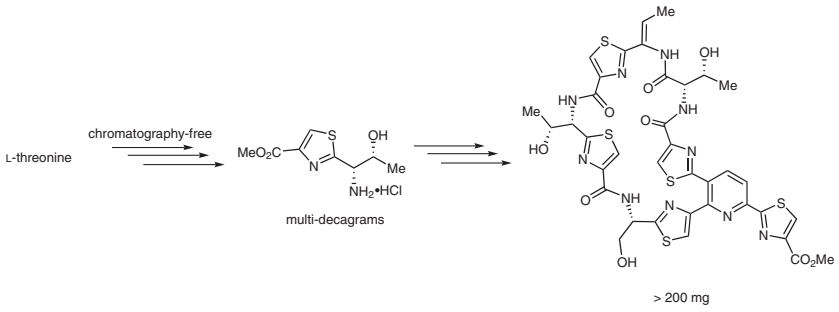
Synthesis T. C. Johnson et al. Feature

Synthesis of the 26-Membered Core of Thiopeptide Natural Products by Scalable Thiazole-Forming Reactions of Cysteine Derivatives and Nitriles

Trevor C. Johnson[a]
Mitchell P. Christy[a]
Dionicio Siegel*[b]

[a] Department of Chemistry & Biochemistry, University of California-San Diego, 9500 Gilman Drive, La Jolla, California 92093, United States
[b] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California-San Diego, 9500 Gilman Drive, La Jolla, California 92093-0934, United States
drsiegel@health.ucsd.edu



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Abstract The increased resistance of bacteria to clinical antibiotics is one of the major dilemmas facing human health and without solutions the problem will grow exponentially worse. Thiopeptide natural products have shown promising antibiotic activities and provide an opportunity for the development of a new class of antibiotics. Attempts to directly translate these compounds into human medicine have been limited due to poor physiochemical properties. The synthesis of the core structure of the 26-membered class of thiopeptide natural products is reported using chemistry that enables the synthesis of large quantities of synthetic intermediates and the common core structure. The use of cysteine/nitrile condensation reactions followed by oxidation to generate thiazoles has been key in enabling large academic scale reactions that in many instances avoided chromatography further aiding in accessing large amounts of key synthetic intermediates.

Key words thiopeptide, natural product, thiazole, scalable, antibiotic, pharmacophore

Resistance to clinical antibiotics has emerged as one of the major challenges confronting global health. As a result, this problem has become a top priority for international governing bodies. The Centers for Disease Control and Prevention estimates annually, in the United States, 2 million adults infected with drug-resistant bacteria and, of those infected, there is a 12% morbidity rate.[1] These numbers will increase yearly. Antibiotic resistance mechanisms, many of which can be transferred between different bacteria, have greatly outpaced drug development, as a result of this disease being deprioritized by the pharmaceutical industry. Government efforts to incentivize the development of new antibiotics have been approached in large part by rejuvenating existing clinically employed antibiotics providing a lower barrier for entering the market. However, the development of new chemical entities represent a significantly better solution and is expected to have the largest impact on the current resistance dilemma.

An approach to develop a new class of antibiotics for use in humans has been inspired by the natural product nosiheptide. Nosiheptide, initially named multhiomycin, was first identified as a member of the thiazolyl peptide (thiopeptide) class of antibiotics and reported to possess potent antibiotic activity in 1970.[2] The bactericidal activity occurs through the inhibition of ribosomal protein synthesis in a manner distinct from other clinical protein synthesis inhibitors.[3] Nosiheptide has pronounced antibiotic effects against a large number of clinically relevant strains including those derived from patients.[4] Potent activity is present against numerous methicillin-resistant *Staphylococcus aureus* (MRSA) and other multidrug resistant strains. Single digit, nanomolar activity is achieved against *Clostridium difficile* (*C. difficile*), a difficult bacterium to kill. Remarkably no toxicity is seen when dosing animals at the extremely high level of 2.5 g/kg.[5] As a result, the compounds have been used outside of the United States to increase feed conversion in livestock.[6] With desirable activity and no apparent toxicity, chemistry is now needed to deliver derivatives that possess improved solubility but maintain the potent antibacterial effects. With the characterization of the potential pharmacophore of nosiheptide and related thiazolyl peptide natural products, total syntheses achieved for some of the most structurally complex members, and paths forward for development, now is the opportune time to advance this compound class through chemistry.[7,8]

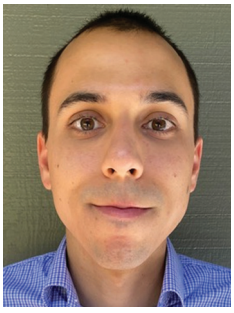
Toward the goal of developing new derivatives the structural requirements for antibiotic activity are required. Through significant effort by multiple research groups an understanding of the pharmacophore of thiazolyl peptides

similar to nosiheptide has been developed. Key structural similarities across this class are highlighted in red. In addition to the compounds shown in Figure 1, the related natural products thiostrepton, nocathiacins, and siomycins also possess a similar core, a 26-membered macrocycle with multiple thiazoles. Recently identified or prepared members of this class include lactocillin[9] from the human microbiome (absolute configuration yet to be reported) and QN3323A (YM-266183),[10] a compound undergoing development.

Important structure-activity relationships have been achieved accessing unnatural thiocillins and thiostrepton derivatives through prepeptide gene replacement. These compounds were tested for structural modifications to the antibiotics and were achieved by exchanging amino acids within the natural products precursors and determining the new, designed compounds' activity.[11] Remarkably the use of prepeptide gene replacement techniques has even yielded thiocillin derivatives with variations in the size of the macrocycle, however, as expected these compounds were devoid of antibiotic activity.[12] Evolved cross-resistance to the parent natural products has provided information with respect to interactions with the ribosome.[13] Semisynthesis of active analogues, starting from QN3323A (YM-266183), have undoubtedly built upon these findings.[8,10]

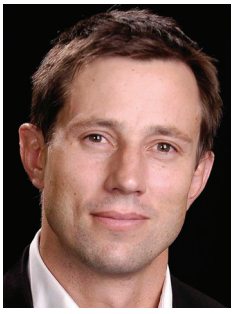
The total syntheses of thiazolyl peptide antibiotics have been achieved with synthetic creativity and through these studies numerous transformations have been developed and adapted. To date, arguably, the most structurally challenging thiazolyl peptide natural product to be synthesized is thiostrepton by Nicolaou and co-workers.[14] The total synthesis of nosiheptide has also been achieved[15] after significant efforts were made in an attempt to prepare it through total synthesis.[16] In addition, many notable accomplishments have been made through the total syntheses of other thiazolyl peptide natural products.[17] While a great deal of chemistry has been learned from these syntheses the application of these findings to drug development is still devel-

Biographical Sketches



Trevor Johnson was born in Sacramento, California. He completed his undergraduate degree at the University of California, Santa Cruz in Biochemistry and Molecular Biology and completed research under the mentorship of Professor Needhi Bhalla (Molecular Biology). He joined the labs of Professor Dionicio Siegel in 2012, where his research focused on the total synthesis of complex natural products, bioactive small molecules, and reaction methodology and he received his Ph.D. in chemistry in 2016. He joined the Process Chemistry Department at Gilead Sciences in 2016 where he is currently working on the development of innovative therapeutics for patients worldwide.

Mitchell P. Christy was born in Houston, Texas and completed his undergraduate degree in chemistry (B.S. 2014) from the University of Texas at Austin where he undertook research in organometallic reaction method development under Dr. Guangbin Dong. He then moved to California to complete his Ph.D. in organic chemistry at UC San Diego under the direction of Dr. Dionicio Siegel where he worked on the total synthesis of natural products and drug development in the areas of malaria and cancer in collaboration with the Winzeler and Ideker labs in the school of medicine. He completed his Ph.D. in 2019 and is currently a postdoc in the Gerwick lab at the Scripps Institute of Oceanography in La Jolla where he is pursuing the isolation and synthesis of novel marine natural products and medicinal chemistry development of protease inhibitors against SARS-CoV-2.



