

Cite this: *Chem. Sci.*, 2022, 13, 3447

All publication charges for this article have been paid for by the Royal Society of Chemistry

The convergent total synthesis and antibacterial profile of the natural product streptothricin F†

Matthew G. Dowgiallo,^a Brandon C. Miller,^a Mintesinot Kassu,^a Kenneth P. Smith,^{ef} Andrew D. Fetigan,^a Jason J. Guo,^{acd} James E. Kirby^{def} and Roman Manetsch^{id*abc}

A convergent, diversity-enabling total synthesis of the natural product streptothricin F has been achieved. Herein, we describe the potent antimicrobial activity of streptothricin F and highlight the importance of a total synthesis that allows for the installation of practical divergent steps for medicinal chemistry exploits. Key features of our synthesis include a Burgess reagent-mediated 1,2-*anti*-diamine installation, diastereoselective azidation of a lactam enolate, and a mercury(II) chloride-mediated desulfurization-guanidination. The development of this chemistry enables the synthesis and structure–activity studies of streptothricin F analogs.

Received 18th November 2021
Accepted 16th February 2022

DOI: 10.1039/d1sc06445b

rsc.li/chemical-science

Introduction

The streptothricins are a class of natural products exhibiting potent antimicrobial activity against multidrug-resistant, Gram-negative bacteria. Streptothricins were first isolated in 1942 by Waksman and Woodruff from *Streptomyces lavendulae*¹ and have since been identified under a variety of pseudonyms from other *Streptomyces* species.^{2–9} Isolates of streptothricin generally exist as complex mixtures of homologs A–F, and X (Fig. 1). These mixtures are typically referred to as “nourseothricin” and contain varying ratios of the component streptothricins, with streptothricin F (1) being the principal component. Nourseothricin attracted initial interest because of the impressive Gram-positive and Gram-negative antimicrobial activity^{1,6,10–13} and high water solubility^{10,14–16} of the streptothricins. However, this natural product class has not been pursued as a therapeutic due to inherent toxicity.^{7,17–20} Additionally, isolation of the individual streptothricin components of nourseothricin has proven to be challenging, with limited reports of biological characterization on demonstrated pure material.^{6,19,21} The streptothricin backbone consists of a carbamoylated gulosamine sugar core (Fig. 1, black) affixed with a streptolidine lactam moiety (red)

and β-lysine homopolymer (blue) attached to the C7 and C8 amines, respectively. Streptolidine is an unusual guanidine-containing amino acid that has been isolated as a streptothricin hydrolysis product and appears to be unique to this natural product class.²² Additionally, the rarity of β-amino acids adds another layer of structural peculiarity, synthetic challenge, and a unique opportunity for medicinal chemistry discovery.²³

Streptothricins were discovered to be vulnerable to resistance through two mechanisms. The β-amine of the β-lysine moiety is susceptible to an acylation-based resistance mechanism in bacteria containing streptothricin acetyl transferases^{24–30} while enzymatic hydrolysis of the streptolidine moiety proceeds through a less-prominent resistance pathway.³¹ Streptothricin F was previously found to inhibit prokaryotic ribosomal translocation and also induce significant miscoding. That is, like aminoglycosides, they cause incorrect amino acids to be added to the growing peptide chains during protein synthesis and thereby poison the bacterial cell leading to cell death. Experimentally streptothricin F was previously found not to effect nucleic acid synthesis.^{32,33} Our main attraction to this natural product class derives from previous reports that have demonstrated streptothricin F (1) to be less toxic than other

^aDepartment of Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA. E-mail: r.manetsch@northeastern.edu

^bDepartment of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA

^cCenter for Drug Discovery, Northeastern University, Boston, MA, USA

^dBarnett Institute for Chemical and Biological Analysis, Northeastern University, Boston, MA, USA

^eDepartment of Pathology, Beth Israel Deaconess Medical Center, Boston, MA, USA

^fHarvard Medical School, Boston, MA, USA

† Electronic supplementary information (ESI) available: Detailed experimental procedures and characterization data for all new compounds. See DOI: 10.1039/d1sc06445b

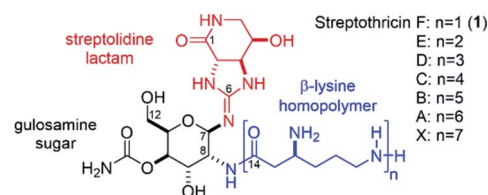
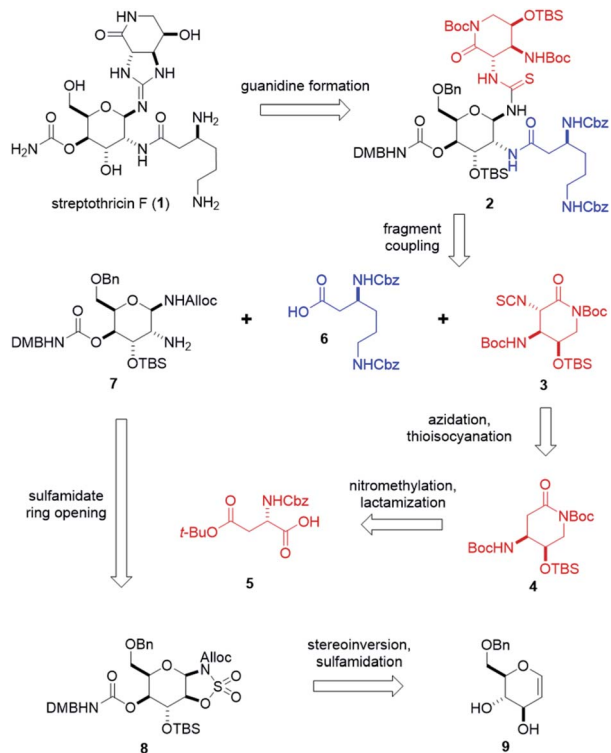


