A platform for the discovery of new macrolide antibiotics


Natural products have provided critical starting points for the development of a majority of the antibiotic drugs listed as essential medicines by the World Health Organization. During the period from about 1940 to 1960, sometimes described as the golden age of antibiotics research, academic and industrial laboratories identified the natural products that went on to define many of the major classes of modern antibiotics. Few if any natural products prove to be optimal for safety, efficacy or oral use in humans, however, for these were probably not evolutionary pressures for the microbes in which they developed. For six decades a primary tool by which new antibiotics have been discovered and manufactured is semisynthesis, or the chemical modification of natural products derived from fermentation. Semisynthesis is inherently limited because it is challenging to modify structurally complex natural products selectively, and typically few positions of any given scaffold can be modified effectively. Macrolide antibiotics (macrocyclic lactones with one or more pendant glycosidic residues), which have proven to be safe and effective for use in treating human infectious diseases such as community-acquired bacterial pneumonia, gonorrhoea and others, provide a compelling case in point. Since the discovery of erythromycin from a Philippine soil sample in 1949, in spite of extraordinary efforts leading to fully synthetic and modified biosynthetic routes to macrolide antibiotics, all members of this class approved or in clinical development for use in humans have been manufactured by chemically modifying erythromycin (erythromycin itself is unstable in the stomach, and rearranges to form a product with gastrointestinal side-effects). Thus, azithromycin is prepared from erythromycin in four steps, clarithromycin requires six steps, and the advanced clinical candidate solithromycin is currently produced from erythromycin by a 16-step linear sequence. New macrolides are badly needed, for resistance to approved macrolide antibiotics such as clarithromycin and azithromycin is now widespread in both hospitals and the community. Here we present a practical, fully synthetic platform for the preparation of macrolide antibiotics, providing both a discovery engine for structurally novel antibiotic candidates that would be difficult or impossible to obtain from erythromycin, as well as a basis for their eventual manufacture. Using simple building blocks and a highly convergent assembly process, we have prepared more than 300 structurally diverse macrolide antibiotic candidates, as well as the approved drug telithromycin and the clinical candidate solithromycin. We have identified molecules with diverse macrocyclic scaffolds that exhibit potent activities against bacterial strains resistant to erythromycin, azithromycin and other current antibiotics of different classes.

Synthesis of 14-membered azaketolides

To illustrate our approach to the synthesis of macrolide antibiotics, we begin by detailing a route to new and highly active hybrid antibiotics, 14-membered azaketolides (Fig. 2), and then show how the approach can be easily expanded to subsume many other active macrocyclic scaffolds, with broad latitude for substitutional variation. Ketolides replace the C3-cladinose carbohydrate residue of erythromycin with a carbonyl group, a chemical modification that allows them to evade certain inducible forms of macrolide resistance, whereas the azalide azithromycin incorporates a nitrogen atom within an expanded (15-membered) macrolactone ring, features that have been proposed to impart favourable pharmacological properties, including increased efficacy against certain Gram-negative pathogens. The hybrid ‘azaketolides’, or macrolides that contain both a C3 carbonyl group and a nitrogen atom within the macrolactone ring, have been little explored; one such compound has been reported and was found to be essentially inactive. We were particularly interested in 14-membered azaketolides that would arise from formal replacement of the carbonyl group at position C9 with a nitrogen atom.
Figure 1  |  Summary of macrolide antibiotic development by semisynthesis. To date, all macrolide antibiotics are produced by chemical modification (semisynthesis) of erythromycin, a natural product produced on the ton scale by fermentation. Depicted are erythromycin and the approved semisynthetic macrolide antibiotics clarithromycin, azithromycin, and telithromycin along with the dates of their FDA approval and the number of steps for their synthesis from erythromycin. The previous ketolide clinical candidate cethromycin and the current clinical candidate solithromycin are also depicted. It is evident that increasingly lengthy sequences are being employed in macrolide discovery efforts.