Dionicio Siegel, born in 1974 in Truchas New Mexico, studied chemistry starting at Reed College and earned his Ph.D. in chemistry with Andy Myers at Harvard University. Postdoctoral work with Samuel Danishefsky at the Memorial Sloan-Kettering Cancer Center was followed, from 2007 to 2014, with a faculty position at The University of Texas at Austin. In 2014 he transitioned to the University of California, San Diego in the Skaggs School of Pharmacy and Pharmaceutical Sciences where he is currently Chair of the Division of Pharmaceutical Chemistry. His research interests focus on synthetic organic chemistry applied to natural product-based drug discovery and medicinal chemistry.

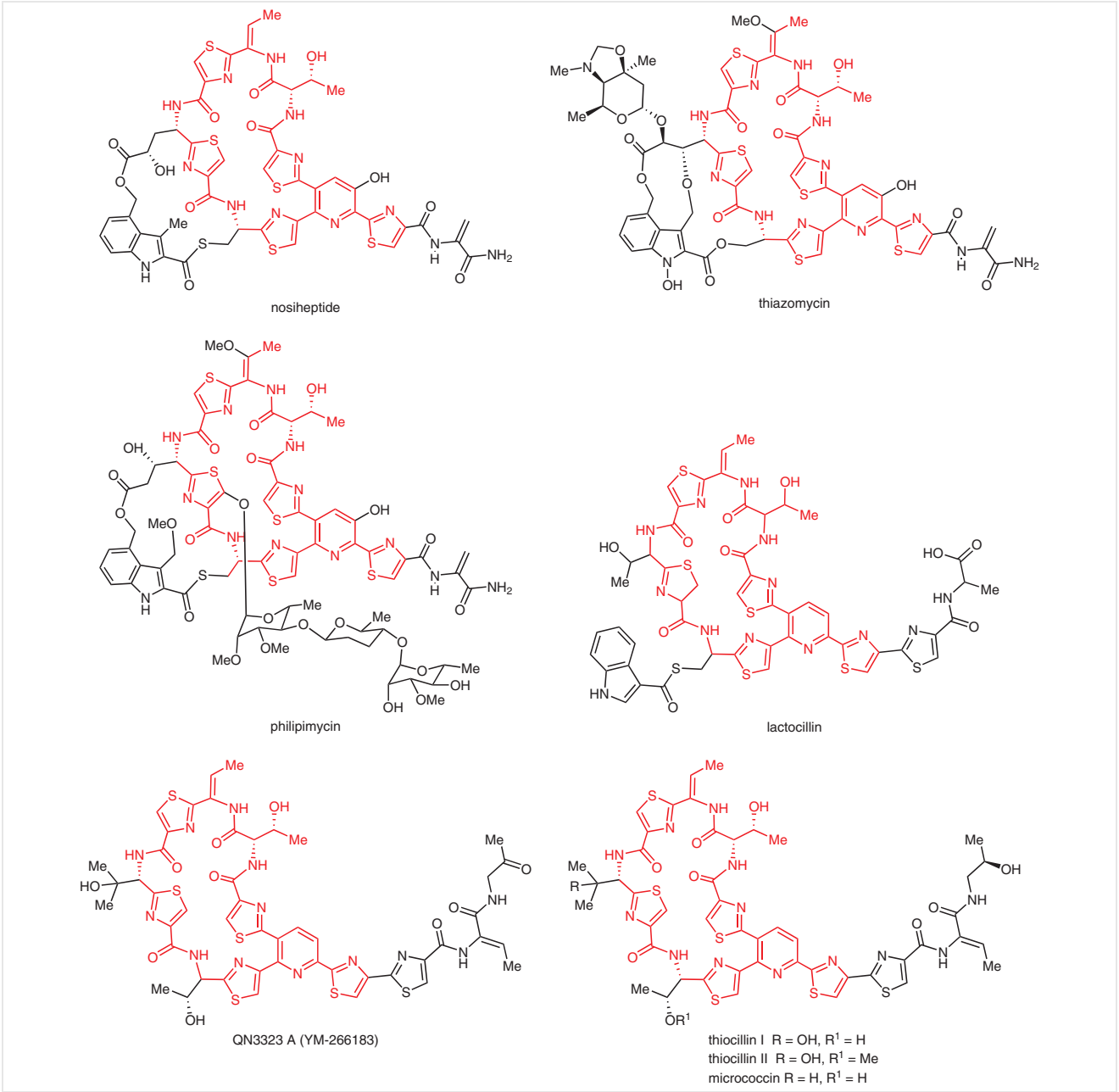


Figure 1 Structures of 26-membered thiazolyl peptides known or predicted to target the L11 protein/23S rRNA region of the ribosome. Related core structures are highlighted in red.

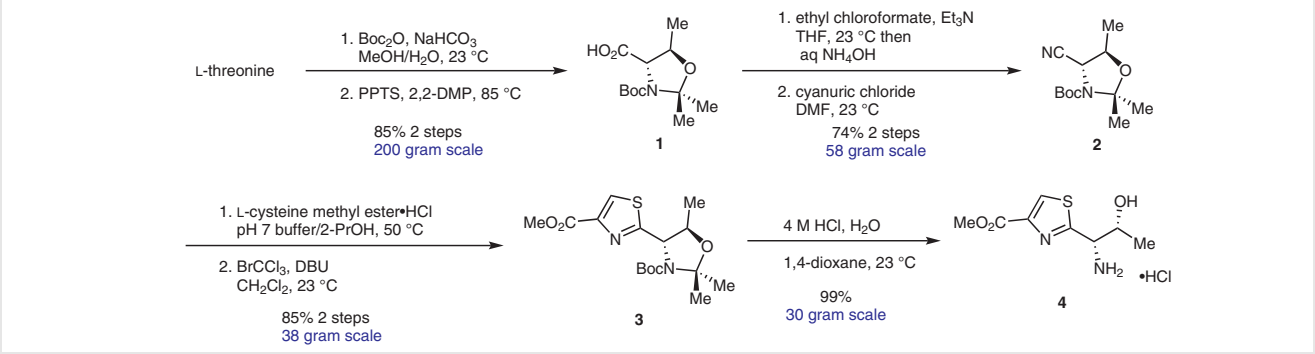
oping. In our group, we have succeeded synthesizing micrococcin P1 implementing cysteine nitrile condensation reactions to form thiazoles.[18] This provided guidance in the synthesis of the common core of the 26-membered thiopeptides that we believe to be a fundamentally important substrate to assess structure-activity relationships present in this class of natural products as they are related to antiproliferative effects.

Our approach to synthesizing the fragments of thiazolyl peptides, chiral carboxyaminothiazoles, used a general strategy that has proven both reliable and scalable; cysteine-nitrile condensation reactions[19] followed by aromatization.[20] For comparison, the Hantzch thiazole synthesis has proven useful in the past for the syntheses of this class of natural products as the method is highly reliable, however, conducting these thiazole forming reactions on the multidecagram scale proved difficult in our hands in maintaining

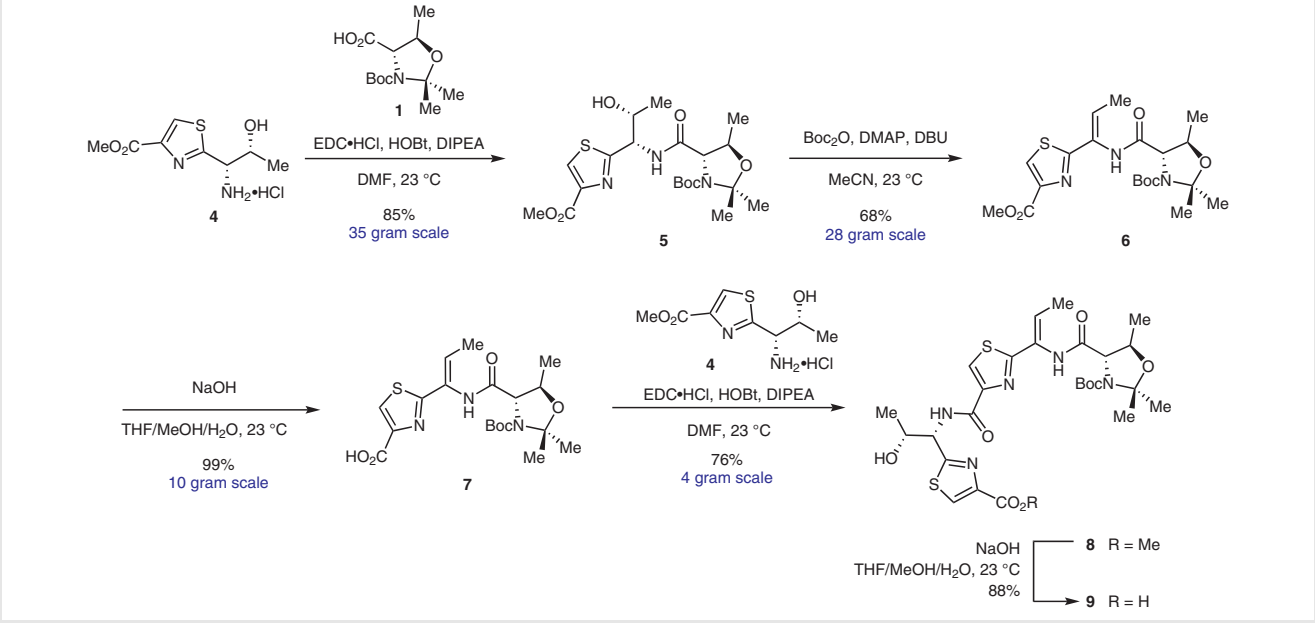
yields achieved at smaller scales. As the cysteine-nitrile condensation links easily into the syntheses of all thiazoles except for one we also utilized the addition of thiazole-based Grignard reagents into chiral *N*-sulfinylimines, which has similarly proven reliable and scalable.[21]

