

A platform for the discovery of new macrolide antibiotics

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The chemical modification of structurally complex fermentation products, a process known as semisynthesis, has been an important tool in 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 effective, because bacterial resistance to these compounds has developed. Here we present a practical, fully synthetic route to macrolide antibiotics by the convergent assembly of simple chemical building blocks, enabling the synthesis of diverse structures not accessible by traditional semisynthetic approaches. More than 300 new macrolide antibiotic candidates, as well as the clinical candidate solithromycin, have been synthesized using our convergent approach. Evaluation of these compounds against a panel of pathogenic bacteria revealed that the majority of these structures had antibiotic activity, some efficacious against strains resistant to macrolides in current use. The chemistry we describe here provides a platform for the discovery of new macrolide antibiotics and may also serve as the basis for their manufacture.

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 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 antibiotics¹. 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^{2,3}. 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 1949⁴, 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 steps¹⁴, clarithromycin requires six steps¹⁵, and the advanced clinical candidate solithromycin is currently produced from erythromycin by a 16-step linear sequence (Fig. 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 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 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 that have been proposed to impart favourable 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 inactive²⁵. We were particularly interested in 14-membered azaketolides that would arise from formal replacement of the carbonyl group at position C9

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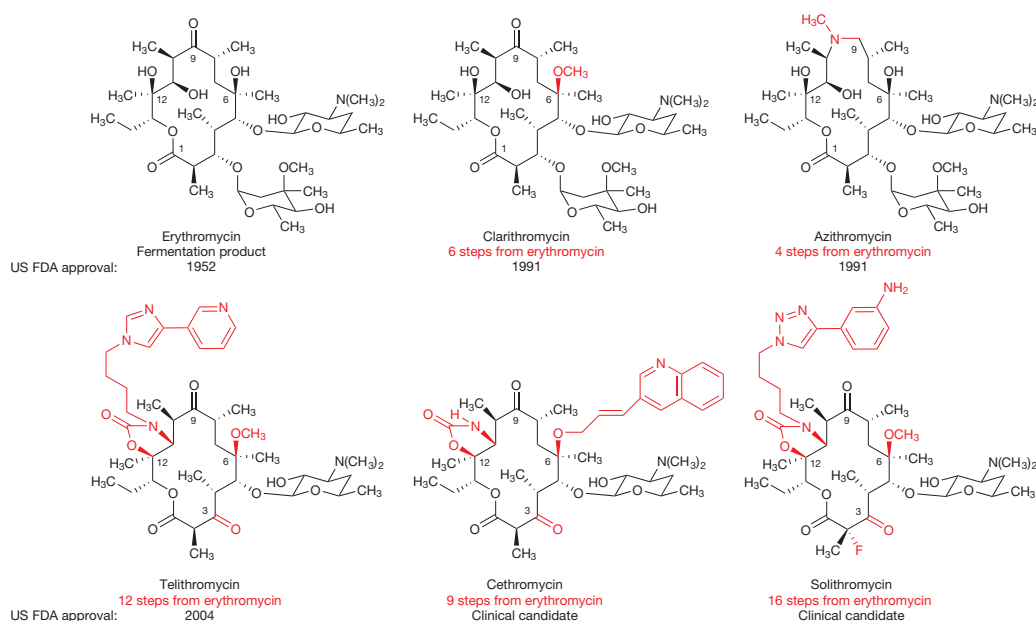


Figure 1 | Summary of macrolide antibiotic development by semisynthesis. To date, all macrolide antibiotics are produced by chemical modification (semisynthesis) of erythromycin, a natural product produced on the ton scale by fermentation. Depicted are erythromycin and the approved semisynthetic macrolide 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.

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 of which are commercially available and six of which are synthesized in sequences of 3–6 steps in 30–150-g amounts, are assembled in a highly convergent manner (Fig. 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. (Here left and right refer to the position in the graphical representation of the target molecule, Fig. 2a.) The left- and right-hand intermediates are then joined in a highly stereoselective and efficient reductive coupling reaction (82% yield, diastereomeric ratio (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 towards the natural products diploidalide 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 strategies²⁸. 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 brings the putative acyl ketene function (produced from the dioxolenone function upon thermolysis) and the reactive secondary alcohol into favourable 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 before the macrocyclization reaction merit brief discussion (see Fig. 2). The key aldol coupling reaction that unites building blocks 1³⁵ and 2³⁶, 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 diastereomer³⁶. 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-catalysed ene reaction developed by Mikami and co-workers, and proceeds in 93% yield and 92% enantiomeric excess (e.e.)³⁷. Two convergent coupling reactions follow three linear functional group interconversions, the first coupling being a Mukaiyama³⁸ aldol reaction (to incorporate building block 6³⁹) 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 erythromycin⁵. 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

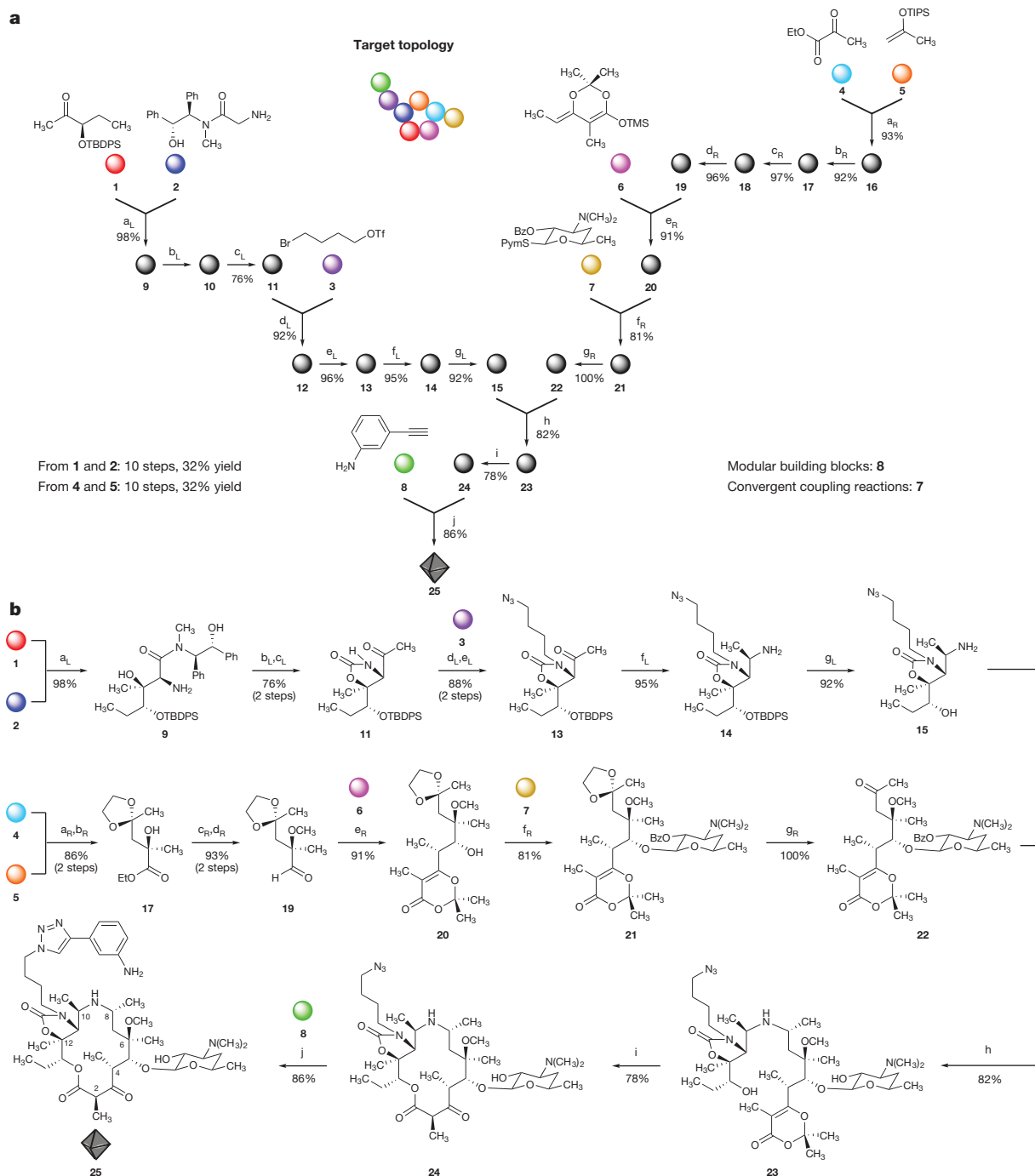


Figure 2 | A convergent, fully synthetic route to the 14-membered azaketolide 25. **a**, Graphical representation of the convergent synthesis of azaketolide 25 from eight variable building blocks (represented by coloured spheres). Downward, 'Y'-shaped arrows signify convergent coupling reactions. **b**, Synthesis of azaketolide 25, reagents and conditions (subscripts L and R indicate left and right halves, respectively) as follows: (a_L) LiHMDS, LiCl, 98%; (b_L) Et^tPr₂N, COCl₂; (c_L) ^tPrMgCl, 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(OⁱPr)₄, NaBH₄, 95%; (g_L) Bu₄NF, 92%; (a_R) Pd[(*S*)-SegPhos]Cl₂, AgSbF₆, 93%, 92% e.e.; (b_R) ethylene glycol, PPTS, 92%; (c_R) KH, MeI, 97%; (d_R) (^tBu)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%.

