Published in final edited form as:

Bioorg Med Chem Lett. 2013 October 15; 23(20): 5654–5659. doi:10.1016/j.bmcl.2013.08.026.

Efforts toward broadening the spectrum of arylomycin antibiotic activity

Jian Liu^a, Peter A. Smith^b, Danielle Barrios Steed^a, and Floyd Romesberg^{a,*}

^aDepartment of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 USA

^bRQx Pharmaceuticals, Inc., 11099 North Torrey Pines Road, La Jolla, CA 92037 USA

Abstract

New antibiotics are needed, and one source may be ‘latent’ antibiotics, natural products whose once broad-spectrum activity is currently limited by the evolution of resistance in nature. We have identified a potential class of latent antibiotics, the arylomycins, which are lipopeptides with a C-terminal macrocycle that target signal peptidase and whose spectrum is limited by a resistance-conferring mutation in many bacteria. Herein, we report the synthesis and evaluation of several arylomycin derivatives, and demonstrate that both C-terminal homologation with a glycylaldehyde and addition of a positive charge to the macrocycle increase the activity and spectrum of the arylomycin scaffold.

Keywords

Signal peptidase; Protein secretion; Latent antibiotic; C-terminal electrophile

The ever-growing threat of antibiotic resistant bacteria continues to motivate efforts to discover and develop new antibiotics for clinical use, in particular, those acting via novel mechanisms and possessing a broad-spectrum of activity.^{1,2} Natural product antibiotics benefit from eons of evolution and thus provide ideal scaffolds for optimization, and indeed, the majority of clinically used antibiotics were derived from natural sources.^{3,4} However, new classes of natural product antibiotics with broad-spectrum activity have become difficult to identify,⁵ and the more commonplace, narrow-spectrum compounds are commonly thought to be compromised by intrinsic limitations of their scaffold, such as poor penetrance, or of their target, such as insufficient conservation or essentiality. Thus, it is inferred that these scaffolds never had broad-spectrum activity, and that it would likely be challenging to optimize them to have sufficient spectrum for development. However, some of these scaffolds may be narrow spectrum due to the previous evolution of resistance during the same natural arms race that drove their development. We predict that these formerly more broad-spectrum scaffolds may be more amenable to optimization, just as antibiotics compromised during clinical use have been successfully ‘re-optimized,’ resulting

© 2013 Elsevier Ltd. All rights reserved.

*Corresponding author. Tel.: 1-858-784-7290; fax: 1-858-784-7472; floyd@scripps.edu (F. Romesberg).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary Material

Synthetic procedures and spectroscopic data (¹H, ¹³C, MS, and HRMS).

in the many next-generation variants used in the clinic today. We refer to such natural products as ‘latent’ antibiotics.^{6,7}

We recently reported the synthesis of several members of a novel class of natural product antibiotics, the arylomycins, as well as several derivatives.^{8–10} Discovered as secondary metabolites of a strain of *Streptomyces* in 2002,^{11–13} the arylomycins are five or six residue peptides with a C-terminal hydroxyphenyl glycine (Hpg)-alanine-tyrosine tripeptide macrocycle and an N-terminal aliphatic chain (Fig. 1). *In vitro*, the arylomycins were shown to inhibit type I bacterial signal peptidase (SPase), an essential Ser-Lys dyad protease that is highly conserved, positioned on the outer leaflet of the inner membrane, and essential due to its role in removing the N-terminal leader peptide that targets proteins for secretion via the general secretory pathway.^{14–18} Although only a few strains of bacteria were tested, the arylomycins were deemed to have a narrow spectrum of activity and were not considered to be promising for development. However, after a wider survey of activity, we discovered not only that the arylomycins have potent activity against *Staphylococcus epidermidis*,⁸ but also that this pathogen evolves resistance to the arylomycins by mutation of Ser29 to Pro in the target SPase and that most bacteria that are inherently resistant already possess an SPase with Pro at the analogous position (*i.e.* position 84 in *E. coli*).^{6,19} Moreover, we found that a wide variety of both Gram-positive and Gram-negative bacteria with SPases lacking an analogous Pro are sensitive to the arylomycins, and that mutation of the Pro to Ser is sufficient to render *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* sensitive to the arylomycins. Finally, a broad survey of diverse bacteria revealed that sensitivity is most correlated with the absence of an analogous Pro and not with phylogenetic relatedness. Based on this data, we hypothesized that the arylomycins are latent antibiotics and that their scaffold might be optimized for broad-spectrum activity.

