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Dissecting the Structure of Thiopeptides: Assessment of Thiazoline and Tail Moieties of Baringolin and Antibacterial Activity Optimization

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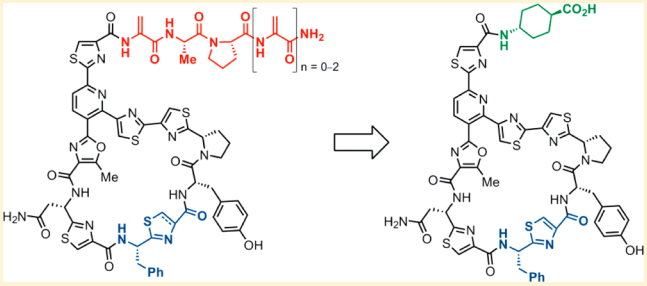
Supporting Information

ABSTRACT: Several analogues of baringolin (**1**) were prepared to evaluate the role of its characteristic thiazoline ring and pentapeptidic tail with the aim of defining structure−activity relationships for these moieties. The thiazoline ring appeared as a crucial moiety to maintain a broad scope of activities against different Gram-positive bacteria. Further modifications were performed to simplify the structure of the natural product and assess the role of its tail, resulting in an enhanced in vitro performance. Analogue **25**, with the thiazole-containing macrocycle and a 4-aminocyclohexane-1-carboxylic acid moiety in place of the pentapeptidic tail, was identified as a much more potent analogue, capable of overcoming the absence of the thiazoline ring and performing extraordinarily well against all strains tested. This is the first library of thiopeptide analogues produced by chemical synthesis alone, which demonstrates the robustness and convenience of the synthetic strategy used.



INTRODUCTION

Antibiotic resistance to marketed drugs is an increasing concern in the clinic and requires the development of new compounds that can overcome this phenomenon.[1] The discovery of new molecules with new modes of action is key to avoid cross-resistance. In this context, thiopeptide antibiotics have arisen as promising candidates due to their good performance in in vitro assays against various microorganisms. Despite the good activities reported, their lack of aqueous solubility has limited their use to the treatment of skin infections, regardless of the huge efforts carried out for the synthesis of more soluble analogues.[2−4]

The complex architecture of thiopeptides[5] has prompted many groups to develop sophisticated and robust synthetic strategies to achieve the total synthesis of many members of this family of antibiotics.[6] These approaches have scarcely been applied to the synthesis of analogues, with most of the reports focusing on fragment and synthetic intermediates screening.[7−10] In this communication we report the first de novo synthesis of a library of thiopeptide analogues[11] to assess the impact of different moieties in baringolin (**1**), leading to the preparation of various derivatives with general structure **2** (Figure 1). Alternative approaches to the preparation of modified thiopeptides have been previously explored, all taking advantage of the biosynthetic pathway that produces the parent natural products.[12] On one hand, engineering of the biosynthetic pathway has shown its potential for obtaining analogues arising from different kinds of modifications: residue replacement, enzyme knockout, and feeding with non-natural precursors.[13] Replacement of putative Ile, which is oxidized to form 2-hydroxy-4-methylpyrrolidine in the parent peptide sequence of GE37468A (**3**),[14] with Pro produced the mutasynthetic analogue **4**,[15] which has a macrocycle identical to that of baringolin (**1**) (Figure 1). On the other hand, semisynthesis permits chemical modification of the product at its most reactive sites, giving rise to a wide variety of transformations such as the conversion of thiomuracin A (**5**)[16] into its derivative **6**.[17]

Despite the very limited presence of fully synthetic thiopeptide derivatives in the literature, our recent studies on the total synthesis of **1** aimed to develop a modular and

