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Synthesis and Biological Characterization of Arylomycin B Antibiotics

Tucker C. Roberts, **Peter A. Smith**, and **Floyd E. Romesberg***
Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Abstract

Antibiotics are virtually always isolated as families of related compounds, but the evolutionary forces underlying the observed diversity are generally poorly understood, and it is not even clear whether they are all expected to be biologically active. The arylomycin class of antibiotics is comprised of three related families that are differentiated by nitration, glycosylation, and hydroxylation of a conserved core scaffold. Previously, we reported the total synthesis of an A series member, arylomycin A_2, as well as the A series derivative arylomycin C_{16}, and showed that both are active against a broader spectrum of bacteria then previously appreciated. We now report the total synthesis of a B series analogue, arylomycin B–C_{16}, and its aromatic amine derivative. While the aromatic amine loses activity against all bacteria tested, the B series compound shows activities that are similar to the A series compounds, except that it also gains activity against the important pathogen *Streptococcus agalactiae*.

Bacteria produce a large assortment of compounds that kill other bacteria, possibly to gain advantage over competing microorganisms for limited resources.[1–7] Most if not all of these antibiotics are produced as families of related compounds; however the biological relevance of this diversity is not well understood.[8] It has been suggested that many of the related compounds might have important biological functions, and thus that their presence is a result of selection.[9,10] Conversely, it has also been argued that the diversity results from the action of enzymes with broad substrate tolerance functioning in non-specific biosynthetic pathways or from selection for diversity itself.[8,11,12] Characterizing the biological activity of the related compounds, as well as intermediates within their biosynthetic pathways, is expected to provide insight into how and why antibiotics evolved. From a chemical perspective, nitro substitution is a particularly interesting modification, especially at aromatic positions, due to the large effects expected on the physiochemical properties of the compounds.[13,14] While nitro substituents are generally rare among natural products,[15] they are relatively more common among antibiotics,[13,16,17] such as chloramphenicol and pyrrolnitrin, which both have aromatic nitro groups that are thought to be biosynthesized from the corresponding aromatic amines.[14,18–20]

The arylomycin family of antibiotics is comprised of three related series of compounds, each possessing a conserved core lipohexapeptide containing a C-terminal tripeptide macrocycle and a variable N-terminal fatty acid.[21,22] The A series compounds have an unmodified core, the B series compounds are nitrated (Figure 1), and the lipoglycopeptides are differentiated by glycosylation, and in some cases hydroxylation, of the core hydroxyphenylglycine. The arylomycins were first isolated in 2002 from a strain of Streptomyces[23] and were shown to

*To whom correspondence should be addressed. floyd@scripps.edu.

Supporting Information Available. ^1H and ^{13}C NMR spectra of compounds **1**, **8**, **10**, **11**, **12** and arylomycin B–C_{16}. This material is available free of charge via the Internet at http://pubs.acs.org.

act via the novel mechanism of inhibiting type I signal peptidase (SPase).[22,24,25] Nonetheless, the development of these compounds as therapeutics was abandoned after it was concluded that they have activity against only a few Gram-positive bacteria,[22,23] and due to poor penetration, no activity against any Gram-negative bacteria.[22] However, after reporting the first synthesis of an arylomycin, the A series member arylomycin A_2 and its derivative arylomycin C_{16} (Figure 1),[26] we recently reported that these A series compounds actually have a remarkably broad spectrum of activity, including potent activity against both Gram-positive and Gram-negative bacteria, but which is limited by the natural presence of a resistance-conferring Pro residue in the signal peptidase of some bacteria (including most bacteria originally tested).[25] We also observed that several bacteria lacking the resistance-conferring Pro, for example, *Streptococcus agalactiae*, are resistant to the arylomycins, suggesting that in some bacteria additional mechanisms of resistance exist.

Because it has been suggested that the arylomycin B series of antibiotics may have a different spectrum of activity,[23] we were interested in the synthesis and evaluation of a B series compound, as any differences in activity might shed more light on potential resistance mechanisms and also further elucidate the potential of the arylomycin scaffold as a therapeutic. Moreover, we envisioned that the synthesis would provide access to the amino derivative **1** (Figure 1), which based on the known biosynthetic pathways of other antibiotics[14,18–20] could represent a biosynthetic precursor to the arylomycin B compounds.