To begin to probe the potential of the arylomycins for optimization, we initially focused on modifications of the lipopeptide tail,⁹ because it is interactions with the lipopeptide tail that are disrupted by the resistance-conferring mutation.⁶ While derivatives were identified that gained activity against *S. aureus*,⁹ a more general strategy for the development of peptidase inhibitors is the introduction of a suitably positioned moiety that mimics the acylation transition state or an electrophilic moiety that forms a covalent bond with the catalytic nucleophile. However, while the C-terminus of the arylomycins binds SPase in a position that is proximal to the catalytic nucleophile, the naturally present carboxylate engages in hydrogen-bonding and ionic interactions^{10,20} that are predicted to be essential for binding,²¹ suggesting that the C-terminus might not be amenable to modification.

In addition to target affinity, target accessibility can limit the spectrum of an antibiotic.²² With Gram-positive bacteria, the cell wall is comprised predominantly of peptidoglycan and negatively polymers such as teichoic acids,^{23,24} and is the only barrier separating SPase from potential inhibitors. While the cell wall is not usually thought to act as a barrier to small molecules (<~50,000 Da),²⁵ several studies suggest that the physicochemical properties of a small molecule can affect its rate of diffusion through the cell wall, and thus it is possible that optimization of an inhibitors physicochemical properties could broaden its Gram-positive spectrum.²⁶ Target access is particularly challenging with Gram-negative bacteria, and although we previously reported that the arylomycins penetrate the Gram-negative outer membrane,⁶ the efficiency of this process is unclear, as is the mechanism. In this work we report the first studies directed at modifying the arylomycin C-terminus to optimize interactions with the catalytic residues of SPase and at exploring the properties affecting penetration and access to SPase in whole cells.

C-terminal derivatization

To begin to explore the potential of C-terminal modifications of the arylomycin scaffold, we derivatized arylomycin A-C₁₆ (Fig. 1), as it has been the most extensively characterized.⁹ We thus synthesized compounds **1** – **6**, where the C-terminal carboxylate is replaced with a phosphonate or aldehyde moiety (Scheme 1 and Supplementary Data). Compounds **1** – **4** were synthesized via coupling of the protected arylomycin A-C₁₆ with the corresponding aminomethylphosphonate (or the benzyl phosphonic ester in the case of compound **1**), followed by deprotection via Pd-catalyzed hydrogenation. Compound **5** was prepared from protected arylomycin A-C₁₆ by coupling with (±)-3-amino propane-1,2-diol, followed by Pd-catalyzed hydrogenation, and NaIO₄ mediated oxidative cleavage. Compound **6** was similarly prepared from the homologated arylomycin derivative, which was synthesized from the protected macrocycle via Arndt-Eistert homologation, followed by global deprotection with BBr₃ and oxidative cleavage with NaIO₄ (Scheme 2).

The biochemical activity (Table 1) of each derivative was evaluated by determining the half maximal inhibitory concentration (IC₅₀) with *E. coli* and *S. aureus* SPases. The IC₅₀ for the parental arylomycin A-C₁₆ is 70 nM with the *E. coli* enzyme and 1800 nM with the *S. aureus* enzyme. Relative to the parent arylomycin, the phosphonic acid **1**, a potential transition state mimic, lost ~10-fold activity against both proteins. Phosphonates, particularly those with electrophilic substituents such as diaryl phosphonates, can potentially react irreversibly with active site nucleophiles; however, all of the phosphonates tested, including diaryl phosphonate **4**, lost virtually all affinity for both proteins. In contrast, compound **5**, which introduces a glycyl aldehyde at a position analogous to that occupied by the scissile bond of natural peptide substrate, was 5-fold more potent than arylomycin A-C₁₆ against the *E. coli* protein and 40-fold more potent against the *S. aureus* protein. This gain in activity is clearly dependent on the specific positioning of the aldehyde, as the homologated variant **6** lost virtually all activity.

