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A Platform for the Discovery of New Macrolide Antibiotics

Ian B. Seiple^{1,*†}, Ziyang Zhang^{1,*}, Pavol Jakubec¹, Audrey Langlois-Mercier^{1,†}, Peter M. Wright^{1,†}, Daniel T. Hog^{1,†}, Kazuo Yabu^{1,†}, Senkara Rao Allu¹, Takehiro Fukuzaki¹, Peter N. Carlsen¹, Yoshiaki Kitamura^{1,†}, Xiang Zhou¹, Matthew L. Condakes^{1,†}, Filip T. Szczypiński^{1,†}, William D. Green^{1,†}, and Andrew G. Myers¹

¹Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, USA

Abstract

The chemical modification of structurally complex fermentation products, a process known as semisynthesis, has been an important tool for the discovery and manufacture of antibiotics for the treatment of various infectious diseases. However, many of the therapeutics obtained in this way are no longer efficacious, as bacterial resistance to many of these compounds has developed. In this manuscript, we describe a practical, fully synthetic route to macrolide antibiotics via the convergent assembly of simple chemical building blocks, with diverse structures not possible by traditional semisynthesis. More than 300 new macrolide antibiotic candidates, as well as the investigational drug solithromycin, were synthesized by our convergent approach. Evaluation of the novel compounds against a panel of pathogenic bacteria revealed many antibiotic candidates,

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Correspondence and requests for materials should be addressed to A.G.M. (myers@chemistry.harvard.edu).

[†]Present addresses: School of Pharmacy, University of California, San Francisco, California 94158, USA (I.B.S.); McKinsey & Company, 55 East 52nd Street, 21st Floor, New York, New York 10022, USA (P.M.W.); Novartis Pharma AG, Chemical and Analytical Development, CH-4002 Basel, Switzerland (A.L.); Bayer Pharma AG, Medicinal Chemistry, Müllerstr. 178, 13353 Berlin, Germany (D.T.H.); Medicinal Chemistry Research Laboratories, Daiichi Sankyo Co., Ltd., Shinagawa R&D Center, 1-2-58 Hiromachi, Shinagawa, Tokyo 140-8710, Japan (K.Y.); Department of Chemistry and Biomolecular Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan (Y.K.); Department of Chemistry, University of California, Berkeley, CA 94720, USA (M.L.C.); Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK (F.T.S.); Trinity College, Cambridge CB2 1TQ, UK (W.D.G.).

*These authors contributed equally to this work.

Author Contributions I.B.S., Z.Z. and A.G.M. identified the targets and designed the synthetic routes; I.B.S. and Z.Z. executed and optimized the synthetic routes described in the main text; I.B.S., Z.Z., P.J., P.M.W., A.L. and D.T.H. executed the synthetic routes shown in the Extended Data; I.B.S., Z.Z., P.J., P.M.W., A.L., D.T.H., K.Y., T.F., P.N.C., X.Z., M.L.C., F.T.S. and W.D.G. synthesized individual macrolide analogs; I.B.S., Z.Z., Y.K. and S.R.A. synthesized and scaled the building blocks. I.B.S., Z.Z. and A.G.M. wrote the paper. All authors discussed the results and commented on the manuscript.

Competing Financial Interests The authors I.B.S., Z.Z., and A.G.M. have filed three provisional patents and an international patent application: U.S. 62/061571, “14-Membered Ketolides and Methods of Their Preparation and Use”; U.S. 62/138198, “Macrolides with Modified Desosamine Sugars and Uses Thereof”; U.S. 62/214774, “Right Half Synthesis of 14-Membered Azaketolides”; PCT/US2014/033025, “Macrolides and Methods of Their Preparation and Use”. A.G.M. declares that he is a founder, board member, and chairman of the scientific advisory board of Macrolide Pharmaceuticals, and Z.Z. and I.B.S. declare that they serve as scientific consultants to Macrolide Pharmaceuticals.

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature.

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with efficacy against strains resistant to macrolides in current use. The chemistry we describe herein provides a platform for the discovery of new macrolide antibiotics and may also serve as a basis for their manufacture.

Introduction

Natural products have provided critical starting points for the development of a majority of the antibacterial drugs listed as essential medicines by the World Health Organization. During the period ~1940–1960, 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 antibiotics in our modern day armamentarium.¹ Few if any natural products prove to be optimal for safety, efficacy, or oral bioavailability in humans, however, for these were likely 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 chemically modifying natural products derived from fermentation. Semisynthesis is inherently limited because it is challenging to selectively modify structurally complex natural products, and typically few positions of any given scaffold can be modified effectively.^{2,3} Macrolide antibiotics (macrocylic 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, gonorrhea, and others, provide a compelling case in point. Since the discovery of erythromycin from a Philippine soil sample in 1949,⁴ in spite of extraordinary efforts leading to fully synthetic^{5–8} and modified biosynthetic routes^{9–12} 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).¹³ Thus, azithromycin is prepared from erythromycin in four steps,¹⁴ clarithromycin requires six steps,¹⁵ and the advanced clinical candidate solithromycin is currently produced from erythromycin by a 16-step linear sequence (Figure 1).^{16,17} 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.^{18–20} 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 macrocylic 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 (Figure 2), and then show how the approach can be easily expanded to subsume many other active

macrolide 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,^{21,22} whereas the azalide azithromycin incorporates a nitrogen atom within an expanded (15-membered) macrolactone ring, features which have been proposed to impart favorable pharmacological properties, including increased efficacy against certain Gram-negative pathogens.^{23,24} 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.²⁵ We were particularly interested in 14-membered azaketolides that would arise from formal replacement of the carbonyl group at position C9 of ketolides with an amino group, a transformation that would not be feasible by semisynthesis.

An overview is helpful as it emphasizes our synthetic strategy. Eight simple building blocks (**1-8**), two commercially available and six synthesized in 3–6-step sequences in 30–150-g amounts, are assembled in a highly convergent manner (Figure 2). As depicted, building blocks **1-3** are transformed by a seven-step sequence featuring two convergent coupling reactions to provide a key left-hand intermediate (**15**, an amine) in 57% yield, while building blocks **4-7** are transformed in a seven-step sequence featuring three convergent coupling reactions to form a key right-hand intermediate (**22**, a ketone) in 58% yield. The left- and right-hand intermediates are then joined in a highly stereoselective and efficient reductive coupling reaction (82% yield, d.r. > 20:1) that also serves to remove the benzoate protective group within the desosamine residue. The next step is the key macrocyclization reaction, for which we employ a method developed by Boeckman *et al.* in the context of their approaches toward the natural products diplodialide A (a 10-membered macrolactone), kromycin (a 14-membered macrolactone), and ikarugamycin (a 17-membered macrolactam).^{26,27} We found that thermolysis of intermediate **23** (1 mM in chlorobenzene) at 132 °C afforded the macrolactone **24** in 78% yield (1-g scale). There are a number of notable features of this transformation, not least its efficacy and, as we will show, generality. Many pioneering efforts to synthesize macrolide antibiotics failed at the stage of macrocyclization; successful macrocyclizations were shown to require carefully designed, rigidifying protective group strategies.²⁸ We believe that the success of the present cyclization (which we note occurs in the presence of a free secondary amine as well as another secondary alcohol) is attributable in part to the rigid structuring induced by the cyclic carbamate function on the left-hand side of the molecule, which we suspect favorably brings the putative acyl ketene function (produced from the dioxolenone function upon thermolysis) and the reactive secondary alcohol into proximity. A single operation then transforms the product of macrocyclization into a fully synthetic antibiotic candidate (**25**) while incorporating the final building block using Sharpless’ optimized conditions for azide-alkyne dipolar cycloaddition²⁹ (**8**, 86% yield). This final step is by design a convergent coupling reaction, and permits optimal late-stage diversification of a key side-chain residue that is known to occupy a novel pocket in the bacterial ribosome.^{30–34}

Aspects of certain steps prior to the macrocyclization reaction merit brief discussion. The key aldol coupling reaction that unites building blocks **1**³⁵ and **2**,³⁶ initiating the synthesis of

the left-half intermediate, involves new methodology that was developed specifically for the present purpose (though it proves to be a general transformation) and proceeds in 98% yield on a 33-g scale to form a single, crystalline diastereomer.³⁶ In a subsequent step, which transforms the pseudoephedrine amide function into a methyl ketone in one operation, the pseudoephedrine auxiliary is recovered by a simple aqueous extraction procedure in 92% yield. The convergent coupling reaction that unites building blocks **4** and **5** to begin the construction of the right-half intermediate is based on a palladium-catalyzed ene reaction developed by Mikami and coworkers, and proceeds in 93% yield and 92% ee.³⁷ Two convergent coupling reactions follow three linear functional group interconversions, the first coupling being a Mukaiyama³⁸ aldol reaction (to incorporate building block **6**³⁹) whose diastereoselectivity was observed to be dependent upon the reaction conditions. The conditions employed here (magnesium bromide etherate as Lewis acid, triethylamine as base, dichloromethane as solvent, and a reaction temperature of -78 °C) provided maximum selectivity (d.r. >20:1, 91% yield) for the desired syn-aldol product. The next convergent coupling reaction introduced the protected desosamine carbohydrate residue and for this purpose we employed the thioglycoside **7**,^{40,41} a donor very similar to that employed by Woodward *et al.* in their synthesis of erythromycin.⁵ In the present case, we found that the use of a benzoyl group to shield the secondary alcohol on the glycosidic donor in lieu of methoxycarbonyl improved the yield and diastereoselectivity of the glycosidation reaction. The overall yield of the fully synthetic azaketolide antibiotic candidate **25** was 32% from initial building blocks **1** and **2** (a 10-step sequence) or 32% from initial building blocks **4** and **5** (also a 10-step sequence).