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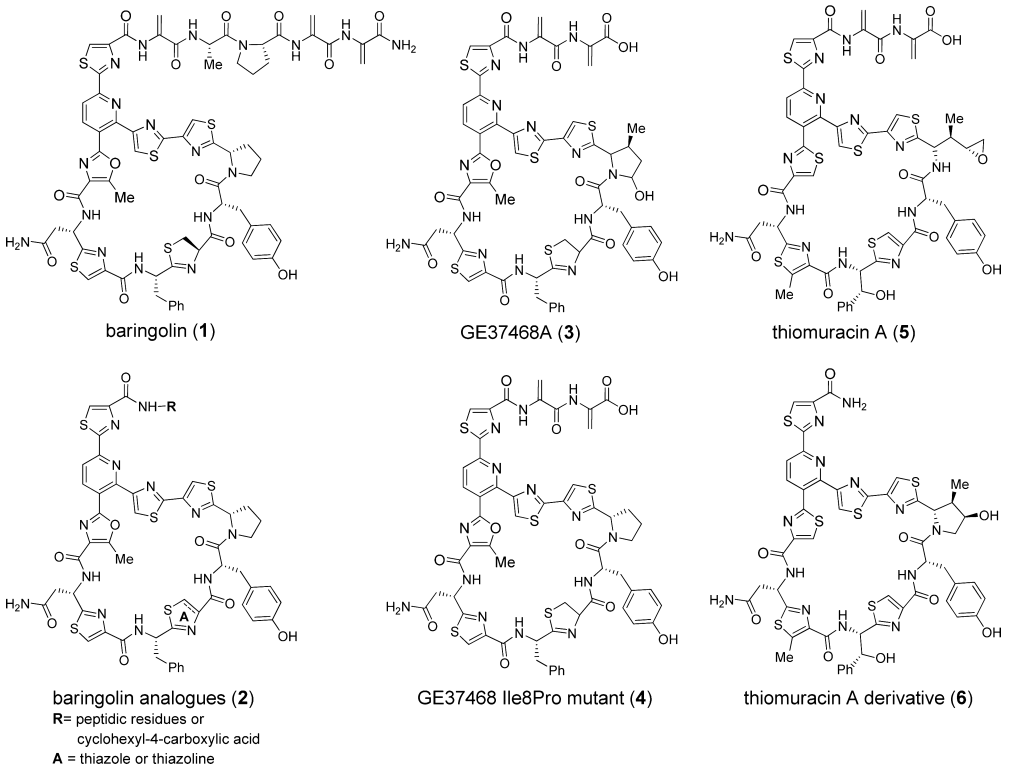
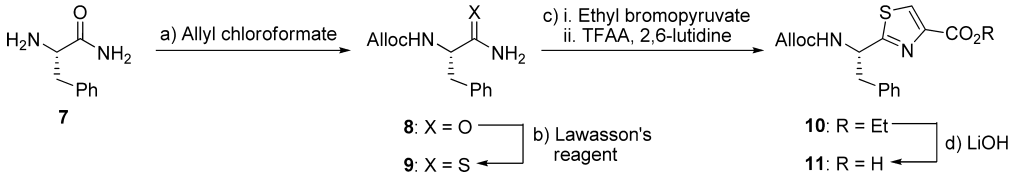


Figure 1. Thiopeptide antibiotics of the *d* series (top) and analogues of diverse origin derived from them (bottom). Baringolin analogues with general structure **2**, reported herein, were produced by chemical synthesis alone, **4** was produced by mutasynthesis, and **6** was obtained by semisynthesis.

Scheme 1. Synthesis of 11[a]



[a]Reagents and conditions: (a) allyl chloroformate, NEt_3, CH_2Cl_2, 0 °C, 2 h, 86%; (b) Lawesson's reagent, THF, rt, 4.5 h, 88%; (c) (i) ethyl bromopyruvate, $KHCO_3$, DME, 0 °C, 2.5 h, (ii) TFAA, 2,6-lutidine, DME, −20 °C, 2.5 h, 99% (95% ee); (d) LiOH, H_2O, THF, rt, 15 h, 86%. DME = dimethoxyethane, TFAA = trifluoroacetic anhydride.

convergent strategy that should facilitate the preparation of its analogues.[18] In the present study, our goal was to assess the role of both the thiazoline ring and the pentapeptidic tail of **1**. To do so, we first aimed at the synthesis of a new macrocycle in which a thiazole moiety replaces the naturally occurring thiazoline. Second, shorter peptidic tails were to be introduced to assess their impact in antibacterial activity. Some of these modifications led to analogues with improved activity. Herein we present the first library of thiopeptide analogues obtained solely by chemical synthesis.

