Bioorganic & Medicinal Chemistry Letters 20 (2010) 5936–5938

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Natural product derivatives with bactericidal activity against Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*

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ARTICLE INFO

Article history:
Received 1 March 2010
Revised 23 June 2010
Accepted 29 June 2010
Available online 7 July 2010

Keywords:
Antibiotic
Biosynthesis
Nonactin
Triazoloester

ABSTRACT

We have shown that the intentional engineering of a natural product biosynthesis pathway is a useful way to generate stereochemically complex scaffolds for use in the generation of combinatorial libraries that capture the structural features of both natural products and synthetic compounds. Analysis of a prototype library based upon nonactic acid lead to the discovery of triazole-containing nonactic acid analogs, a new structural class of antibiotic that exhibits bactericidal activity against drug resistant, Gram-positive pathogens including *Staphylococcus aureus* and *Enterococcus faecalis*.

Antibiotic resistance among common bacterial pathogens is a serious public health problem as it compromises our ability to treat infectious disease. The occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE) in hospitals has risen markedly.[1,2] Unfortunately, the great need for new antibacterial agents is contrasted by the low numbers of new antibiotics that have been recently approved for use.

The majorities of antibacterial agents are either natural products, are made from natural products or have structures that are inspired by natural products.[3] The very properties of natural products that make them suited as antibacterial agents (topological and stereochemical complexity)[4] are the properties that complicate their synthesis, the generation of their structural analogs and their development as useful drugs.

To overcome the inherent discovery and development hurdles in natural product research much work has been done to understand the biosynthesis of antibiotics, particularly those of polyketide[5] and peptide origin.[6–8] Such work has lead to exciting possibilities for the generation of 'non-natural' natural products through combinatorial biosynthesis.[5,6,9] Often, however, the genetic manipulations needed to achieve combinatorial biosynthesis are neither general nor straightforward. Furthermore, the genetic manipulation of antibiotic-producing organisms often results in product titers from fermentative cultures that are below practical levels.

We have been interested for some time in developing a straightforward approach to leverage advances in polyketide biosynthesis for the introduction of complex natural product-derived building blocks into combinatorial libraries.[10] Our aim has been to modify polyketide biosynthesis to make downstream synthetic manipulations easier rather than to generate new compounds directly. We recognized at an early stage that our experience in the biosynthesis of the macrotetrolide antibiotic nonactin would make this an ideal model system.[11] Nonactin is a cyclic tetramer made from both enantiomers of nonactic acid, **3**, arranged in an alternating pattern so that nonactin has S4 symmetry.[12] Nonactic acid is an outstanding natural product scaffold for library development.[10] The C-8 alcohol and C-1 carboxyl groups can be readily diversified with little requirement for protecting group manipulations. The central core of the scaffold is available in both enantiomeric series allowing for the straightforward introduction of stereochemical diversity into a library of compounds.

Nonactin was obtained from an optimized fermentation of *Streptomyces griseus* PB1 as a mixture primarily composed of nonactin **1** and its homologue monactin **2** (Fig. 1). Methanolysis of the mixture, followed by a lipase-mediated separation[13] of the methyl esters of nonactic **3** and homononactic acid **4** gave us enantiomerically enriched **3** (~30% ee). The resolution of nonactic acid on a useful scale was an intractable issue for us until we discovered that *Rhodococcus*

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doi:10.1016/j.bmcl.2010.06.146

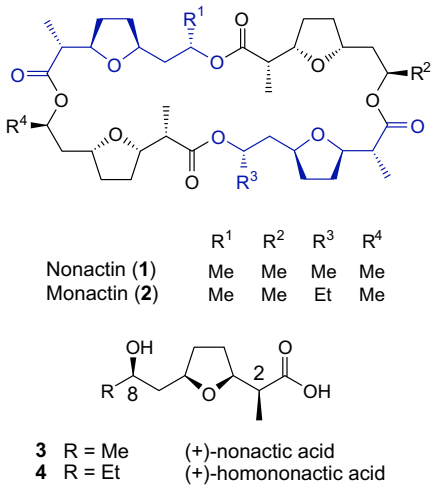
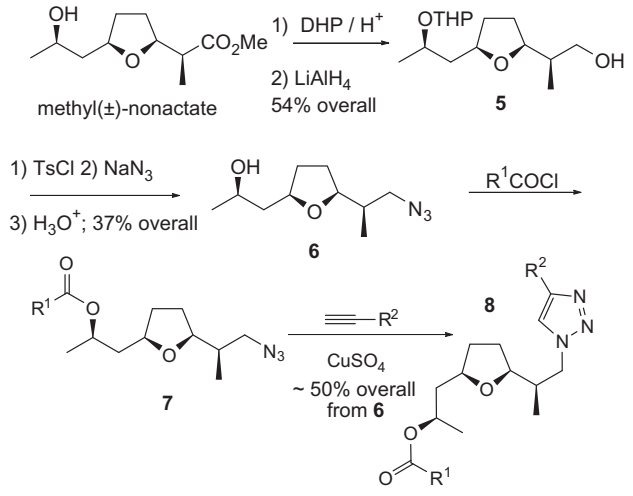