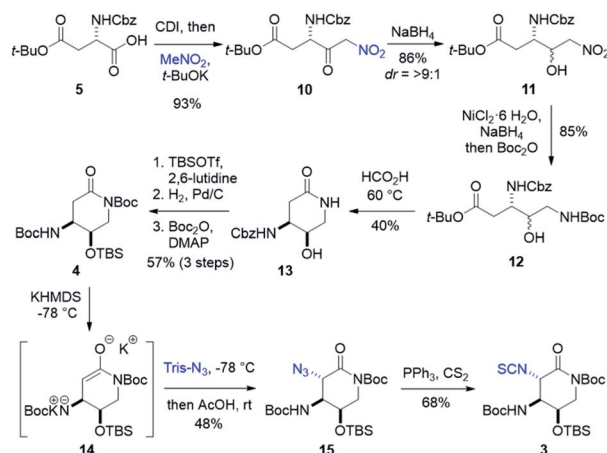
Fig. 1 Structures of the streptothricins A–F, and X. The streptolidine lactam is shown in red, the gulosamine core is shown in black, and the β-lysine homopolymer is shown in blue.





Scheme 1 Retrosynthetic analysis of streptothricin F (1).

streptothricins. Studies of purified streptothricins in mice indicate that toxicity is influenced directly by the unit length (n) of the β -lysine homopolymer. Streptothricin F, ($n = 1$, LD₅₀: 300 mg kg⁻¹) shows remarkably less toxicity than streptothricin E ($n = 2$, LD₅₀: 26 mg kg⁻¹), streptothricin D ($n = 3$, LD₅₀: ~10 mg kg⁻¹), and streptothricin C ($n = 4$, LD₅₀: ~10 mg kg⁻¹).^{6,15,34} Conversely, antimicrobial activity favors longer β -lysine homopolymer chains, with nourseothricin (*K. pneumoniae* Nevada strain AR-0636 MIC: 0.15 μ g mL⁻¹) and streptothricin D (MIC: 0.19 μ g mL⁻¹) exhibiting approximately 4-fold more activity (per mol) than streptothricin F (MIC: 1 μ M).³⁴

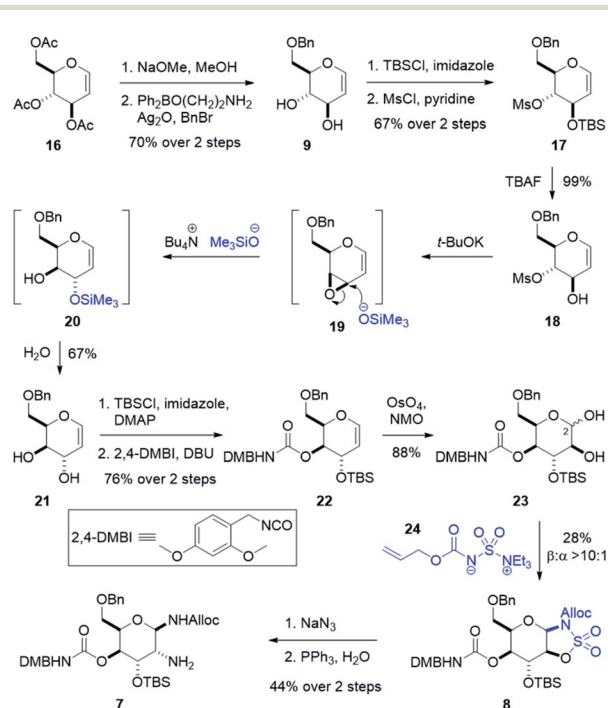


Scheme 2 Synthesis of streptolidine isothiocyanate 3.

Results and discussion

To fully explore the therapeutic potential of this promising scaffold, it is important to develop an efficient and robust total synthesis to produce significant quantities of streptothricins and analogs. A single total synthesis of streptothricin F has been reported in the literature by Shiba and co-workers,³⁵ and no other streptothricin has been attained through synthetic means exclusively. While a landmark for its time, Shiba's synthesis contains over 46 total steps with a longest linear sequence of 25 steps and an overall yield of less than 0.28%.³⁶ While Shiba's synthesis contains elements of convergence, 12 synthetic steps take place after the first fragment coupling, including the installation of a stereocenter. Drawing inspiration from Shiba's efforts and the promising attributes of streptothricin F, we have designed a total synthesis of streptothricin F that readily enables SAR exploration. Through the incorporation of late-stage fragment coupling, we believe independent modification of the three structural components of streptothricin F is possible. The intended design of our synthesis is to facilitate rapid, combinatorial-like library generation of streptothricin F analogs that are targeted to evade known resistance pathways and maintain, or further reduce, low toxicity. Herein, we report our highly convergent, diversity-enabling streptothricin F total synthesis consisting of 35 total steps, with a longest linear sequence of 19 steps and an overall yield of 0.40%.