RESULTS AND DISCUSSION

Substitution of the thiazoline moiety of the macrocycle in **1** with the corresponding thiazole was regarded as a modification likely to introduce rigidity and stability to the macrocyclic scaffold.[15] Furthermore, this modification would substitute a thiazoline building block with a more robust thiazole and should also enhance the chemical stability of the final compound. Such a modification was expected to retain activity against *Staphylococcus aureus* because the thiomuracins display the same fully unsaturated thiazole on the equivalent position of their similar macrocycle.[16] To obtain the desired analogue, a suitable building block was synthesized (Scheme 1). Phenylalaninamide (**7**) was protected with the Alloc group, yielding **8**, which was then converted into the corresponding thioamide (**9**) and subsequently transformed into the desired thiazole (**10**) by means of a two-step Hantzsch cyclization.[19−21] Ester hydrolysis produced carboxylic acid (**11**), a suitably functionalized fragment for further condensation. The use of Alloc-protected fragment **11** will facilitate the deprotection step prior to macrocyclization in subsequent stages of the synthesis.

To evaluate the role of the dehydroaminoacid tail of baringolin, the precursor tri- and tetrapeptides were synthesized to be used in the preparation of analogues of various tail lengths. The phenylselecnocysteine-containing peptides, **12** and **13**, were synthesized by solid-phase peptide synthesis (SPPS) on a Rink-Amide/Chem-Matrix resin[22] using the methodology described by van der Donk et al.[23] The corresponding C-terminal amides were thus obtained using the same methodology described for the preparation of the baringolin pentapeptide precursor (**14**)[18,24] (Scheme 2).