Antibacterial activity (Table 2) was measured for each derivative by determining the minimal inhibitory concentration (MIC) against wild type strains of *S. epidermidis* (RP62A), *S. aureus* (methicillin sensitive I (MSSA) strain 8325 and methicillin resistant *S. aureus* (MRSA) strain USA300) and *E. coli* (MG1655), as well as against mutant strains of *S. aureus* 8325 and *E. coli* MG1655 that were rendered sensitive to the arylomycins via mutation of the resistance-conferring SPase Pro residue to Ser (*S. aureus*: *spsB*(P29S)) or Leu (*E. coli*: *lepB*(P84L)). As reported previously, arylomycin A-C₁₆ is potently active against *S. epidermidis*, but not against MSSA, MRSA, or *E. coli*.⁶ Relative to the parent arylomycin A-C₁₆, derivatives **1** – **4** lost activity against all of the strains of bacteria tested, although the loss was somewhat smaller with **1** against *S. epidermidis*, as well as against the sensitized strains of *E. coli* and *P. aeruginosa*. In contrast, derivative **5** retained activity against wild-type *S. epidermidis* (2 µg/mL), both of the sensitized strains (MICs of 8 and 4 µg/mL, respectively), and most significantly, gained activity against MRSA (16 µg/mL, relative to no detectable activity for the parent arylomycin A-C₁₆).

Hpg derivatization

The most interesting result from the C-terminal modified derivatives is the significant increase in biochemical activity of aldehyde of **5**; however, while this translates into increased activity against MRSA, and activity is generally retained against other bacteria, it does not translate into increased activity against the other strains tested. It is possible that the removal of the sole charged group of the arylomycin scaffold affects its physicochemical properties in a way that decreases target access. To test this hypothesis, and also to explore modifications at a second site within the macrocycle, three pairs of compounds with

modifications to the macrocycle core Hpg were synthesized (Schemes 3 – 5 and Supplementary Data). Compounds **7** and **8** were synthesized to examine the effects of an added negative charge; **9** and **10**, to examine the effects of an added positive charge; and **11** and **12**, to examine the effects of an added hydroxyl group. In general, synthesis of these derivatives proceeded via phosphorylation or alkylation of the protected macrocycle, followed by coupling to the lipopeptide using DEPBT, and then global deprotection via Me₃SnOH mediated hydrolysis and Pd/C catalyzed hydrogenation. The synthesis of **7** required C-terminal benzyl (as opposed to methyl) protection to prevent decomposition of the phosphate group during hydrolysis. In the case of **8**, a modified route was employed that involved first installing the C-terminal allylamine, followed by phosphorylation and lipopeptide coupling, and then finally dihydroxylation of the olefin and NaIO₄ mediated cleavage to produce the desired aldehyde. For **10** and **12**, the aldehyde moiety was installed as described above prior to hydrogenation.

The biochemical activity of compounds **7** – **12** was again determined for both the *E. coli* and *S. aureus* SPases (Table 1). Relative to arylomycin A-C₁₆, the derivatives installed on the natural C-terminal carboxylic acid scaffold (**7**, **9**, and **11**) had little effect on binding against either SPase (IC₅₀s within ~2-fold). Similarly, when installed on the glycyl aldehyde backbone, the hydroxy-ethyl moiety of **12** had little effect on the affinity for either SPase. However, the ethyl amine derivative **10** bound to both SPases with 6-fold lower affinity, and the phosphate derivative **8** bound to the *S. aureus* SPase with 4-fold lower affinity and to the *E. coli* SPase with 15-fold lower affinity.