Retrosynthetically, our synthesis stems from two key disconnections at the C7 and C8 amines on the gulosamine core (Scheme 1). This approach hedged the production of streptothricin F (1) on a Lewis acid-catalyzed guanidine closure of thiourea 2, followed by global, stepwise deprotection. We



Scheme 3 Synthesis of the gulosamine 7.



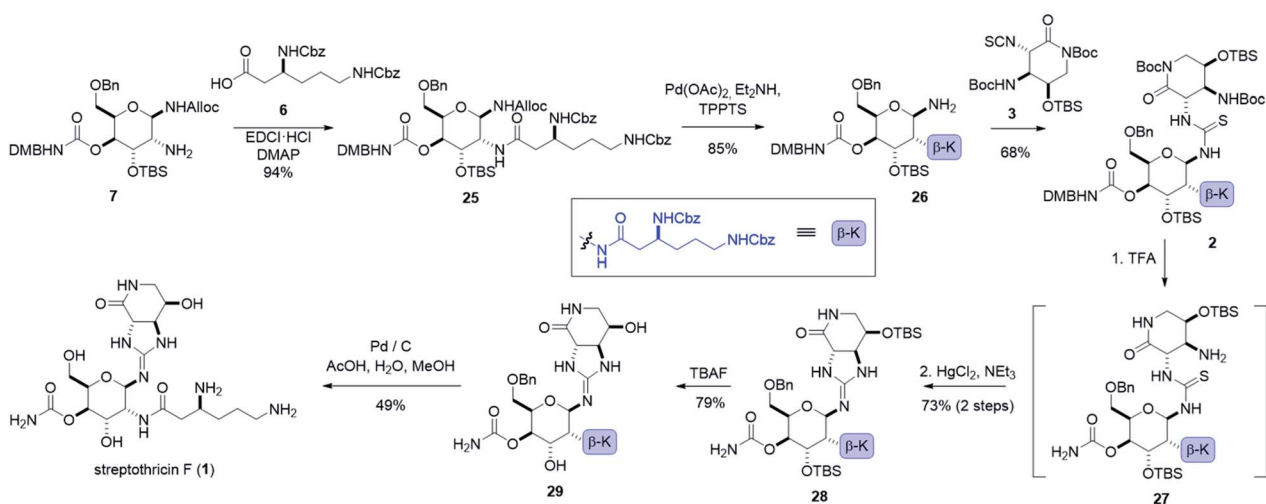
envisioned thiourea **2** as the product of fragment couplings of isothiocyanate **3**, partially protected β -lysine **6**, and gulosamine **7**. The first of these three fragments, isothiocyanate **3**, can be generated through stereoselective azidation and tandem Staudinger–aza-Wittig thioisocyanation of lactam **4**. Preparation of lactam **4** relied on nitromethylation of protected aspartic acid **5**, followed by a formic acid-promoted deprotective-lactamization. Prior to coupling with isothiocyanate **3**, partially protected β -lysine **6** and gulosamine **7** would be coupled at the C8 amine. We anticipated that access to gulosamine **7** could arise from nucleophilic ring-opening of β -sulfamidate **8**. Burgess reagent-sulfamidation to obtain β -sulfamidate **8** was readily performed following stereoinversion and dihydroxylation of glucal sugar **9**, which is obtained in decagram quantities from commercially available starting material.

Forward synthesis began with construction of the streptolidine moiety of streptothricin F (Scheme 2). Commercially available *N*-Cbz-L-aspartic acid 4-*tert*-butyl ester **5** was treated with carbonyl diimidazole producing an activated anhydride that reacted smoothly with a solution of excess nitromethane and stoichiometric *t*-BuOK³⁷ to yield nitroketone **10**.^{38,39} Diastereoselective reduction was carried out under Felkin–Ahn conditions, allowing hydride attack from the less hindered face of **10** resulting in >9 : 1 dr of the desired *erythro* (3*S*,4*R*) nitroalcohol **11**.^{39–41} Nitro reduction through generation of nickel boride followed by immediate addition of di-*tert*-butyl dicarbonate in one pot produced dicarbamate **12** as a mixture of diastereomers.^{42,43} Warming in formic acid deprotected the previously installed Boc group and promoted lactamization to afford **13**, where the diastereomeric mixture could be separated. Silylation of **13** with TBS triflate, followed by hydrogenation to remove benzyl carbamate and subsequent Boc protection produced dicarbamate lactam **4** in good yield over three steps. Deprotonation of **4** to lactam enolate **14** and treatment with trisyl azide provided α -azidolactam **15** as a single diastereomer.^{44,45} The complete selectivity of this reaction is likely directed through the synergistic steric effects of a congested top

face of enolate **14** and the bulky nature of trisyl azide. A moderate yield of **15** was observed as a consequence of enolate formation and substrate stability. To avoid stepwise isothiocyanate formation and risk lactam hydrolysis, we looked to the tandem Staudinger–aza-Wittig method for a one pot conversion of azides to isothiocyanates.^{46,47} Adhering to this protocol, α -azidolactam **15** was treated with triphenylphosphine and excess carbon disulfide to yield isothiocyanate **3**, with conservation of our lactam ring.^{46,47}