Results and Discussion

The arylomycins are naturally lipidated with fatty acids of varying alkyl chain length (ranging in length from 12 to 16), but because we found previously that the C_{16} tail of arylomycin C_{16} optimizes activity, and because the majority of data available for comparison is with this compound, our initial efforts to synthesize a B series derivative targeted the analogous derivative, denoted arylomycin B–C_{16} (Figure 1). Our synthesis (Scheme 1) drew in part from our previously reported synthesis of arylomycin A_2,[26] as well as the synthesis of Dufour and colleagues,[27] and commenced with construction of the nitrated tyrosine building block **5**. Boc protection of 3-nitro-tyrosine, followed by iodination of the phenol using benzyltrimethylammonium dichloroiodate[28] gave compound **3**, which was transformed to compound **4** in 82% yield over 3 steps. After attempts to transform **4** into the corresponding boronic ester using Miyaura's boration conditions failed, we found that the desired tripeptide **10** could be readily prepared by coupling **5** to dipeptide **9**, which was synthesized from the iodinated N-Me-hydroxyphenylglycine **6**, which was prepared as described by Dufour.[27] The tripeptide was then subjected to Suzuki-Miyaura coupling conditions ($PdCl_2$(dppf)/$NaHCO_3$ in DMF) and Boc deprotected to give compound **11** in 42% yield over 2 steps. Compound **11** was then coupled to the lipotripeptide tail **12** using DEPBT,[29,30] yielding the protected arylomycin analogue in 44% yield.

As reported with arylomycin A_2, treatment of the protected B series analogue with EtSH and 1.0 M $AlBr_3$ in CH_2Br_2 under inert atmosphere at 50 °C resulted in global deprotection. However, in this case, deprotection proceeded concomitantly with reduction of the aromatic nitro group,[31–33] yielding the deprotected, aminated arylomycin **1** as the major product (19% yield). Pleasingly, fully deprotected arylomycin B–C_{16} was obtained in 67% yield after 1.0 M $AlBr_3$/CH_2Br_2 was added to a stirring solution of the protected natural product under air atmosphere at ambient temperature in 10% EtSH/$CHCl_3$.

During the preparation of this manuscript, Dufour and colleagues reported the synthesis of arylomycin B_2,[27] which differs from our approach mainly in the method used for macrocyclization. The resistance of **5** to Miyaura's boration conditions is possibly due to reduced electron density within the aryl iodide bond. As described above, we circumvented

this problem by installing the boronic ester at the MeHpg center, while Dufour *et al.* appear to have elegantly circumvented the same problem by not protecting the phenolic oxygen of the iodo-nitro-tyrosine. Under the conditions of the reaction, the free phenol is deprotonated and perhaps this facilitates oxidative insertion by increasing electron density at the aryl-iodide bond or by chelating boron. However, an advantage to our route is that the Suzuki-Miyaura coupling may proceed with a protected phenol (the phenol groups cannot be protected after installation of the boronic ester[27]), which increases the yield of this critical step with the arylomycin A series compounds, and also eliminates a post-cyclization protection step that might be less attractive for the future synthesis of derivatives.

The biological activity of arylomycin B–C_{16} and its derivative **1** was characterized by determining the minimal inhibitory concentration (MIC) required to inhibit the growth of wild type *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Table 1). In addition, for comparison with the previously reported activity of arylomycin C_{16}, MICs were also determined against isogenic strains of *S. aureus*, *E. coli*, and *P. aeruginosa* that were rendered sensitive to the arylomycins by mutation of the resistance-conferring Pro to a residue that does not confer resistance (P29S in the *S. aureus* protein, and P84L in the *E. coli* and *P. aeruginosa* proteins).[25] We also examined activity against a mutant strain of *S. epidermidis* that was evolved to be resistant to arylomycin C_{16}.[34]

As with the A series derivative arylomycin C_{16}, arylomycin B–C_{16} has potent activity against wild type *S. epidermidis* (Table 1) and no activity against wild type *S. aureus*, *E. coli*, or *P. aeruginosa* (MIC >128 mg/mL). Also like the A series compound, arylomycin B–C_{16} has activity against the mutant strains of *S. aureus*, *E. coli*, and *P. aeruginosa* and significantly less activity against the mutant strain of *S. epidermidis*. In fact, the level of arylomycin B–C_{16} activity against virtually all strains tested is indistinguishable from that of arylomycin C_{16} (Table 1). Thus, we conclude that the activity of the B series compound is limited via the same mechanism that limits the A series compounds, the presence of a resistance-conferring Pro in SPase.[25]

The similar activities observed for the arylomycin A and B series compounds was somewhat surprising given a previous report that the B series derivatives have greater activity against several species of Gram-positive soil bacteria.[23] To generate a better assessment of the relative activities of the A and B series arylomycins, we examined a broad range of bacteria that have been reported to be sensitive to the arylomycins, including *B. brevis*, which was previously reported to be significantly more sensitive to the B series compounds.[23,25] However, we found that the A and B series arylomycins displayed nearly identical activities against almost all of these bacteria as well, suggesting, that in contrast to previous reports, nitration does not generally increase the activity of the arylomycins. Interestingly, under the conditions we employed, neither the A nor B series arylomycins demonstrated appreciable activity against *B. brevis* (MIC >64 μg/ml). To determine whether the disagreement with the literature is a result of a difference in growth conditions, we replicated the conditions of the previous report (0.8% nutrient broth + 0.5% NaCl, grown with aeration by shaking).[23] Although, we found that the arylomycins do display some activity under these conditions (MIC ~ 2 μg/ml), the growth observed in the absence of drug was very poor, and importantly, the activities of the A and B series arylomycins were again indistinguishable. Removing the 0.5% NaCl restored robust growth but also eliminated the arylomycin sensitivities, suggesting that poor growth conditions can predispose some bacteria to arylomycin sensitivity.