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features of both the azalide antibiotic azithromycin and the ketolide clinical candidate solithromycin (Fig. 3) required modification of just three of the eight building blocks that were used to assemble 14-membered azaketolides (Fig. 2). The left-hand amine intermediate (15) was the same in both 14- and 15-membered azaketolide scaffold syntheses and thus construction of the 15-membered azaketolides simply required access to the homologated right-hand intermediate, aldehyde 35. Here, too, we employed a seven-step sequence (38% yield) featuring three convergent coupling reactions to assemble four simple building blocks. The first coupling, which unites building blocks 26 and 27 to form the tertiary alcohol 29, emerged only after extensive experimentation, in which we discovered that incorporating the large tert-butylidiphenylsilyl protective group within component 27 was key to achieve high diastereoselectivity. After two subsequent functional group interconversions, building blocks 6 (as before) and 28 (the original glycosyl donor of Woodward et al.1) which proved to be effective in this scaffold synthesis) were incorporated by successive convergent coupling reactions. Two linear steps then provided the right-hand
intermediate, aldehyde 35. Reductive coupling of the left-hand amine (15) and the right-hand aldehyde (35) in the presence of sodium cyanoborohydride proceeded without detectable epimerization to afford the macrocyclization precursor 36 in 86% yield. Thermolysis of 36 at 132 °C in chlorobenzene then afforded the 15-membered macrolide 37 in 94% yield on a 1.9-g scale. Incorporation of the final building block (8, as before) and cleavage of the methoxycarbonyl protective group were achieved in a single, final step to provide the azaketolide antibiotic candidate 38. The overall yield of the fully synthetic macrolide 38 (FSM-20707, see below) was 43% from initial building blocks 1 and 2 (a 10-step linear sequence) or 33% from initial building blocks 26 and 27 (also a 10-step linear sequence).

**Synthesis of 14-membered ketolides**

We next adapted our convergent assembly strategy to synthesize 14-membered ketolides, a class that includes the clinical candidate solithromycin as well as the approved drug telithromycin, with the goal of preparing diverse structural analogues. Towards this end we envisioned coupling of the same right-hand intermediate employed in the synthesis of 15-membered azaketolides (aldehyde 35) with a Grignard reagent derived from hydromagnesiation of the acetylenic alcohol 35 in the presence of sodium cyanoborohydride. Towards this end we envisioned coupling of the same right-hand intermediate employed by the addition of 1-lithiopropyne (14) to ketone 39 by the addition of 1-lithiopropyne (39) to ketone 40 in the presence of lithium (1S,2R)-1-phenyl-2-(pyrrolidin-1-yl)-1-propanolate 45. Macrocyclization, as before, then provided the 14-membered azaketolide antibiotic candidate 38.

**Target topology**

**Convergent coupling reactions:**

(aL) LiHMDS, LiCl, 98%; (bL) Et₂N, COCl₂; (cL) NaHMDS; (dL) NaN₃, 88% over 2 steps; (eL) NaN₃, 88% over 2 steps; (fL) NH₃, Ti(O₂CMe)₂, 97%; (gL) Bu₂NF, 92%; (hL) NaN₃, 88% over 2 steps; (iL) NaCNBH₃, 86%; (jL) CuSO₄, sodium l-ascorbate, 93%.

**Modular building blocks:**

8 12 13 14 15

From 1 and 2: 10 steps, 43% yield

From 26 and 27: 10 steps, 33% yield

**Figure 3 | A convergent, fully synthetic route to the 15-membered azaketolide 38.** a. Graphical representation of the convergent synthesis of azaketolide 38 from eight variable building blocks (represented by coloured spheres). Downward, 'Y'-shaped arrows signify convergent coupling reactions. b. Synthesis of azaketolide 38, reagents and conditions (subscripts L and R indicate left and right halves, respectively): (aL) LiHMDS, LiCl, 98%; (bL) Et₂N, COCl₂; (cL) iPr₂N, COCl₂; (dL) NaN₃, 88% over 2 steps; (eL) NaN₃, 88% over 2 steps; (fL) NH₃, Ti(O₂CMe)₂, 97%; (gL) Bu₂NF, 92%; (hL) NaN₃, 88% over 2 steps; (iL) NaCNBH₃, 86%; (jL) CuSO₄, sodium l-ascorbate, 93%.

76% over 2 steps, 92% of recovered (R,R)-pseudoephedrine; (dR) NaHMDS; (eR) NaN₃, 88% over 2 steps; (fR) NH₃, Ti(O₂CMe)₂, NaBH₄, 95%; (gR) Bu₂NF, 92%; (aR) iPr₂N, COCl₂; MeMgBr, 81%; (bR) KH, MeI, 99%; (cR) H₂SO₄, 99%; (dR) ZnCl₂, 91%; (eR) AgOTf, 70%; (fR) HF (aq.); (gR) Dess–Martin periodinane, 87% over 2 steps; (h) NaN₃, 88% over 2 steps; (i) 132 °C, 1 mM, PhCl, 94%; (j) CuSO₄, sodium l-ascorbate, 93%.
macrolactone 46 in 66% yield (1.7-g scale). Fluorination at position C2 proceeded in 85% yield. Transformation of the C12 tertiary alcohol within the latter intermediate (47) to the corresponding acyl imidazolidine (carbonyl diimidazole (Im$_2$CO), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)) and trapping with amine 49 then afforded fully synthetic solithromycin in one operation (87% yield, the methoxycarbonyl protective group was cleaved concomitantly under these conditions).