The antibacterial activity of compounds **7** – **12** was determined using the same strains of bacteria described above (Table 2). In addition, to more directly examine the effects on penetration through the Gram-negative outer membrane, we measured activity of arylomycin A-C₁₆ and each derivative against a hyperpermeable strain of *E. coli* harboring an *lptD* mutation, which renders the outer-membrane permeable to large molecules such as vancomycin and rifampin.²⁷ Also, to examine the combined effects of outer-membrane disruption and SPase sensitization, we measured activity of each compound against a strain of *E. coli* that harbors both the *lptD* mutation and the *lepB*(P84L) mutation that confers arylomycin sensitivity. Arylomycin A-C₁₆ shows significant activity against these permeabilized strains (MICs = 16 and 0.02 µg/mL, respectively), clearly demonstrating that the resistance conferring Pro and compound exclusion by the outer-membrane both play significant roles in the low susceptibility of wild-type *E. coli*.

Compounds **7**, **9**, and **11** generally retained activity against the bacteria against which the parent compound is active. The only exception where activity was lost was with compound **7** against sensitized *S. aureus*. An exception where activity was gained was compound **9**, which gained measurable activity against MRSA. In addition, while **9** did not gain activity against either sensitized Gram-negative strain, it gained more activity than the parent compound or any other derivative against both the sensitized and unsensitized strains upon permeabilization. As predicted from the biochemical data, the same modifications installed within the C-terminal aldehyde scaffold of **5** (compounds **8**, **10** and **12**) generally resulted in similar or decreased antibacterial activity.

To examine the optimization of the arylomycin scaffold for broad-spectrum antibacterial activity we pursued two routes. The first route was based on increasing the affinity of the scaffold for its peptidase target via introduction of a phosphonate transition state mimic or an electrophile that might form a covalent bond with the catalytic nucleophile. The second route was based on increasing target access via the introduction of a charged or hydrogen-bonding substituent to the macrocycle. In general, C-terminal homologation with an aminomethylphosphonic acid or ester ablated virtually all affinity of the arylomycin scaffold

for SPase. This suggests that the modifications either eliminate an essential interaction mediated by the free carboxylate, or introduce steric clashes within the binding site. However, homologation with a glycyl aldehyde increased affinity by 5- and 40-fold for the *E. coli* and *S. aureus* SPases, respectively, resulting in low nM inhibition of both enzymes and also imparting the scaffold with antibacterial activity against MRSA.

It is unclear whether the glycyl aldehyde mediates its effects via the formation of a covalent bond with SPase. However, the data suggest that its presence at least induces a rather different mode of binding. This conclusion is drawn from the very different effects on SPase binding of adding a substituent to the Hpg of the natural and glycyl aldehyde homologated scaffolds. The natural scaffold is generally insensitive to the modifications, which is consistent with crystal structure data that show that the Hpg phenolic hydroxyl group is directed away from the protein and into solvent.^{10,28} However, both the phosphate and ethyl amine derivatizations result in significantly reduced affinity for SPase when made within the glycyl aldehyde scaffold. Regardless of the exact mechanism by which the glycyl aldehyde influences SPase binding, it is clear that the free C-terminal carboxylate is not required for binding, and that other C-terminal modifications should be explored.

Among the Hpg modifications examined, the most promising is the ethyl amine of **9**, which despite not significantly altering the affinity of the scaffold for *S. aureus* SPase, bestowed it with modest anti-MRSA activity. This activity may result from the positively charged primary amine introducing ionic interactions with the negatively charged teichoic acids of the Gram-positive cell wall and thus increasing the effective concentration of the arylomycin proximal to its target. This is also consistent with the loss of anti-*S. aureus* activity observed with the negatively charged phosphate derivative **7** relative to arylomycin A-C₁₆, despite similar affinity for the I SPase. Further exploration of this effect is warranted and should include varying the position of the charge and the addition of multiple positively charged moieties, for example, to the macrocycle and throughout the peptide backbone. Regardless, along with the previously reported lipopeptide modifications that bestow the scaffold with Gram-positive activity, the identification of two macrocycle derivatives with broadened Gram-positive activity (compounds **5** and **9**) is consistent with the hypothesis that the arylomycins are latent antibiotics and are well suited for further optimization of Gram-positive activity.