Although the nitration of the arylomycin core observed in the B series arylomycin does not generally appear to increase activity, we did find that arylomycin B–C_{16} shows significant

activity against *S. agalactiae*, which stands in sharp contrast to arylomycin C_{16} which has no activity against *S. agalactiae*. Unlike the activity observed against *B. brevis*, the activity of arylomycin B–C_{16} against *S. agalactiae* was independent of the media employed (cation-adjusted Mueller Hinton II Broth or Todd Hewitt Broth). From the perspective of the potential development of the arylomycins as antibiotics, this is significant as *S. agalactiae*, also known as group B streptococcus, is a leading cause of morbidity and mortality among newborns[35,36] and costs the United States alone an estimated $60 million annually.[37] The activity against *S. agalactiae* is also particularly noteworthy because this pathogen is predicted to be sensitive to the arylomycins[25] (the sequenced strain of this species encodes two SPases, neither of which possess the resistance-conferring Pro), but grows in the presence of >128 μg/ml arylomycin C_{16}. Interestingly, the sensitivity of *S. agalactiae* to arylomycin B–C_{16} is very similar to the sensitivity of the related species *S. pneumoniae* and *S. pyogenes* to both the A and B series variants. Thus, within the context of these related organisms, it does not appear that the B series compound gained activity against *S. agalactiae*, but rather that the A series compound lost activity against *S. agalactiae*. An explanation consistent with this data is that *S. agalactiae* possesses other resistance mechanisms that are effective against the A series derivative but not the B series derivative, such as a modifying enzyme, although other factors such as differences in affinity that are specific to *S. agalactiae* or differences in cell wall penetration cannot be ruled out. Regardless, the increased activity of the B series compound against *S. agalactiae* suggests that the arylomycin nitration may have evolved as an adaptation.

In contrast, relative to arylomycin C_{16} and arylomycin B–C_{16}, we found that the amino derivative **1** is significantly less active against all bacteria tested (Table 1), suggesting either that it is not an intermediate during arylomycin B synthesis, or that potent activity was not required for the evolution of the biosynthetic pathway. The loss in activity is slightly larger against the Gram-positive bacteria (32-fold) than against the Gram-negative pathogens (8-fold). This loss in activity is perhaps surprising considering that when bound to SPase, the amino group is expected to be oriented into solvent. A variety of possible causes may underlie this loss in activity. Perhaps the *ortho* amino group induces changes in solvation, either directly by interacting with water molecules or indirectly by hydrogen-bonding with the adjacent hydroxyl group, which disfavors binding. Alternatively, the amino group may stabilize interactions within a different environment where the arylomycin is not active, such as the plasma membrane or micelles.

In conclusion, the synthesis of arylomycin B–C_{16} was achieved via a modification of published protocols[26,27,29] in 9 steps from 3-nitro tyrosine and in 8% overall yield. Generally, the spectrum of antibiotic activity of arylomycin B–C_{16} is limited by the same specific mechanism of resistance as the A series compounds, which is widespread in nature and reduces the practical utility of these compounds as therapeutics. However, just as many clinically used therapeutics have been reoptimized to overcome specific mechanisms of resistance that evolved during their clinical use, it is possible that derivatization of the arylomycin scaffold may be able to re-optimize it for broad-spectrum activity. Indeed, at least regarding *S. agalactiae*, the B series compounds have a broader spectrum of activity than the A series compounds and may represent the natural manifestation of this re-optimization. Whatever the evolutionary history of the arylomycins, the results support the possibility that the spectrum of the arylomycins can be optimized by derivatization. The current and previously reported syntheses of the A and B series arylomycins should provide for ready access to different derivatives, which should allow us to begin testing this hypothesis.

Experimental Section

General Experimental Procedures

Dry solvents were purchased from Acros. Commercially available amino acids were purchased from Bachem (Torrence, CA), Chem-Impex (Wood Dale, IL) or Novabiochem (EMD Chemicals, Gibbstown, NJ). Celite 545 filter aid (not acid washed) was purchased from Fisher. Anhydrous 1-hydroxybenzotriazole (HOBt) was purchased from Chem-Impex. All other chemicals were purchased from Fisher/Acros or Aldrich. Abbreviations: THF, tetrahydrofuran; EtOH, ethanol; MeOH, methanol; AcOH, acetic acid; DCM, dichloromethane; DMF, N,N-dimethylformamide; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate; Hex, hexanes; Ar, argon; TFA, trifluoroacetic acid; BTMA ICl_2, benzyltrimethylammonium dichloroiodate; MeI, iodomethane; DEPBT, 3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm Whatman pre-coated silica gel (with fluorescence indicator) plates. Flash chromatography was performed with silica gel (particle size 40–63 μm, EMD chemicals). ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 500, or Bruker DRX 600 spectrometers. Chemical shifts are reported relative to either chloroform (δ 7.26) or methanol (δ 3.31) for ^{1}H NMR and either chloroform (δ 77.16) or methanol (δ 49.00) for ^{13}C NMR. High resolution time-of-flight mass spectra were measured at the Scripps Center for Mass Spectrometry. ESI mass spectra were measured on either an HP Series 1100 MSD or a PESCIEX API/Plus. For all compounds exhibiting atropisomerism or isolated as semi-pure mixtures, NMR spectra are provided in Supporting Information. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated.