Synthesis of 15-membered azaketolides

By design, the convergent building block strategy we employ enables rapid access to structurally related scaffolds; for example, synthesis of a 15-membered hybrid azaketolide scaffold comprising structural features of both the azalide antibiotic azithromycin and the ketolide clinical candidate solithromycin (Figure 3) required modification of just three of the eight building blocks that were used to assemble 14-membered azaketolides (Figure 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**⁴² and **27**^{43,44} to form the tertiary alcohol **29**, emerged only after extensive experimentation, in which we discovered that incorporating the large *tert*-butyldiphenylsilyl 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.*,⁵ 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 in Figure 5) 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 analogs. Toward 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 **42**. Intermediate **42** was obtained in one step (14:1 d.r., 85% yield) by the addition of 1-lithiopropyne (**39**) to ketone **40**³⁵ in the presence of lithium (1*S*,2*R*)-1-phenyl-2-(pyrrolidin-1-yl)-1-propanolate.⁴⁵ Hydromagnesiation of **42** with cyclopentylmagnesium bromide and bis-cyclopentadienyltitanium dichloride⁴⁶ proceeded regioselectively to furnish a vinyl Grignard reagent that was captured in situ with aldehyde **35**. The resulting diastereomeric mixture of allylic alcohols (obtained in 80% yield on a 3-g scale) was oxidized directly (Dess-Martin periodinane, 97%) and the α,β -unsaturated ketone produced was desilylated (*n*-Bu₄NF, 95%) to afford the keto alcohol **45**. Macrocyclization, as before, then provided the 14-membered macrolactone **46** in 66% yield (1.7-g scale). Fluorination at position C2 proceeded in 85% yield.⁴⁷ Transformation of the C12 tertiary alcohol within the latter intermediate (**47**) to the corresponding acyl imidazolide (Im₂CO, 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 reported¹⁶) 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 prior to the penultimate step in the published semisynthetic route to solithromycin.¹⁶ 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 (*vide infra*).

Construction of a Library of Macrolide Antibiotics

We prepared an initial library of >300 fully synthetic macrolide (FSM) antibiotic candidates by varying the building blocks in concert with modifying readily diversifiable elements (e.g., an azido group, an amino group, a β -keto lactone function, an allyl group, etc.) 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 coupling

components (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 Figures 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

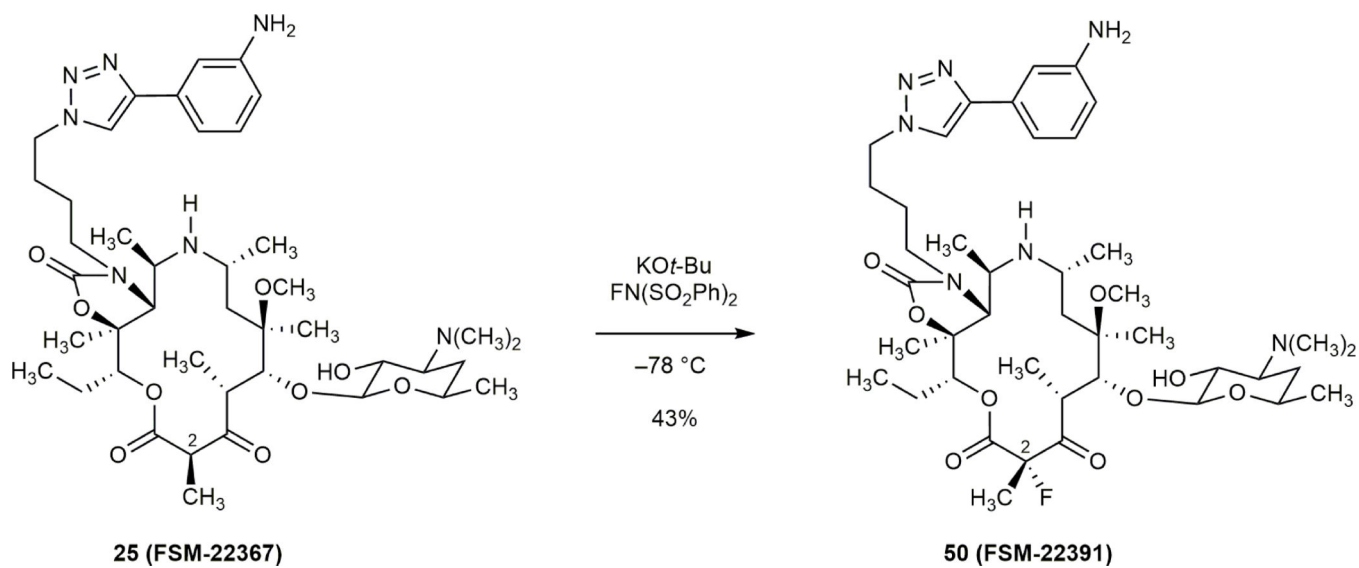
To evaluate our fully synthetic macrolides for antibiotic activity we screened 305 of them against a panel of pathogens comprising standardized Gram-positive and Gram-negative strains (See Supplementary Information). For the most promising antibiotic candidates the panel was expanded to include bacteria with genetically characterized resistance mechanisms to erythromycin as well as other antibiotics. These include 3 different strains of *S. aureus*, two of them clinically isolated methicillin-resistant (MRSA) pathogens, a clinically isolated strain of *S. pneumoniae* with both ribosome-modifying methyltransferase (*ermB*) and efflux (*mefA*) genes, and finally a clinically isolated strain of *E. faecalis* with an *ermB* gene, a strain resistant to all approved macrolides as well as vancomycin (VRE) (Figure 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 an MIC \leq 4 $\mu\text{g/mL}$ against wild-type *S. pneumoniae*, which is known to be highly susceptible to inhibition by macrolides (Supplementary Information). Among the most promising compounds (Figure 5) are represented variously 14-, 15-, and 16-membered azaketolide scaffolds (e.g., FSM-22391, FSM-20707, and FSM-21397, respectively), a 15-membered azacethromycin hybrid antibiotic (FSM-20919), and a number of fully synthetic 14-membered ketolides (most notably, FSM-100573 and FSM-100563). The latter 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's \leq 0.03 $\mu\text{g/mL}$), a VRE with an *ermB* gene (MIC's 1 and 2 $\mu\text{g/mL}$, respectively), a MRSA with a constitutively-expressed erythromycin ribosome methyltransferase (*c-erm*) gene (MIC's 16 $\mu\text{g/mL}$), and *P. aeruginosa* (MIC's 16 and 32 $\mu\text{g/mL}$, 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.⁴⁸

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

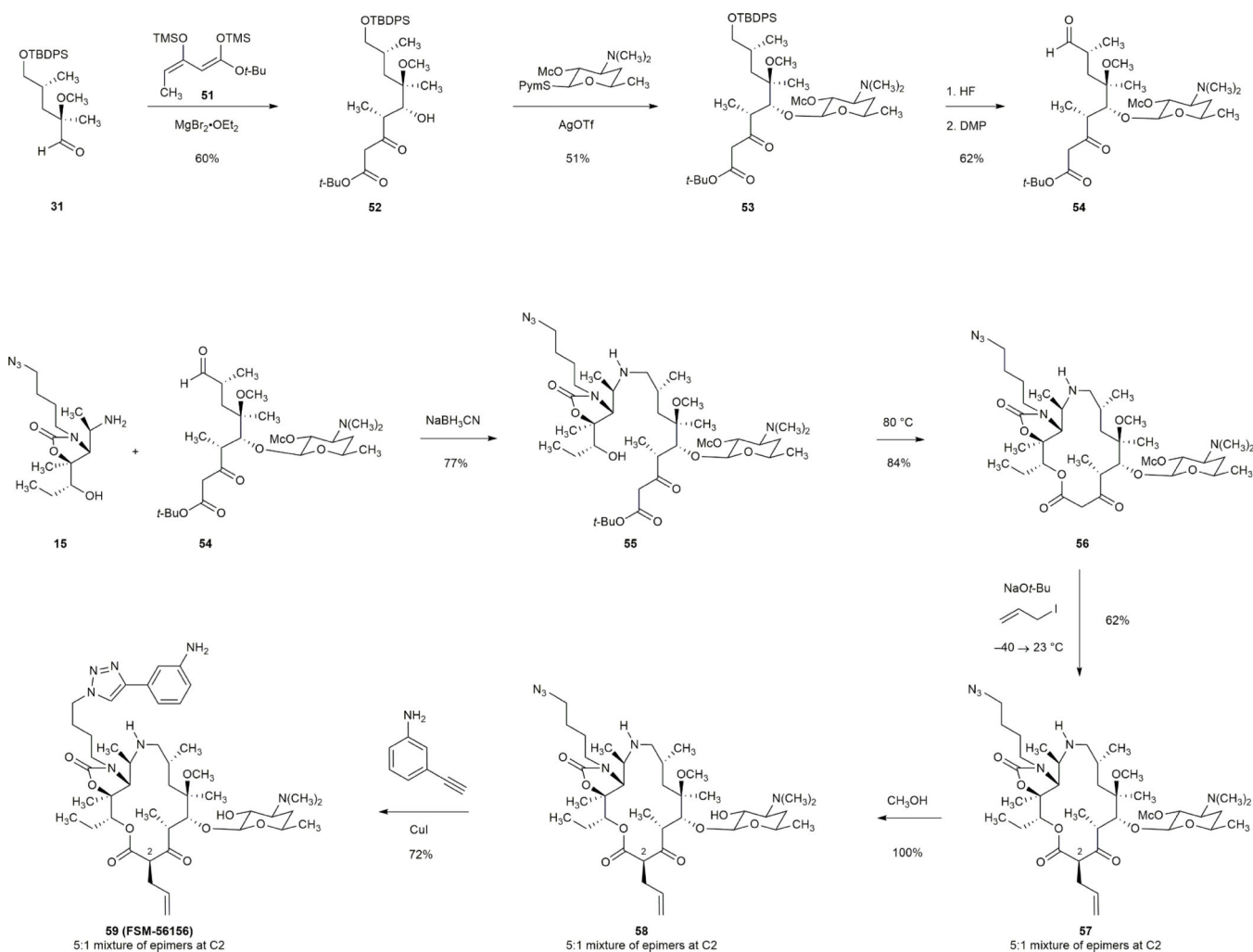
assembly process. As with our earlier convergent synthesis of tetracycline antibiotics,^{49,50} we anticipate that many thousands of novel macrolide structures can be prepared for evaluation as potential antibiotics using the present synthetic platform. In light of this, it seems logical to conclude that developing similar convergent routes to other naturally occurring antibiotic families may accelerate the discovery of new therapeutic agents for human infectious disease.