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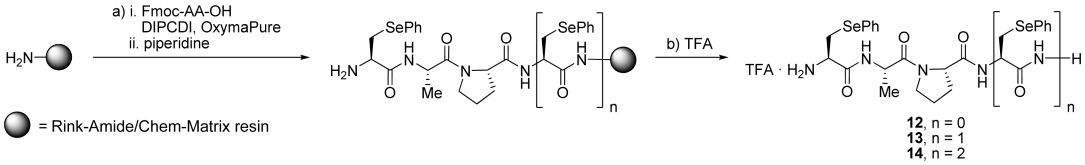
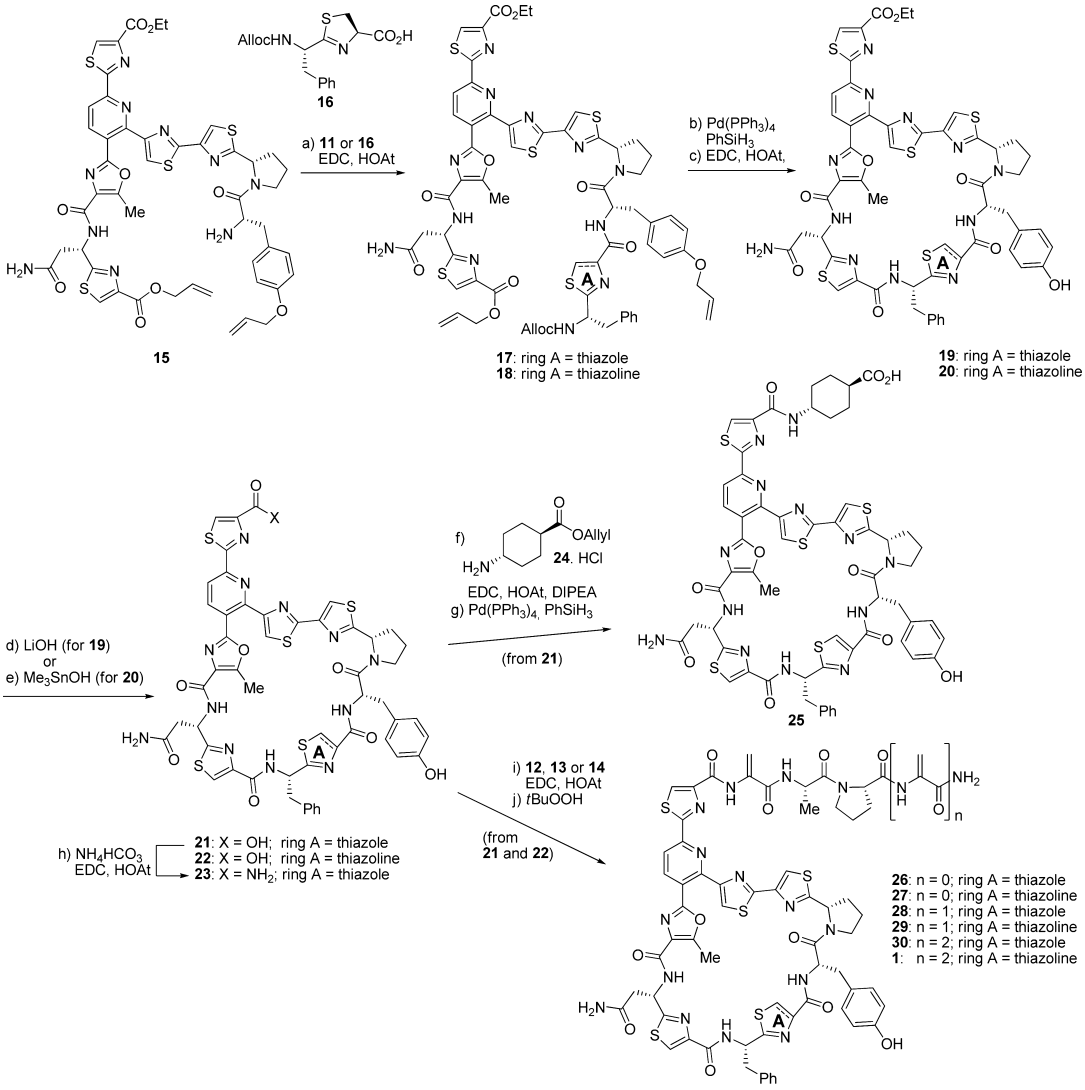
Scheme 2. Solid Phase Synthesis of Tri-, Tetra-, and Pentapeptide Precursors, 12, 13, and 14, Respectively[a]



[a]Reagents and conditions: (a) (i) Fmoc-AA-OH, DIPCDI, OxymaPure, DMF, rt, 1.5 h, (ii) 20% piperidine in DMF, rt (4 treatments); (b) 95% TFA in CH_2Cl_2, rt (4 treatments). Yields: **12** (quant), **13** (quant), **14** (89%). DIPCDI = *N*,*N*′-diisopropylcarbodiimide.

Scheme 3. Synthesis of Analogues 19−21, 23, and 25−30[a]



[a]Reagents and conditions: (a) **11** or **16**, EDC, HOAt, DIPEA, DMF, 0 °C to rt, 3 h, 85% (**17**), 68% (**18**); (b) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; (c) EDC, HOAt, DMF (1 mM), rt, 61% (**19**), 30% (**20**); (d) Me_3SnOH, $ClCH_2CH_2Cl$, 60 °C, 19 h; (e) LiOH, H_2O/THF, rt, 17 h, 99% (**21**); (f) **24**, EDC, HOAt, DIPEA, DMF, 0 °C to rt, 5 h; (g) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt, 2 h, 39% (2 steps); (h) NH_4HCO_3, EDC, HOAt, DIPEA, DMF, 0 °C to rt, 28 h, 68%; (i) **12**, **13**, or **14**, EDC, HOAt, DIPEA, DMF, 0 °C to rt; (j) *t*BuOOH, CH_2Cl_2, rt, 39% (**26**, 2 steps), 63% (**27**, 3 steps), 33% (**28**, 2 steps), 55% (**29**, 3 steps), 50% (**30**, 2 steps), 53% (**1**, 3 steps).