All preparative reverse phase chromatography was performed using Dynamax SD-200 pumps connected to a Dynamax UV-D II detector (monitoring at 220 nm) on a Phenomenex Jupiter C_{18} column (10 μm, 2.12 × 25 cm, 300 Å pore size). All solvents contained 0.1% TFA; Solvent A, H_2O; Solvent B, 90% acetonitrile/10% H_2O. All samples were loaded onto the column at 0% B, and the column was allowed to equilibrate ~10 min before a linear gradient was started. Retention times are reported according to the linear gradient used and the % B at the time the sample eluted.

Synthesis of Compound 4

A solution of 3-nitro-tyrosine (1 g, 4.4 mmol, 1 eq) was dissolved in acetone:H_2O (1:1, 10 mL) and treated with $NaHCO_3$ (554 mg, 1.5 eq) and Boc_2O (946 μL, 1 eq) and allowed to stir overnight. The reaction was acidified with 5% citric acid (pH 3) and extracted 3× with EtOAc, then the combined organic fractions were washed with brine, dried over sodium sulfate and concentrated. The crude material was then iodinated by a modification of a procedure by Kajigaeshi *et al*.[28] The crude material (1.37 g, 4.2 mmol, 1 eq) was taken up in a 5:2 mixture of DCM:MeOH (56 mL), treated with BTMA-ICl_2 (1.6 g, 1.1 eq) and $NaHCO_3$ (2.47 g, 7 eq) and allowed to stir overnight. The solid $NaHCO_3$ was then filtered, and the filtrate was concentrated and acidified with 5% citric acid (pH 3). The aqueous layer was extracted 3× with EtOAc, and the combined organic layers were dried over sodium sulfate and concentrated. The crude material (1.89 g, 4.19 mmol, 1 eq) was dissolved in acetone, treated with K_2CO_3 (2.9 g, 5 eq) and MeI (1.3 mL, 5 eq), and heated to reflux over two days. The reaction mixture was then allowed to cool to room temperature, quenched with a small amount of water, and the volatiles were evaporated. 5% citric acid (pH 3) and EtOAc were added, then separated, and the aqueous layer was extracted 2× with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The crude material was purified via column chromatography (0 – 0.5% MeOH in DCM) to yield compound **4** (1.67 g, 82% yield over 3 steps). ^{1}H NMR ($CDCl_3$, 500

MHz) (ppm) 7.80 (d, *J* = 1.5 Hz, 1H), 7.56 (d, *J* = 1.5 Hz, 1H), 5.12 (d, *J* = 6.5 Hz, 1H), 4.54-4.53 (m, 1H), 3.94 (s, 3H), 3.76 (s, 3H), 3.18 (dd, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H) 2.98 (dd, *J* = 6.5 Hz, *J* = 14.0 Hz, 1H) 1.41 (s, 9H) ^{13}C NMR ($CDCl_3$, 500 MHz) (ppm) 171.5, 155.0, 152.1, 144.9, 143.8, 135.1, 126.4, 94.3, 80.5, 62.8, 54.2, 52.8, 37.0, 28.4. MS (ESI) *m/z* ($M + Na^+$) 503.0. Compound **4** (127 mg, 0.27mmol, 1 eq) was then dissolved in DCM (2.5 mL) and treated with TFA (0.5 mL). When TLC analysis indicated the complete consumption of starting material, the volatiles were evaporated and the residue was dried under vacuum. The residue was then taken up in EtOAc, and saturated $NaHCO_3$, the aqueous layer was extracted 3× with EtOAc, and the combined organic layers were dried over sodium sulfate and concentrated. The resulting compound **5** (101 mg) was used without characterization or further purification.