To assemble the gulosamine core, the first task was to generate the rare gulal sugar core from readily obtained starting material while enabling discrete alcohol functionalization (Scheme 3). Construction of gulal sugars has not been widely explored; however, two reliable methods have been reported. The first, pioneered by the Danishefsky laboratory, used a thiophenol Ferrier-type/Mislow–Evans [2,3] sigmatropic rearrangement starting from tri-*O*-acetyl-D-galactal.^{48–50} The second method, reported by Crotti and co-workers, started from tri-*O*-acetyl-D-glucal and relied on a regioselective epoxide ring-opening to invert both the 9- and 10-position alcohols.^{51,52} Our route hewed more closely to the Crotti approach due to scalability and availability of inexpensive starting material. Deacylation of **16** and regioselective benzylation of the primary alcohol using Taylor's aminoethyl diphenylborinate catalyst⁵³ and silver oxide as an activator gave benzyl glucal **9**. Silylation with good regioselectivity and subsequent mesylation produced fully functionalized glucal **17**. Treatment with TBAF yielded our stereoinversion precursor **18**, which was treated with *t*-BuOK to form epoxide **19**. When exposed to freshly prepared tetrabutylammonium trimethylsilylanolate,⁵² epoxide **19** is selectively opened at the allylic position to give TMS alcohol **20** which is hydrolyzed upon work-up to give 6-*O*-benzyl-D-gulal **21**. Silylation of **21** was performed with high regioselectivity and subsequent treatment with freshly prepared 2,4-dimethoxybenzyl isocyanate^{49,54} yielded the carbamoylated gulal **22**.

To install our 1,2-diamine functionality on the gulal sugar, we employed Burgess reagent chemistry pioneered by Nicolaou



Scheme 4 The convergent total synthesis of streptothricin F.



and co-workers.⁵⁵ Among other uses for the Burgess reagent, a method for preparation of 1,2-diamino sugars was reported, which we adopted for our synthesis. Dihydroxylation of gulal **22** gave diol **23** as an 8 : 1 mixture of anomers favoring the α -anomer, as implicated by the coupling constant of the anomeric proton ($J_{1,2} = 11.3$ Hz). Addition of the alloc-modified Burgess reagent **24** produced a disulfamate intermediate that reacts *via* C2 delivery to give β -sulfamidate **8** (>10 : 1 dr), (see ESI†).⁵⁵ Ring opening of β -sulfamidate **8** with sodium azide followed by Staudinger reduction yielded gulosamine **7**.^{55,56} To generate our C8 linkage, gulosamine **7** was coupled to partially protected β -lysine **6** (attained through homologation of α -lysine, see the ESI†) to give protected β -lysyl-gulosamine **25** (Scheme 4). Allyl carbamate deprotection of **25** proceeded through a catalytic allyl transfer mechanism to avoid anomerization. This mild deprotection method uses the palladium–TPPTS complex in tandem with diethyl amine as an allyl acceptor to generate β -lysyl-gulosamine **26**.^{55,57} Coupling of β -lysyl-gulosamine **26** with previously prepared isothiocyanate **3** yielded thiourea **2**, and completed our C7 linkage. Coupling of β -lysyl-gulosamine **26** with previously prepared isothiocyanate **3** yielded thiourea **2**, and completed our C7 linkage.

At this stage (four steps from the completion of the synthesis), all streptothricin F stereocenters are set, and our three synthetic routes have converged. Treatment of thiourea **2** with TFA removed both Boc groups as well as the 2,4-dimethoxybenzyl moiety to yield thiourea **27** which was unstable to purification methods. The crude reaction mixture containing **27** was therefore directly cyclized to guanidine **28** through mercury(II) chloride mediated desulfurization with triethylamine (for detailed optimization attempts and alternative approaches to prepare guanidine **28** see the ESI†). Two consecutive deprotections from guanidine **28** would complete our total synthesis. Silyl removal with TBAF provided partially deprotected streptothricin F **29** and under conditions similar to the Shiba total synthesis, benzyl and carboxybenzyl groups were removed in

a single step through hydrogenolysis in acidic solvent.³⁵ This hydrogenolysis provided streptothricin F (**1**) as an acetate salt; however, we desired to convert streptothricin F acetate to the sulfate to compare the activity and spectral data of synthetic streptothricin F more accurately to streptothricin F sulfate isolated from commercially available nourseothricin sulfate. Purified streptothricin F acetate was acidified to a pH of 2 in H₂SO₄, precipitated from methanol-diethyl ether, and collected *via* centrifugation.⁵⁸

The comparison of antimicrobial activity of synthetic streptothricin F sulfate to isolated streptothricin F sulfate purified in our laboratory is shown in Table 1. Our purification method was adopted from Taniyama *et al.* with modifications to column length and flow rate.⁶ Upon loading a ~300 mg sample of commercially available nourseothricin sulfate onto a glass column (150 × 2.4 cm) packed with Sephadex LH-20 afforded ~75 mg of pure streptothricin F and ~15 mg of pure streptothricin D, as well as mixed fractions. The microbes highlighted in Table 1 represent a diverse panel of Gram-positive and Gram-negative pathogens, many of which have been designated as either urgent or serious threats by the CDC and the WHO for their resistance capabilities,^{59–61} or are surrogates for CDC category A or B biothreat pathogens. Many of these species are also members of the so-called ESKAPE pathogens⁶² for which emerging antibiotic resistance threatens to eliminate effectiveness of all currently available antibiotics. Of note, both natural and synthetic streptothricin F were found to be active against the following: vancomycin-resistant *Staphylococcus aureus*; *Bacillus anthracis* (the cause of anthrax); multi-drug-resistant species of Gram-negative pathogens including the pan-resistant *K. pneumoniae*,⁶³ *Escherichia coli* expressing the colistin resistance gene *mcr-1*, and *Acinetobacter baumannii*; *Yersinia pestis* (the cause of bubonic plague); and *Francisella tularensis* (the cause of tularemia). However, there was low activity against *Burkholderia* and *Pseudomonas* strains tested. As a useful metric of comparison, the minimum inhibitory