The preparation of the macrocycle-containing analogues proceeded from **15**, an intermediate in the total synthesis of baringolin.[18] The condensation of **15** with either thiazole building block **11** or the corresponding thiazoline (**16**)[18] furnished the protected macrocycle precursors **17** and **18**, respectively (Scheme 3). Next, removal of all allyl-based protecting groups and macrocyclization under diluted conditions (1 mM) afforded the desired macrocycles **19** and **20**. At this point, different conditions were required for hydrolysis of the ethyl ester of the two macrocycles. While the ester in **19** was hydrolyzed under more conventional basic conditions to obtain carboxylic acid **21**, **20** contains a thiazoline ring and necessitated the use of trimethyltin hydroxide to ensure a mild and epimerization-free saponification to yield **22**.[25] With both **21** and **22** in hand, manipulation of the carboxylic acids was performed to introduce a series of substituents. The amide of

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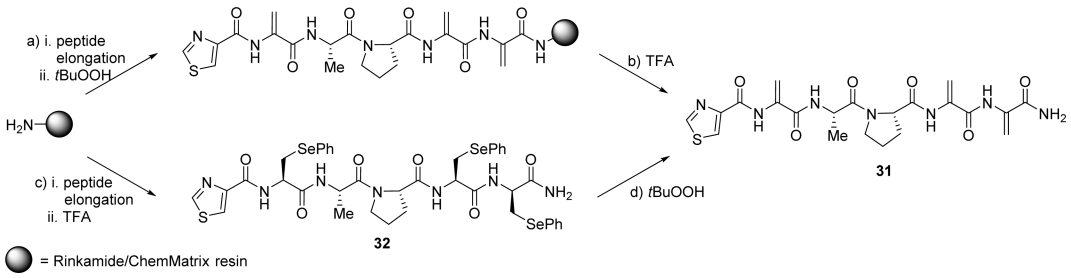
Scheme 4. Synthesis of 31[a]



[a]Reagents and conditions: (a) (i) Fmoc-AA-OH, DIPCDI, OxymaPure, DMF, rt, 1.5 h, 20% piperidine in DMF, rt (4 treatments), (ii) *t*BuOOH, CH_2Cl_2, rt; (b) 95% TFA in CH_2Cl_2, rt (4 treatments), 36% (2 steps); (c) (i) Fmoc-AA-OH, *N*,*N*′-diisopropylcarbodiimide, OxymaPure, DMF, rt, 1.5 h, 20% piperidine in DMF, rt (4 treatments), (ii) 95% TFA in CH_2Cl_2, rt (4 treatments), 47%; (d) *t*BuOOH, CH_2Cl_2, rt, 72%. DIPCDI = *N*,*N*′-diisopropylcarbodiimide.

the thiazole−macrocycle analogue was also synthesized in an analogous manner, giving rise to **23**. Moreover, allyl *trans*-4-aminocyclohexanoate (**24**) was also condensed with **21** to generate the corresponding cyclohexanoic acid derivative **25** after deprotection. The cyclohexanoic acid moiety has been previously installed into other thiopeptides through different linkers with satisfactory results.[3] Peptides **12**−**14** were condensed with acids **21** and **22** to obtain analogues **25**−**29** and **1**[18] in order to assess the impact of both thiazoline and thiazole rings as well as the role of the different peptidic tails.

In addition to the macrocyclic analogues, fragment **31**, consisting of the pentapeptide present in baringolin bearing a C-terminal 4-thiazolecarboxylic acid, was synthesized in order to assess whether the peptidic tail possess antibiotic activity in the absence of the macrocyclic scaffold. Two alternative approaches were used for the preparation of **31**: a solid-phase strategy analogous to the one described above (Scheme 4) and also the solution-phase oxidation-elimination step of the solid-support-cleaved phenylselenocysteine peptide (**32**). The two methods were equally efficient, and **31** was obtained in 34−36% overall yield.