Synthesis of Compound 7

To a solution of compound **6**[27] (300 mg, 0.74 mmol, 1 eq) in DMF (7.4 mL) was added sequentially H-Ala-OBn HCl (160 mg, 1 eq), EDC (170 mg, 1.2 eq), HOBt (100 mg, 1 eq) and $NaHCO_3$ (71 mg, 1.15 eq), and the reaction was allowed to stir overnight. Dilute $NaHCO_3$ was added and the aqueous phase was extracted 3× with EtOAc. The combined organic layers were washed with 5% citric acid (pH 3), water, and brine, and then dried over sodium sulfate and concentrated. The crude material (353 mg, 0.62 mmol, 1 eq) was taken up in acetone (6.2 mL) and to this solution was added K_2CO_3 (428 mg, 5 eq) and MeI (386 μL, 10 eq). The mixture was allowed to stir overnight at reflux in a sealed vial, then the solvent was evaporated, water was added, and the aqueous phase was extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated. The crude material was purified via column chromatography (0.75% MeOH in DCM) to give the product (189 mg, 44% yield over 2 steps). ^{1}H NMR ($CDCl_3$, 600 MHz) (ppm) 7.80 (s, 1H), 7.37-7.29 (m, 6H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.30 (d, *J* = 7.2 Hz, 1H), 5.74 (br s, 1H), 5.22-5.15 (m, 2H), 4.70-4.66 (m, 1H) 3.87 (s, 3H) 2.70 (s, 3H) 1.48 (s, 9H), 1.44 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 600 MHz) (ppm) 172.6, 169.3, 158.2, 140.4, 135.4, 130.6, 129.3, 128.8, 128.6, 128.4, 114.2, 110.7, 86.2, 80.9, 67.4, 56.6, 55.4, 53.6, 48.5, 31.7, 28.5, 18.3. MS (ESI) *m/z* ($M + Na^+$) 605.1.

Synthesis of Compound 8

To a solution of compound **7** (185 mg, 0.36 mmol, 1 eq) in DMSO (7 mL) under Ar was added sequentially bispinacolatodiboron (95 mg, 1.05 eq), potassium acetate (353 mg, 10 eq) and $PdCl_2$(dppf) (15 mg, 0.05 eq). The mixture was allowed to stir for 2.5 hrs at 80° C, then cooled to room temperature, diluted with water, and extracted 3× with EtOAc. The combined organic layers were washed with brine dried over sodium sulfate and concentrated. The crude material was purified by abbreviated column chromatography (35% EtOAc in Hex) (to minimize the time of exposure to silica) giving compound **8** as a mixture of boronic acid and ester (118 mg, 64% yield). NMR spectra showed two sets of overlapping signals in a 3:1 ratio. ^{1}H NMR ($CDCl_3$, 600 MHz) (ppm) 7.61-7.59 (m, 1H), 7.37-7.31 (m, 5H), 6.81-6.76 (m, 1H), 6.31-6.18 (m, 1H), 5.74 (br, s), 5.20-5.12 (m, 2H), 4.73-4.66 (m, 1H), 3.83-3.80 (m, 3H), 2.68-2.67 (m, 3H) 1.47-1.40 (m, 12H), 1.34-1.33 (m, 9H). MS (ESI) *m/z* ($M + Na^+$) 605.3.

Synthesis of Compound 10

Compound **8** (118 mg, 0.19 mmol, 1 eq) was taken up in 95% EtOH (2 mL), 10% Pd/C (38 mg, 1/3 by weight) was added, and the mixture was placed under an atmosphere of H_2. The reaction was allowed to proceed until TLC analysis indicated the complete consumption of starting material. The mixture was then filtered through Celite and concentrated. To a solution of this crude compound **9** (94 mg, 0.19 mmol, 1 eq) and compound **5** (101 mg, 0.27 mmol, 1.4 eq) in AcCN:DMF (2.2:1, 2mL) was added sequentially HOBt (64 mg, 2.5 eq)

and EDC (80 mg, 2.2eq) and the reaction was allowed to stir overnight. Dilute $NaHCO_{3(aq)}$ was then added to the reaction, and the aqueous phase was extracted 3× with EtOAc. The combined organic layers were washed with 5% citric acid, water and brine, then dried over sodium sulfate and concentrated. The crude material was purified via abbreviated column chromatography (3% MeOH in DCM) to provide **10** as a mixture of boronic acid and ester (130 mg, 80%). MS (ESI) *m/z* ($M + Na^+$) 877.2 (ester).

Synthesis of Compound 11

A solution of compound **10** (118 mg, 0.14 mol, 1 eq) and $NaHCO_3$ (118 mg, 10 eq) in DMF (4.2 mL) was purged several times via cycling with vacuum and Ar and sealed with a crimped septum. To this solution was added, via syringe, a solution of $PdCl_2$(dppf) (23.0 mg, 0.2 eq) in DMF (2.8 mL) that had been sparged with Ar for ~15 minutes. The resulting mixture was submitted to several more cycles of vacuum and Ar, then heated to 80 °C. The mixture was then cooled to room temperature, and water was added. The aqueous phase was extracted with EtOAc 3×, then washed with water and brine, dried over sodium sulfate, and concentrated. The crude material was subjected to abbreviated column chromatography (4% MeOH in DCM) to remove most of the Pd species, then used without further purification. The resulting semi-pure material (83 mg) was taken up in DCM (4.0 mL) and treated with TFA (0.8 mL). The reaction was monitored via TLC, and when starting material was no longer present the volatiles were evaporated under a stream of nitrogen. DCM was added and evaporated under nitrogen twice more and the crude residue was dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$, dried over sodium sulfate, and concentrated. The crude material was purified via pipette column chromatography (9% MeOH in DCM) to give the product (29.7 mg, 42% yield). Multiple species were observed by NMR due to atropisomerism. MS (ESI) *m/z* ($M + H^+$) 501.1.

