022.

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