Table 1 Antimicrobial activity comparison of synthetic and isolated streptothricin F

Organism	Phenotype	MIC values ($\mu\text{g mL}^{-1}$)	
		Isolated S-F	Synthetic S-F
Gram-positive	<i>S. aureus</i> ATCC 29213	4	4
	<i>S. aureus</i> (VRSA) NR46422	4	8
	<i>S. aureus</i> (VRSA) NR49120	4	16
	<i>S. aureus</i> (VRSA) NR46420	4	16
Gram-negative	<i>A. baumannii</i> ATCC 17978	2	4
	<i>A. baumannii</i> MSRN 1450	16	8
	<i>B. cenocepacia</i> clinical isolate K56-2 (ET-12 clone)	>64	>64
	<i>B. thailandensis</i> NR9908	>64	>64
	<i>B. thailandensis</i> NR9909	>64	>64
	<i>E. coli</i> ATCC 25922	1	2
	<i>E. coli</i> FDA-CDC 346 (MCR-1)	1	4
	<i>B. anthracis</i> Sterne 9131	8	8
	<i>F. tularensis</i> LVS	0.125	0.125
	<i>K. pneumoniae</i> Nevada strain AR-0636	1	1
	<i>P. aeruginosa</i> ATCC 27853	64	>64
	<i>Y. pestis</i> Yokohama NR4693	1	4



concentration (MIC) values for ampicillin, gentamicin, and tetracycline were 2, 0.5, and 2 $\mu\text{g mL}^{-1}$ for *Escherichia coli*; 16, 0.5, and 8 $\mu\text{g mL}^{-1}$ for *Acinetobacter baumannii*; and 128 (defined for benzylpenicillin), 0.125, and 0.5 $\mu\text{g mL}^{-1}$ for methicillin-resistant *Staphylococci aureus*, respectively.⁶⁴ MIC values for Gram-negative multidrug-resistant pathogens are typically much higher and are dependent upon the antibiotic and species of interest.^{65–67} Importantly, the MIC values of synthetic streptothricin F and streptothricin F purified from nourseothricin natural product were essentially identical within the expected experimental error, providing biological confirmation of the anticipated activity for our synthetic natural product.

Conclusions

The streptothricin scaffold has been overlooked by the synthetic chemistry community until now. Our disclosed work represents the second total synthesis of streptothricin F (1), and the first through a diversity-enabling convergent route. We have prepared streptothricin F in a longest linear sequence of 19 steps, with 35 total steps, and 0.40% overall yield. Additionally, our convergent total synthesis includes the ability to install practical, divergent synthetic steps and facilitate manipulations of each of the three independent structural moieties. We have shown that streptothricin F has great promise as a broadly active antibiotic scaffold ripe for optimization. Accordingly, through the fully developed, diversity-enabling total synthesis of streptothricin F, we are pursuing medicinal chemistry-guided analog generation of streptothricin F to explore synthetic analogs for potential therapeutic development.

Author contributions

R. M. and J. E. K. conceptualized and supervised the work as well as edited the manuscript. M. G. D., B. C. M., M. K., K. P. S., and A. D. F. designed and performed experiments. J. J. G. performed some NMR spectral acquisition. The first draft of the manuscript was written by M. G. D. and B. C. M. All authors contributed to manuscript review.

Conflicts of interest

The authors declare no competing financial interest. The HP D300 digital dispenser and TECAN M1000 used for MIC analysis in Table 1 were provided for our use by TECAN (Morrisville, NC). TECAN had no role in study design, data collection/interpretation, manuscript preparation, or decision to publish.

Acknowledgements

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under awards R21AI140212 and R01AI157208. K. P. S. was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number F32 AI124590. We gratefully acknowledge and thank Clarissa Santori and G. Marco Rossi for contributions to the synthetic

studies early in development. We thank Donna Baldisseri from Bruker Biospin Corporation for recording NMR data of select late-stage intermediates. We thank Charles Sheahan from Harvard Medical School for assistance in recording NMR data of select late-stage intermediates.