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 26⁴² 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

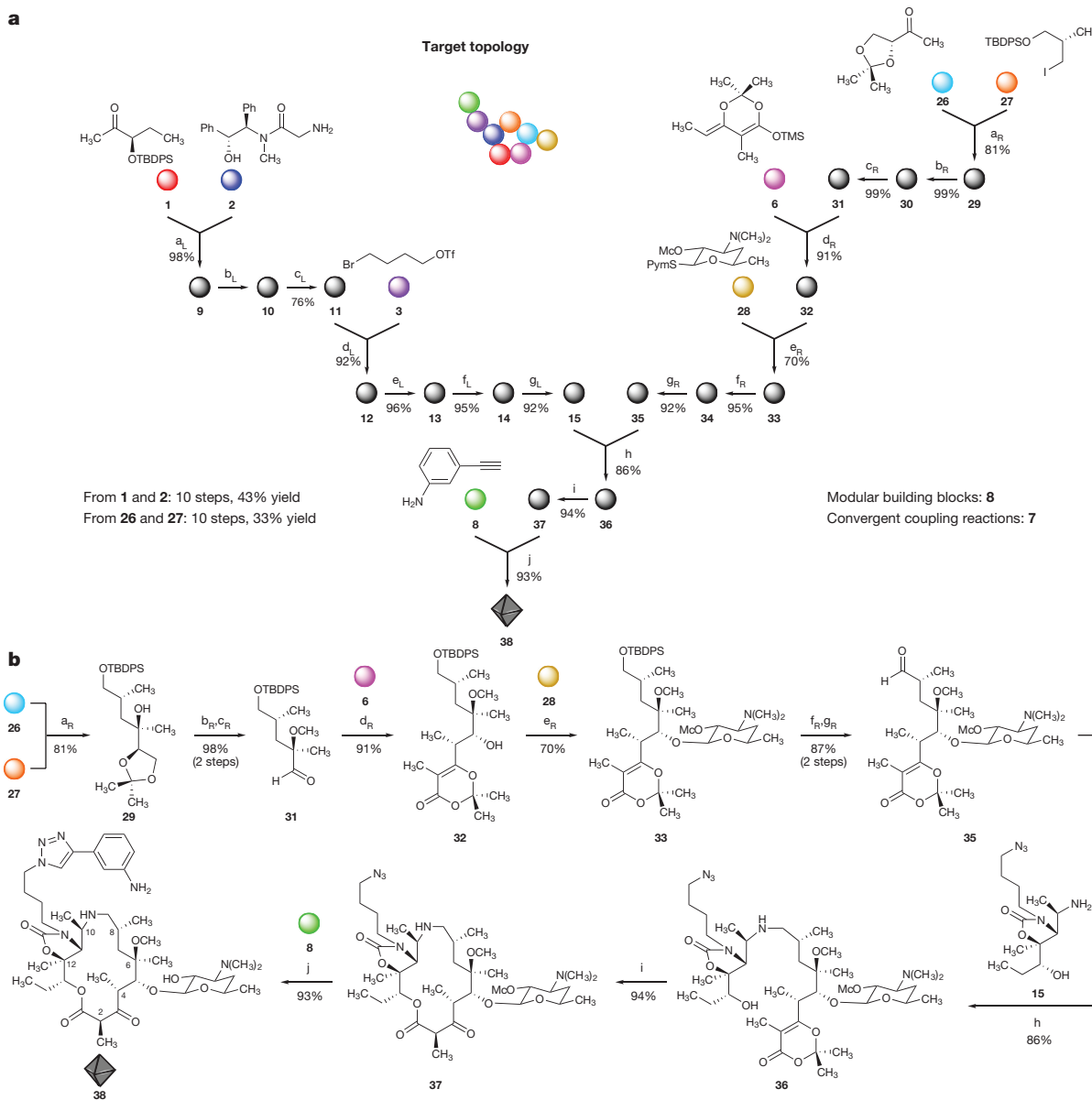


Figure 3 | A convergent, fully synthetic route to the 15-membered azaketolide 38. **a**, Graphical representation of the convergent synthesis of azaketolide 38 from eight variable building blocks (represented by coloured spheres). Downward, 'Y'-shaped arrows signify convergent coupling reactions. **b**, Synthesis of azaketolide 38, reagents and conditions (subscripts L and R indicate left and right halves, respectively): (a_L) LiHMDS, LiCl, 98%; (b_L) Et'Pr₂N, COCl₂; (c_L) 'PrMgCl, 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) 'BuLi, MgBr₂, 81%; (b_R) KH, MeI, 99%; (c_R) H₅IO₆, 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%.

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 42 (Fig. 4). Intermediate 42 was obtained in one step (14:1 d.r., 85% yield) by the addition of 1-lithiopropene (39) to ketone 40³⁵ in the presence of lithium (1*S*,2*R*)-1-phenyl-2-(pyrrolidin-1-yl)-1-propanolate⁴⁵. 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 (tetrabutylammonium fluoride (Bu₄NF), 95%) to afford the keto alcohol 45. Macrocyclization, as before, then provided the 14-membered

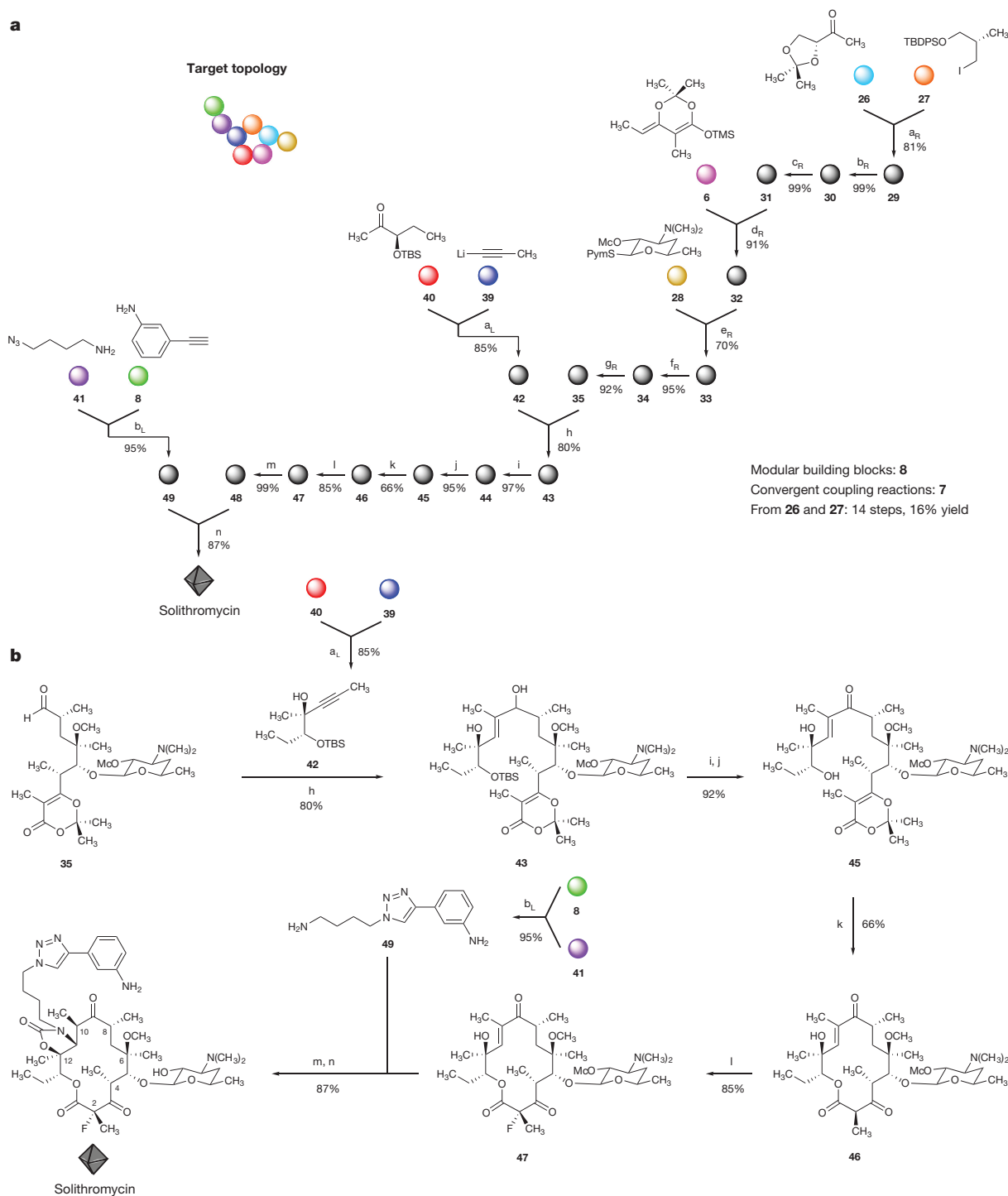


Figure 4 | A convergent, fully synthetic route to solithromycin.