The antibacterial activity of all prepared baringolin analogues was evaluated in vitro against different strains of Gram-positive bacteria: *Staphylococcus aureus*, *Propionibacterium acnes*, *Bacillus subtilis*, and *Micrococcus luteus*. **1** displayed good potency against all strains (Table 1), while analogues with shorter tails, such as **27** and **29**, performed similarly to **1**, indicating that the unusually long tail of baringolin is not essential for its activity. Interestingly, analogues **26**, **28**, and **30** that possess the same variable-length peptidic tails but that incorporate a thiazole ring instead of a thiazoline, retained potency against *S. aureus* but exhibited generally diminished activity. Such results indicate that the more conformationally flexible analogues incorporating a thiazoline ring might be more readily accommodated into the binding site of their biological target, presumably elongation factor Tu (EF-Tu).[26] While ethyl esters **19** and **20** were devoid of any remarkable activity, carboxylic acid **21** and amide **23** retained theirs against *S. aureus*. Such behavior was in accordance with the results obtained for the thiazole series analogues mentioned above. Surprisingly, and to our delight, analogue **25** showed an improved profile when compared to baringolin. Despite the presence of the thiazole ring in place of thiazoline, **25** remained active toward all tested strains and showed higher potencies against *S. aureus*, *P. acnes*, and *B. subtilis*. These results point out to key interactions of the newly introduced carboxylic acid,[2,3] which were able to overcome the presumably increased rigidity of the non-natural macrocycle.

Table 1. Antibacterial Activity and Solubility of Baringolin Analogues

	MIC[a] (μg/mL)				solubility[b] (mg/mL)	
compd	*S. aureus*	*P. acnes*	*B. subtilis*	*M. luteus*	H_2O	PB 0.1 M
baringolin (**1**)	0.25	0.125	0.25	0.5	BLD[c]	BLD[c]
19	>8	4	>8	2	BLD[c]	BLD[c]
20	>8	4	8	4	BLD[c]	BLD[c]
21	2	2	8	>8	BLD[c]	0.023
23	1	8	8	>8	BLD[c]	0.007
25	0.03	0.06	0.03	0.5	BLD[c]	0.018
26	0.5	4	8	2	BLD[c]	BLD[c]
27	0.25	0.125	0.25	0.5	BLD[c]	BLD[c]
28	0.5	8	0.5	2	BLD[c]	BLD[c]
29	0.5	0.5	0.5	1	BLD[c]	BLD[c]
30	0.5	8	1	2	BLD[c]	BLD[c]
31	>8	8	>8	2	4.661	6.654

[a]MIC = minimum inhibitory concentration. [b]Solubility was determined by measuring the concentration of a saturated solution of compounds. [c]BLD = below limit of detection.

Despite the higher solubility of **31** (Table 1), its poor biological profile reinforces the hypothesis of the limited impact of the peptidic tail and the otherwise key role of the macrocyclic scaffold to exert its antibacterial activity.

A robust and convergent strategy consisting of a combination of solution and solid-phase modes has facilitated the construction of the first fully synthetic library of thiopeptide analogues. The modifications introduced have helped us identify the thiazoline moiety as responsible for the broader activity profile of baringolin when compared to its less saturated analogue **29**. Moreover, the role of the tail region has also been evaluated, showing a very limited impact of tail length on activity and potency. Using the thiazole-containing macrocycle analogue as a more robust and accessible platform, the *trans*-4-aminocyclohexanoic acid moiety was introduced to furnish **25**; this modification restored the activity profile and highly improved the potency of baringolin toward most strains.

The use of a fully synthetic approach such as the one presented herein could be used to further assess the role of other regions of thiopeptides not easily modified by alternative methods of analogue production.