The present route to solithromycin proceeds in 14 steps and 16% yield from building blocks 26 and 27, and differs from the semisynthetic route (16 steps from erythromycin, yields not reported) in that the linker and its attached side-chain heterocycle are introduced in the final step of our synthesis and thus modification of these residues is quite facile, whereas the linker residue is introduced 6 steps before the penultimate step in the published semisynthetic route to solithromycin.

Figure 4 | A convergent, fully synthetic route to solithromycin.

a. Graphical representation of the convergent synthesis of solithromycin, which was previously only accessible by semisynthesis. This route has been adapted for the synthesis of >30 novel ketolide antibiotic candidates, as well as the FDA-approved ketolide telithromycin. Downward, ‘Y’-shaped arrows signify convergent coupling reactions. b. Synthesis of solithromycin, reagents and conditions (subscripts L and R indicate left and right portions, arrows signify convergent coupling reactions.

From 26 and 27: 14 steps, 16% yield.
We prepared a number of fully synthetic 14-membered ketolides by taking advantage of this feature of our synthesis to simultaneously vary the linker and side-chain heterocycle, and many of these products show potencies that are superior to solithromycin in microbiological assays (see below).

Construction of a library of macrocyclic antibiotics

We prepared an initial library of >300 fully synthetic macrocyclic (FSM) antibiotic candidates by varying the building blocks in concert with modifying readily diversifiable elements (for example, an azido group, an amino group, a β-keto lactone function, an allyl group) that we introduced into and around the macrolide ring, a powerful tactical combination. In addition to members of the three primary macrocyclic scaffolds discussed in detail above, we prepared a number of other unique scaffolds by modifying the principal component fragments (left- and right-halves) and their modes of coupling using straightforward alternative chemical transformations (see Supplementary Information for a complete list of structures synthesized and Extended Data Figs 1–10 for exemplary schemes for their preparation). This strategy not only provided novel scaffolds to explore, but also permitted deep-seated variations of positions within these scaffolds, thus enabling access to molecules that could not be prepared using semisynthetic methods.

Microbiological testing

To evaluate our FSMs for antibiotic activity, we screened 305 of them against a panel of pathogens comprising standardized Gram-positive and Gram-negative strains (see Supplementary Information). For the most promising antibiotic candidates, the panel was expanded to include bacteria with genetically characterized resistance mechanisms to erythromycin as well as other antibiotics. These include three different strains of Staphylococcus aureus, two of them clinically isolated methicillin-resistant (methicillin-resistant Staphylococcus aureus, MRSA) pathogens, a clinically isolated strain of Streptococcus...
pneumoniae with both ribosome-modifying methyltransferase (ermB) and efflux (mefA) genes, and finally a clinically isolated strain of Enterococcus faecalis with an ermB gene, a strain resistant to all approved macrolides as well as vancomycin (vancomycin-resistant Enterococcus, VRE) (Fig. 5). The data show that a majority of compounds in the library exhibit demonstrable antibiotic activity. For example, 83% of compounds in the collection displayed a minimum inhibitory concentration (MIC) ≤ 4 μg ml⁻¹ against wild-type S. pneumoniae, which is known to be highly susceptible to inhibition by macrolides (see Supplementary Information). Among the most promising compounds (Fig. 5) are represented variously 14-, 15- and 16-membered azaketolide scaffolds (for example, FSM-22391, FSM-20707 and FSM-21397, respectively), a 15-membered azachromycin hybrid antibiotic (FSM-20919), and a number of fully synthetic 14-membered ketolides (most notably, FSM-100573 and FSM-100563). The last two substances display superior potencies relative to any macrolide in current clinical use in the following extremely challenging strains: an S. pneumoniae with both ermB and mefA genes (MIC for both FSM-100573 and FSM-100563 ≤ 0.03 μg ml⁻¹), a VRE with an ermB gene (MICs 1 and 2 μg ml⁻¹, respectively), an MRSA with a constitutively-expressed erythromycin ribosome methyltransferase (c-erm) gene (MICs 16 μg ml⁻¹), and Pseudomonas aeruginosa (MICs 16 and 32 μg ml⁻¹, respectively). Clearly, further work will be required to drive potencies in the latter two strains to clinically efficacious levels, but we believe that demonstration of even modest activity in these challenging strains by a macrolide is significant. It is interesting and encouraging to note that FSM-100573 also displays improved Gram-negative activity relative to many other macrolides; further advances in this regard would address an important area of unmet medical need.