Synthesis of Compound 12

Compound **12** was synthesized via standard Fmoc/piperidine solid phase peptide synthesis. Fmoc-Gly-OH was loaded onto chlorotrityl chloride resin with DIEA at a loading density of 0.61 mmol/g, then the constituent amino acids, Fmoc-D-Ala-OH and Fmoc-N-Me-D-Ser(OBn)-OH were coupled to the resin using HCTU/DIEA (3 eq:6 eq) in DMF followed by palmitic acid coupling with HCTU/DIEA (3 eq:6 eq) in DMF and enough DCM to completely dissolve the acid. Cleavage from the resin was achieved using 1% TFA in DCM using protocols supplied by Novabiochem. The product was purified via HPLC (linear gradient, 0.66% B per minute, product eluted at 97% B) to give compound **12** (173 mg, 30% yield from after loading of Gly).

Synthesis of Arylomycin B–C_{16}

To a solution of compound **11** (29.2 mg, 58.4 μmol) and compound **12** (50 mg, 1.5 eq) in THF (0.5 mL) at 0° C was added DEPBT (28.0 mg, 1.6 eq) and $NaHCO_3$ (5.0 mg, 1 eq). The reaction was then allowed to warm to room temperature and stirred overnight. The THF was then evaporated under a stream of nitrogen and the reaction was dried under vacuum. The crude reaction mixture was taken up in EtOAc, washed 2× with saturated $NaHCO_3$, then brine, dried over sodium sulfate and concentrated. The crude was purified via column chromatography (3% MeOH in DCM then 4.5% MeOH in DCM) to give the protected arylomycin (14.7 mg, 44%). The protected arylomycin (10.0 mg, 9.4 μmol, 1 eq) was dissolved in $CHCl_3$ (2 mL) treated with ethanethiol (180 μL, 250 eq) and 1.0 M $AlBr_3$ in CH_2Br_2 (189 μL, 20 eq) and stirred in a vial open to air at room temperature for 6 hrs. The reaction was quenched by the addition of MeOH and the volatiles were evaporated under a stream of nitrogen. The crude material was taken up in MeOH and dried twice more to remove any remaining ethanethiol, then it was dissolved in MeOH, centrifuged, and purified via HPLC (linear gradient, 1.0% B per minute, product eluted at 82% B) to give the product

(5.8 mg, 67% yield). Multiple species were observed by NMR due to atropisomerism. ESI HRMS *m/z* [(M + H)$^+$] 926.4873 (calcd for $C_{47}H_{70}N_6O_{11}$ 926.4869).

Synthesis of Compound 1

The protected arylomycin (6.3 mg, 6.0 μmol, 1 eq) was dissolved in ethanethiol (300 μL) and 1.0 M $AlBr_3$ in CH_2Br_2 (120 μL, 20 eq) and stirred in a vial for 5 hrs under Ar at 50 °C. The reaction was quenched by the addition of MeOH and the volatiles were evaporated under a stream of nitrogen. The crude material was taken up in MeOH and dried twice more to remove any lingering ethanethiol, then it was dissolved in MeOH centrifuged and purified via HPLC (linear gradient, 1.0% B per minute, product eluted at 75% B) to give the product (1.0 mg, 19% yield). Multiple species were observed by NMR due to atropisomerism. ESI HRMS *m/z* [(M + H)$^+$] 896.5123 (calcd for $C_{47}H_{70}N_6O_{11}$, 896.5128).