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CONCLUSION

Two of the most characteristic moieties of baringolin were modified to assess their structure−activity profile. Thiazoline was substituted by its aromatic counterpart using the corresponding Phe-derived thiazole. Peptidic tail variants were obtained using solid-phase synthesis. Several thiazole-4-carboxylic acid derivatives were also synthetized in the absence of a peptidic tail. Testing of all analogues against various Gram-positive bacterial strains showed that substitution of the thiazoline ring in the macrocycle with thiazole could affect the scope of antibacterial activity. By contrast, the peptidic tail did not appear as a crucial moiety, and its substitution for a *trans*-4-aminocyclohexane-1-carboxilyc acid moiety in compound **25** improved the antibacterial potency against most strains and overcame the restrictions of the thiazole series of analogues.

Notably, this is the first fully synthetic library of thiopeptide analogues ever reported. This fact, combined with the good activity results obtained, are excellent evidence to validate the synthetic strategy as a suitable one for the assessment of structure−activity relationships of such complex molecules.

EXPERIMENTAL SECTION

MIC Assays. MIC assays were performed using *Staphylococcus aureus* and *Propionibacterium acnes* from our collection, isolated from clinical samples, *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633. Isolates were taken from the freezer and transferred at least twice on supplemented Brucella agar for anaerobes and on sheep blood agar for aerobes to ensure purity and good growth. Anaerobes were incubated for 48 h and aerobes for 24 h prior to testing. Inocula were prepared by direct suspensions of cells into saline solution to achieve the turbidity of the 0.5 McFarland standard. For facultative and aerobic bacteria (*S. aureus*, *B. subtilis*, and *M. luteus*), MIC was performed by microdilution method in Mueller−Hinton broth according to CLSI guideleness (M7-A9)[28] incubated at 35 °C for 24h. For anaerobic bacteria (*P. acnes*), MIC was performed in Brucella broth supplemented with hemin (5 μg/mL), vitamin K1 (1mcg/mL), and lysed horse blood (5%) as described in CLSI-M11-A8[29] incubated at anaerobic conditions, at 35 °C for 24h.

Solubility Determination. An amount of approximately 1 mg of compound was weighed, and a known volume of the solvent was added to ensure a saturated solution would result. Vigorous vortexing for 1 min and shaking for 48 h followed. After centrifugation at 10000 rpm during 3 min, the supernatant was analyzed using a spectrophotometer at a reading wavelength of 304 nm.

Synthesis. Tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were dried using a PureSolv solvent purification system. All other solvents and reagents were used as purchased without further purification. Flash column chromatography was performed on SDS silica gel (60A 35−70 μm) as stationary phase. Analytical thin layer chromatography was performed using aluminum backed plates coated with Merck Kieselgel 60 F_{254}; compounds were visualized under a UV lamp (254 nm). Melting points were determined in a Buchi melting point B540 apparatus in open capillaries. Reverse-phase analytical HPLC was performed on a Waters Alliance separation module 2695 equipped with a Waters XBridge C18 column (4.6 mm × 75 mm, 2.5 μm) and a Waters 996 PDA with a photodiode array detector, using MeCN (0.036% TFA) and H_2O (0.045% TFA) as mobile phases in 8 min runs. Enantiomeric excess (ee) was determined by HPLC on the same separation module with a chiral stationary phase Chiralpak IA 250 mm × 4.6 mm 5 μm analytical column, flow rate 1 mL min^{-1} in 40 min runs. Polarimetry studies were performed on a Perkin−Elmer 241 or Jasco P-2000 polarimeter. IR spectra were recorded on a Thermo Nicolet FT-IR Nexus spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz or Bruker 600 MHz spectrometer. Multiplicity of the carbons was assigned with gHSQC experiments. Standard abbreviations for off-resonance decoupling were employed: (s) singlet, (d) doublet, (t) triplet, and (q) quartet. The same abbreviations were also used for the multiplicity of signals in ^1H NMR, plus: (m) multiplet, (dd) double doublet, (ddd) double doublet of doublets, (dq) double quartet and (bs) broad singlet. Spectra were referenced to appropriate residual solvent peaks ($CDCl_3$, DMSO-d_6, acetone-d_6, or pyridine-d_5). High-resolution mass spectroscopy (HRMS) was performed on either a LTQ-FT Ultra (Thermo Scientific) or a LCT-Premier (Waters) high resolution mass spectrometer by the Mass Spectrometry Service of the Institute for Research in Biomedicine (IRB). Purity of tested compounds was assessed by HPLC to be >95%.