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.

macrolactone **46** in 66% yield (1.7-g scale). Fluorination at position C2 proceeded in 85% yield⁴⁷. Transformation of the C12 tertiary alcohol within the latter intermediate (**47**) to the corresponding acyl imidazolide (carbonyl diimidazole (Im₂CO), 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 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 before the penultimate step in the published semisynthetic route to solithromycin¹⁶.

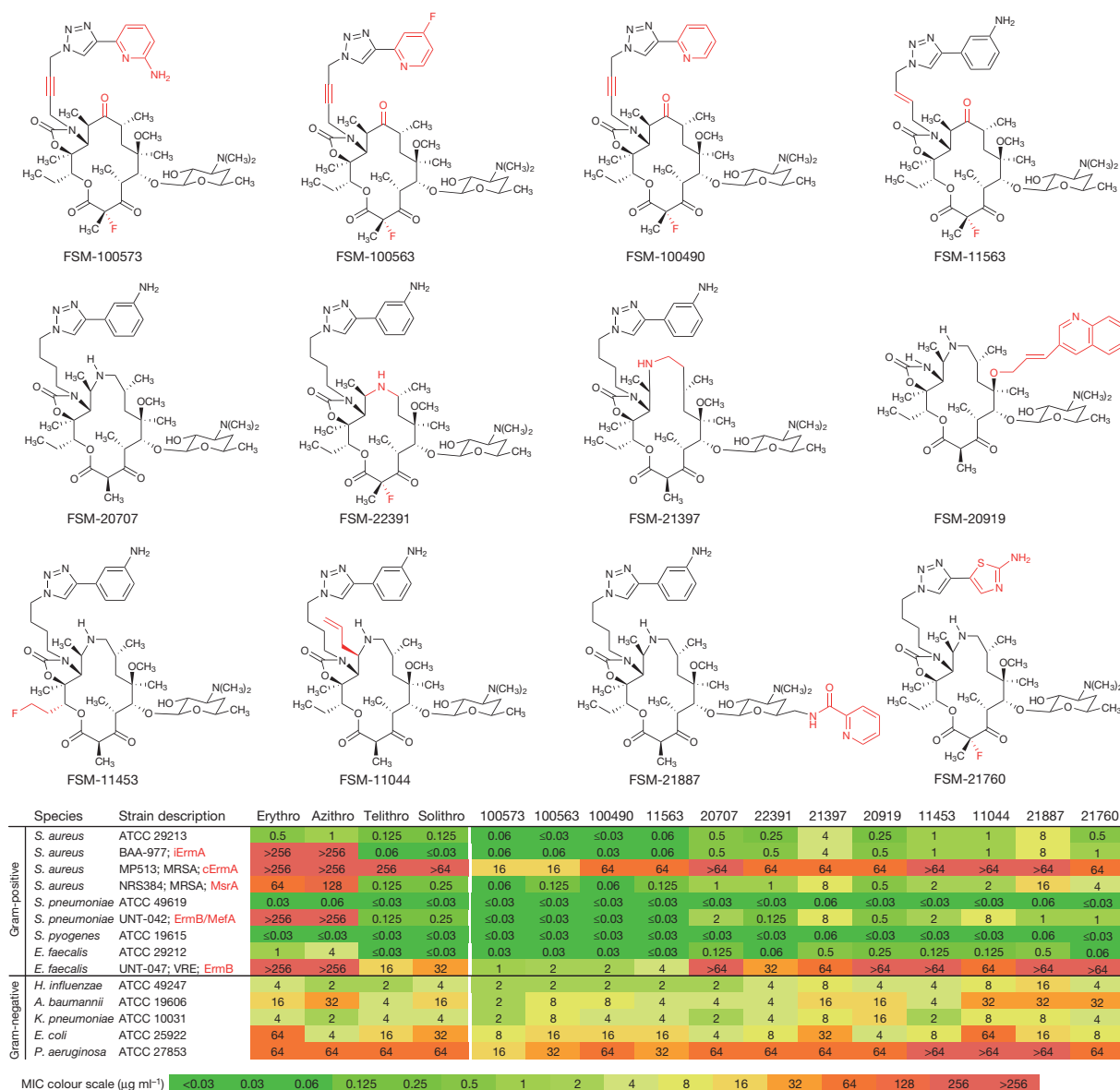


Figure 5 | Structures of selected fully synthetic macrolide antibiotics, and their MICs compared to those of natural and semisynthetic macrolide antibiotics. Top three rows, structural formulae. Bottom row, table showing MICs (values in $\mu\text{g ml}^{-1}$ colour coded, key at bottom) of current macrolide antibiotics and of selected analogues 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.

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 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 (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 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 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) $\leq 4 \mu\text{g ml}^{-1}$ 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 azacethromycin 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 $\leq 0.03 \mu\text{g ml}^{-1}$), a VRE with an *ermB* gene (MICs 1 and $2 \mu\text{g ml}^{-1}$, respectively), an MRSA with a constitutively-expressed erythromycin ribosome methyltransferase (*c-erm*) gene (MICs $16 \mu\text{g ml}^{-1}$), and *Pseudomonas aeruginosa* (MICs 16 and $32 \mu\text{g ml}^{-1}$, 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 need⁴⁸.

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 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.

Received 7 December 2015; accepted 23 March 2016.

- Walsh, C. *Antibiotics: Actions, Origins, Resistance* (American Society for Microbiology Press, 2003).
- Fischbach, M. A. & Walsh, C. T. Antibiotics for emerging pathogens. *Science* **325**, 1089–1093 (2009).
- Wright, P. M., Seiple, I. B. & Myers, A. G. The evolving role of chemical synthesis in antibacterial drug discovery. *Angew. Chem. Int. Ed.* **53**, 8840–8869 (2014).
- Mcguire, J. M. *et al.* Ilotycin, a new antibiotic. *Antibiot. Chemother.* **2**, 281–283 (1952).
- Woodward, R. B. *et al.* Asymmetric total synthesis of erythromycin. 3. Total synthesis of erythromycin. *J. Am. Chem. Soc.* **103**, 3215–3217 (1981).
- Martin, S. F., Hida, T., Kym, P. R., Loft, M. & Hodgson, A. The asymmetric synthesis of erythromycin B. *J. Am. Chem. Soc.* **119**, 3193–3194 (1997).
- Kim, H. C. & Kang, S. H. Total synthesis of azithromycin. *Angew. Chem. Int. Ed.* **48**, 1827–1829 (2009).
- Andrade, R. B. Total synthesis of desmethyl macrolide antibiotics. *Synlett* **26**, 2199–2215 (2015).