Notes and references

- 1 S. A. Waksman and H. B. Woodruff, Streptothricin, a new selective bacteriostatic and bactericidal agent, particularly active against gram-negative bacteria, *Proc. Soc. Exp. Biol. Med.*, 1942, **49**(2), 207–210.
- 2 M. Lumb, N. Chamberlain, T. Cross, P. E. Macey, J. Spyvee, J. M. Uprichard and R. D. Wright, An antibiotic complex (A4788) containing streptothricin and having activity against powdery mildews, *J. Sci. Food Agric.*, 1962, **13**(6), 343–352.
- 3 A. W. Johnson and J. W. Westley, The streptothricin group of antibiotics. Part I. the general structural pattern, *J. Chem. Soc.*, 1962, **1042**, 1642.
- 4 R. K. Sinha and P. N. Nandi, A new antibacterial agent produced by *streptomyces Sp.* AC6-569, *Experientia*, 1968, **24**(8), 795–796.
- 5 R. K. Sinha, Boseimycin—a new streptothricin-like antibiotic, *J. Antibiot.*, 1970, **23**(7), 360–364.
- 6 H. Taniyama, Y. Sawada and T. Kitagawa, Characterization of racemomycins, *Chem. Pharm. Bull.*, 1971, **19**(8), 1627–1634.
- 7 J. Fried and O. Wintersteiner, Crystalline reineckates of streptothricin and streptomycin, *Science*, 1945, **101**(2633), 613–615.
- 8 S. Hosoya, M. Soeda, N. Komatsu, S. Imamura, M. Iwasaki, Y. Sonoda and K. Okada, The antibiotic substances produced by *streptomyces*, *Jpn. J. Exp. Med.*, 1949, **20**, 121–133.
- 9 S. Hosoya, M. Soeda, N. Komatsu and S. Imamura, Antibiotic substances produced by a *streptomyces* H-277 strain, *Jpn. J. Exp. Med.*, 1950, **20**, 481–488.
- 10 H. J. Robinson, O. E. Graessle and D. G. Smith, Studies on the toxicity and activity of streptothricin, *Science*, 1944, **99**(2583), 540–542.
- 11 D. M. Reynolds, A. Schatz and S. A. Waksman, Grisein, a new antibiotic produced by a strain of *streptomyces griseus*, *Proc. Soc. Exp. Biol. Med.*, 1947, **64**(1), 50–54.
- 12 K. Akasaki, H. Abe, A. Seino and S. Shirato, Yazumycin, a new antibiotic produced by *streptomyces lavendulae*, *J. Antibiot.*, 1968, **21**(2), 98–105.
- 13 A. S. Khokhlov, Streptothricins and related antibiotics, *J. Chromatogr. Libr.*, 1978, **15**, 617–713.
- 14 Y. Inamori, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada, H. Taniyama and H. Matsuda, Toxicological approaches to streptothricin antibiotics. II. The developmental mechanism of delayed toxicity in mice and rats, *Chem. Pharm. Bull.*, 1979, **27**(1), 230–234.
- 15 Y. Inamori, Y. Kato, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada and H. Taniyama, Toxicological approaches to streptothricin antibiotics. I. Implications of delayed toxicity in mice, *Chem. Pharm. Bull.*, 1978, **26**(4), 1147–1152.