Extended Data



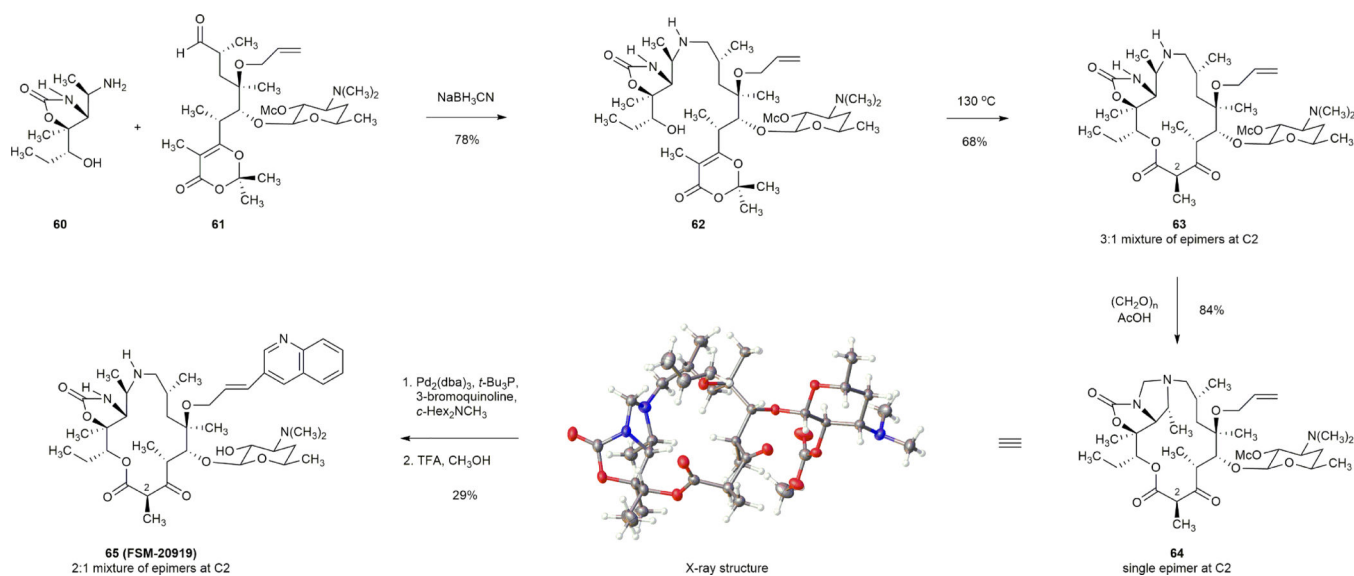
Extended Data Figure 1 |. Synthesis of a C2-fluoro 14-membered azaketolide by a late-stage fluorination reaction.

Subjection of β -keto lactone **25** to potassium *tert*-butoxide (1.0 equiv) at -78 °C followed by *N*-fluorobenzenesulfonimide (1.0 equiv) afforded FSM-22391 in 43% yield.



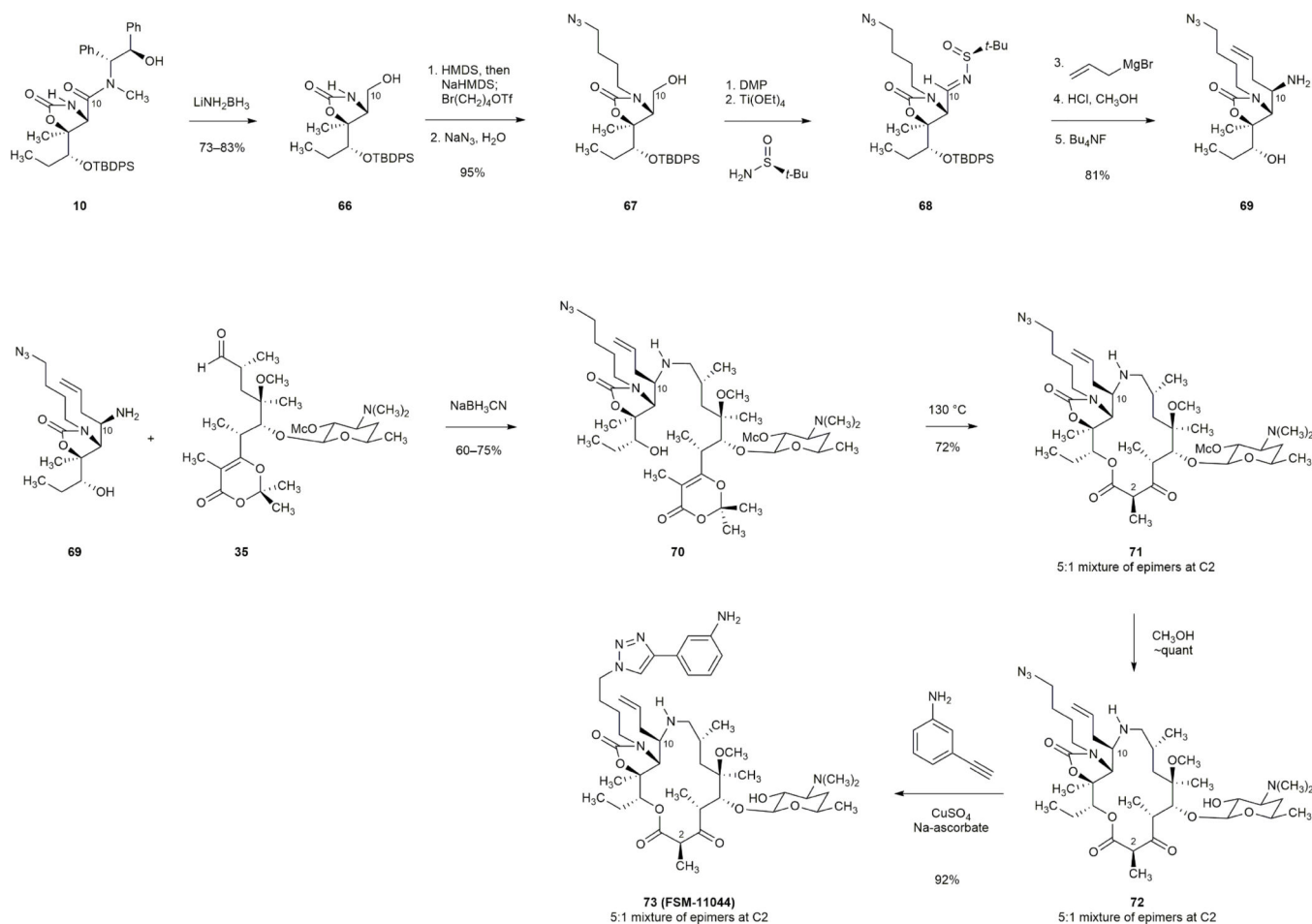
Extended Data Figure 2 |. Synthesis of a 15-membered azaketolide with a modified C2-substituent.

Thermolysis of a β -keto *tert*-butyl ester substrate (**55**) proceeded at a lower temperature (80 °C) and afforded a 15-membered macrocycle without substitution at C2 (**56**). 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 $-40\text{ }^\circ\text{C}$ followed by warming the reaction solution to $23\text{ }^\circ\text{C}$. The product **57** (obtained in 62% yield) was then transformed to FSM-56156 in two steps (72% yield).