As an example, starting from threonine in *a seven-step sequence that does not require chromatography* we accessed 30 grams of amino alcohol **4** as its hydrochloride salt (Scheme 1). The cysteine/nitrile condensation onto nitrile **2** followed by aromatization with trichlorobromomethane and DBU was conducted on a 38 gram scale to generate pure thiazole **3**. The reaction was both clean and the product readily separated from reactants and by-products. Notably, the purification is greatly simplified compared to the Hantzch thiazole synthesis that employs Lawesson's reagent and results in the generation of poorly behaved spent reagents. Simple acidic liberation of the amino alcohol provides **4** as the hydrochloride salt with the acid masked as the methyl ester.

Coupling of the amino alcohol **4** to a compound generated earlier in the route, carboxylic acid **1**, proceeded uneventfully using EDC and HOBt to generate amide **5** (Scheme 2). Dehydrative elimination to form alkene **6** on large scale failed to provide good yields under multiple conditions used for the syntheses dehydroamino acids, however, in situ formation of the *tert*-butyl carbonate followed by reaction with DBU led to facile elimination and the reaction scaled well, conducted as shown on 28 grams of material.[22] Saponification of the ester of **6** generated carboxylic acid **7** that was combined with the previously synthesized amino alcohol **4** using EDC/HOBt coupling to yield amide **8**. The resulting amide product **8** was then hydrolyzed at the thiazole methyl ester to yield carboxylic acid **9**.



Scheme 1 Chromatography-free, multi-decagram synthesis of chiral carboxyaminothiazole **4**



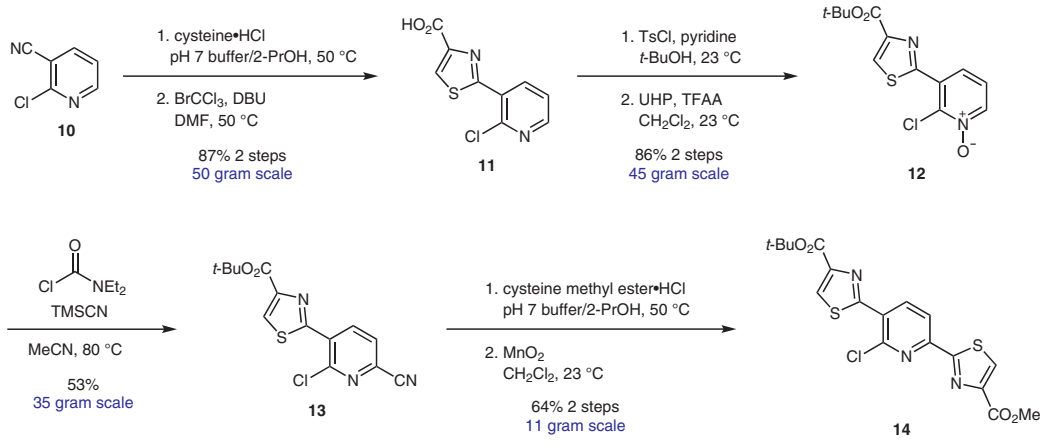
Scheme 2 Five step conversion of amino alcohol **4** into acid **9**

Synthesis of the double thiazole-substituted pyridyl core was also greatly simplified using the cysteine/nitrile condensation-aromatization sequence (Scheme 3). Starting from inexpensive 2-chloro-3-pyridinecarbonitrile (**10**) seven steps arrived at the appropriately modified pyridine with the differentially protected esters, compound **14**. Condensation of cysteine and 2-chloro-3-pyridinecarbonitrile (**10**) and oxidation of the thiazoline generated thiazole **11**. Protection of the free acid as the *tert*-butyl ester and oxidation of the pyridine nitrogen to the *N*-oxide proceeded smoothly[23] setting up a modified Reissert reaction with trimethylsilyl cyanide and diethylcarbamoyl chloride to provide nitrile **13** as a crystalline, off-white solid. The sequence was scalable with all intermediates crystallized from the reaction mixture or purified by trituration. A second cysteine/nitrile condensation followed by MnO_2 oxidation (proved optimal for this system) generated the second appended thiazole ester.

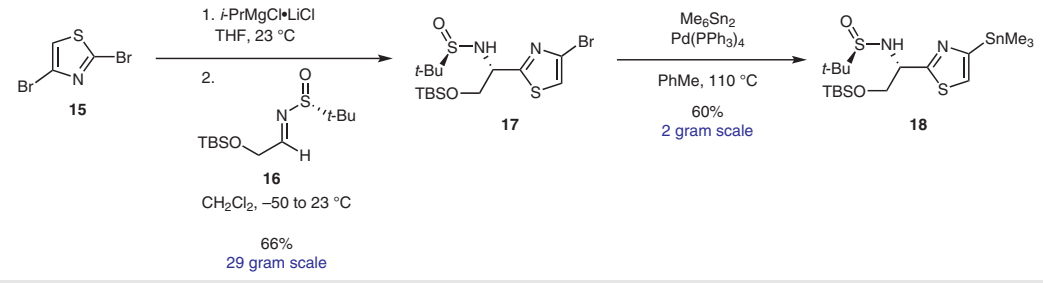
The last required fragment possessed a thiazole with an alternative substitution pattern. Although a Hunsdiecker reaction could, in theory, generate this compound following a cysteine/nitrile condensation-aromatization the broad utility of the Ellman auxiliary[21] led us to the route shown in Scheme 4 for the synthesis of the coupling fragment, stannane **18**. Diastereoselective delivery of the Grignard derived from 2,4-dibromothiazole (Turbo Grignard exchange[24]) provided a 4:1 ratio of diastereomers and a 66% isolated yield of the major diastereomer.

Coupling of the three fragments chloropyridine **14**, stannane **18**, and acid **9** followed by macrocyclization generated the core structure triol ester **21** (Scheme 5). Stille coupling of chloropyridine **14** and stannane **18** was optimal using $Pd_2(dba)_3$ with Cy-JohnPhos, cleanly generating the tri-thiazole bearing pyridine **19**. Selective cleavage of the *tert*-butylsulfinyl group (and TBS) with hydrochloric acid provided an amine that was directly coupled to carboxylic acid **9** using HATU to provide the cyclization precursor in protected form, compound **20**. Deprotection of both Boc groups and acetonide was followed by cyclization, again using HATU, providing the desired triol compound **21** in 27% yield over two steps, conducted on the gram scale.