- Khosla, C. Harnessing the biosynthetic potential of modular polyketide synthases. *Chem. Rev.* **97**, 2577–2590 (1997).
- Cane, D. E., Walsh, C. T. & Khosla, C. Harnessing the biosynthetic code: combinations, permutations, and mutations. *Science* **282**, 63–68 (1998).
- Marsden, A. F. A. *et al.* Engineering broader specificity into an antibiotic-producing polyketide synthase. *Science* **279**, 199–202 (1998).
- Park, S. R. *et al.* Genetic engineering of macrolide biosynthesis: past advances, current state, and future prospects. *Appl. Microbiol. Biotechnol.* **85**, 1227–1239 (2010).
- Kurath, P., Jones, P. H., Egan, R. S. & Perun, T. J. Acid degradation of erythromycin A and erythromycin B. *Experientia* **27**, 362 (1971).
- Bright, G. M. *et al.* Synthesis, *in vitro* and *in vivo* activity of novel 9-deoxy-9a-aza-9a-homoerythromycin A derivatives – a new class of macrolide antibiotics, the azalides. *J. Antibiot. (Tokyo)* **41**, 1029–1047 (1988).
- Morimoto, S., Takahashi, Y., Watanabe, Y. & Omura, S. Chemical modification of erythromycins. 1. Synthesis and antibacterial activity of 6-O-methylerythromycins-A. *J. Antibiot. (Tokyo)* **37**, 187–189 (1984).
- Fernandes, P. B. Methods for treating gastrointestinal diseases. WO patent 2010/048599 (2010).
- Putnam, S. D., Castanheira, M., Moet, G. J., Farrell, D. J. & Jones, R. N. CEM-101, a novel fluoroketolide: antimicrobial activity against a diverse collection of Gram-positive and Gram-negative bacteria. *Diagn. Microbiol. Infect. Dis.* **66**, 393–401 (2010).
- Leclercq, R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.* **34**, 482–492 (2002).
- Gaynor, M. & Mankin, A. S. Macrolide antibiotics: binding site, mechanism of action, resistance. *Curr. Top. Med. Chem.* **3**, 949–961 (2003).
- Subramanian, S. L., Ramu, H. & Mankin, A. S. in *Antibiotic Discovery and Development* (eds Dougherty, T. J. & Pucci, M. J.) 455–484 (Springer, 2012).
- Bryskier, A. Ketolides-telithromycin, an example of a new class of antibacterial agents. *Clin. Microbiol. Infect.* **6**, 661–669 (2000).
- Zhanel, G. G. *et al.* The ketolides: a critical review. *Drugs* **62**, 1771–1804 (2002).
- Retsema, J. *et al.* Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against Gram-negative organisms. *Antimicrob. Agents Chemother.* **31**, 1939–1947 (1987).
- Girard, A. E. *et al.* Pharmacokinetic and *in vivo* studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. *Antimicrob. Agents Chemother.* **31**, 1948–1954 (1987).
- Denis, A. & Agouridas, C. Synthesis of 6-O-methyl-azithromycin and its ketolide analog via Beckmann rearrangement of 9(E)-6-O-methyl-erythromycin oxime. *Bioorg. Med. Chem. Lett.* **8**, 2427–2432 (1998).
- Boeckman, R. K., Jr & Pruitt, J. R. A new, highly efficient, selective methodology for formation of medium-ring and macrocyclic lactones via intramolecular ketene trapping: an application to a convergent synthesis of (–)-kromycin. *J. Am. Chem. Soc.* **111**, 8286–8288 (1989).
- Boeckman, R. K. Jr & Perni, R. B. Studies directed toward the synthesis of naturally occurring acyltetramic acids. 2. Preparation of the macrocyclic subunit of ikarugamycin. *J. Org. Chem.* **51**, 5486–5489 (1986).
- Woodward, R. B. *et al.* Asymmetric total synthesis of erythromycin. 2. Synthesis of an erythronolide A lactone system. *J. Am. Chem. Soc.* **103**, 3213–3215 (1981).
- Rostovtsev, V. V., Green, L. G., Fokin, V. V. & Sharpless, K. B. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective “ligation” of azides and terminal alkynes. *Angew. Chem. Int. Ed.* **41**, 2596–2599 (2002).
- Berisio, R. *et al.* Structural insight into the antibiotic action of telithromycin against resistant mutants. *J. Bacteriol.* **185**, 4276–4279 (2003).
- Tu, D., Blaha, G., Moore, P. B. & Steitz, T. A. Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. *Cell* **121**, 257–270 (2005).
- Llano-Sotelo, B. *et al.* Binding and action of CEM-101, a new fluoroketolide antibiotic that inhibits protein synthesis. *Antimicrob. Agents Chemother.* **54**, 4961–4970 (2010).
- Bulkley, D., Innis, C. A., Blaha, G. & Steitz, T. A. Revisiting the structures of several antibiotics bound to the bacterial ribosome. *Proc. Natl Acad. Sci. USA* **107**, 17158–17163 (2010).
- Eyal, Z. *et al.* Structural insights into species-specific features of the ribosome from the pathogen *Staphylococcus aureus*. *Proc. Natl Acad. Sci. USA* **112**, E5805–E5814 (2015).
- Seiple, I. B., Hog, D. T. & Myers, A. G. Practical protocols for the preparation of highly enantioenriched silyl ethers of (R)-3-hydroxypentan-2-one, building blocks for the synthesis of macrolide antibiotics. *Synlett* **27**, 57–60 (2016).
- Seiple, I. B., Mercer, J. A. M., Sussman, R. J., Zhang, Z. & Myers, A. G. Stereocontrolled synthesis of syn-β-hydroxy-α-amino acids by direct aldolization of pseudophenamine glycinamide. *Angew. Chem. Int. Ed.* **53**, 4642–4647 (2014).
- Mikami, K., Kawakami, Y., Akiyama, K. & Aikawa, K. Enantioselective catalysis of ketoester-ene reaction of silyl enol ether to construct quaternary carbons by chiral dicationic palladium(II) complexes. *J. Am. Chem. Soc.* **129**, 12950–12951 (2007).
- Mukaiyama, T., Narasaka, K. & Banno, K. New aldol type reaction. *Chem. Lett.* **2**, 1011–1014 (1973).

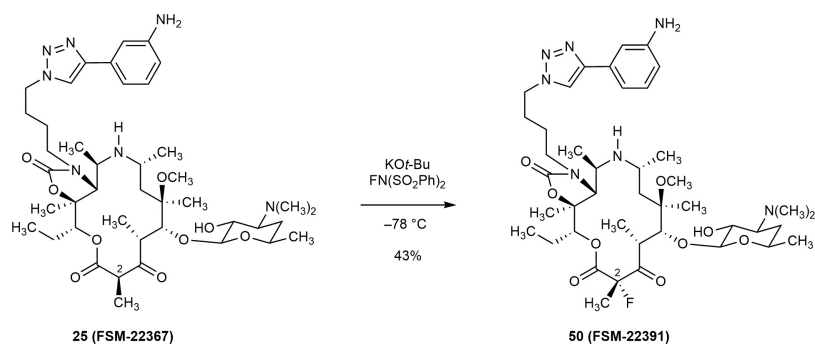
39. Zhang, Z., Kitamura, Y. & Myers, A. G. An efficient directed Claisen reaction allows for rapid construction of 5,6-disubstituted 1,3-dioxin-4-ones. *Synthesis* **47**, 2709–2712 (2015).
40. Hauske, J. R. & Schulte, G. R. Desosamino derivatives of macrolides as immunosuppressants and antifungal agents. WO patent 1993018042 (1993).
41. Zhang, Z., Fukuzaki, T. & Myers, A. G. Synthesis of D-desosamine and analogs by rapid assembly of 3-amino sugars. *Angew. Chem. Int. Ed.* **55**, 523–527 (2016).
42. Leyes, A. E. & Poulter, C. D. Synthesis of (*R*)-[2-²H]isopentenyl diphosphate and determination of its enantiopurity by ²H NMR spectroscopy in a lyotropic medium. *Org. Lett.* **1**, 1067–1070 (1999).
43. Tan, Z. & Negishi, E.-i. An efficient and general method for the synthesis of α,ω -difunctional reduced polypropionates by Zr-catalyzed asymmetric carboalumination: synthesis of the scyphostatin side chain. *Angew. Chem. Int. Ed.* **43**, 2911–2914 (2004).
44. Santaniello, E., Ferraboschi, P. & Grisenti, P. An efficient chemo-enzymatic approach to the enantioselective synthesis of 2-methyl-1,3-propanediol derivatives. *Tetrahedr. Lett.* **31**, 5657–5660 (1990).
45. Thompson, A. S., Corley, E. G., Huntington, M. F. & Grabowski, E. J. J. Use of an ephedrine alkoxide to mediate enantioselective addition of an acetylide to a prochiral ketone: asymmetric synthesis of the reverse transcriptase inhibitor L-743,726. *Tetrahedr. Lett.* **36**, 8937–8940 (1995).
46. Sato, F., Ishikawa, H., Watanabe, H., Miyake, T. & Sato, M. Specific hydromagnesiation of prop-2-ynyl alcohols. A simple and specific route to terpenoids. *J. Chem. Soc. Chem. Commun.* 718–720 (1981).
47. Ashley, G. *et al.* Ketolide antibacterials. US patent 6,590,083 (2003).
48. Antibiotic Resistance Threats in the United States, 2013. <http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf> (CDC, US Dept Health and Human Services, 2013).
49. Charest, M. G., Lerner, C. D., Brubaker, J. D., Siegel, D. R. & Myers, A. G. A convergent enantioselective route to structurally diverse 6-deoxytetracycline antibiotics. *Science* **308**, 395–398 (2005).
50. Liu, F. & Myers, A. G. Development of a platform for the discovery and practical synthesis of new tetracycline antibiotics. *Curr. Opin. Chem. Biol.* **32**, 48–57 (2016).