Arylomycin A-C₁₆, as well as every derivative examined, is significantly more active against *E. coli* harboring the *lepB*(P84L) mutation compared to their isogenic counterpart. Interestingly, other methods of membrane permeabilization, such as moderate concentrations of polymyxin B nonapeptide, have little effect on arylomycin sensitivity,⁶ suggesting the penetrance is complex and bimodal, with relative little penetration across an intact outer-membrane and increased penetrance occurring only after significant disruption of the lipopolysaccharide barrier. Due to the detection limits of assay, the precise fold-changes in sensitivity are not available for all compounds, but in general, the hyperpermeable phenotype results in 25 to 125-fold greater susceptibility. Interestingly, the relative increase in sensitivity upon permeabilization is larger for the derivatives than for arylomycin A-C₁₆ itself. The relative increase is the greatest with compound **9**, and because the biochemical activity and the antibacterial activity against the unpermeabilized strains of arylomycin A-C₁₆ and **9** are similar, the difference appears to result from increased penetration of **9** with the hyperpermeable strain. This again may result from ionic interactions that localize the inhibitor to the cell wall, possibly predisposing it to take advantage of its disruption, and/or the Donnan effect,²⁹⁻³¹ which drives the accumulation of positively charged compounds within the periplasmic space. Regardless of the exact mechanism of arylomycin penetration, the data emphasize that the barriers posed by the

Gram-negative outer-membrane remain a clear challenge for developing arylomycins with potent Gram-negative activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Office of Naval Research (Awards N000140310126 and N000140810478) and the National Institutes of Health (AI081126).

References and notes

1. Hancock RE. Mol. Syst. Biol. 2007; 3:142. [PubMed: 17940532]
2. Fischbach MA, Walsh CT. Science. 2009; 325:1089. [PubMed: 19713519]
3. von Nussbaum F, Brands M, Hinzen B, Weigand S, Habich D. Angew. Chem. Int. Ed. 2006; 45:5072.
4. Newman DJ, Cragg GM. J. Nat. Prod. 2007; 70:461. [PubMed: 17309302]
5. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Nat. Rev. Drug. Discov. 2007; 6:29. [PubMed: 17159923]
6. Smith PA, Roberts TC, Romesberg FE. Chem. Biol. 2010; 17:1223. [PubMed: 21095572]
7. Tan YX, Romesberg FE. MedChemComm. 2012; 3:916.
8. Roberts TC, Smith PA, Cirz RT, Romesberg FE. J. Am. Chem. Soc. 2007; 129:15830. [PubMed: 18052061]
9. Roberts TC, Schallenger MA, Liu J, Smith PA, Romesberg FE. J. Med. Chem. 2011; 54:4954. [PubMed: 21630667]
10. Liu J, Luo C, Smith PA, Chin JK, Page MG, Paetzel M, Romesberg FE. J. Am. Chem. Soc. 2011; 133:17869. [PubMed: 21999324]
11. Holtzel A, Schmid DG, Nicholson GJ, Stevanovic S, Schimana J, Gebhardt K, Fiedler HP, Jung G. J. Antibiot. 2002; 55:571. [PubMed: 12195963]
12. Schimana J, Gebhardt K, Holtzel A, Schmid DG, Sussmuth R, Muller J, Pukall R, Fiedler HP. J. Antibiot. 2002; 55:565. [PubMed: 12195962]
13. Kulanthaivel P, Kreuzman AJ, Strega MA, Belvo MD, Smitka TA, Clemens M, Swartling JR, Minton KL, Zheng F, Angleton EL, Mullen D, Jungheim LN, Klimkowski VJ, Nicas TI, Thompson RC, Peng SB. J. Biol. Chem. 2004; 279:36250. [PubMed: 15173160]
14. Paetzel M, Dalbey RE, Strynadka NC. Pharmacol. Ther. 2000; 87:27. [PubMed: 10924740]
15. Paetzel M, Karla A, Strynadka NC, Dalbey RE. Chem. Rev. 2002; 102:4549. [PubMed: 12475201]
16. Date T. J. Bacteriol. 1983; 154:76. [PubMed: 6339483]
17. Cregg KM, Wilding I, Black MT. J. Bacteriol. 1996; 178:5712. [PubMed: 8824617]
18. Zhang YB, Greenberg B, Lacks SA. Gene. 1997; 194:249. [PubMed: 9272867]
19. Smith PA, Powers ME, Roberts TC, Romesberg FE. Antimicrob. Agents Chemother. 2011; 55:1130. [PubMed: 21189343]
20. Paetzel M, Goodall JJ, Kania M, Dalbey RE, Page MGP. J. Biol. Chem. 2004; 279:30781. [PubMed: 15136583]
21. Li T, Froeyen M, Herdewijn P. J. Mol. Graph. Model. 2008; 26:813. [PubMed: 17532654]
22. Poole K. J. Antimicrob. Chemother. 2005; 56:20. [PubMed: 15914491]
23. Weidenmaier C, Peschel A. Nat. Rev. Microbiol. 2008; 6:276. [PubMed: 18327271]
24. Swoboda JG, Campbell J, Meredith TC, Walker S. Chembiochem. 2010; 11:35. [PubMed: 19899094]
25. Demchick P, Koch AL. J. Bacteriol. 1996; 178:768. [PubMed: 8550511]