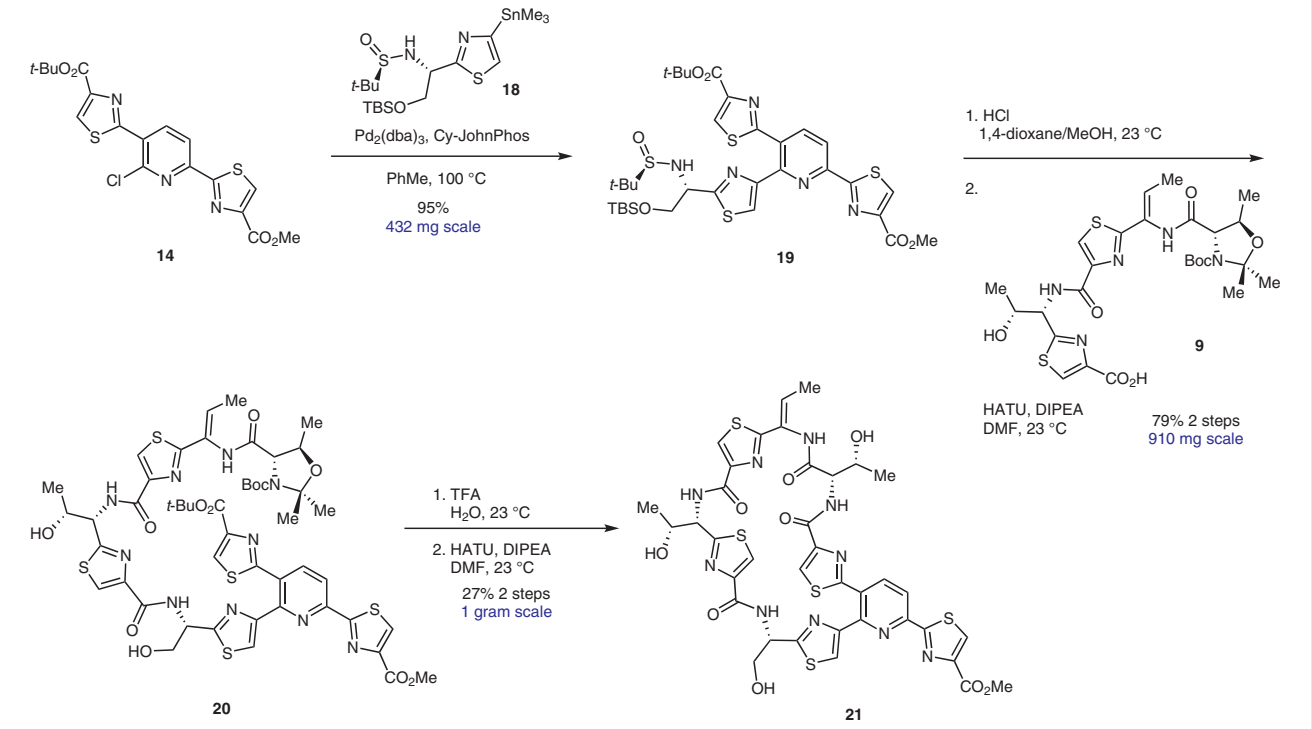
With access to ample quantities of core compound **21**, which we envision to be the key pharmacophore for the 26-membered thiopeptide natural products functionalization can be achieved through modifications to the ester position and the primary alcohol. This follows from previous efforts to increase the water solubility of thiazolyl peptides, which succeeded in developing an antibiotic that reached Phase II clinical trials for the treatment of *C. difficile*, LFF571.[25] The compound, developed by Norvartis, function through a



Scheme 3 Tri-substituted thiazole-pyridine **14** synthesis from commercial pyridine **10**



Scheme 4 Diastereoselective Grignard addition into chiral *N*-sulfinylimine **16** and stannylation of bromide **17** generating **18**



Scheme 5 Fragment coupling and completion of the synthesis of the 26-membered macrocycle **21**

different mechanism of ribosome inhibition and like most of the thiazolyl peptides was initially too insoluble for development.

All reactions were performed in flame-dried round-bottomed flasks fitted with rubber septa under a positive pressure of argon or N_2, unless otherwise indicated. Air and moisture sensitive liquids and solutions were transferred via syringe or cannula. Organic solutions were concentrated by rotary evaporation at 20 torr in a water bath heated to 40 °C, unless otherwise noted. Et_2O, CH_2Cl_2, THF, and toluene (PhMe) were purified using a Pure-Solv MD-5 Solvent Purification System (Innovative Technology). MeCN, DMF, and MeOH were purchased from Acros (99.8%, anhyd) and EtOH was purchased from Pharmco-Aaper (200 proof, absolute). The molarity of *n*-BuLi was determined by titration against diphenylacetic acid. All other reagents were used directly from the supplier without further purification, unless otherwise noted. Analytical TLC was carried out using 0.2 mm commercial silica gel plates (silica gel 60, F254, EMD chemical) and visualized using a UV lamp and/or aqueous ceric ammonium molybdate (CAM), aqueous $KMnO_4$ stain, or ethanolic vanillin. IR spectra were recorded on a Nicolet 380 FTIR using neat thin film technique. High-resolution mass spectra (HRMS) were recorded on a Karatos MS9 or Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS and are reported as *m*/*z* (relative intensity). Accurate masses are reported for the molecular ion $[M + Na]^+$, $[M + H]^+$, [M], or [M – H]. ^1H and ^{13}C NMR) were recorded on a Varian Gemini spectrometer [(400 MHz, ^1H at 400 MHz, ^{13}C at 100 MHz), (500 MHz, ^1H at 500 MHz, ^{13}C at 125 MHz), (600 MHz, ^1H at 600 MHz, ^{13}C at 150 MHz)]. For $CDCl_3$ solutions the chemical shifts are reported as ppm referenced to residual protium or carbon of the solvent; $CHCl_3$ (δ_H = 7.26) and $CDCl_3$ (δ_C = 77.0). For DMSO-d_6 solutions the chemical shifts are reported as ppm referenced to residual protium or carbon of the solvents; $(CD_3)(CHD_2)SO$ (δ_H = 2.50) or $(CD_3)_2SO$ (δ_C = 39.5). For CD_3OD solutions the chemical shifts are reported as ppm referenced to residual protium or carbon of the solvents; CHD_2OD (δ_H = 3.31) or CD_3OD (δ_C = 49.0). Coupling constants are reported in hertz (Hz). Data for ^1H NMR spectra are reported as follows: chemical shift [ppm, referenced to protium; multiplicity (standard abbreviations), coupling constant (Hz), and integration]. Melting points were measured on a MEL-TEMP device without corrections.

(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic Acid (1)

Solid L-threonine (100 g, 839 mmol, 1 equiv) was dissolved in 1.7:1 THF/2 M NaOH and cooled in an ice bath. Boc anhydride was added as a solid portionwise (220g, 1.01 mol, 1.2 equiv) over 10 min and the reaction mixture was stirred for 1 h. The reaction vessel was removed from ice bath and stirred at 23 °C for 48 h. THF was removed under vacuum, the residue taken up in aq 2 M HCl (2 L), and the mixture was extracted with EtOAc (3 × 1 L). The combined organic layers were washed with H_2O (750 mL) and brine (750 mL), dried (Na_2SO_4) and concentrated to provide a colorless oil. The crude material (175.4 g) was used directly in the next reaction without further purification.

Crude Boc-L-threonine was dissolved in 2,2-dimethoxypropane (1.03 L) and PPTS (recrystallized from acetone) was added as a solid in one portion (60.3 g, 0.3 equiv) and the reaction mixture was heated to reflux for 14 h. The mixture was cooled to 23 °C and then concentrated and the residue was dissolved in EtOAc (2 L). The EtOAc solution was washed with H_2O (500 mL) and brine (500 mL), dried (Na_2SO_4), and

concentrated. Recrystallization of the residue from hexanes yielded 113.1 g of product and an additional 58 g collected from a 2nd recrystallization to provide **1** as a white solid (85% yield over 2 steps).

^{1}H NMR (600 MHz, MeOD): δ (rotamers) = 4.21–4.13 (m, 1 H), 3.90–3.80 (m, 1 H), 1.58 (br s, 3 H), 1.54 (br s, 3 H), 1.45 (br d, 9 H), 1.37 (d, *J* = 6.1 Hz, 3 H).

tert-Butyl (4*R*,5*R*)-4-Cyano-2,2,5-trimethyloxazolidine-3-carboxylate (2)

Solid **1** (58 g, 224 mmol, 1 equiv) was dissolved in THF (447 mL, 0.5 M) and cooled in an ice bath. Ethyl chloroformate (25.8 mL, 269 mmol, 1.2 equiv) was added followed by dropwise addition of Et_3N (37.5 mL, 269 mmol, 1.2 equiv) with vigorous stirring over 20 min to ensure stirring was not hindered. After the addition of ethyl chloroformate, the reaction mixture was warmed to 23 °C and stirred for 4 h when full consumption of starting material was observed by TLC (ninhydrin stain). The mixture was cooled again in an ice bath and 25% aq NH_4OH (48.8 mL, 1.4 equiv) was added in a single portion and the mixture was stirred with slow warming over 12 h. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (1 L). The combined extracts were washed with H_2O (2 × 350 mL) and brine (350mL), dried (Na_2SO_4), and concentrated to provide an amber oil (48.8 g). This crude material was used in the next reaction without further purification.