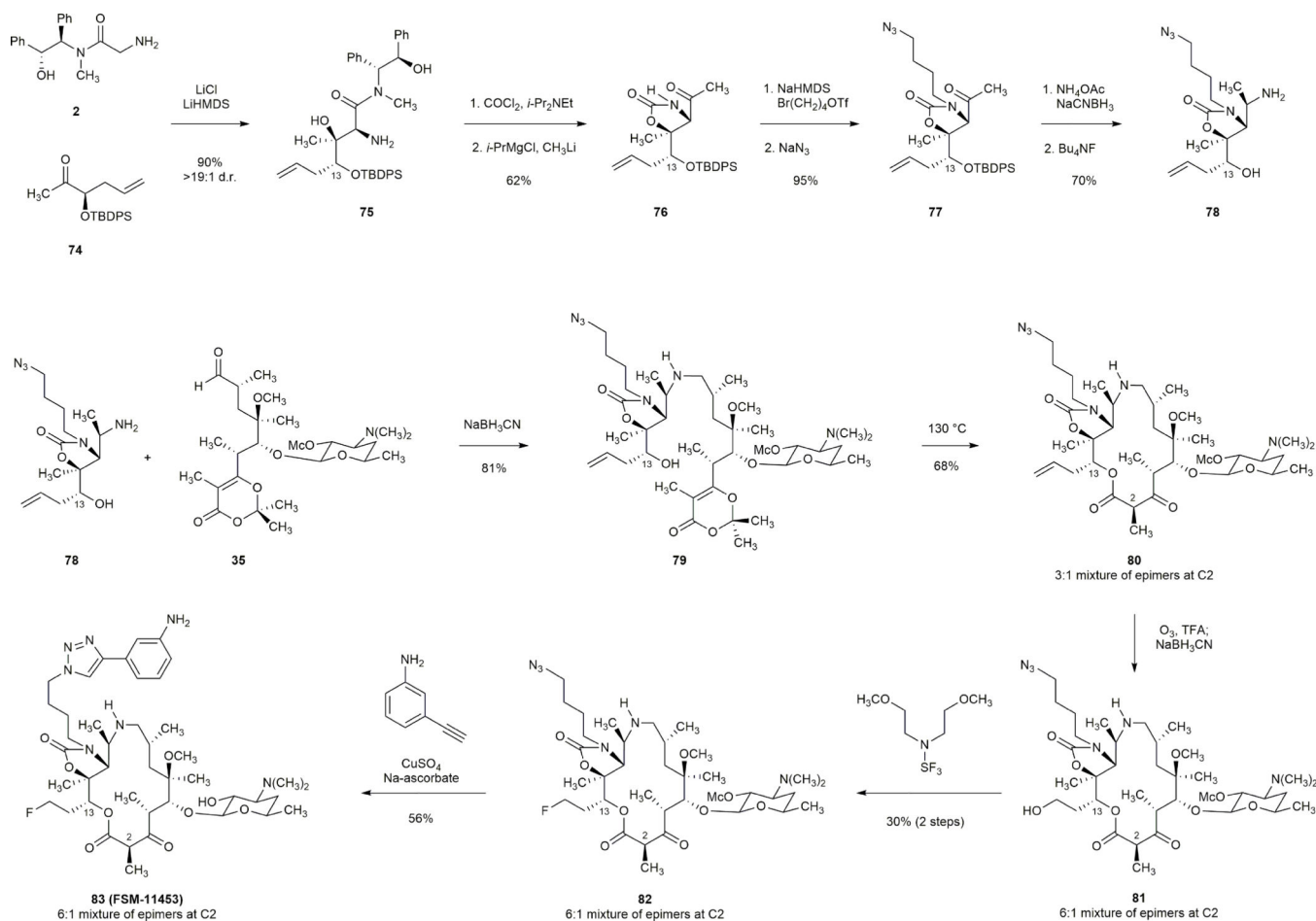
Extended Data Figure 3 | Synthesis of a 15-membered azacethromycin hybrid.

Macrolactone **63** was prepared from amine **60** and aldehyde **61** in two steps 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). 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 FSM-20919 (29%, two-step yield).



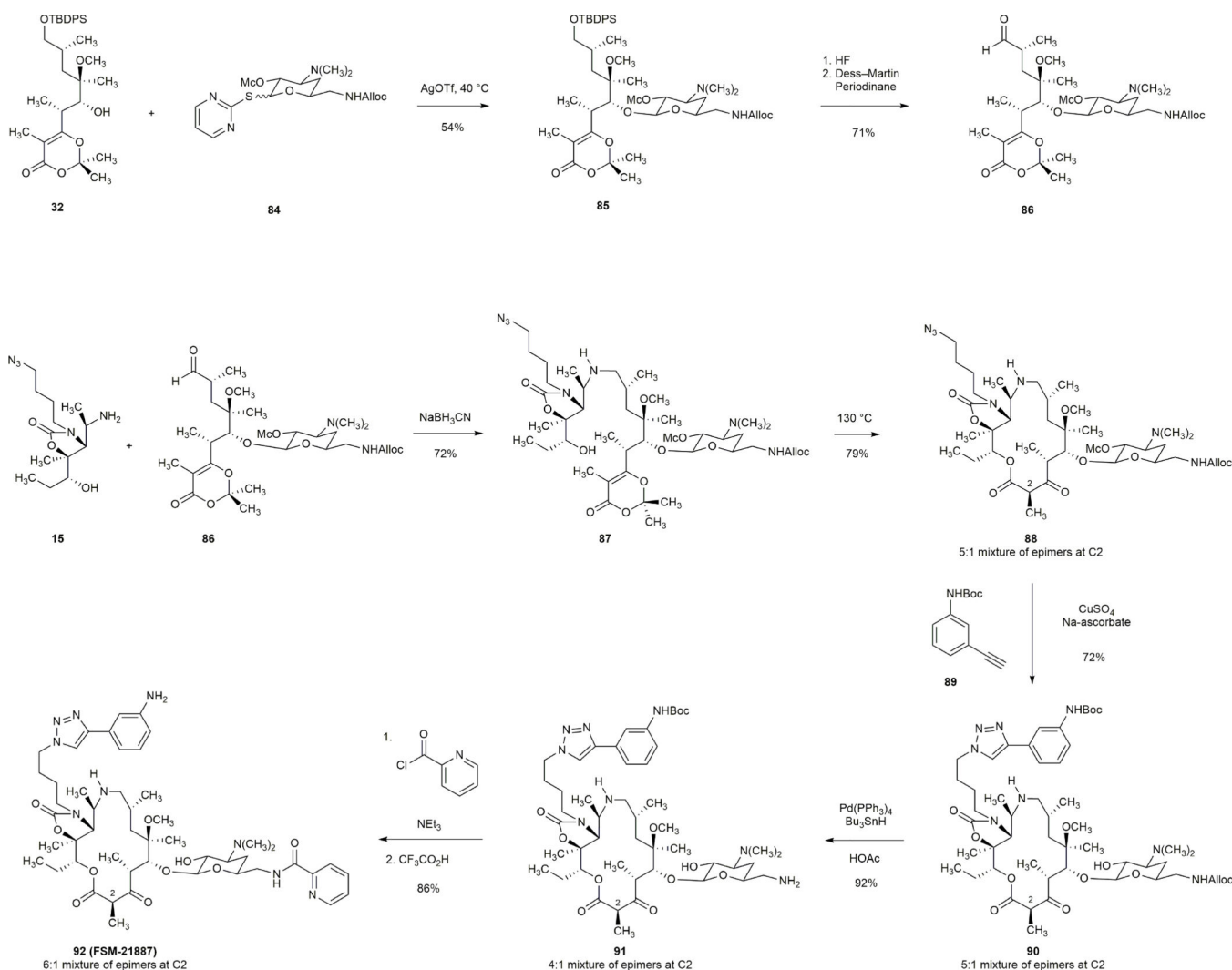
Extended Data Figure 4 |. Synthesis of a 15-membered azaketolide with a modified C10-substituent.

N-tert-butylsulfinyl imine **68** (prepared in five steps from amide **10**) allowed for the stereocontrolled introduction of various C10-substituents. For example, addition of allylmagnesium bromide proceeded with >20:1 stereoselectivity to furnish the adduct depicted; 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 FSM-11044 in a three-step sequence that consisted of a macrocyclization reaction (72% yield), a methanolysis reaction (quantitative yield) and lastly a [3+2] dipolar cycloaddition reaction (92% yield).



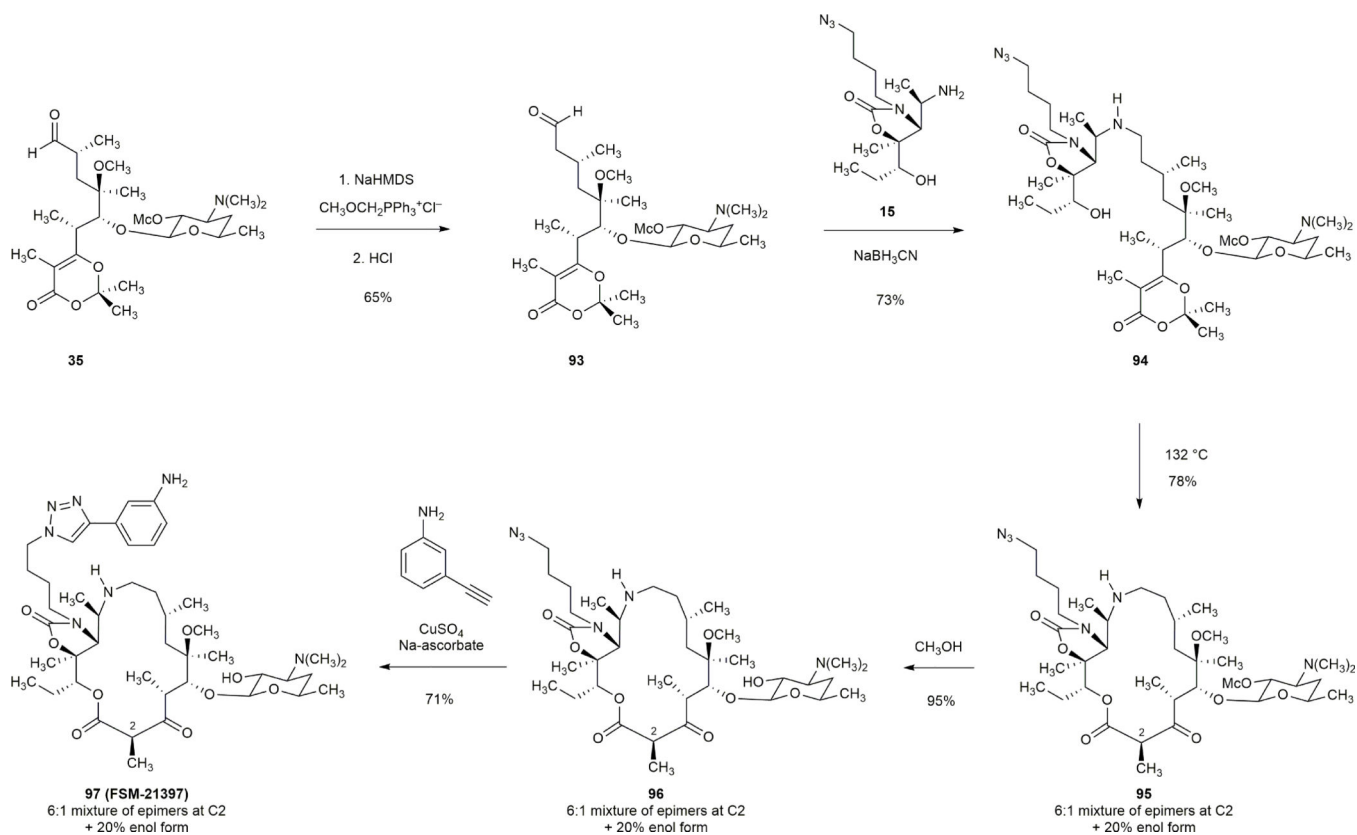
Extended Data Figure 5 | Synthesis of a 15-membered azaketolide with a modified C13-substituent.