- 16 H. Taniyama, Y. Sawada and S. Tanaka, Chemical modification of streptothricin group antibiotics. II. Some amino derivatives in racemomycin-A and their biological activity, *Chem. Pharm. Bull.*, 1974, **22**(2), 337–341.
- 17 Y. Kono, S. Makino, S. Takeuchi and H. Yonehara, Sclerothricin, a new basic antibiotic, *J. Antibiot.*, 1969, **22**(12), 583–589.
- 18 H. E. Carter, R. K. Clark, P. Kohn, J. W. Rothrock, W. R. Taylor, C. A. West, G. B. Whitfield and W. G. Jackson, Streptothricin. I. preparation, properties and hydrolysis products, *J. Am. Chem. Soc.*, 1954, **76**(2), 566–569.
- 19 M. I. Horowitz and C. P. Schaffner, Paper chromatography of streptothricin antibiotics. Differentiation and fractionation studies, *Anal. Chem.*, 1958, **30**(10), 1616–1620.
- 20 M. Gan, X. Zheng, Y. Liu, Y. Guan and C. Xiao, Three new 12-carbamoylated streptothricins from *streptomyces Sp.* I08A 1776, *Bioorg. Med. Chem. Lett.*, 2012, **22**(19), 6151–6154.
- 21 Z. Ji, M. Wang, J. Zhang, S. Wei and W. Wu, Two new members of streptothricin class antibiotics from *streptomyces qinlingensis Sp. Nov.*, *J. Antibiot.*, 2007, **60**(12), 739–744.
- 22 B. W. Bycroft and T. J. King, Crystal structure of streptolidine, a guanidine-containing amino-acid, *J. Chem. Soc., Chem. Commun.*, 1972, **81**(11), 652.
- 23 F. Kudo, A. Miyanaga and T. Eguchi, Biosynthesis of natural products containing β -amino acids, *Nat. Prod. Rep.*, 2014, **31**(8), 1056–1073.
- 24 R. M. Burckhardt and J. C. Escalante-Semerena, Insights into the function of the *N*-acetyltransferase SATa that detoxifies streptothricin in *Bacillus subtilis* and *Bacillus anthracis*, *Appl. Environ. Microbiol.*, 2019, **85**(6), 1–20.
- 25 K. R. Farley and W. W. Metcalf, The streptothricin acetyltransferase (SAT) gene as a positive selectable marker for methanogenic archaea, *FEMS Microbiol. Lett.*, 2019, **366**(17), 1689–1699.
- 26 J. Jacob, S. Evers, K. Bischoff, C. Carlier and P. Courvalin, Characterization of the SAT4 gene encoding a streptothricin acetyltransferase in *Campylobacter coli* bel g4, *FEMS Microbiol. Lett.*, 1994, **120**(1–2), 13–17.
- 27 S. Keeratipibul, M. Sugiyama and R. Nomi, Mechanism of resistance to streptothricin of a producing microorganism, *Biotechnol. Lett.*, 1983, **5**(7), 441–446.
- 28 K. Smalla, R. Prager, M. Isemann, R. Pukall, E. Tietze, J. D. Elsas and H. Tschäpe, Distribution of streptothricin acetyltransferase encodi determinants among environmental bacteria, *Mol. Ecol.*, 1993, **2**(1), 27–33.
- 29 H. E. Webb, F. J. Angulo, S. A. Granier, H. M. Scott and G. H. Loneragan, Illustrative examples of probable transfer of resistance determinants from food animals to humans: streptothricins, glycopeptides, and colistin, *F1000Research*, 2017, **6**, 1805.
- 30 R. M. Burckhardt and J. C. Escalante-Semerena, In *bacillus subtilis*, the SATa (formerly YYAR) acetyltransferase detoxifies streptothricin *via* lysine acetylation, *Appl. Environ. Microbiol.*, 2017, **83**(21), 1–11.
- 31 Y. Hamano, N. Matsuura, M. Kitamura and H. Takagi, A novel enzyme conferring streptothricin resistance alters the toxicity of streptothricin d from broad-spectrum to bacteria-specific, *J. Biol. Chem.*, 2006, **281**(25), 16842–16848.
- 32 I. Haupt, J. Jonák, I. Rychlik and H. Thrum, Action of streptothricin F on ribosomal functions, *J. Antibiot.*, 1980, **33**(6), 636–641.
- 33 I. Haupt, R. Hübener, H. Thrum and F. Streptothricin, an inhibitor of protein synthesis with miscoding activity, *J. Antibiot.*, 1978, **31**(11), 1137–1142.
- 34 K. P. Smith, Y.-S. Kang, A. B. Green, M. G. Dowgiallo, B. C. Miller, L. Chiaraviglio, K. A. Truelson, K. E. Zulauf, S. Rodriguez, R. Manetsch and J. E. Kirby, Profiling the *in vitro* and *in vivo* activity of streptothricin-F against carbapenem-resistant enterobacterales: A historic scaffold with a novel mechanism of action, *bioRxiv*, 2021, DOI: 10.1101/2021.06.14.448463.
- 35 S. Kusumoto, S. Imaoka, Y. Kambayashi and T. Shiba, Total synthesis of antibiotic streptothricin F, *Tetrahedron Lett.*, 1982, **23**(29), 2961–2964.
- 36 The step counts and overall yield of Shiba's synthesis are approximated, not all synthetic methods and purified yields were fully described.
- 37 It was key that the nitroketone formation occur under dilute, ambient conditions to avoid racemization of the Cbz-protected amino stereocenter.
- 38 J. Rudolph, F. Hannig, H. Theis and R. Wischnat, Highly efficient chiral-pool synthesis of (2*S*,4*R*)-4-hydroxyornithine, *Org. Lett.*, 2001, **3**(20), 3153–3155.
- 39 Y. Yuasa, Y. Yuasa and H. Tsuruta, A facile synthesis of (2*R*,3*S*)-1-amino-3-*tert*-butoxycarbonylamino-2-hydroxy-4-phenylbutane; a useful component block of HIV protease inhibitor, *Synth. Commun.*, 1998, **28**(3), 395–401.
- 40 C. H. Heathcock, S. D. Young, J. P. Hagen, M. C. Pirrung, C. T. White and D. VanDerveer, Acyclic stereoselection. 9. Stereochemistry of the addition of lithium enolates to α -alkoxy aldehydes, *J. Org. Chem.*, 1980, **45**(19), 3846–3856.
- 41 A. Guaragna, D. D'Alonzo, C. Paoella, C. Napolitano and G. Palumbo, Highly stereoselective *de novo* synthesis of L-hexoses, *J. Org. Chem.*, 2010, **75**(11), 3558–3568.
- 42 N. J. Adderley, D. J. Buchanan, D. J. Dixon and D. I. Lainé, Highly diastereoselective oxy-michael additions of enantiopure δ -lactol anions to nitroalkenes: asymmetric synthesis of 1,2-amino alcohols, *Angew. Chem., Int. Ed.*, 2003, **42**(35), 4241–4244.
- 43 Boc protection was only employed in this instance to increase the recovery of product from the aqueous phase as the unprotected amino alcohol of **12** had high water solubility.
- 44 T. Stark, M. Suhartono, M. W. Göbel and M. Lautens, A palladium-catalyzed domino reaction as key step for the synthesis of functionalized aromatic amino acids, *Synlett*, 2013, **24**(20), 2730–2734.
- 45 D. A. Evans, T. C. Britton, J. A. Ellman and R. L. Dorow, The asymmetric synthesis of α -amino acids. electrophilic azidation of chiral imide enolates, a practical approach to