Figure 1. The structures of the naturally occurring macrotetrolides and the monomer, nonactic acid.

erythropolis was an efficient biocatalyst that oxidizes the methyl ester of **3** at C-8 in a highly stereoselective fashion[14] to give us (+)-**3**-OMe directly. Although we are able to obtain (−)-**3** by this approach we have recently developed a far more efficient process. Disruption of the chromosomal copy of the nonactin biosynthesis gene *nonD* (a cocaine esterase homologue) generated a mutant *S. griseus* strain deficient in nonactin production. The mutant, however, does accumulate (−)-nonactic acid (>98% ee) to levels of ~500 mg/L. With the mutant *S. griseus* strain and the *Rhodococcus*-mediated biotransformation we had access to significant quantities of both (+)- and (−)-nonactic acid.

Among the many library designs that we have generated based upon the nonactic acid scaffold, the most straightforward to date has been to employ an esterification-dipolar cycloaddition sequence upon the azidoalcohol **6** (Scheme 1). The latter alcohol is efficiently generated from methyl nonactate via protection of the C-8 alcohol as a THP-ether and reduction of the ester to a primary alcohol **7**. The azide is introduced via tosylation and displacement followed by deprotection to afford the scaffold (**5** to **6**). We prepared both enantiomers of the scaffold **6**. As a proof of principal we generated a small panel of 23 compounds through esterification of **6** followed by the Cu(I)-catalyzed dipolar cycloaddition of a series of terminal alkyne derivatives to the azide, thereby generating the final triazole



Scheme 1. Synthesis of a nonactate-derived compound library.

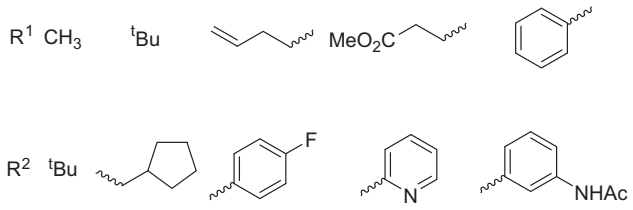


Figure 2. Building blocks used for diversification of the nonactate-derived scaffold **6**.

Table 1
MIC (μg/mL) values against Gram-positive pathogens

	PBP-14E1			PBP-14E5		
	(+)	(−)	Racemate	(+)	(−)	Racemate
B. subtilis	20	>630	40	91	>730	180
B. anthracis	320	>630	630	180	>730	370
S. aureus	320	>630	630	370	>730	370
S. aureus (MRSA)	210	>630	—	122	>730	—
E. faecalis (VRE)	26	>630	—	61	>730	—

8. This small, prototype library combines functional group diversity (ester and triazole substitution (Fig. 2)) with complex stereochemical diversity (nonactic acid core).

It was a natural extension of this work to assay the prototype library for antibiotic activity (Table 1). We were excited to find initial hits, especially given the small size of the prototype library; the most active compounds being PBP-14E1 and PBP-14E5 (Fig. 3). The activity resides in compounds derived from (+)-nonactic acid; the (−)-nonactic acid-derived compounds were inactive. These data show that the stereochemistry of the core scaffold is critical for activity and underscores the importance of exploring stereochemical diversity in compound libraries. To further investigate the antibiotic activity we conducted time-kill assays where a culture of *Bacillus subtilis* was exposed to the test compound and at subsequent times samples were recovered, diluted and plated for counting to differentiate between bacteriostatic and bactericidal effects. Compound PBP-14E5 clearly exerted a bactericidal effect reducing viable bacterial cfu by >3 $\log_{10}$ within 30 min at concentrations as low as 2 × MIC. Again, the bactericidal effect is found solely in compounds derived from (+)-nonactic acid. Since we found that PBP-14E1 and PBP-14E5 were effective against *E. faecalis* and *S. aureus* we conducted further assays against vancomycin-resistant *E. faecalis* (VRE) and methicillin-resistant *S. aureus* (MRSA). Both PBP-14E1 and PBP-14E5 were more active against the important resistant pathogens than their antibiotic susceptible counterparts. The data suggest that the nonactate derivatives likely have a different mechanism of action than vancomycin or β-lactam antibiotics.