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; subsequent thermal macrocyclization provided macrolactone **80**. The allyl group within intermediate **80** was cleaved upon ozonolysis (O_3 , 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 FSM-11453 by a [3+2] dipolar cycloaddition reaction.



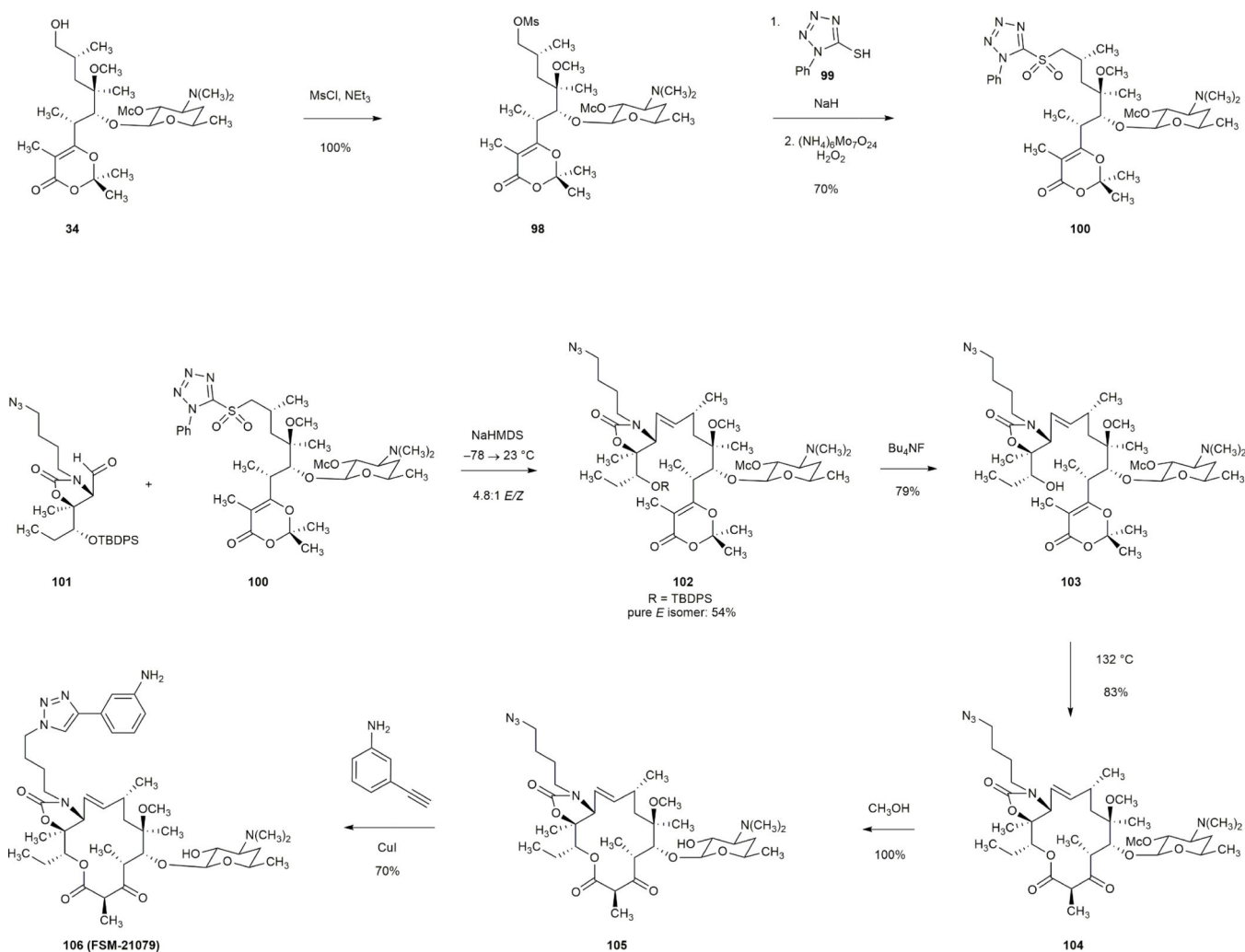
Extended Data Figure 6 |. Synthesis of a 15-membered azaketolide with a modified desosamine sugar residue.

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 allyloxycarbonyl 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 FSM-21887 (86%, two-step yield).



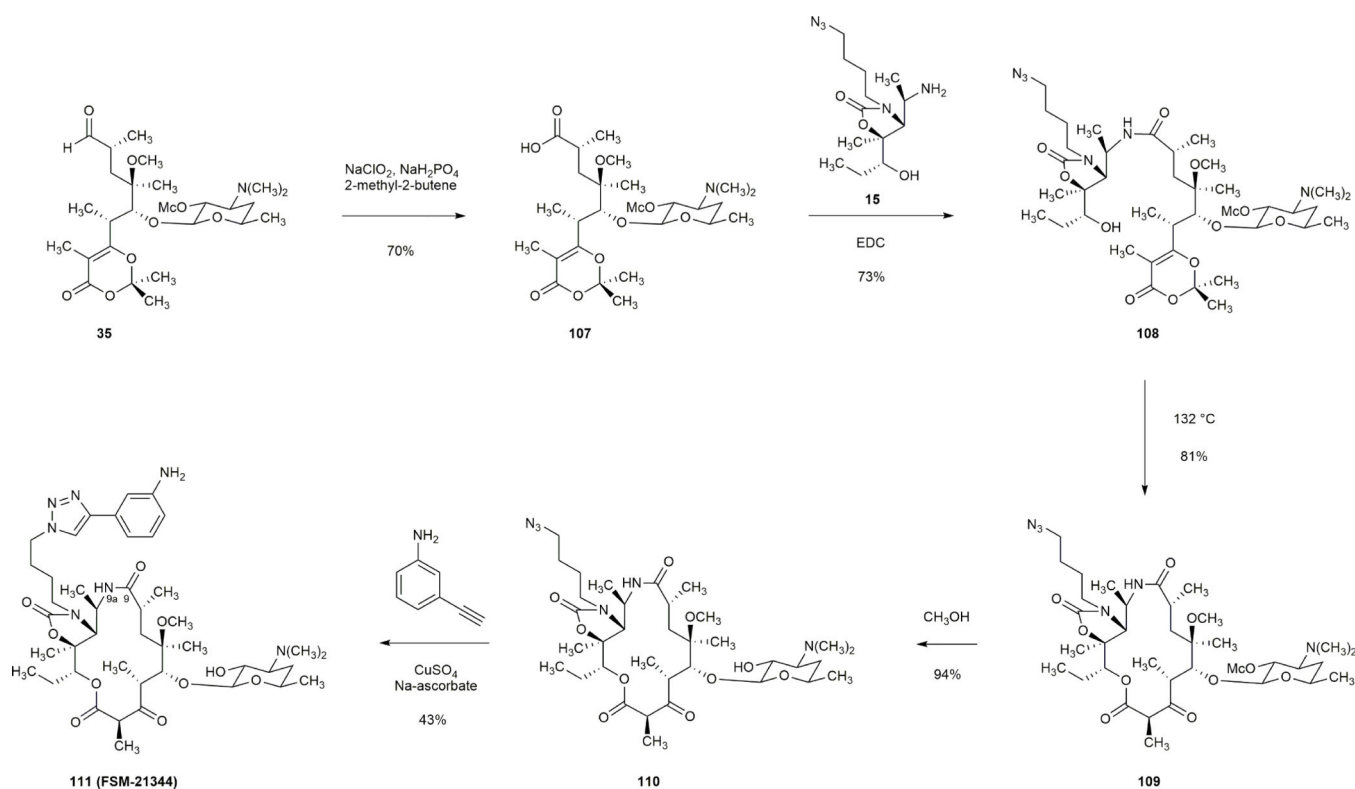
Extended Data Figure 7 |. Synthesis of a 16-membered azaketolide.

Homologation of aldehyde **35** was achieved by a Wittig olefination reaction ($CH_3OCH_2PPh_3^+Cl^-$, 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** (1mM, 132 °C). Two additional steps transformed **95** to the 16-membered azaketolide FSM-21397.