26. Herbert S, Bera A, Nerz C, Kraus D, Peschel A, Goerke C, Meehl M, Cheung A, Gotz F. PLoS Pathog. 2007; 3:e102. [PubMed: 17676995]
27. Sampson BA, Misra R, Benson SA. Genetics. 1989; 122:491. [PubMed: 2547691]
28. Luo C, Roussel P, Dreier J, Page MG, Paetzel M. Biochemistry. 2009; 48:8976. [PubMed: 19655811]
29. Donnan FG, Harris AB. J. Chem. Soc. Trans. 1911; 99:1554.
30. Tanford, C. Physical Chemistry of Macromolecules. New York: Wiley; 1961.
31. Bolton GR, Boesch AW, Basha J, Lacasse DP, Kelley BD, Acharya H. Biotechnol. Prog. 2011; 27:140. [PubMed: 21312362]

Arylomycin A: $R^1 = R^2 = R^3 = R^4 = H$

$$R^5 = iso-C_{10,11,13}, n-C_{11}, anteiso-C_{12}$$

Arylomycin B: $R^1 = \text{NO}_2$, $R^2 = R^3 = R^4 = \text{H}$

$$R^5 = n\text{-}C_{11}, \textit{anteiso}\text{-}C_{12,14}, \textit{iso}\text{-}C_{10-13}$$

Arylomycin C: $R^1 = R^2 = H$, $R^3 = \text{deoxy-}\alpha\text{-mannose}$
(Lipoglycopeptides) $R^4 = H, OH$, $R^5 = \text{iso-C}_{13-15}$, $n\text{-C}_{13-15}$

Arylomycin D: R^3 = deoxy- α -mannose
(actinocarbasin)

Figure 1.
The arylomycin family of natural product antibiotics

Scheme 1.

(a) (i) 20% TFA/DCM; (ii) R'OH, DEPBT, NaHCO₃, THF; (iii) Me₃SnOH, DCE, 80 °C; (b) (i) (1) For **1** and **4**, HOBt, EDC, TEA, H₂NCH₂PO(OR¹) (**1**: R¹ = Bn; **4**: R¹ = Ph), DMF; (2) For **2** and **3**, DEPBT, NaHCO₃, H₂NCH₂PO(OR¹) (**2**: R¹ = Me; **3**: R¹ = Et), THF. (ii) 10% Pd/C, H₂, AcOH/MeOH; (c) (i) ¹BuOCOCl, NMM, 3-aminopropane-1,2-diol, THF; (ii) Pd/C, H₂, AcOH/MeOH (iii) NaIO₄, THF/H₂O.

Scheme 2.