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

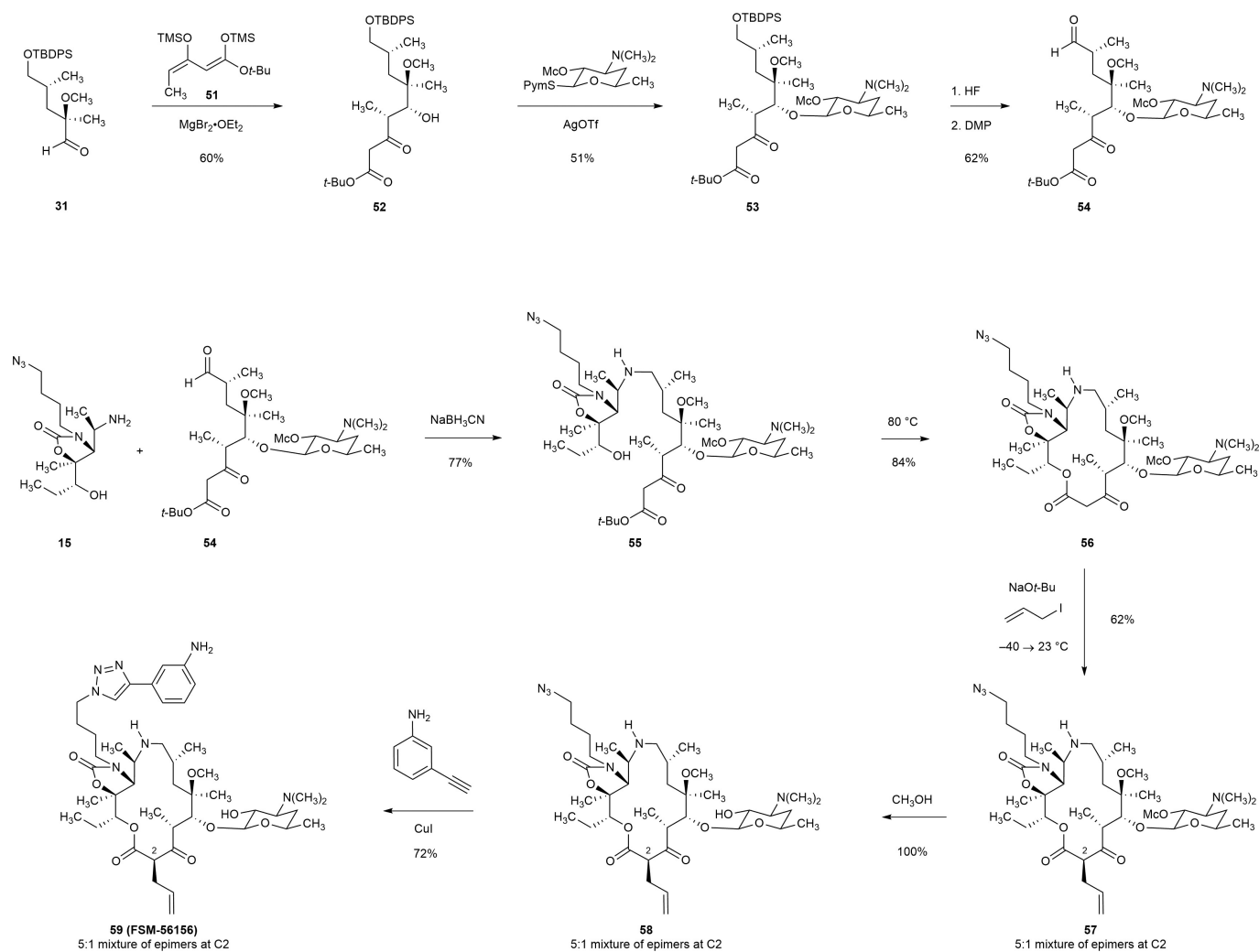
Acknowledgements We acknowledge funding from Alistair and Celine Mactaggart, from the Gustavus and Louise Pfeiffer Research Foundation, and from the Blavatnik Biomedical Accelerator Program at Harvard University. We thank NERCE (NIH project number U54 AI057159), W. Weiss (Univ. North Texas), and R. Alm and S. Lahiri (Macrolide Pharmaceuticals) for measuring MIC values, R. Alm and T. Dougherty (Harvard Medical School) for genetic characterization of a microbial resistance gene, and S.-L. Zheng (Harvard University) for conducting X-ray crystallographic analyses. I.B.S. acknowledges postdoctoral fellowship support from the National Institutes of Health (F32GM099233); Z.Z. is a Howard Hughes Medical Institute International Student Research fellow; A.L.-M. acknowledges postdoctoral fellowship support from the Swiss National Science Foundation (PBGEPE2-139864) and the Novartis Foundation; D.T.H. is indebted to the Deutsche Forschungsgemeinschaft (DFG) for a postdoctoral fellowship (HO 5326/1-1); T.F. acknowledges Daiichi-Sankyo Co., Ltd, for financial support; and Y.K. acknowledges support from the Engineering Promotion Fund of Gifu University.

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.-M. 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.-M., 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 analogues; 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.

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).

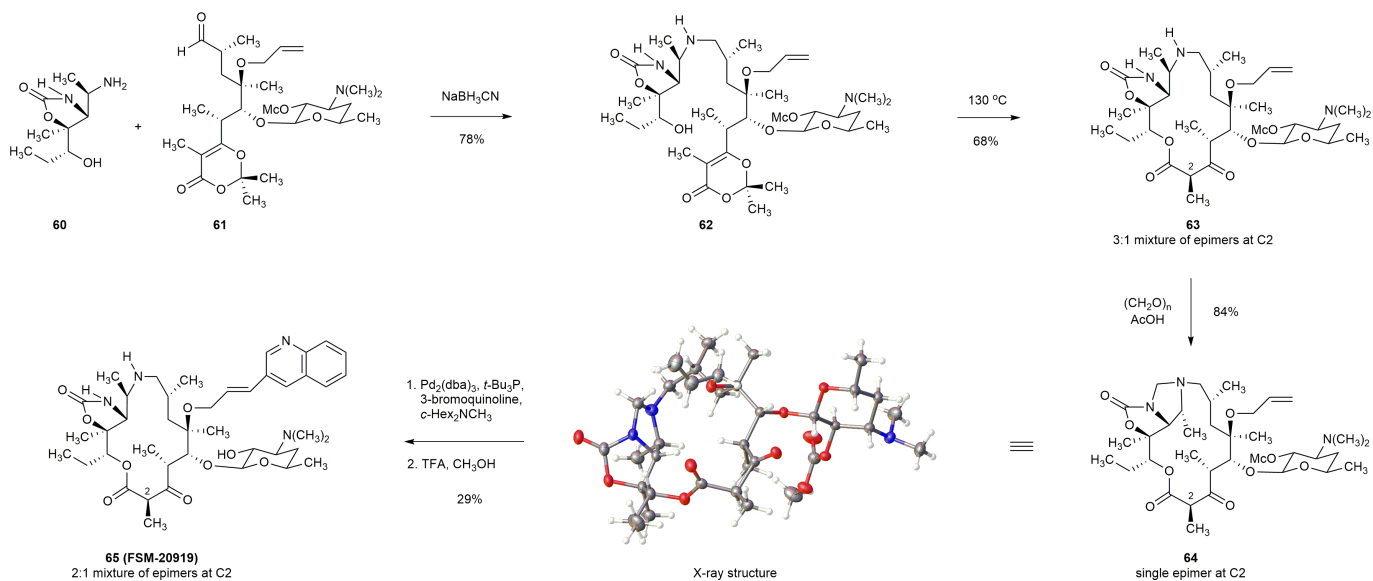


Extended Data Figure 1 | Synthesis of a C2-fluoro 14-membered azaketolide by a late-stage fluorination reaction. Subjection of β -keto lactone **25** (FSM-22367) to potassium *tert*-butoxide (1.0 equiv.) at $-78\text{ }^{\circ}\text{C}$ followed by *N*-fluorobenzenesulfonimide (1.0 equiv.) afforded **50** (FSM-22391) in 43% yield.



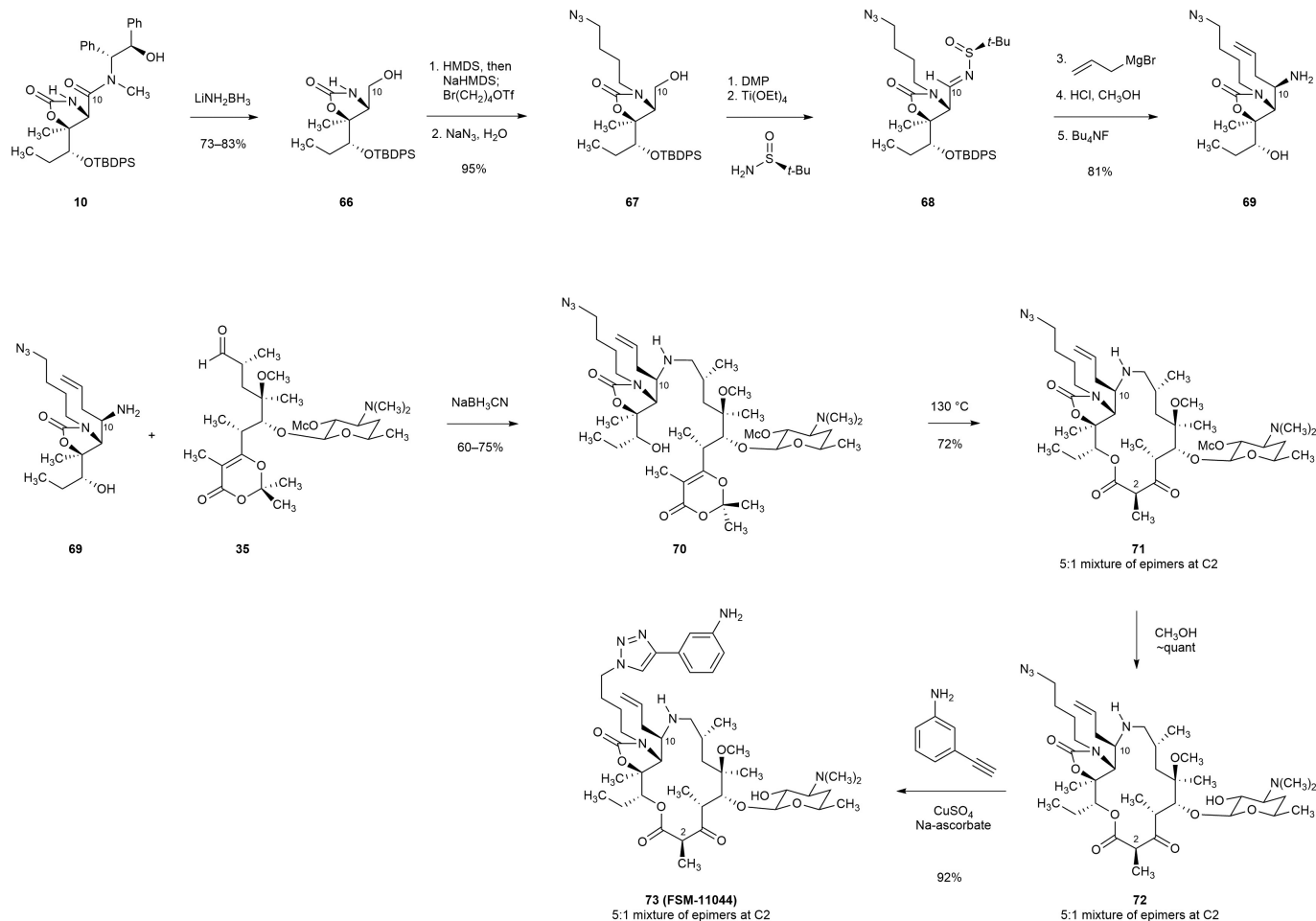
Extended Data Figure 2 | Synthesis of a 15-membered azaketolide with a modified C2-substituent. Thermolysis of a β -keto *tert*-butyl ester substrate (55) proceeded at a lower temperature (80 °C) than that for dioxolenone substrates (132 °C) and afforded a 15-membered macrocycle without substitution 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).