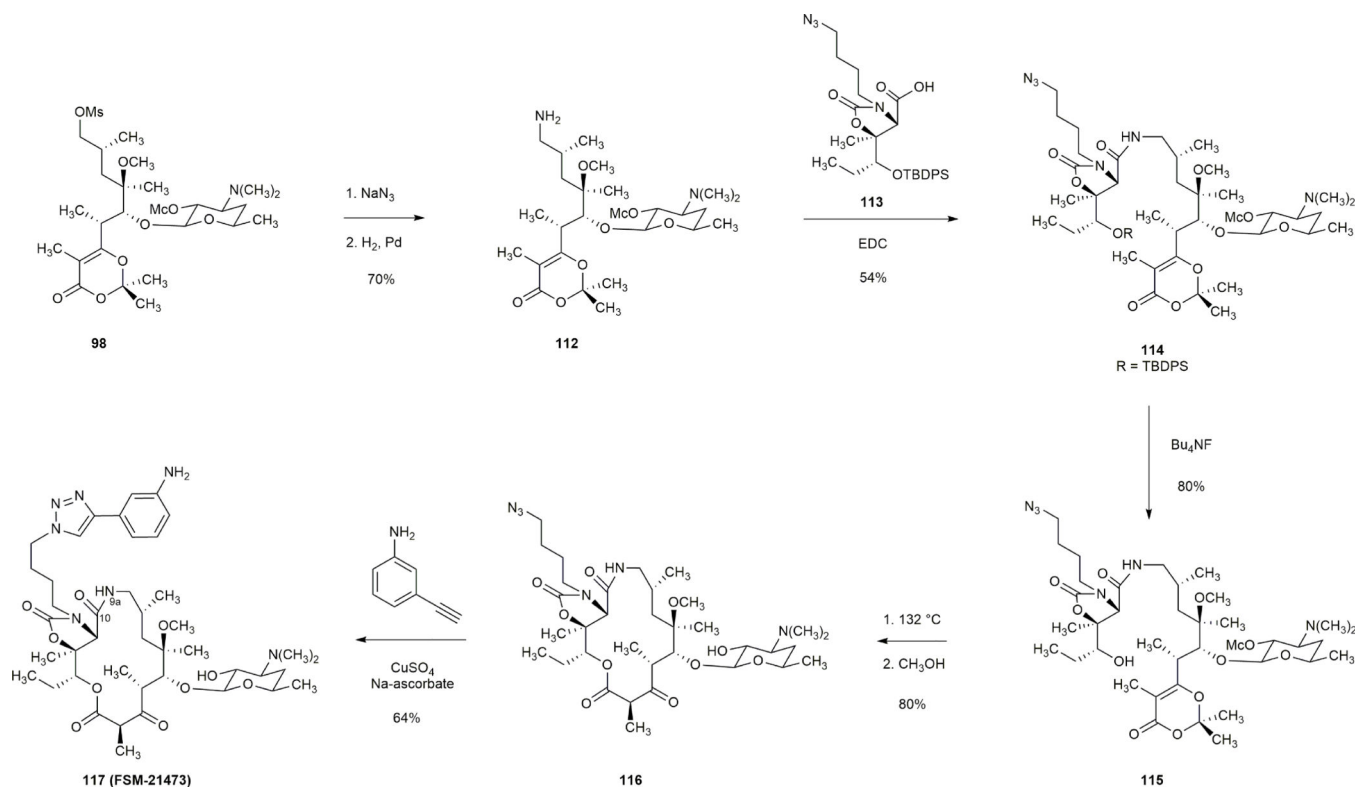
Extended Data Figure 8 |. Synthesis of a 14-membered macrolide with a *trans*-olefin linkage.

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 mesylate **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, $-78 \rightarrow 23^\circ\text{C}$) 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 FSM-21079 in two additional steps (methanolysis and [3+2] dipolar cycloaddition).



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, $132\text{ }^\circ\text{C}$) proceeded in 81% yield to afford macrolactam **109**. Methanolysis and [3+2] dipolar cycloaddition then transformed **109** to FSM-21344 in two steps.



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 mesylate **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-catalyzed [3+2] dipolar cycloaddition then provided FSM-21473 in 64% yield.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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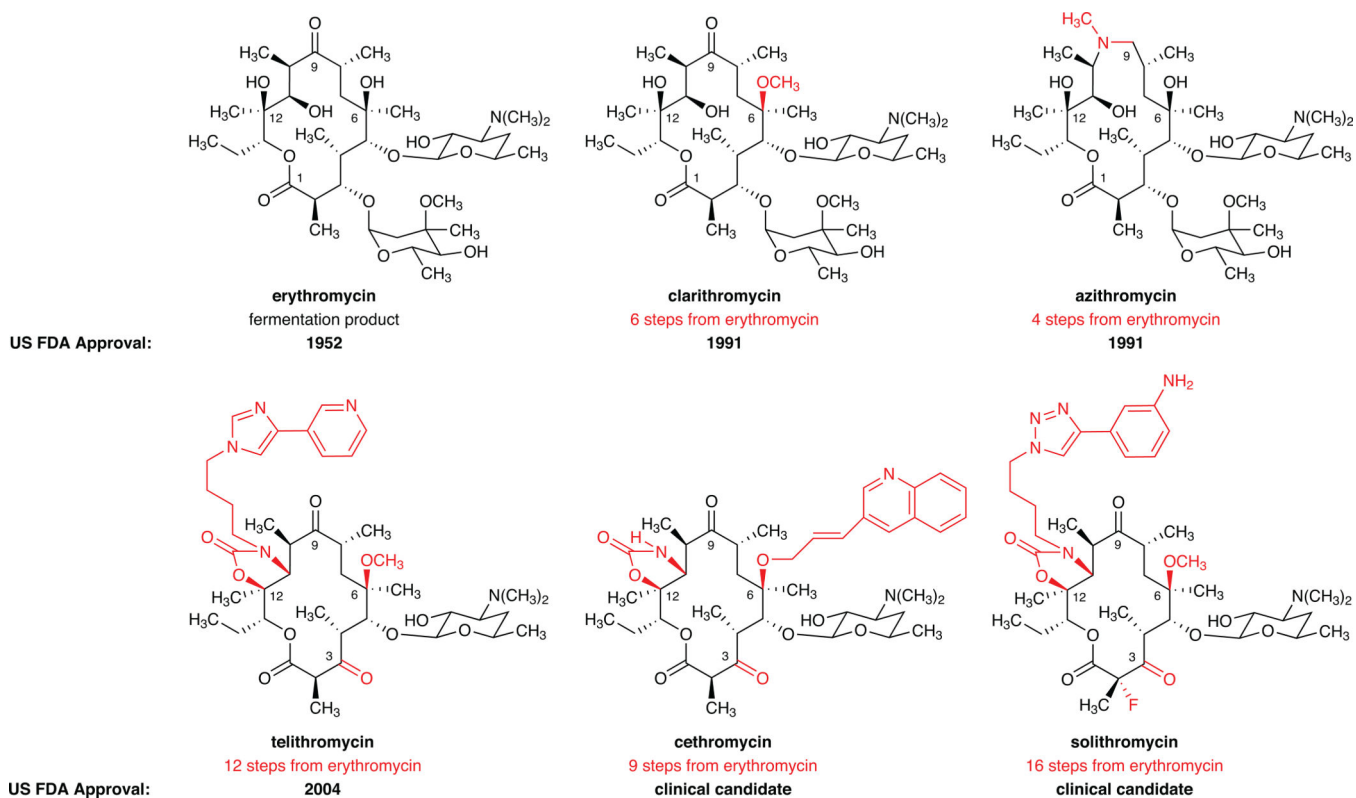


Figure 1 | Summary of macrolide antibiotic development by semisynthesis.

To date, all macrolide antibiotics are produced by chemical modification (semisynthesis) of erythromycin, a natural product produced on ton-scale by fermentation. Depicted are 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.

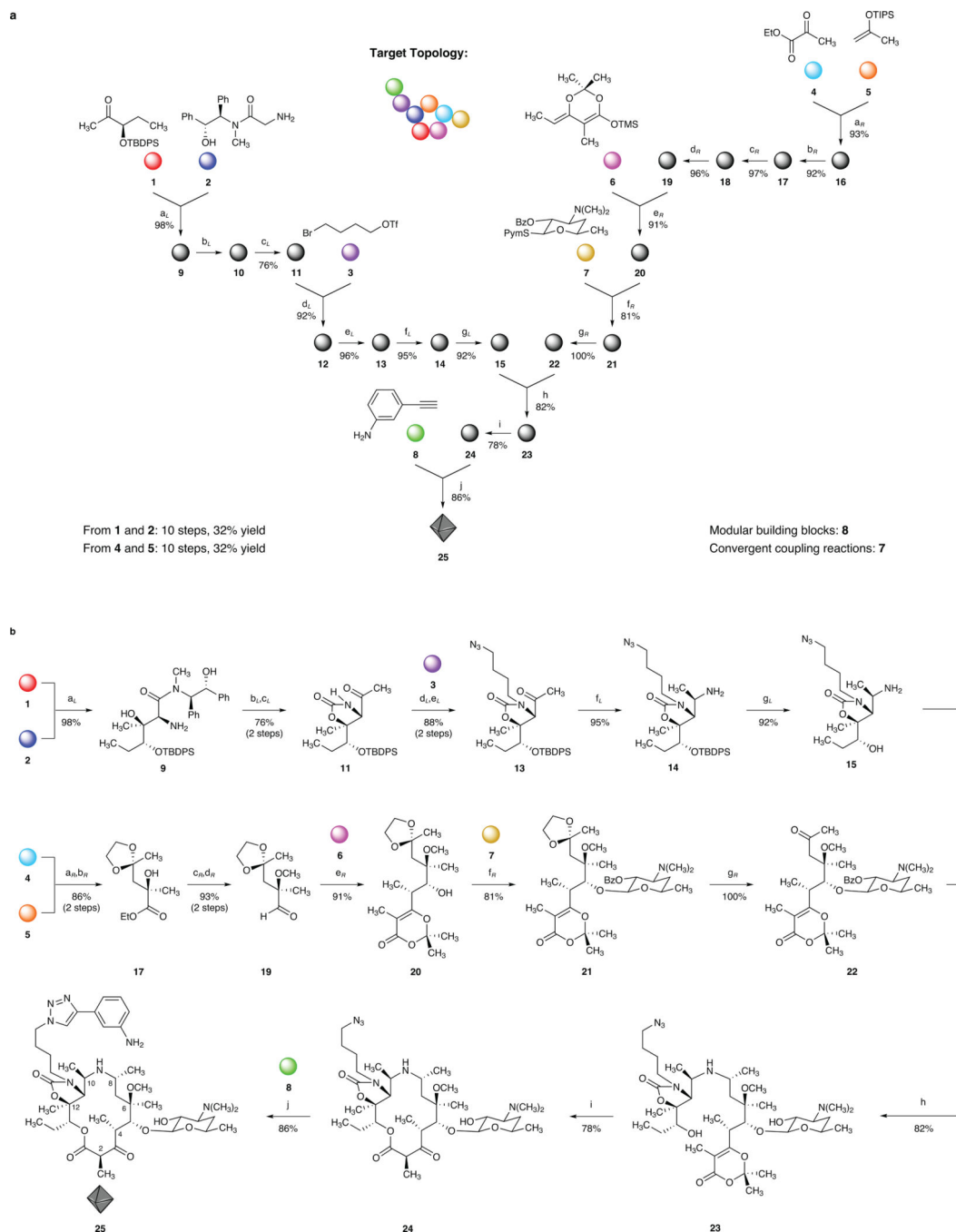


Figure 2 | A convergent, fully synthetic route to the 14-membered azaketolide 25.