Crude amide (51.4 g, 199 mmol, 1 equiv) was dissolved in DMF (200 mL, 1.0 M) and the reaction flask was immersed in a 23 °C water bath. Solid cyanuric chloride (18.44 g, 100 mmol, 0.5 equiv) was added in portions over 10 min and stirred for 1 h. The reaction mixture was poured slowly into ice water (2 L) with vigorous stirring and the solids were collected by filtration. The precipitate was washed with cold H_2O (3 × 250 mL) and dried in vacuo to give the nitrile **2** as a white solid (35.4 g, 147 mmol, 74% over two steps); mp 41–43 °C.

IR (film): 2358, 1715 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.40 (pent, *J* = 6.2 Hz, 1 H), 3.99 (m, 1 H), 1.59 (br s, 3 H), 1.52 (br s, 4 H), 1.48 (s, 9 H), 1.40 (d, *J* = 6.1 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 150.4, 117.1, 95.7, 82.0, 74.1, 52.9, 28.1, 26.4, 24.4, 18.2.

HRMS (ESI): *m*/*z* calcd for $C_{12}H_{20}N_2O_3Na$ [M + Na]$^+$: 263.1366; found: 263.1366.

tert-Butyl (4*S*,5*R*)-4-[4-(Methoxycarbonyl)thiazol-2-yl]-2,2,5-trimethyloxazolidine-3-carboxylate (3)

Nitrile **2** (37.7 g, 157 mmol, 1.0 equiv) was dissolved in a 1.5:1 mixture of *i*-PrOH/pH 7 phosphate buffer (785 mL, 0.2 M buffer, 0.1 M concentraion) and solid cysteine methyl ester hydrochloride was added in a single portion (40.4 g, 236 mmol, 1.5 equiv). The reaction mixture was stirred and heated to 50 °C for 14 h. The solvent was removed under reduced pressure and the residue was partitioned between H_2O (1 L) and EtOAc (500 mL) and the aqueous layer was extracted with EtOAc (3 × 250 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give the thiazoline (50.9 g) as a colorless oil that solidifies upon standing. This crude material was used directly in the next reaction without further purification.

Crude thiazoline was dissolved in DCM (475 mL, 0.3 M) and cooled in an ice bath. $BrCCl_3$ was added (21 mL, 188 mmol, 1.2 equiv) followed by DBU (25.4 mL, 188 mmol, 1.2 equiv) dropwise over several min. The reaction was allowed to warm to 23 °C as the ice bath melts. After completion, the mixture was poured into aq 1 M HCl (500 mL) and extracted with additional DCM (3 × 200 mL). The combined organic layers were washed with H_2O (250 mL) and brine (250 mL), dried (Na_2SO_4), and concentrated to give 47.8 g of thiazole **3** as an off-white solid (134 mmol, 85% over 2 steps); mp 120–123 °C. The crude material was of sufficient purity to be used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.17 (br s, 1 H), 4.78 (m, 1 H), 4.16 (m, 1 H), 3.94 (s, 3 H), 1.69 (br s, 6 H), 1.42 (br s, 9 H), 1.18 (br s, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 173.3, 161.3, 151.0, 146.1, 127.3, 95.0, 80.4, 77.6, 65.7, 52.1, 27.8, 26.2, 25.6, 17.6.

HRMS (ESI): *m*/*z* calcd for $C_{16}H_{24}N_2O_5SNa$ [M + Na]$^+$: 379.1298; found: 379.1295.

Methyl 2-[(1*S*,2*R*)-1-Amino-2-hydroxypropyl]thiazole-4-carboxylate Hydrochloride (4)

1,4-Dioxane (1 mL/g) was added to thiazole **3** (30 g, 84 mmol, 1 equiv) to solubilize the material. A 4 M solution of HCl in 1,4-dioxane was added (105 mL, 5 equiv) followed by dropwise addition of distilled H_2O (11 mL, 10% v/v). The reaction mixture was stirred at 23 °C for 2 h. An oil or solid might appear to precipitate from the reaction, which was the desired HCl salt. The mixture was concentrated from 4:1 benzene/MeOH (3 × 100 mL). The solid crude material was of sufficient purity to be used directly in the next reaction without further purification (99%).

^{1}H NMR (600 MHz, MeOD): δ = 8.54 (s, 1 H), 4.88 (s, 1 H), 4.76 (d, *J* = 6.6 Hz, 1 H), 4.32–4.26 (m, 1 H), 3.92 (s, 3 H), 1.23 (d, *J* = 6.4 Hz, 3 H).

^{13}C NMR (150 MHz, MeOD): δ = 165.2, 162.9, 147.3, 131.7, 68.5, 58.8, 53.0, 19.9.

HRMS (ESI): *m*/*z* calcd for $C_{16}H_{24}N_2O_5SNa$ [M + Na]$^+$: 239.0461; found: 239.0463.

tert-Butyl (4*S*,5*R*)-4-({(1*S*,2*R*)-2-Hydroxy-1-[4-(methoxycarbonyl)thiazol-2-yl]propyl}carbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (5)

Amine **4** (35.3 g, 140 mmol, 1 equiv) was dissolved in DMF (280 mL, 0.5 M) along with protected threonine **1** (39.9 g, 154 mmol, 1.1 equiv), EDC hydrochloride (32.2 g, 168 mmol, 1.2 equiv), and HOBt (22.7 g, 168 mmol, 1.2 equiv, 80% w/w). DIPEA was added dropwise over 5 min (73 mL, 420 mmol, 3 equiv) and the reaction mixture was stirred to completion at 23 °C for 14 h. The mixture was diluted with H_2O (2 L) and extracted with EtOAc (3 × 500 mL). The combined organic layers were washed with aq 3 M LiCl (3 × 300 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography with 60 → 80% EtOAc/hexane on a short length column; the desired product **5** was collected pure after chromatography (54.5 g, 119 mmol, 85%); white foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.98 (s, 1 H), 5.15 (br s, 1 H), 4.44 (m, 1 H), 4.13 (br s, 1 H), 3.82 (d, *J* = 7.4 Hz, 1 H), 3.76 (s, 3 H), 1.48 (s, 3 H), 1.46 (s, 3 H), 1.26 (br s, 9 H), 1.15 (d, *J* = 6.5 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.1, 170.1, 161.2, 151.9, 145.9, 127.6, 94.5, 80.7, 73.7, 68.7, 67.1, 55.8, 52.0, 28.0, 27.4, 25.1, 19.3, 18.7.

HRMS (ESI): *m*/*z* calcd for $C_{20}H_{31}N_3O_7SNa$ [M + Na]$^+$: 480.1775; found: 480.1778.

tert-Butyl (4*S*,5*R*)-4-({(*Z*)-1-[4-(Methoxycarbonyl)thiazol-2-yl]prop-1-en-1-yl}carbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (6)

Solid **5** (28.1 g, 61.4 mmol, 1 equiv) was dissolved in MeCN (205 mL, 0.3 M). Boc anhydride (16.1 g, 73.7 mmol, 1.2 equiv) was added in a single portion followed by DMAP (1.5 g, 12.3 mmol, 0.2 equiv) and the reaction mixture was stirred until all starting materials were consumed. After full conversion of starting materials by TLC, DBU (45.8 mL, 307.0 mmol, 5 equiv) was added dropwise at 23 °C and the mixture was stirred for 14 h. The mixture was diluted with EtOAc (500 mL) and washed with aq 1 M HCl (200 mL), H_2O (200 mL) and brine (200 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography with 15 → 35% EtOAc/hexane on a medium length column to give 18.38 g of intermediate **6** as a colorless oil (41.8 mmol, 68%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.99 (s, 1 H), 7.96 (br s, 1 H), 6.54 (br s, 1 H), 4.32 (br s, 1 H), 3.97 (d, *J* = 7.7 Hz, 1 H), 3.85 (s, 3 H), 1.82 (d, *J* = 6.6 Hz, 3 H), 1.61 (br s, 6 H), 1.44 (d, *J* = 6.1 Hz, 3 H), 1.40 (s, 9 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.4, 167.3, 161.7, 152.3, 146.7, 127.9, 127.1, 95.1, 81.1, 74.2, 67.8, 52.3, 28.3, 27.7, 25.5, 19.0, 14.4.