Conclusion

Employing as a design strategy the multiply convergent assembly of simple chemical building blocks, we have developed a platform of unprecedented versatility for the discovery and practical synthesis of novel macrolide antibiotics. Varying the building blocks as well as (at a later stage) diversifiable elements incorporated within them permits an almost exponential expansion of variability within any given scaffold. In addition, our work shows that variant scaffolds can be obtained by straightforward perturbations of our generalized assembly process. As with our earlier convergent synthesis of tetracycline antibiotics, we anticipate that many thousands of novel macrolide structures can be prepared for evaluation as potential antibiotics using the present synthetic platform. In light of this, it seems logical to conclude that developing similar convergent routes to other naturally occurring antibiotic families may accelerate the discovery of new therapeutic agents for human infectious diseases.

Online Content

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Atomic coordinates and structure factors for the crystal structure reported have been deposited with the Cambridge Crystallographic Database under accession number CCDC 1440650. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.G.M. (myers@chemistry.harvard.edu).
Extended Data Figure 1 | Synthesis of a C2-fluoro 14-membered azaketolide by a late-stage fluorination reaction. Subjection of β-keto lactone 25 (FSM-22367) to potassium tert-butoxide (1.0 equiv.) at −78 °C followed by N-fluorobenzenesulfonimide (1.0 equiv.) afforded 50 (FSM-22391) in 43% yield.
Extended Data Figure 2 | Synthesis of a 15-membered azaketolide with a modified C2-substituent. Thermolysis of a β-keto tert-butyl ester substrate (55) proceeded at a lower temperature (80 °C) than that for dioxolenone substrates (132 °C) and afforded a 15-membered macrocycle without substitution at C2 (56). (Here and elsewhere we omit mention of the intermediate compounds for clarity.) This macrocycle served as a nearly ideal intermediate for preparation of macrolides with diverse C2-substitutions. For example, an allyl group was introduced at C2 by treatment of 56 with sodium tert-butoxide (1.1 equiv.) and allyl iodide (1.1 equiv.) at −40 °C followed by warming the reaction solution to 23 °C. The product 57 (obtained in 62% yield) was then transformed to 59 (FSM-56156) in two steps (via 58, 72% yield).
Extended Data Figure 3 | Synthesis of a 15-membered azacethromycin hybrid. Macrolactone 63 was prepared from amine 60 and aldehyde 61 in two steps (via 62) by a reductive amination–macrocyclization sequence. Treatment of 63 with paraformaldehyde (6.0 equiv.) and acetic acid (10.0 equiv.) furnished adduct 64 as a crystalline solid (84% yield; X-ray structure shown). The imidazolidine group within 64 served to protect both the secondary amine and the cyclic carbamate functions, and permitted the introduction of a quinoline heterocycle via a Heck reaction. Methanolysis (TFA, CH₃OH) cleaved the imidazolidine group, affording 65 (FSM-20919; 29%, two-step yield).
Extended Data Figure 4 | Synthesis of a 15-membered azaketolide with a modified C10-substituent. N-tert-butylsulfinyl imine 68 (prepared in five steps via 66 and 67 from amide 10) allowed for the stereocontrolled introduction of various C10-substituents. For example, addition of allylmagnesium bromide proceeded with >20:1 stereoselectively to establish the stereocentre at C10; subsequent cleavage of the sulfinyl (HCl, CH3OH) and tert-butyldiphenylsilyl (Bu4NF) groups within the adduct then furnished left-hand intermediate 69 (81% yield). Amine 69 and aldehyde 35 were coupled by a reductive amination reaction (NaBH3CN, 60%–75% yield). The product (70) was then transformed to 73 (FSM-11044) in a three-step sequence that consisted of a macrocyclization reaction (giving 71; 72% yield), a methanolysis reaction (giving 72 in quantitative yield) and lastly a [3 + 2] dipolar cycloaddition reaction (giving 73 in 92% yield).
Extended Data Figure 5 | Synthesis of a 15-membered azaketolide with a modified C13-substituent. Modification of position C13 was achieved by modification of a single component, in this case the ketone building block 74 depicted above. Reductive coupling of 78 and 35 united the left- and right-halves to give 79; subsequent thermal macrocyclization provided macrolactone 80. The allyl group within intermediate 80 was cleaved upon ozonolysis (O3, trifluoroacetic acid); reductive workup with sodium cyanoborohydride afforded alcohol 81. Subjection of 81 to bis(2-methoxyethyl)aminosulfur trifluoride afforded the fluoroethyl-substituted macrocycle 82 (30%, two-step yield), which was transformed to 83 (FSM-11453) by a [3 + 2] dipolar cycloaddition reaction.
Extended Data Figure 6 | Synthesis of a 15-membered azaketolide with a modified desosamine sugar residue. The 15-membered macrolactone 90 was synthesized using thioglycoside 84 and alkyne 89 as building blocks (in lieu of building blocks 28 and 8 used for the synthesis of 15-membered azaketolide FSM-20707). Treatment of 90 with tributyltin hydride (2.0 equiv.), acetic acid (5.0 equiv.), and tetrakis(triphenylphosphine)palladium (2 mol%) led to cleavage of the allyloxyacarbonyl protective group, giving rise to amine 91 (92% yield). The latter intermediate has been transformed into a number of fully synthetic macrolides with modified desosamine sugar residues. For example, acylation of the primary amino group of intermediate 91 with pyridine-2-carbonyl chloride (2.0 equiv.) in the presence of trimethylamine (3.0 equiv.) followed by removal of the tert-butoxycarbonyl group afforded 92 (FSM-21887, 86%, two-step yield).
Extended Data Figure 7 | Synthesis of a 16-membered azaketolide.