Determination of Antimicrobial Activity

Antimicrobial activity was examined using eighteen bacterial strains, *Staphylococcus epidermidis* RP62A, *Staphylococcus aureus* NCTC 8325, *Escherichia coli* MG1655, *Pseudomonas aeruginosa* PAO1, *Staphylococcus epidermidis* RP62A SpsIB(S29P) (PAS9001), *Staphylococcus epidermidis* RP62A SpsIB(S31P) (PAS9002),[25] *Staphylococcus aureus* NCTC 8325 SpsB(P29S) (PAS8001),[25] *Escherichia coli* MG1655 LepB(P84L) (PAS0260),[25] *Pseudomonas aeruginosa* PAO1 LepB(P84L) (PAS2008),[25] *Brevibacillus brevis* ATCC 8246, *Rhodococcus equi* ATCC 6939, *Rhodococcus opacus* DSM 1069, *Streptococcus agalactiae* COH-1, *Streptococcus pyogenes* M1-5448, *Streptococcus pneumoniae* R800, *Corynebacterium glutamicum* ATCC 44475, and *Lactococcus lactis* ATCC 19257. Minimum Inhibitory Concentrations (MICs) were determined from at least three independent experiments using the CLSI broth microdilution method. Briefly, inocula were prepared by suspending bacteria growing on solid media into the same type of broth used in the MIC experiment and diluting to a final concentration of 1×10^7 colony forming units/ml. 5 μl of this suspension were added to the wells of a 96-well plate containing 100 μl of media with the appropriate concentrations of compound. The MICs of *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *R. equi*, *R. opacus*, *C. glutamicum*, *B. brevis*, were determined in Cation-adjusted Mueller Hinton II broth. MICs of *S. pyogenes*, and *S. pneumoniae* were determined in Todd Hewitt broth. The MICs of *S. agalactiae* were determined in Cation-adjusted Mueller Hinton II broth and in Todd Hewitt broth (MIC values differed by at most two fold between these two media). The MIC of *L. lactis* was determined in Trypticase Soy Yeast broth. To replicate previous measurements of arylomycin A and B series compounds against *B. brevis*, 10^6 cfu of *B. brevis* were inoculated into 14 ml culture tubes containing 1 ml 0.8% nutrient broth supplemented with 0.5% NaCl, the appropriate concentration of compounds, and DMSO at a final concentration of 1%. Cultures were shaken at 28 °C for 24 hours. Identical experiments were also performed using Cation-adjusted Mueller Hinton II broth and using 0.8% nutrient broth without NaCl. In all cases MICs were defined as the lowest concentration of compound to inhibit visible growth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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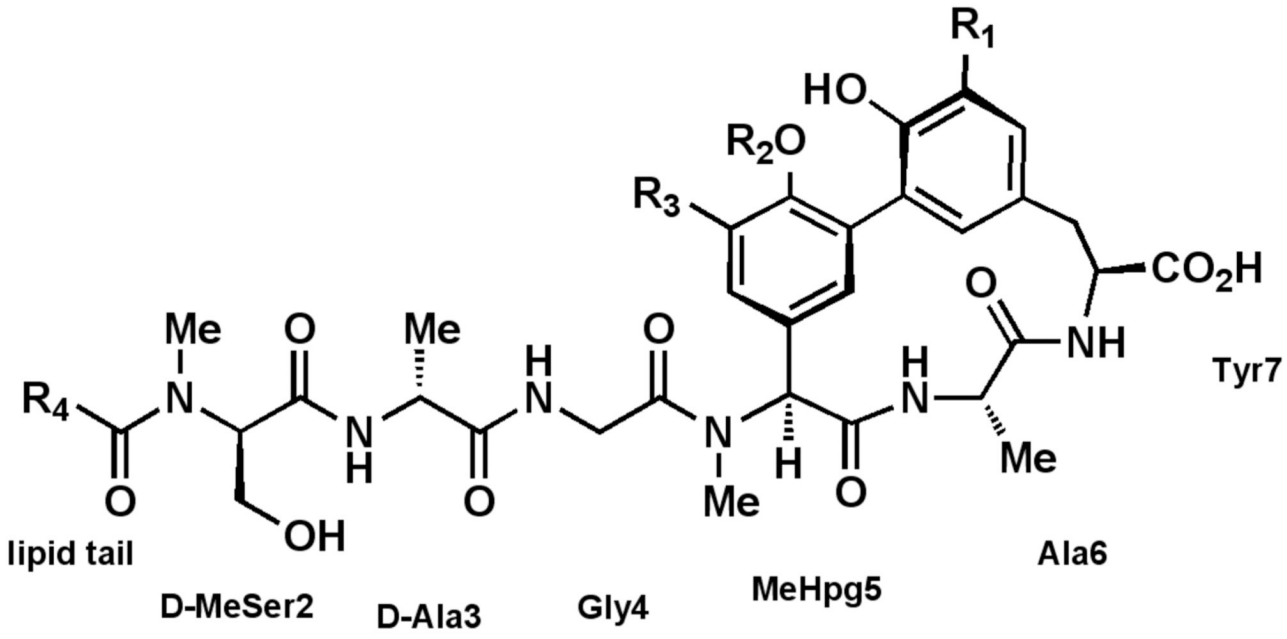
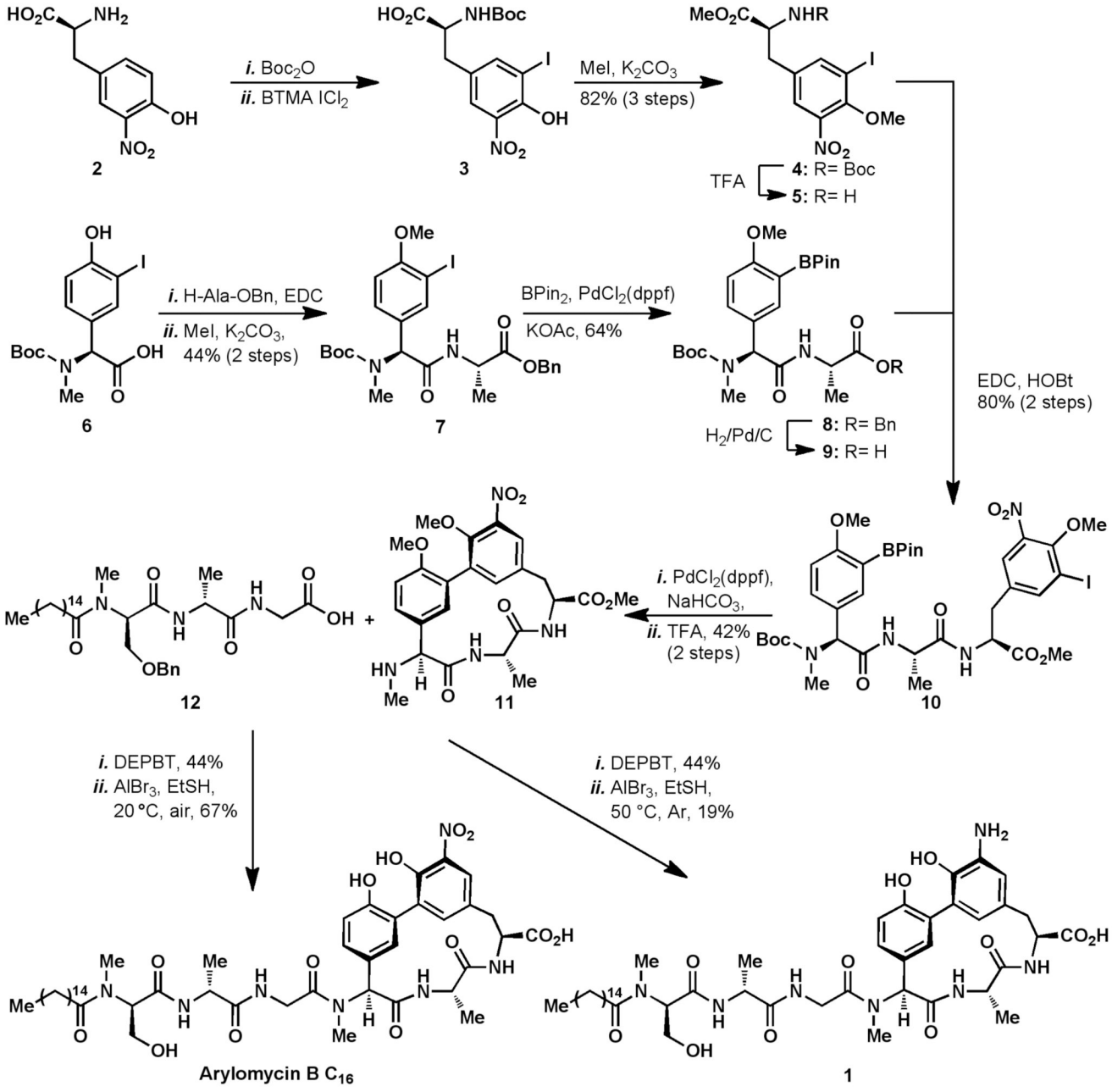