HRMS (ESI)$^+$: *m*/*z* calcd for $C_{20}H_{29}N_3O_6SNa$ [M + Na]$^+$: 462.1669; found: 462.1665.

tert-Butyl (4*S*,5*R*)-4-({(*Z*)-1-[4-({(1*S*,2*R*)-2-Hydroxy-1-[4-(methoxycarbonyl)thiazol-2-yl]propyl}carbamoyl)thiazol-2-yl]prop-1-en-1-yl}carbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (8)

Methyl ester **6** (10.0 g, 22.8 mmol, 1 equiv) was dissolved in a mixture of 3:1 THF and MeOH (11.4 mL, 0.2 M) and aq 10% NaOH was added (22.8 mL, 2.28 g, 2.5 equiv). The reaction mixture was stirred for 1 h and poured into aq 2 M HCl (100 mL), extracted with EtOAc (3 × 50 mL); the combined organic layers were dried (Na_2SO_4) and concentrated. The crude acid **7** (clear oil was used directly in the next reaction without further purification (99%).

Crude acid **7** (3.83 g, 9.0 mmol, 1 equiv) was dissolved in DMF (45 mL, 0.2 M) and solid amine hydrochloride **4** was added (2.50 g, 9.9 mmol, 1.1 equiv) followed by HATU (2.07 g, 10.8 mmol, 1.2 equiv). Neat DIPEA was added dropwise (7.84 mL, 45.0 mmol, 5 equiv) and the reaction mixture was stirred for 12 h. The mixture was poured into brine (500 mL) and extracted with EtOAc (3 × 125 mL). The combined organic layers were washed with aq 3 M LiCl (3 × 100 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography with 55 → 75% EtOAc/hexane on a medium length column to give 4.25 g of **8** as a white solid (6.81 mmol, 76% over 2 steps).

^{1}H NMR (600 MHz, MeOD): δ (rotamers) = 8.34 (s, 1 H), 8.19 (s, 1 H), 6.89–6.53 (m, 1 H), 5.34 (s, 1 H), 4.53 (s, 1 H), 4.25 (s, 1 H), 4.12–4.03 (m, 1 H), 3.92 (s, 3 H), 1.91 (dd, *J* = 6.8, 2.2 Hz, 3 H), 1.59–1.26 (m, 21 H).

2-[(1*S*,2*R*)-1-(2-{(*Z*)-1-[(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxamido]prop-1-en-1-yl}thiazole-4-carboxamido)-2-hydroxypropyl]thiazole-4-carboxylic Acid (9)

Methyl ester **8** (462 mg, 1.17 mmol, 1 equiv) was dissolved in a 3:1 mixture of THF and MeOH (7.5 mL) and aq 2 M NaOH was added (0.74 mL, 2.93 mmol, 2.5 equiv) and the reaction mixture was stirred for 1 h. After full conversion of starting material, the mixture was acidified with aq 2 M HCl (40 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give the carboxylic acid **9** as a white solid. This crude material was analytically pure and was used in the next reaction without further purification (>99%).

^{1}H NMR (600 MHz, MeOD): δ = 8.30 (s, 1 H), 8.19 (s, 1 H), 6.88–6.54 (m, 1 H), 5.35 (br s, 1 H), 4.55 (br s, 1 H), 4.27–4.22 (m, 1 H), 4.05 (d, *J* = 8.0 Hz, 1 H), 1.91 (d, *J* = 7.2 Hz, 3 H), 1.59–1.39 (m, 18 H), 1.28 (d, *J* = 6.4 Hz, 3 H).

2-(2-Chloropyridin-3-yl)thiazole-4-carboxylic Acid (11)

2-Chloronicotinonitrile (**10**; 50 g, 361 mmol, 1 equiv) was dissolved in 1.5:1 *i*-PrOH/pH 7 phosphate buffer (722 mL, 0.5 M) and solid L-cysteine hydrochloride (65.6 g, 542 mmol, 1.5 equiv) was added in one portion. The reaction mixture was heated to 50 °C and stirred for 16 h. The reaction was then terminated by removing *i*-PrOH under reduced pressure and diluting with EtOAc (500 mL) and aq 2 M HCl until the solution was acidic (pH >2). The mixture was extracted with EtOAc (3 × 400 mL), the combined extracts were dried (Na_2SO_4), and concentrated to give the intermediate thiazoline as a yellow solid (76.9 g, 317 mmol, 88%); mp 156–158 °C. The crude material was of sufficient purity to be used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, MeOD): δ = 8.49 (dd, *J* = 4.9, 1.8 Hz, 1 H), 8.10 (dd, *J* = 7.7, 1.8 Hz, 1 H), 7.49 (dd, *J* = 7.7, 4.9 Hz, 1 H), 5.36 (t, *J* = 9.2 Hz, 1 H), 3.87–3.80 (m, 2 H).

Crude thiazoline (76.9 g, 317 mmol, 1 equiv) and $BrCCl_3$ (46.9 mL, 476 mmol, 1.5 equiv) were dissolved in DMF (317 mL, 1 M) in a flask and the flask was immersed in an ice bath. DBU (99 mL, 666 mmol, 2.1 equiv) was added dropwise via an addition funnel over 20 min. On large scale this addition was significantly exothermic. After addition, the reaction mixture was then brought to 50 °C and stirred for 3 h. After completion, the mixture was slowly introduced dropwise into a vigorously stirring aq 1 M HCl (2 L) at 0 °C to ensure a uniform precipitate. The mixture was stirred for 10 min and the fine brown precipitate was filtered and dried under vacuum at 50 °C for 18 h to give 76.0 g (99%, 87% over 2 steps) of crude thiazole product **11** as a grey-brown solid; mp > 200 °C. The crude material was of sufficient purity to be used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, MeOD): δ = 8.76 (dd, *J* = 7.9, 1.8 Hz, 1 H), 8.58 (s, 1 H), 8.50 (dd, *J* = 4.7, 1.9 Hz, 1 H), 7.58 (dd, *J* = 7.9, 4.7 Hz, 1 H).

^{13}C NMR (150 MHz, DMSO-d_6): δ = 162.4, 161.6, 151.3, 148.0, 147.6, 140.2, 131.1, 128.3, 124.4.

HRMS (ESI): *m*/*z* calcd for $C_9H_5ClN_2O_2S$ [M – H]$^-$: 238.9687; found: 238.9689.

3-[4-(*tert*-Butoxycarbonyl)thiazol-2-yl]-2-chloropyridine 1-Oxide (12)

Thiazolyl pyridine **11** (45.4 g, 189 mmol, 1 equiv) was dissolved in 3:1 *t*-BuOH/pyridine (756 mL, 0.25 M). TsCl (71.9 g, 2 equiv) was added slowly at 23 °C and the reaction mixture was then stirred at 60 °C for 4 h. The mixture was slowly poured into vigorously stirring distilled H_2O (3 L) and stirred for 10 min. The precipitate was filtered, washed with cold distilled H_2O (2 × 500 mL), and dried on the filter for 4 h to give the *t*-Bu ester as a brown solid (51.83 g, 93%); mp 115–117 °C.

Crude *t*-Bu ester (10.36 g, 34.9 mmol, 1 equiv) and urea-H_2O_2 complex (6.57 g, 69.8 mmol, 2 equiv) were dissolved in anhyd DCM (175 mL, 0.2 M) in a flame-dried flask equipped with a stir bar. Trifluoroacetic anhydride (TFAA; 9.71 mL, 69.8 mmol, 2 equiv) was added dropwise over 30 min via an addition funnel to the reaction mixture

in an ice bath. The mixture was then warmed to 23 °C and stirred to completion over 12 h. After completion, the reaction was diluted with additional DCM (175 mL) and aq 10% K_2CO_3 (175 mL). The organic layer was washed with H_2O (2 × 100 mL), sat. aq $Na_2S_2O_3$ (2 × 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated to give 10.1 g of crude *N*-oxide as a yellow solid (32.1 mmol, 92%; 86% over 2 steps); mp 134–136 °C. The crude material was of sufficient purity to be used directly in the next reaction without further purification.