a. Graphical representation of the convergent synthesis of azaketolide **25** from eight variable building blocks (represented by colored spheres). Downward, Y-shaped arrows signify convergent coupling reactions. **b.** Synthesis of azaketolide **25**, reagents and conditions (subscripts *L* and *R* indicate left and right halves, respectively): (*a_L*) LiHMDS, LiCl, 98%; (*b_L*) Et'*Pr*₂N, COCl₂; (*c_L*) '*Pr*MgCl, CH₃Li, 76% over 2 steps, 92% of recovered (*R,R*)-pseudoephedrine; (*d_L*) NaHMDS; (*e_L*) NaN₃, 88% over 2 steps; (*f_L*) NH₃, Ti(*O*'*Pr*)₄, NaBH₄, 95%; (*g_L*) Bu₄NF, 92%; (*a_R*) Pd[(*S*)-SegPhos]Cl₂, AgSbF₆, 93%, 92% ee; (*b_R*)

ethylene glycol, PPTS, 92%; (c_R) KH, MeI, 97%; (d_R) (*t*Bu)₂AlH, 96%; (e_R) Et₃N, MgBr₂•OEt₂, 91%; (f_R) AgOTf, 81%, 16:1 β:α; (g_R) HCl, 100%; (h) NaCNBH₃, 82%; (i) 132 °C, 1 mM, PhCl, 78%; (j) CuSO₄, sodium L-ascorbate, 86%.

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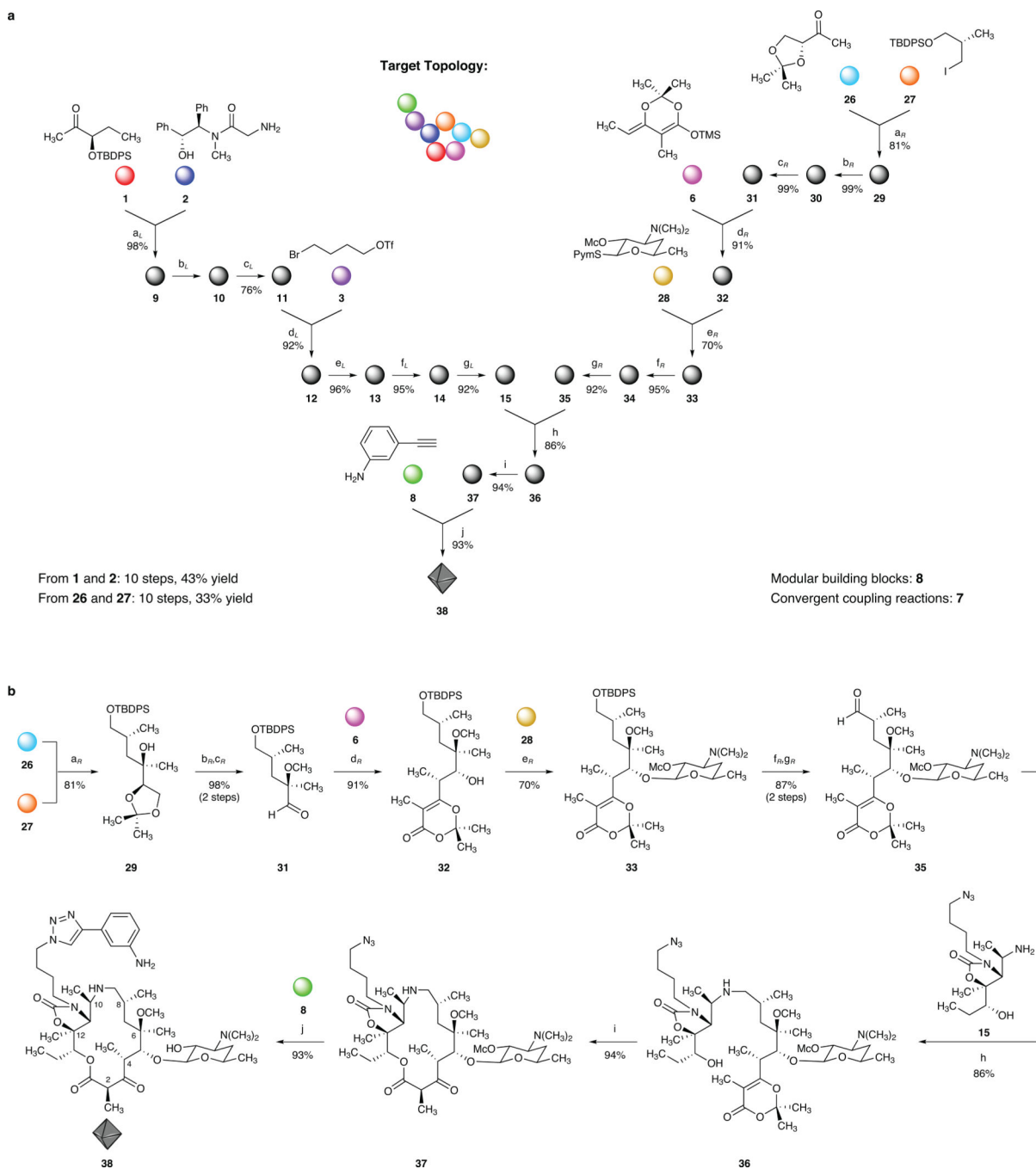


Figure 3 | A convergent, fully synthetic route to the 15-membered azaketolide 38.

a. Graphical representation of the convergent synthesis of azaketolide **38** from eight variable building blocks (represented by colored 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): (a_L) LiHMDS, LiCl, 98%; (b_L) $\text{Et}^i\text{Pr}_2\text{N}$, COCl_2 ; (c_L) $i\text{PrMgCl}$, CH_3Li , 76% over 2 steps, 92% of recovered (R,R)-pseudoephedrine; (d_L) NaHMDS; (e_L) NaN_3 , 88% over 2 steps; (f_L) NH_3 , $\text{Ti}(\text{O}^i\text{Pr})_4$, NaBH_4 , 95%; (g_L) Bu_4NF , 92%; (a_R) $t\text{BuLi}$, MgBr_2 , 81%; (b_R) KH , MeI , 99%; (c_R) H_5IO_6 ,

99%; (d_R) ZnCl₂, 91%; (e_R) AgOTf, 70%; (f_R) HF (aq); (g_R) Dess–Martin periodinane, 87% over 2 steps; (h) NaCNBH₃, 86%; (i) 132 °C, 1 mM, PhCl, 94%; (j) CuSO₄, sodium L-ascorbate, 93%.

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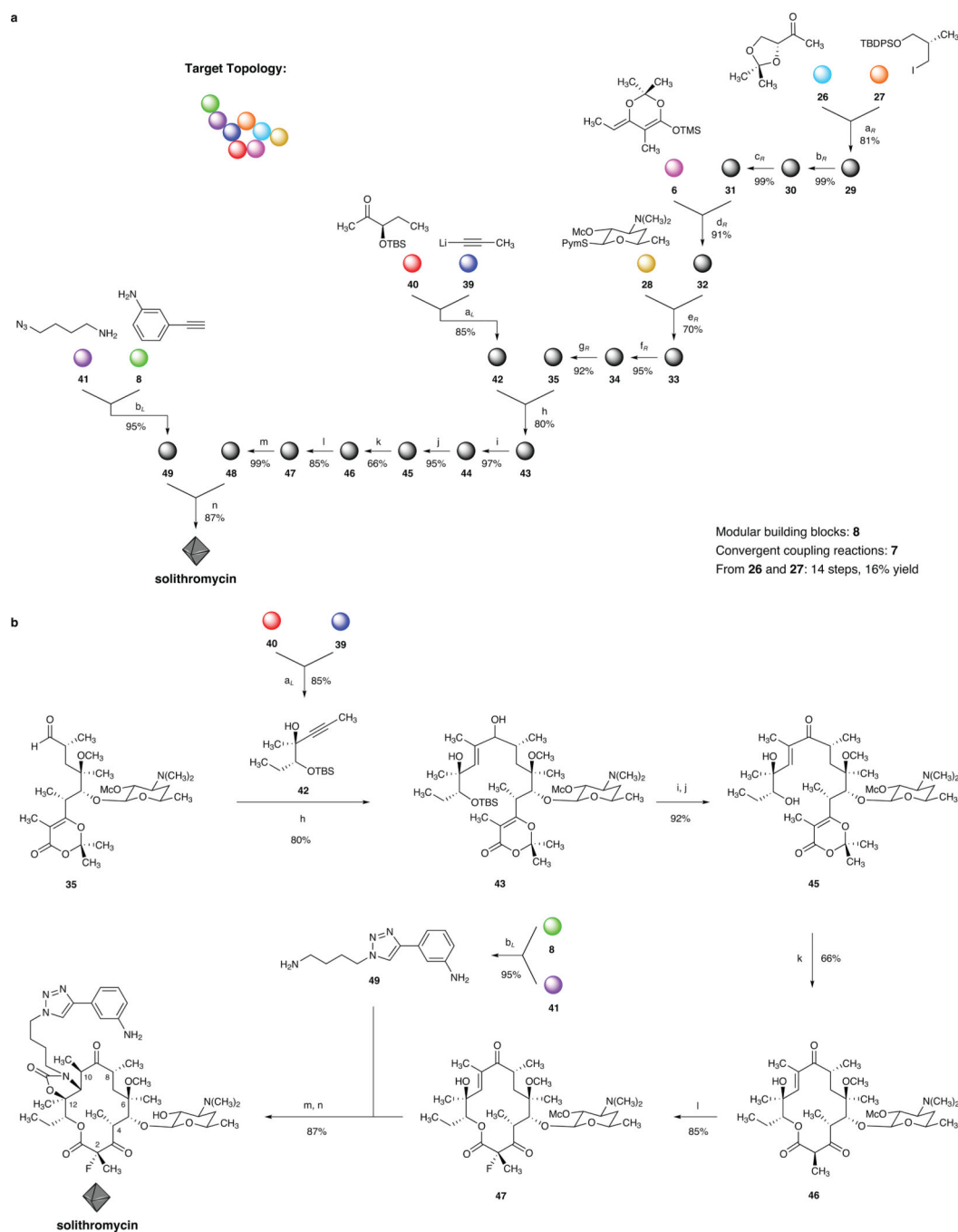