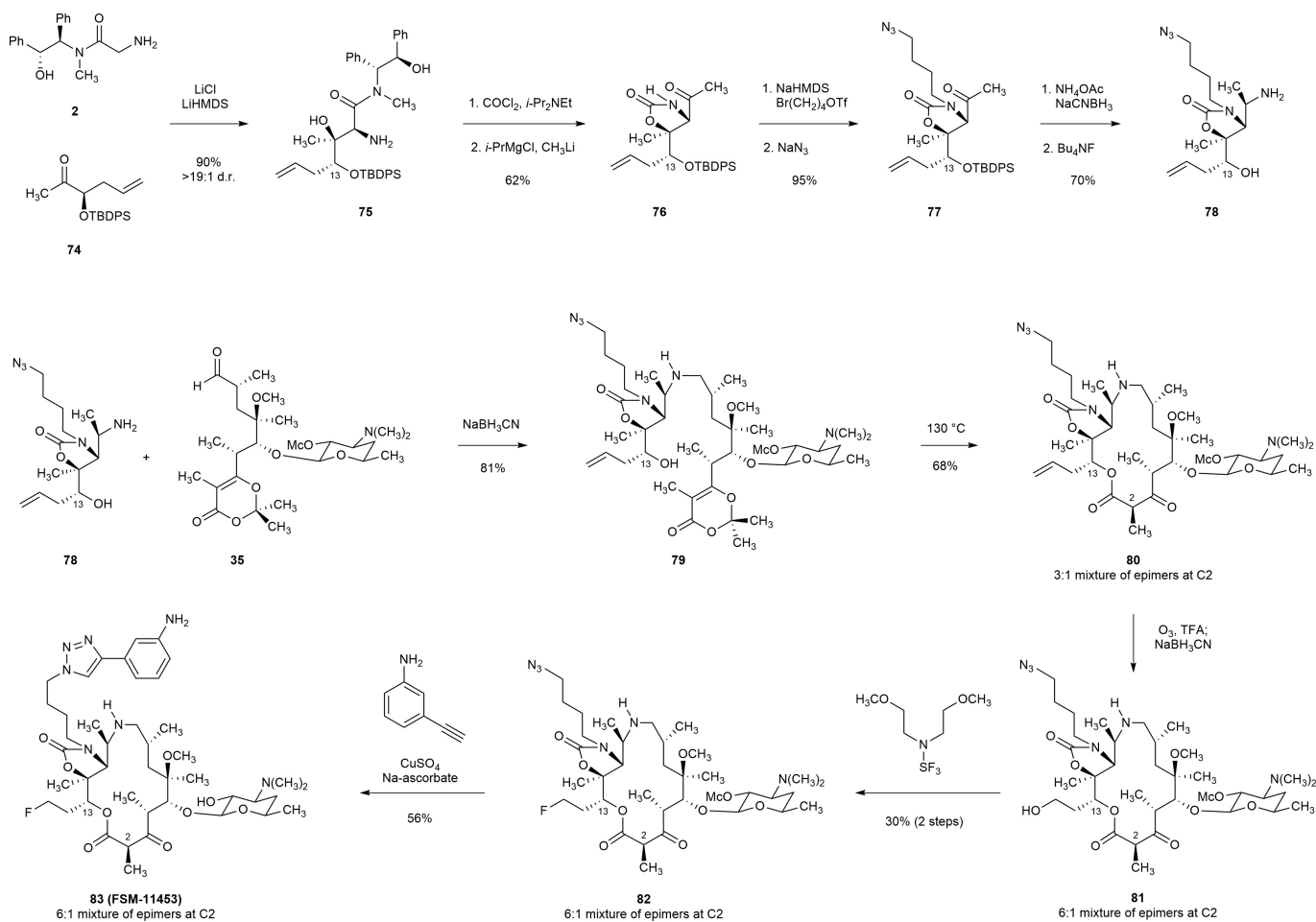
Extended Data Figure 3 | Synthesis of a 15-membered azacethromycin hybrid. Macrolactone **63** was prepared from amine **60** and aldehyde **61** in two steps (via **62**) by a reductive amination–macrocyclization sequence. Treatment of **63** with paraformaldehyde (6.0 equiv.) and acetic acid (10.0 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, CH_3OH) cleaved the imidazolidine group, affording **65** (FSM-20919; 29%, two-step yield).



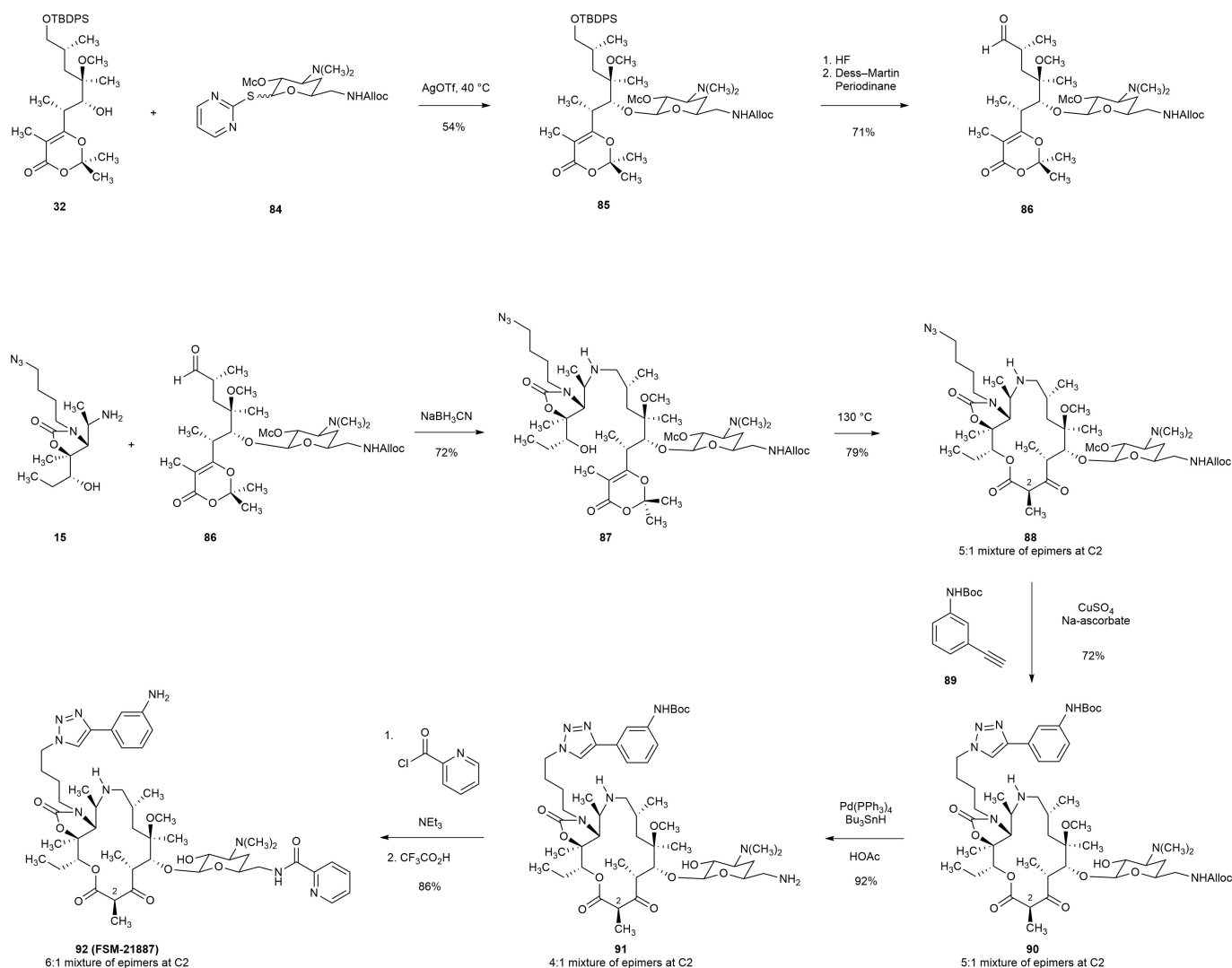
Extended Data Figure 4 | Synthesis of a 15-membered azaketolide with a modified C10-substituent. *N*-*tert*-butylsulfinyl imine **68** (prepared in five steps via **66** and **67** from amide **10**) allowed for the stereocontrolled introduction of various C10-substituents. For example, addition of allylmagnesium bromide proceeded with >20:1 stereoselectivity 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).