- the synthesis of (*R*)- and (*S*)- α -azido carboxylic acids, *J. Am. Chem. Soc.*, 1990, **112**(10), 4011–4030.
- 46 M. I. García-Moreno, P. Díaz-Pérez, J. M. Benito, C. Ortiz Mellet, J. Defaye and J. M. García Fernández, One-step synthesis of non-anomeric sugar isothiocyanates from sugar azides, *Carbohydr. Res.*, 2002, **337**(21–23), 2329–2334.
- 47 P. Singh, V. Mehra, A. Anand, V. Kumar and M. P. Mahajan, β -lactam-synthon-interceded diastereoselective synthesis of novel thioxo-imidazolines: a convenient access to functionally decorated 4,5-dihydro-imidazoles, *Tetrahedron Lett.*, 2011, **52**(39), 5060–5063.
- 48 M. D. Wittman, R. L. Halcomb, S. J. Danishefsky, J. Golik and D. Vyas, A route to glycals in the allal and gular series: synthesis of the thiosugar of esperamicin A1, *J. Org. Chem.*, 1990, **55**(7), 1979–1981.
- 49 S. Buttar, J. Caine, E. Goné, R. Harris, J. Gillman, R. Atienza, R. Gupta, K. M. Sogi, L. Jain, N. C. Abascal, Y. Levine, L. M. Repka and C. M. Rojas, Glycal metallanitrenes for 2-amino sugar synthesis: amidoglycosylation of gular-, allal-, glugal-, and galactal 3-carbamates, *J. Org. Chem.*, 2018, **83**(15), 8054–8080.
- 50 O. Boutureira, M. A. Rodríguez, M. I. Matheu, Y. Díaz and S. Castillón, General method for synthesizing pyranoid glycals. A new route to allal and gular derivatives, *Org. Lett.*, 2006, **8**(4), 673–675.
- 51 V. Di Bussolo, M. Caselli, M. Pineschi and P. Crotti, New stereoselective β -C-glycosidation by uncatalyzed 1,4-addition of organolithium reagents to a glycal-derived vinyl oxirane, *Org. Lett.*, 2003, **5**(12), 2173–2176.
- 52 V. Di Bussolo, M. Caselli, M. R. Romano, M. Pineschi and P. Crotti, Stereospecific uncatalyzed α -O-glycosylation and α -C-glycosidation by means of a new D-gular-derived α vinyl oxirane, *J. Org. Chem.*, 2004, **69**(21), 7383–7386.
- 53 T. M. Beale, P. J. Moon and M. S. Taylor, Organoboron-catalyzed regio- and stereoselective formation of β -2-deoxyglycosidic linkages, *Org. Lett.*, 2014, **16**(13), 3604–3607.
- 54 The introduction of a primary carbamoyl as opposed to the DMB-protected carbamoyl reduced the yields of the dihydroxylation and the Burgess reagent sulfamidation from 88% to 51% and 28% to 15%, respectively.
- 55 K. C. Nicolaou, S. A. Snyder, D. A. Longbottom, A. Z. Nalbandian and X. Huang, New uses for the Burgess reagent in chemical synthesis: methods for the facile and stereoselective formation of sulfamidates, glycosylamines, and sulfamides, *Chem.-Eur. J.*, 2004, **10**(22), 5581–5606.
- 56 E. H. Ryu, A. Ellern and Y. Zhao, High guest inclusion in 3 β -amino-7 α ,12 α -dihydroxycholestan-24-oic acid enabled by charge-assisted hydrogen bonds, *Tetrahedron*, 2006, **62**(29), 6808–6813.
- 57 J. P. Genêt, E. Blart, M. Savignac, S. Lemeune and J. M. Paris, Palladium-catalyzed reactions in aqueous media. an efficient removal of allyloxycarbonyl protecting group from oxygen and nitrogen, *Tetrahedron Lett.*, 1993, **34**(26), 4189–4192.
- 58 J. B. Aggen, P. Dozzo, A. A. Goldblum, D. J. Hildebrandt, T. R. Kane, M. J. Gliedt and M. S. Linsell, Antibacterial aminoglycoside analogs, WO 2011/044501 A2, 2011.
- 59 Centers for Disease Control and Prevention, *Antibiotic Resistance Threats in the United States*, Atlanta, 2019.
- 60 World Health Organization, *Global Action Plan on Antimicrobial Resistance*, Geneva, 2015.
- 61 World Health Organization, *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics*, Geneva, 2017.
- 62 H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg and J. Bartlett, Bad bugs, no drugs: no ESKAPE! an update from the infectious diseases society of America, *Clin. Infect. Dis.*, 2009, **48**(1), 1–12.
- 63 L. Chen, R. Todd, J. Kiehlbauch, M. Walters and A. Kallen, Notes from the field: pan-resistant New Delhi metallo-beta-lactamase-producing *Klebsiella pneumoniae* –Washoe County, Nevada, 2016, *Morb. Mortal. Wkly. Rep.*, 2017, **66**(1), 33.
- 64 *Modal MICs for MIC distributions provided by eucast (European committee on antimicrobial susceptibility testing)*, <https://mic.eucast.org/search/>, accessed 1/29/2022.
- 65 A. D. Kang, K. P. Smith, G. M. Eliopoulos, A. H. Berg, C. McCoy and J. E. Kirby, *In vitro* apramycin activity against multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, *Diagn. Microbiol. Infect. Dis.*, 2017, **88**(2), 188–191.
- 66 T. Brennan-Krohn, K. A. Truelson, K. P. Smith and J. E. Kirby, Screening for synergistic activity of antimicrobial combinations against carbapenem-resistant enterobacteriaceae using inkjet printer-based technology, *J. Antimicrob. Chemother.*, 2017, **72**(10), 2775–2781.
- 67 K. P. Smith and J. E. Kirby, Verification of an automated, digital dispensing platform for at-will broth microdilution-based antimicrobial susceptibility testing, *J. Clin. Microbiol.*, 2016, **54**(9), 2288–2293.