Arylomycin C_{16}: $R_1 = H$, $R_2 = H$, $R_3 = H$, $R_4 = n\text{-}C_{16}$

Arylomycin B-C_{16}: $R_1 = NO_2$, $R_2 = H$, $R_3 = H$, $R_4 = n\text{-}C_{16}$

1: $R_1 = NH_2$, $R_2 = H$, $R_3 = H$, $R_4 = n\text{-}C_{16}$

Figure 1.
Structure of the arylomycin derivatives characterized in this study.



Scheme 1.
Arylomycin B–C_{16} synthesis.

Table 1

MICs of Arylomycin Derivatives (μg/mL)[a]

Strain	Arylomycin C_{16}	Arylomycin B–C_{16}	1
S. epidermidis RP62A	0.25	0.25	8
S. aureus P29S PAS8001	4	4	64
E. coli P84L PAS0260	2	2	16
P. aeruginosa P84L PAS2008	4	4	32
S. epidermidis PAS9002[b]	8	8	nd
B. brevis ATCC 8246	>64	>64	>64
R. equi ATCC 6939	16	32	nd
R. opacus DSM 1069	1	4	nd
S. agalactiae COH-1	>128	8	nd
S. pyogenes M1-5448	8	4	nd
S. pneumoniae R800	8	16	nd
C. glutamicum DSM 44475	2	2	nd
L. lactis ATCC 19257	16	32	nd

[a] nd, not determined.

[b] *S. epidermidis* strain PAS9002 that has been evolved to be resistant to arylomycin C_{16} Ref. 25.