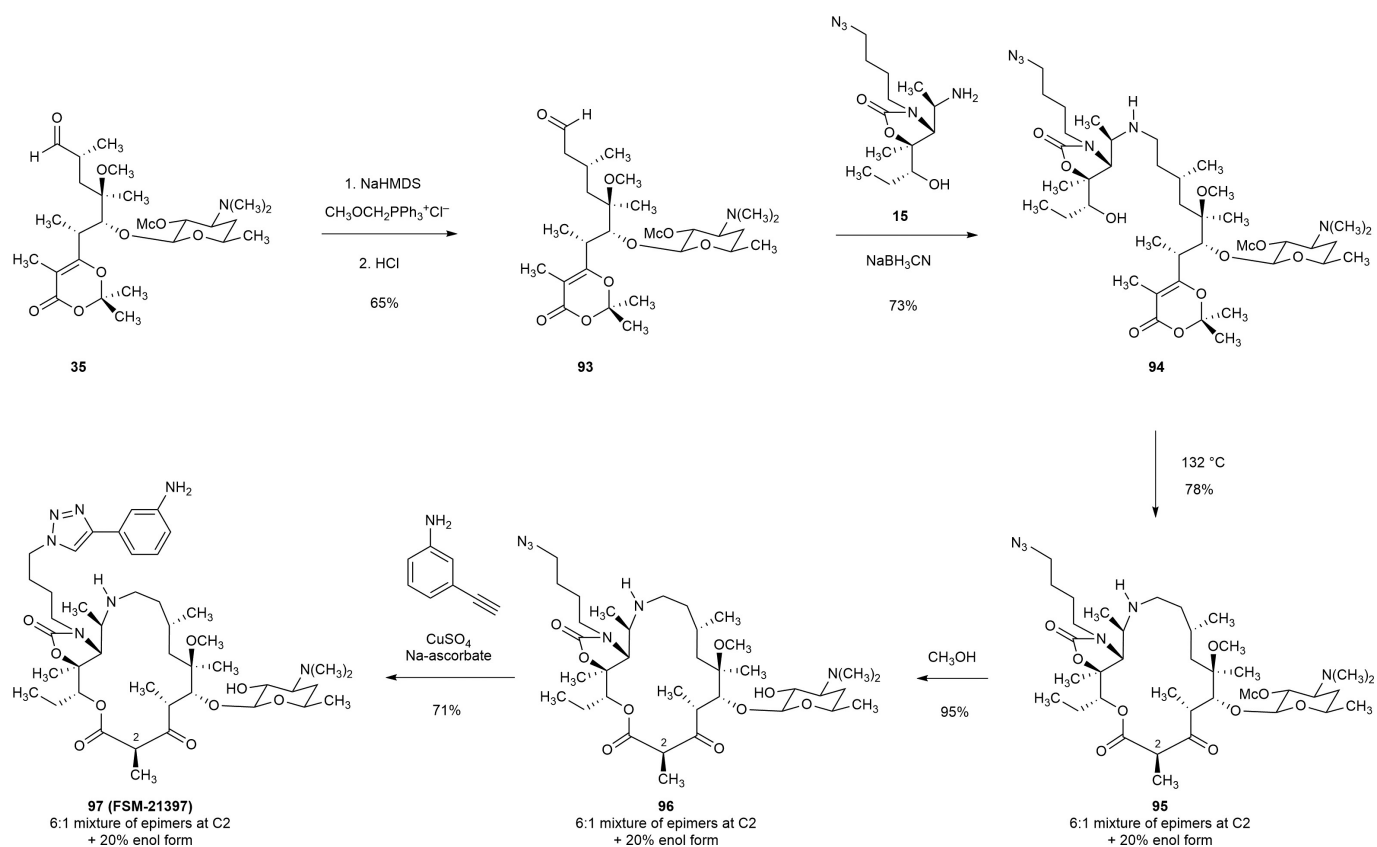
Extended Data Figure 5 | Synthesis of a 15-membered azaketolide with a modified C13-substituent. Modification of position C13 was achieved by modification of a single component, in this case the ketone building block **74** depicted above. Reductive coupling of **78** and **35** united the left- and right-halves to give **79**; subsequent thermal macrocyclization provided macrolactone **80**. The allyl group within intermediate **80** was

cleaved upon ozonolysis (O_3 , trifluoroacetic acid); reductive workup with sodium cyanoborohydride afforded alcohol **81**. Subjecting 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 desamine 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

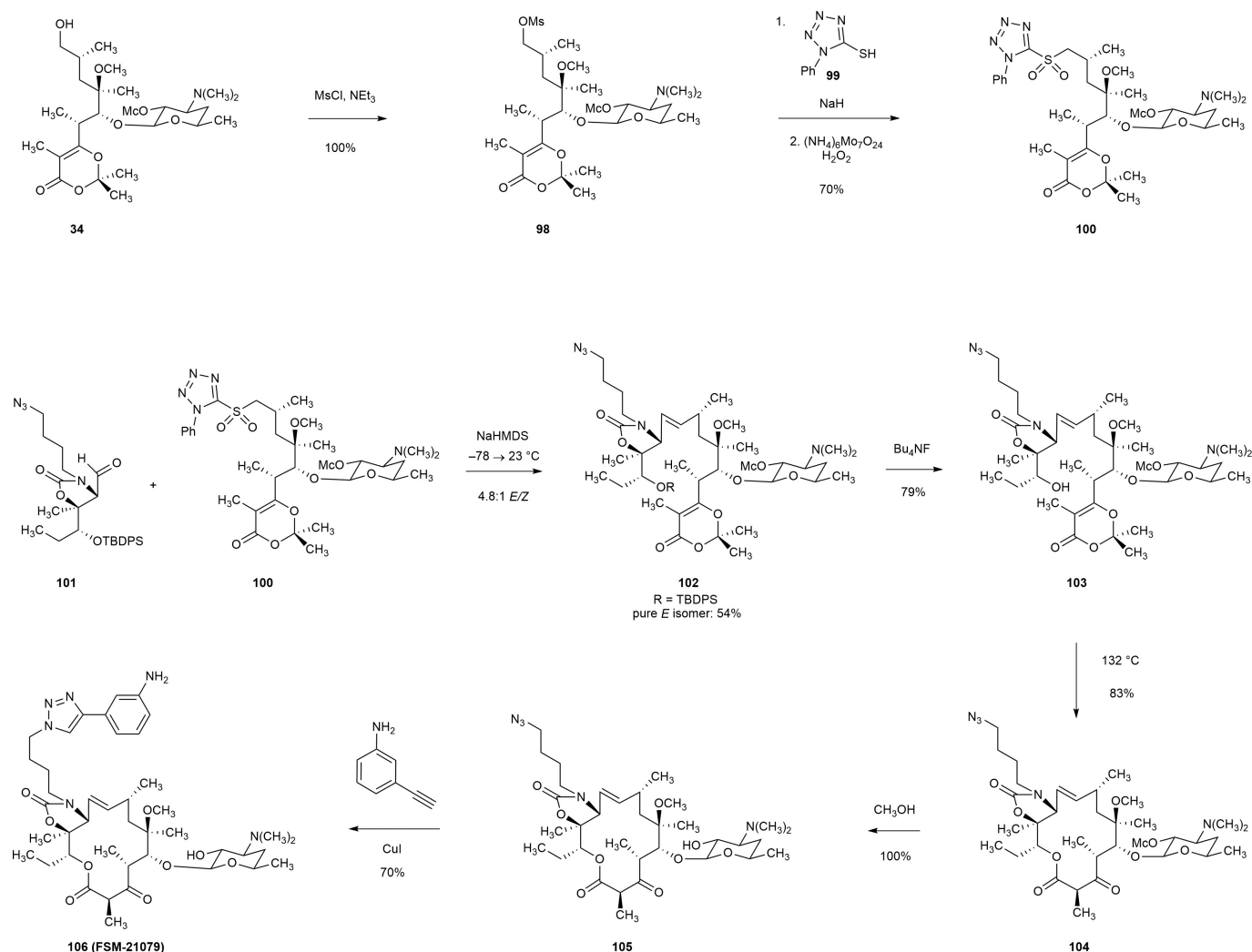
allyloxycarbonyl protective group, giving rise to amine **91** (92% yield). The latter intermediate has been transformed into a number of fully synthetic macrolides with modified desamine sugar residues. For example, acylation of the primary amino group of intermediate **91** with pyridine-2-carbonyl 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.