Figure 4 | A convergent, fully synthetic route to solithromycin.

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, respectively): (a_L) lithium (1*S*,2*R*)-1-phenyl-2-(pyrrolidin-1-yl)-1-propanolate, 85%; (b_L) CuSO₄, sodium L-ascorbate, 95%; (a_R) ^tBuLi, MgBr₂, 81%; (b_R)

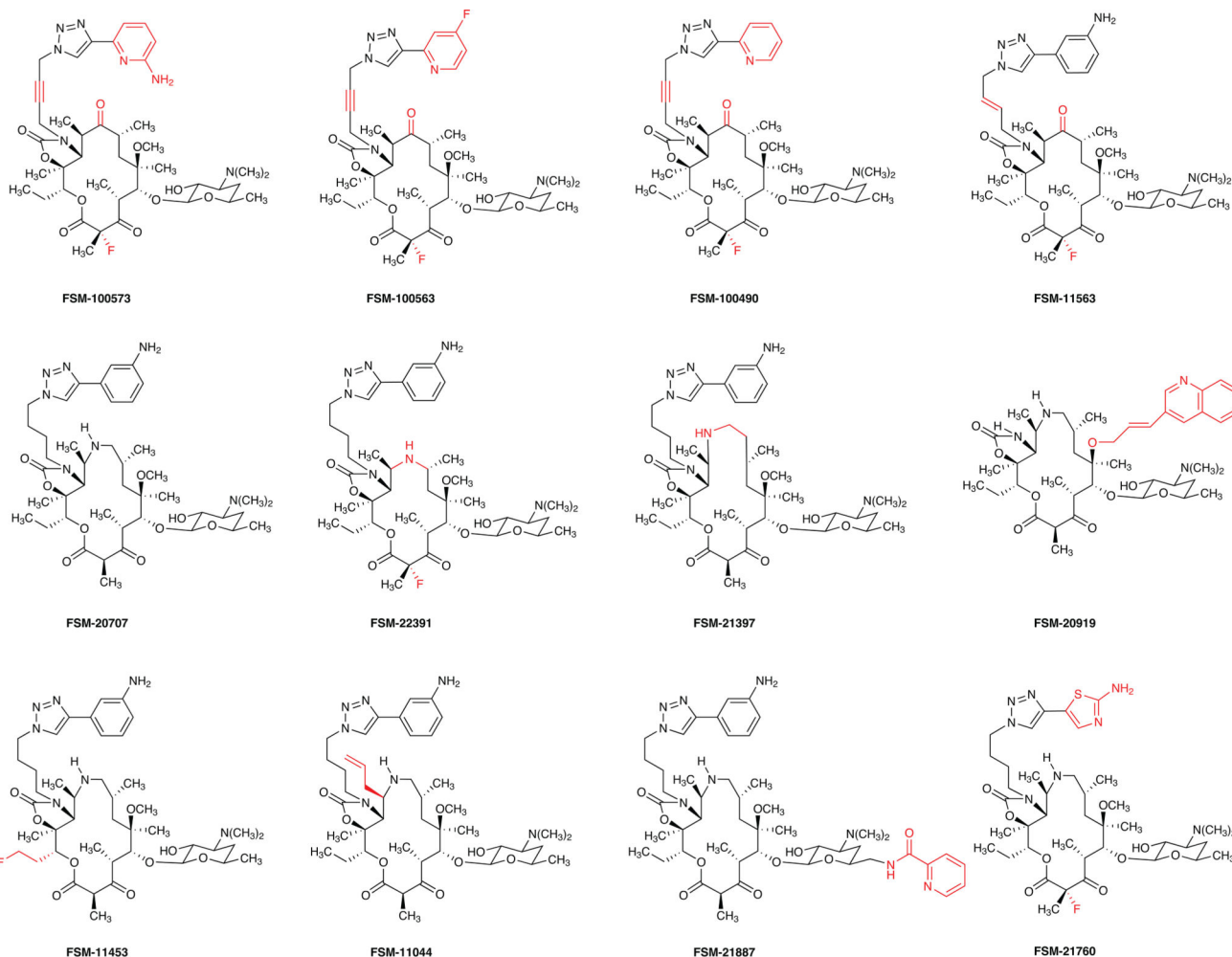
KH, MeI, 99%; (c_R) H₅IO₆, 99%; (d_R) ZnCl₂, 91%; (e_R) AgOTf, 70%; (f_R) HF (aq), 95%; (g_R) Dess–Martin periodinane, 92%; (h) Cp₂TiCl₂, cyclopentylmagnesium bromide, 80%; (i) Dess–Martin periodinane, 97%; (j) Bu₄NF, 95%; (k) 132 °C, 0.5 mM, PhCl, 66%; (l) KO^tBu, FN(SO₂Ph)₂, 85%; (m) Im₂CO, DBU; (n) imidazole hydrochloride, 60 °C, 87% over 2 steps.

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Species	Strain Description	Erythro	Azithro	Telithro	Solithro	100573	100563	100490	11563	20707	22391	21397	20919	11453	11044	21887	21760	
Gram-positive	<i>S. aureus</i> ATCC 29213	0.5	1	0.125	0.125	0.06	≤0.03	≤0.03	0.06	0.5	0.25	4	0.25	1	1	8	0.5	
	<i>S. aureus</i> BAA-977; iErmA	>256	>256	0.06	≤0.03	0.06	0.06	0.03	0.06	0.5	0.5	4	0.5	1	1	8	1	
	<i>S. aureus</i> MP513; MRSA; cErmA	>256	>256	256	>64	16	16	64	64	>64	64	64	64	>64	>64	>64	64	64
	<i>S. aureus</i> NRS384; MRSA; MsrA	64	128	0.125	0.25	0.06	0.125	0.06	0.125	1	1	8	0.5	2	2	16	4	4
	<i>S. pneumoniae</i> ATCC 49619	0.03	0.06	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.06	≤0.03	≤0.03	≤0.03	0.06	≤0.03	≤0.03
	<i>S. pneumoniae</i> UNT-042; ErmB/MefA	>256	>256	0.125	0.25	≤0.03	≤0.03	≤0.03	≤0.03	2	0.125	8	0.5	2	8	1	1	1
	<i>S. pyogenes</i> ATCC 19615	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.06	≤0.03	≤0.03	≤0.03	≤0.03	0.06	≤0.03
	<i>E. faecalis</i> ATCC 29212	1	4	≤0.03	≤0.03	0.03	0.03	0.03	≤0.03	0.125	0.06	0.5	0.25	0.125	0.125	0.5	0.06	0.06
	<i>E. faecalis</i> UNT-047; VRE; ErmB	>256	>256	16	32	1	2	2	4	>64	32	64	>64	>64	>64	>64	>64	>64
	Gram-negative	<i>H. influenzae</i> ATCC 49247	4	2	2	4	2	2	2	2	2	4	8	4	4	8	16	4
<i>A. baumannii</i> ATCC 19606		16	32	4	16	2	8	2	4	4	4	16	16	4	32	32	32	32
<i>K. pneumoniae</i> ATCC 10031		4	2	4	4	2	8	4	4	2	4	8	16	2	8	8	4	4
<i>E. coli</i> ATCC 25922		64	4	16	32	8	16	16	16	4	8	32	4	8	64	16	8	8
<i>P. aeruginosa</i> ATCC 27853		64	64	64	64	16	32	64	32	64	64	64	64	>64	>64	>64	>64	64

MIC Color Scale (µg/mL): <0.03 (dark green), 0.03 (green), 0.06 (light green), 0.125 (yellow-green), 0.25 (yellow), 0.5 (orange), 1 (orange-red), 2 (red), 4 (dark red), 8 (red), 16 (dark red), 32 (dark red), 64 (dark red), 128 (dark red), 256 (dark red), >256 (dark red)

Figure 5 | Minimum inhibitory concentrations (µg/mL) for selected analogs against 9 Gram-positive and 5 Gram-negative microorganisms.

iErmA – inducible erythromycin ribosome methyltransferase A; cErmA – constitutive erythromycin ribosome methyltransferase A; MsrA – macrolide streptogramin resistance efflux pump A; MefA – macrolide efflux protein A; ErmB – erythromycin ribosome methyltransferase B; Erythro – erythromycin; Azithro – azithromycin; Telithro – telithromycin; Solithro – solithromycin.