^{1}H NMR (599 MHz, MeOD): δ = 8.61 (dd, *J* = 6.5, 1.4 Hz, 1 H), 8.56 (s, 1 H), 8.36 (dd, *J* = 8.2, 1.4 Hz, 1 H), 7.60 (dd, *J* = 8.2, 6.5 Hz, 1 H), 1.63 (s, 9 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 160.0, 159.9, 148.8, 140.4, 131.6, 128.8, 126.8, 122.9, 82.6, 28.1.

HRMS (ESI): *m*/*z* calcd for $C_{14}H_{12}ClN_3O_2S$ [M + Na]$^+$: 335.0228; found: 335.0221.

tert-Butyl 2-(2-Chloro-6-cyanopyridin-3-yl)thiazole-4-carboxylate (13)

The *N*-oxide **12** (35.1 g, 112 mmol, 1 equiv) was dissolved in anhyd MeCN (374 mL, 0.3 M) and TMSCN (35.1 mL, 2.5 equiv) and diethylcarbamyl chloride (35.6 mL, 2.5 equiv) were added at 23 °C. The reaction mixture was then brought to reflux and stirred for 14 h to completion. The reaction mixture was slowly poured into a vigorously stirred ice cold solution of aq 10% K_2CO_3 (1.5 L) and stirred for 10 min. The brown precipitate was filtered and washed with additional cold H_2O (3 × 200 mL). The collected solids were dissolved in EtOAc (500 mL), washed with brine (300 mL), dried (Na_2SO_4), and concentrated. The crude product was recrystallized from hexanes and EtOAc to give 19.13 g of **13** as a crystalline, slightly yellow solid. (59.5 mmol; 53%); mp 151–154 °C.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.01 (d, *J* = 8.0 Hz, 1 H), 8.30 (s, 1 H), 7.78 (d, *J* = 8.0 Hz, 1 H), 1.64 (s, 9 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 160.0, 159.6, 149.2, 148.7, 140.4, 133.0, 131.6, 129.4, 127.2, 115.6, 82.8, 28.2.

HRMS (ESI): *m*/*z* calcd for $C_{14}H_{12}ClN_3O_2SNa$ [M + Na]$^+$: 344.0231; found: 344.0227.

tert-Butyl 2-{2-Chloro-6-[4-(methoxycarbonyl)thiazol-2-yl]-pyridin-3-yl}thiazole-4-carboxylate (14)

Cyanopyridine **13** (10.94 g, 34.0 mmol, 1 equiv) was dissolved in 1.5:1 *i*-PrOH/pH 7 phosphate buffer (340 mL, 0.2 M buffer, 0.1 M reaction) followed by solid cysteine methyl ester hydrochloride (8.75 g, 51 mmol, 1.5 equiv) in one portion. The reaction mixture was heated to 50 °C and stirred for 6 h. *i*-PrOH was removed under reduced pressure and the residue was diluted with H_2O (300 mL) and this mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and concentrated to give 7.2 g of crude thiazoline as a yellow solid (9.18 mmol, 65%). The crude material was used directly in the next reaction without further purification.

Crude thiazoline (7.2 g, 22.2 mmol, 1 equiv) was dissolved in anhyd DCM (222 mL, 0.1 M) and activated MnO_2 (42.4 g, 444 mmol, 20 equiv) was added (Alfa Aesar; tech. 90%; LOT: W08D050). The reaction mixture was stirred rapidly for 18 h, then filtered through a pad of silica gel with MeOH to give the desired product as a yellow solid (>99%, 64% over 2 steps); mp > 200 °C. The crude material was of sufficient purity to be used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.97 (d, *J* = 8.2 Hz, 1 H), 8.38 (d, *J* = 8.2 Hz, 1 H), 8.35 (s, 1 H), 8.24 (s, 1 H), 4.01 (s, 3 H), 1.64 (s, 9 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 167.1, 161.6, 161.0, 160.2, 150.7, 148.3, 147.4, 140.8, 130.7, 129.1, 128.5, 119.1, 82.4, 52.6, 28.1.

HRMS (ESI): *m*/*z* calcd for $C_{18}H_{16}ClN_3O_4S_2Na$ [M + Na]$^+$: 438.0344; found: 438.0346.

(*R*)-*N*-{(*S*)-1-(4-Bromothiazol-2-yl)-2-[(*tert*-butyldimethylsilyl)oxy]ethyl}-2-methylpropane-2-sulfinamide (17)

Solid 2,4-dibromothiazole **15** (28.9 g, 119 mmol, 1.5 equiv) was dissolved in THF (50 mL, ?2 mL/g) and cooled in an ice bath. A 1.3 M solution of *i*-PrMgCl·LiCl in THF (98 mL, 127 mmol, 1.6 equiv) was added dropwise over 10 min. The reaction mixture was warmed to 23 °C over 30 min. The resulting solution was added dropwise over 2 h to a separate reaction vessel cooled to approximately –50 °C containing a solution of chiral imine **16** (22 g, 79 mmol, 1.0 equiv) in DCM (793 mL, 0.1 M). The mixture was allowed to warm to 23 °C over 12 h, then poured into brine (2 L), and the aqueous layer was extracted with DCM (3 × 500 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give an amber oil. The crude material was purified by flash chromatography with 10 → 30% EtOAc/hexane on a medium length column to give 23.1 g of the chiral aminothiazole **17** as an amber oil (52.3 mmol, 66%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.16 (s, 1 H), 4.83–4.77 (m, 1 H), 4.66 (d, *J* = 6.4 Hz, 1 H), 4.16 (dd, *J* = 9.8, 3.6 Hz, 1 H), 4.08 (dd, *J* = 9.9, 3.5 Hz, 1 H), 1.30 (s, 9 H), 0.81 (s, 9 H), 0.03 (s, 3 H), –0.08 (s, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 173,7, 125.0, 117.3, 66.0, 59.0, 56.3, 25.6, 22.5, 18.0, –5.5.

HRMS (ESI): *m*/*z* calcd for $C_{15}H_{29}BrN_2O_2S_2SiNa$ [M + Na]$^+$: 463.0515; found: 463.0512

(*R*)-*N*-{(*S*)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-[4-(trimethylstannyl)thiazol-2-yl]ethyl}-2-methylpropane-2-sulfinamide (18)

Bromide **17** (2.2 g, 5.0 mmol, 1 equiv) was added to a flame-dried, N_2-filled flask and dissolved in toluene (25 mL, 0.2 M). Solid $Pd(PPh_3)_4$ (576 mg, 0.5 mmol, 0.1 equiv) and neat Me_6Sn_2 (2.19 mL, 10 mmol, 2 equiv) were added and the reaction mixture was heated to 100 °C for 1 h. The mixture was then cooled to 23 °C and partially concentrated. The remaining residue was loaded directly onto a column and purified by flash chromatography with 10 → 20% EtOAc/hexane to give 1.56 g of pure stannane **18** as a slightly yellow oil (2.97 mmol, 60%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.30–7.27 (m, 1 H), 4.89 (dd, *J* = 10.0, 4.2 Hz, 1 H), 4.70 (d, *J* = 6.1 Hz, 1 H), 4.15 (dd, *J* = 9.7, 4.4 Hz, 1 H), 4.07 (dd, *J* = 9.7, 3.9 Hz, 1 H), 1.29 (s, 9 H), 0.80 (s, 9 H), 0.39–0.28 (m, 9 H), 0.01 (s, 3 H), –0.11 (s, 3 H).

tert-Butyl 2-[2-(2-{(1*S*)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-[(*tert*-butylsulfinyl)amino]ethyl}thiazol-4-yl)-6-[4-(methoxycarbonyl)thiazol-2-yl]pyridin-3-yl]thiazole-4-carboxylate (19)