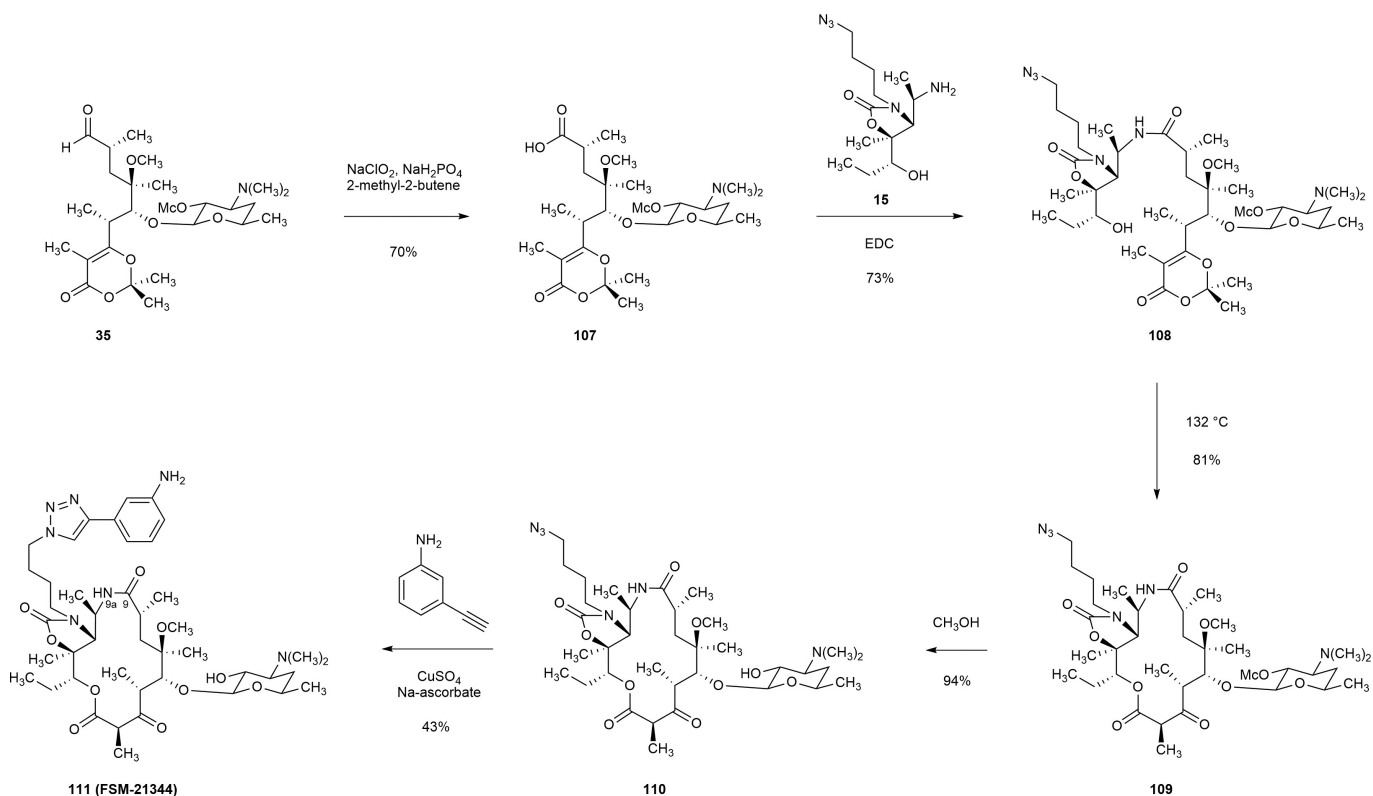
Homologation of aldehyde **35** was achieved by a Wittig olefination reaction ($\text{CH}_3\text{OCH}_2\text{PPh}_3^+\text{Cl}^-$, NaHMDS) followed by hydrolysis of the resulting enol ether to afford aldehyde **93** in 65% yield. Reductive coupling

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).



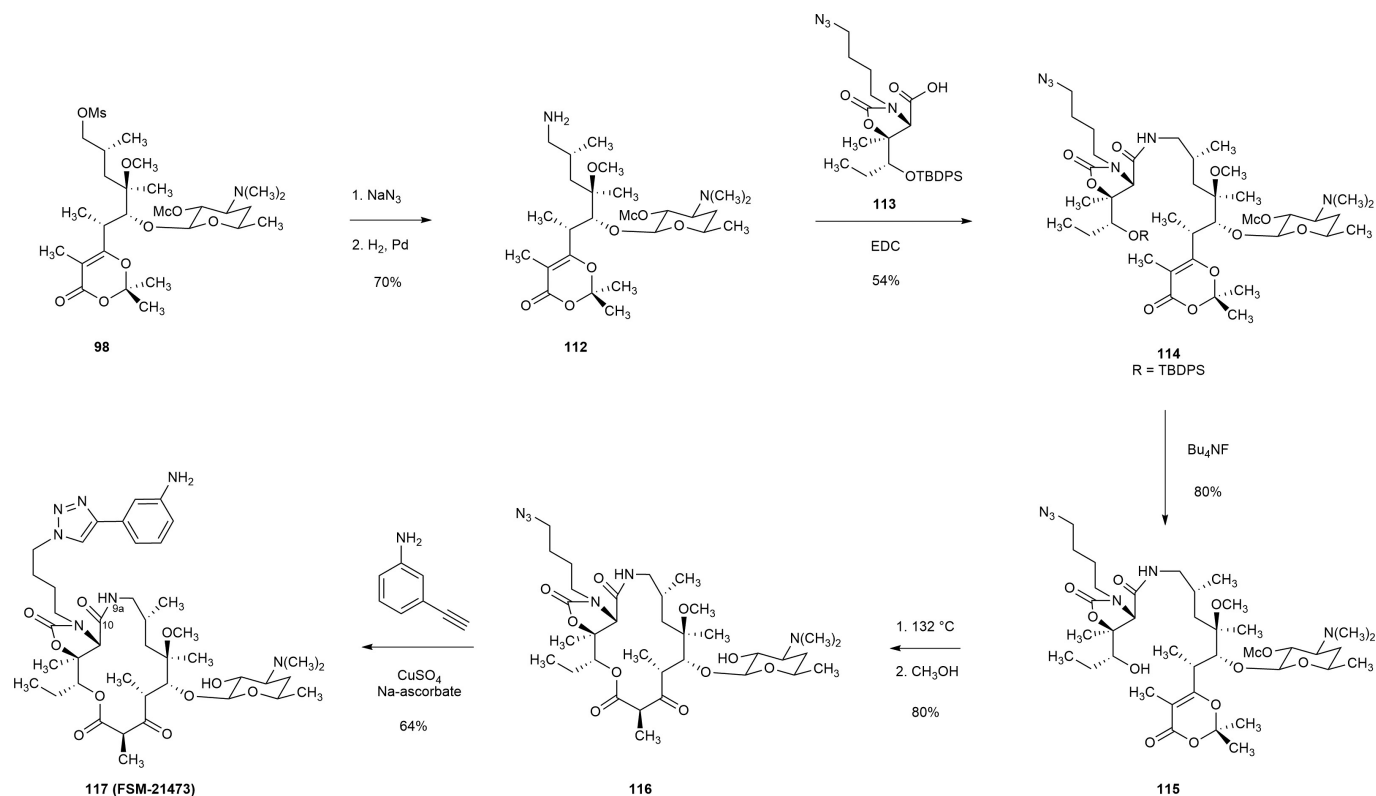
Extended Data Figure 8 | Synthesis of a 14-membered macrolide with a *trans*-olefin linkage. Mesylate **98** was prepared in quantitative yield by treatment of alcohol **34** with methanesulfonyl chloride (1.50 equiv.) and triethylamine (2.0 equiv.). Displacement of the mesylate group in **98** with sodium 1-phenyl-1*H*-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 **106** (FSM-21079) in two additional steps (methanolysis 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 (H_2 , Pd). The coupling of amine **112** and acid **113** proceeded in 54% yield. The product, amide **114**,

was desilylated (Bu_4NF , 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.