In this work we have shown that preparing complex scaffolds from natural products for library generation is a valuable way to approach combinatorial libraries that capture the structural features of both natural products and synthetic compounds. Our targeted biosynthesis manipulation distinguishes this approach from a straight-

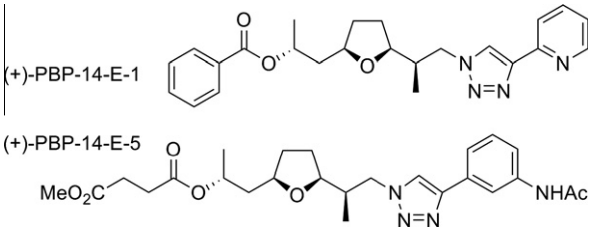


Figure 3. Structures of (+)-PBP-14E1[15] and (+)-PBP-14E5.[16]

forward appropriation of the chiral pool. The generation of larger libraries of these natural product hybrids is now straightforward as the upfront optimization of the biosynthesis pathway provides the complex scaffolds in scale. Access to larger libraries will allow us to improve the antibacterial activity through a more extensive structure search. Furthermore, the discovery that the derivatized nonactate scaffold elicits a clear bactericidal effect can be used for 'reverse chemical genetics' whereby we now have the ability to identify new potential targets in Gram-positive organisms that can be exploited for therapeutic intervention.

Acknowledgments

This work was supported by NIH awards to N.D.P. (CA77347) and D.L.W. (AI070248).

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15. *PBP-14E1*: R_f = 0.19 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (m, 1H), 8.16 (m, 2H), 8.05 (m, 2H), 7.75 (m, 1H), 7.54 (m, 1H), 7.43 (m, 2H), 7.21 (m, 1H), 5.36 (m, 1H), 4.64 (m, 1H), 4.32 (m, 1H), 3.98 (m, 1H), 3.51 (m, 1H), 2.00 (m, 4H), 1.86 (m, 1H), 1.58 (m, 2H), 1.40 (d, J = 6.3 Hz, 3H) and 0.85 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 150.4, 14.3, 148.1. 136.8, 132.7, 130.7, 129.5, 128.3, 122.9, 122.6, 120.2, 80.7, 76.3, 69.6, 53.5, 42.6, 40.4, 31.4, 29.6, 20.8 and 14.3; IR (ATR) 3133, 3065, 2972, 2954, 2935, 2877, 2100, 1736, 1708, 1600, 1593, 1569, 1544, 1472, 1455, 1430, 1420, 1382, 1356, 1319, 1275, 1260, 1235, 1201, 1155, 1108, 1070, 1060, 1031, 844, 790 and 718 cm^{-1}; ESI-TOF MS $[M+H]^+$ calcd for $C_{24}H_{29}N_4O_3$ 421.22, found 421.24.
16. *PBP-14E5*: R_f = 0.38 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.91 (s, 1H), 7.77 (m, 2H), 7.65 (d, J = 7.8 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 5.18 (m, 1H), 4.52 (m, 1H), 4.40 (m, 1H), 3.82 (m, 1H), 3.64 (s, 3H), 3.38 (m, 1H), 2.63 (s, 4H), 2.17 (s, 3H), 1.97 (m, 3H), 1.81 (m, 3H), 1.51 (m, 2H), 1.24 (d, J = 6.3 Hz, 3H) and 0.89 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.0, 168.6, 146.9, 138.6, 131.4, 129.5, 121.4, 121.2, 119.4, 116.6, 80.6, 75.9, 69.4, 53.3, 51.8, 42.4, 40.3, 31.2, 29.8, 29.5, 29.0, 24.5, 20.7 and 14.6; IR (ATR) 3311, 3130, 2974, 2099, 1730, 1693, 1618, 1592, 1569, 1534, 1488, 1438, 1406, 1369, 1316, 1222, 1163, 1045, 997, 884, 847, 792 and 694 cm^{-1}; ESI-TOF MS m/z $[M+H]^+$ calcd for $C_{25}H_{35}N_4O_6$ 487.26, found 487.27.