Solid chloropyridine **14** (432 mg, 0.99 mmol, 1 equiv), stannane **18** (518 mg, 0.99 mmol, 1 equiv), $Pd_2(dba)_3$ (28.4 mg, 0.05 mmol, 0.05 equiv), and CycloJohnPhos (69.1 mg, 0.2 mmol, 0.2 equiv) were added to a flame-dried flask and purged with N_2. Toluene was added (10 mL, 0.1 M) and the reaction mixture was heated to 100 °C and stirred for 18 h. The mixture was then cooled to 23 °C and partially concentrated. The crude residue was loaded directly onto a column and purified by flash chromatography with 35 → 50% EtOAc/hexane on a medium length column to give 716 mg of the coupled product **19** (0.94 mmol, 95%) as a pale-yellow foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.41 (d, *J* = 8.2 Hz, 1 H), 8.38 (d, *J* = 8.2 Hz, 1 H), 8.32 (s, 1 H), 8.07 (s, 1 H), 7.82 (s, 1 H), 4.73 (q, *J* = 5.0 Hz, 1 H), 4.59 (d, *J* = 5.6 Hz, 1 H), 4.01 (s, 3 H), 3.97–3.89 (m, 2 H), 1.62 (s, 9 H), 1.30 (s, 9 H), 0.86 (s, 9 H), 0.06 (s, 3 H), –0.02 (s, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.6, 168.9, 164.8, 161.7, 160.1, 153.0, 150.8, 150.4, 148.3, 148.0, 140.2, 130.3, 129.4, 128.2, 121.5, 119.0, 82.1, 66.0, 56.2, 52.5, 28.1, 27.5, 22.5, 18.0.

HRMS (ESI): *m*/*z* calcd for $C_{33}H_{45}N_5O_6S_4Si$ $[M]^+$: 764.2905; found: 764.2094.

tert-Butyl (4*S*,5*R*)-4-{[(*Z*)-1-(4-{[(1*S*,2*R*)-1-(4-{[(*S*)-1-(4-{3-[4-(*tert*-Butoxycarbonyl)thiazol-2-yl]-6-[4-(methoxycarbonyl)thiazol-2-yl]pyridin-2-yl}thiazol-2-yl)-2-hydroxyethyl]carbamoyl}thiazol-2-yl)-2-hydroxypropyl]carbamoyl}thiazol-2-yl)prop-1-en-1-yl]carbamoyl}-2,2,5-trimethyloxazolidine-3-carboxylate (20)

Trithiazolyl pyridine **19** (910 mg, 1.19 mmol, 1 equiv) was dissolved in MeOH (6 mL, 0.2 M) and 4 N HCl in 1,4-dioxane (1.5 mL, 5.95 mmol, 5 equiv) was added and the reaction mixture was stirred at 23 °C for 2 h. After completion, the mixture was diluted with toluene and concentrated (3 × 25 mL). The crude residue was used directly in the next reaction without further purification.

The crude amine was dissolved in DMF (11.9 mL, 0.1 M) and the fragment **9** was added (800 mg, 1.31 mmol, 1.1 equiv) followed by HATU (498 mg, 1.31 mmol, 1.1 equiv) and DIPEA (0.62 mL, 3.57 mmol, 3 equiv). The reaction mixture was stirred for 14 h at 23 °C. The mixture was diluted with H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with aq 3 M LiCl (3 × 50 mL), dried (Na_2SO_4), and concentrated. The crude material was dry loaded on silica gel and purified by column chromatography with 2 → 6% MeOH/DCM on a long column to give 1.07 g of fully assembled intermediate **20** as a yellow solid (1.07 g, 0.94 mmol; 79% over 2 steps); mp > 200 °C.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.38 (d, *J* = 8.1 Hz, 1 H), 8.32 (s, 1 H), 8.28 (d, *J* = 8.1 Hz, 1 H), 8.13 (s, 1 H), 8.07 (s, 1 H), 8.04 (s, 1 H), 7.98 (s, 1 H), 5.41–5.37 (m, 1 H), 5.31 (dd, *J* = 8.8, 1.8 Hz, 1 H), 4.76–4.68 (m, 1 H), 4.37 (s, 1 H), 4.05 (dd, *J* = 11.6, 2.9 Hz, 1 H), 3.85 (dd, *J* = 11.5, 4.1 Hz, 1 H), 1.85 (d, *J* = 6.7 Hz, 3 H), 1.60–1.58 (m, *J* = 4.5 Hz, 12 H), 1.44 (d, *J* = 6.1 Hz, 3 H), 1.32 (d, *J* = 6.4 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.8, 168.9, 168.8, 168.3, 167.0, 165.1, 161.8, 161.3, 160.7, 160.6, 152.5, 150.7, 150.6, 149.1, 148.6, 148.2, 140.3, 130.5, 129.2, 128.5, 127.7, 124.5, 124.0, 122.2, 119.1, 95.0, 82.6, 81.5, 68.3, 64.2, 56.3, 52.6, 51.6, 28.3, 28.2, 26.0, 20.1, 19.4.

HRMS (ESI): *m*/*z* calcd for $C_{49}H_{56}N_{10}O_{12}S_5Na$ $[M + Na]^+$: 1159.2575; found: 1159.2572.

Methyl 2-{(1^2*Z*,3^2*Z*,7^2*Z*,11^2*Z*,4*S*,8*S*,12*Z*,15*S*)-12-Ethylidene-8,15-bis[(*R*)-1-hydroxyethyl]-4-(hydroxymethyl)-6,10,14,17-tetraoxo-5,9,13,16-tetraaza-1(2,4),3,7,11(4,2)-tetrathiazola-2(3,2)-pyridinocycloheptadecaphane-2^6-yl}thiazole-4-carboxylate (21)

Acyclic precursor **20** (1.07 g, 0.94 mmol, 1 equiv) was dissolved in 3:1 DCM/TFA (5 mL) and H_2O was added (0.5 mL, 10% v/v). The reaction mixture was stirred at 23 °C for 2 h. The mixture was concentrated from toluene (3 × 20 mL) and the residue was used directly in the next reaction without further purification.

The crude, fully deprotected intermediate was dissolved in DMF (94 mL, 0.01 M) and HATU (715 mg, 1.88 mmol, 2 equiv) and DIPEA (0.82 mL, 4.70 mmol, 5 equiv) were added and the reaction mixture was stirred for 16 h to completion. The mixture was diluted with EtOAc (500 mL) and washed with aq 3 M LiCl (3 × 200 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography with 2 → 8% MeOH/DCM on a long column, dry loaded on silica gel. Macrocycle **21** (233 mg) was collected pure after chromatography as a white solid (0.25 mmol, 27% over 2 steps); mp > 200 °C.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.72 (s, 1 H), 8.62 (d, *J* = 8.1 Hz, 1 H), 8.38 (d, *J* = 8.1 Hz, 1 H), 8.33 (s, 1 H), 8.24 (d, *J* = 9.2 Hz, 1 H), 8.17 (s, 1 H), 8.15 (s, 1 H), 8.03 (d, *J* = 8.1 Hz, 1 H), 8.00 (s, 1 H), 7.98 (s, 1 H), 7.92 (d, *J* = 7.7 Hz, 1 H), 6.43 (q, *J* = 7.0 Hz, 1 H), 5.46–5.39 (m, 2 H), 4.89 (dd, *J* = 7.8, 2.4 Hz, 1 H), 4.70–4.65 (m, 1 H), 4.39–4.35 (m, 1 H), 4.01 (s, 3 H), 3.96 (dd, *J* = 11.0, 2.9 Hz, 1 H), 1.82 (d, *J* = 7.0 Hz, 3 H), 1.48 (d, *J* = 6.3 Hz, 3 H), 1.34 (d, *J* = 6.3 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 169.8, 168.9, 168.8, 168.7, 166.2, 165.8, 161.8, 161.2, 161.0, 160.4, 153.7, 150.8, 150.6, 149.8, 149.5, 148.5, 148.2, 140.3, 130.5, 128.9, 128.6, 128.3, 125.1, 124.9, 123.7, 121.5, 118.9, 69.0, 67.9, 63.7, 57.5, 54.6, 52.6, 51.4, 20.1, 19.0, 14.5.

HRMS (ESI): *m*/*z* calcd for $C_{37}H_{34}N_{10}O_9S_5Na$ $[M + Na]^+$: 945.1006; found: 945.1011.

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Supporting Information

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