(a) (i) LiOH(aq.), THF, rt; (ii) t -BuOCOCl, NMM, CH_2N_2 , THF; (iii) CF_3CO_2Ag , MeOH, rt;
 (b) (i) 20% TFA/DCM; (ii) $R'OH$, DEPBT, $NaHCO_3$, THF; (iii) Me_3SnOH , DCE, 80 °C; (c)
 (i) t -BuOCOCl, NMM, 3-aminopropane-1,2-diol, THF; (ii) BBr_3 , DCM, -78 °C – rt; (iii)
 $NaIO_4$, THF/ H_2O .

Scheme 3.

(a) CCl_4 , DMAP, DIEA, $(\text{BnO})_2\text{P}(\text{O})\text{H}$, ACN, rt; (b) (i) 15% TFA/DCM, 0 °C; (ii) R'OH, DEPBT, NaHCO_3 , THF, rt; (c) Pd/C, H_2 , $\text{HCO}_2\text{H}/\text{MeOH}$, rt.

Scheme 4.

(a) (i) LiOH (aq), THF; (ii) NMM, i BuOCOCl, allylamine; (b) CCl_4 , DMAP, DIEA, $(BnO)_2P(O)H$, ACN; (c) (i) 15% TFA/DCM, 0 °C; (ii) R'OH, DEPBT, $NaHCO_3$, THF, rt; (d) $K_2OsO_4 \cdot H_2O$, $K_3Fe(CN)_6$, then $NaIO_4$, THF/ H_2O ; (e) Pd/C, H_2 , $HCO_2H/MeOH$, rt.

Scheme 5.

(a) $\text{ICH}_2\text{CH}_2\text{R}^3$, base, acetone, reflux (For **9** and **10**, $\text{R}^3 = \text{NHChz}$, base = Cs_2CO_3 ; For **11** and **12**, $\text{R}^3 = \text{OBn}$, base = K_2CO_3); (b) (i) 20% TFA/DCM; (ii) $\text{R}'\text{OH}$, DEPBT, NaHCO_3 , THF; (iii) Me_3SnOH , DCE, 80°C ; (c) Pd/C, H_2 , $\text{HCO}_2\text{H}/\text{MeOH}$, rt; (d) (i) $i\text{BuOCOC}$ l, *N*-methylmorpholine, THF, 3-aminopropane-1,2-diol, 0°C – rt; (ii) Pd/C, H_2 , AcOH/MeOH ; (iii) NaIO_4 , THF, H_2O .

Table 1Biochemical activity (IC₅₀s (ng/mL)) of arylomycin derivatives against purified SPase.

Cmpd.	<i>E. coli</i>	<i>S. aureus</i>
1	820	16000
2	20000	>20000
3	>20000	>20000
4	>20000	>20000
5	15	49
6	950	>20000
7	140	1900
8	230	180
9	51	3200
10	84	320
11	70	3900
12	9	61

Table 2

Antibacterial activity (MICs (μg/mL)) of arylomycin derivatives.

Cmpd.	<i>S. epidermidis</i>	<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>S. aureus</i> (<i>spsB</i> (P29S))	<i>E. coli</i> (<i>lepB</i> (P84L))	<i>E. coli</i> (<i>lptD</i>)	<i>E. coli</i> (<i>lptD lepB</i> (P84L))
A-C ₁₆ ^a	0.25	>64	>64	>64	4	0.5	16	0.02
1	8	>64	64	>64	>64	4	>64	0.1
2	8	>64	>64	>64	>64	64	>64	2
3	8	>64	>64	>64	>64	>64	>64	2
4	>64	>64	>64	>64	>64	>64	>64	8
5	2	>64	16	>64	8	4	>64	0.06
6	32	>64	>64	>64	>64	>64	>64	2
7	0.3	>64	>64	>64	>64	1	32	0.03
8	1	>64	>64	>64	32	16	64	0.3
9	0.1	>64	32	>64	4	0.5	4	0.004
10	1	>64	>64	>64	32	2	>64	0.06
11	1	>64	>64	>64	8	1	16	0.02
12	2	>64	64	>64	8	2	16	0.03

^a Arylomycin A-C₁₆, included for reference.