Homologation of aldehyde 35 was achieved by a Wittig olefination reaction (CH$_3$OCH$_3$PPh$_3$Cl$^-$, NaHMDS) followed by hydrolysis of the resulting enol ether to afford aldehyde 93 in 65% yield. Reductive coupling of amine 15 and aldehyde 93 furnished macrocyclization precursor 94 (73% yield). The 16-membered macrolactone 95 was obtained in 78% yield upon thermolysis of 94 (1 mM, 132 °C). Two additional steps transformed 95 via 96 to the 16-membered azaketolide 97 (FSM-21397).
Extended Data Figure 8 | Synthesis of a 14-membered macrocycle with a trans-olefin linkage. Mesylate 98 was prepared in quantitative yield by treatment of alcohol 34 with methanesulfonyl chloride (1.50 equiv.) and triethylamine (2.0 equiv.). Displacement of the mesylate group in 98 with sodium 1-phenyl-1H-tetrazole-5-thiolate (2.0 equiv.) followed by oxidation of the resulting thioether with ammonium molybdate (0.20 equiv.)–hydrogen peroxide (100 equiv.) afforded sulfone 100 in 70% yield. Aldehyde 101 and sulfone 100 were coupled in a Julia–Kocienski olefination reaction (NaHMDS, −78 → 23 °C) to provide a 4.8:1 mixture of E- and Z-olefin isomers. The E-isomer 102 was isolated and desilylated (Bu4NF, 79%). Thermolysis of the product 103 (1 mM, 132 °C) furnished the 14-membered macrocycle 104 in 83% yield. 104 was then transformed to 106 (FSM-21079) in two additional steps (methanolyis and [3 + 2] dipolar cycloaddition) via 105.
Extended Data Figure 9 | Synthesis of a 15-membered macrolide with an amide linkage (C9-N9a). Oxidation of aldehyde 35 with sodium chlorite (10.0 equiv.) in the presence of sodium dihydrogen phosphate (10.0 equiv.) and 2-methyl-2-butene (100 equiv.) afforded carboxylic acid 107 in 70% yield. Acid 107 and amine 15 were coupled in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (2.00 equiv.) to provide amide 108. Macrocyclization of 108 (1 mM, 132 °C) proceeded in 81% yield to afford macrolactam 109. Methanolysis and [3 + 2] dipolar cycloaddition then transformed 109 to 111 (FSM-21344) in two steps via 110.
Extended Data Figure 10 | Synthesis of a 15-membered macrolide with an amide linkage (C10-N9a). Amine 112 was prepared in 70% yield by displacement of the mesylate group in 98 with sodium azide followed by reduction of the resulting alkyl azide (H₂, Pd). The coupling of amine 112 and acid 113 proceeded in 54% yield. The product, amide 114, was desilylated (Bu₄NF, 80%) to afford the macrocyclization precursor 115. Thermal macrocyclization of 115 followed by cleavage of the methoxycarbonyl protective group afforded lactam 116 in 80% yield. Copper-catalysed [3 + 2] dipolar cycloaddition then provided 117 (FSM-21473) in 64% yield.