*(S) N-(Allyloxycarbonyl)phenylalaninamide (**8**).* Allyl chloroformate (1.2 mL, 10.96 mmol) was added slowly to a stirring suspension of hydrochloride salt of **7** (2.0 g, 9.97 mmol) in dry CH_2Cl_2 (200 mL) cooled in an ice bath. Next, NEt_3 (3.1 mL, 21.93 mmol) was added dropwise. After stirring for 2 h at 0 °C, the reaction mixture was poured onto brine (200 mL), fractions were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 3:7). The title product was obtained as a white solid (2.13 g, 86%). The product obtained in this manner was identical to the one described in the literature.[27] ^1H NMR (400 MHz, $CDCl_3$) δ = 3.06 (dd, J = 14.0, 6.8 Hz, 1 H), 3.12 (dd, J = 13.6, 6.8 Hz, 1 H), 4.38−4.48 (m, 1 H), 4.53−4.57 (m, 2 H), 5.21 (ddd, J = 10.4, 2.8, 1.2 Hz, 1 H), 5.27 (ddd, J = 17.2, 2.8, 1.6 Hz, 1 H), 5.36 (bs, 1 H), 5.53 (bs, 1 H), 5.77 (bs, 1 H), 5.82−5.93 (m, 1 H), 7.20−7.28 (m, 3 H), 7.29−7.34 (m, 2 H) ppm. HRMS m/z calcd for $C_{13}H_{17}O_3N_2$ (M + H) 249.1234, found 249.1234.

*(S) N-(Allyloxycarbonyl)phenylalanine thioamide (**9**).* A solution of **8** (1.83 g, 7.37 mmol) and Lawesson's reagent (1.49 g, 3.69 mmol) in dry THF (10 mL) was stirred at rt. After 4.5 h, saturated aq $NaHCO_3$ (50 mL) was added to the reaction vessel. After 1 h, the reaction mixture was poured into saturated aq $NaHCO_3$ (100 mL). Layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 150 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 1:1). The title product was obtained as a colorless oil (1.71 g, 88%). $[\alpha]_D$ +30.6 (c = 1.00, CH_2Cl_2). IR (film) 3302, 3206, 2943, 1700, 1623, 1502, 1438, 1152, 1041 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 3.12 (dd, J = 13.6, 8.2 Hz, 1 H), 3.21 (dd, J = 13.6, 8.2 Hz, 1 H), 4.50−4.56 (m, 2 H), 4.62−4.70 (m, 1 H), 5.21 (ddd, J = 10.4, 2.8, 1.2 Hz, 1 H), 5.28 (ddd, J = 17.2, 2.8, 1.6 Hz, 1 H), 5.58 (bs, 1 H), 5.82−5.93 (m, 1 H), 7.14 (bs, 1 H), 7.22−7.38 (m, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.2 (t), 61.6 (d), 66.4 (t), 118.3 (t), 127.4 (d), 128.9 (d), 129.6 (d), 132.5 (d), 136.5 (s), 156.2 (s) ppm. HRMS m/z calcd for $C_{13}H_{17}O_2N_2S$ (M + H) 265.1005, found 265.1018.

*(S)-Ethyl 2-(1-(Allyloxycarbonylamino)-2-phenylethyl)thiazole-4-carboxylate (**10**).* A mixture of **9** (1.41 g, 5.35 mmol) and $KHCO_3$ (5.9 g, 58.85 mmol) in dry DME (13.4 mL) was stirred at rt. After 15 min, the mixture was placed in an ice bath and ethyl bromopyruvate (2.0 mL, 16.05 mmol) was added dropwise, and the resulting mixture was stirred at 0 °C. After 20 h, the mixture was allowed to reach rt, filtered through celite, and washed with Et_2O. Volatiles were removed, and the crude hydroxythiazoline was redissolved in dry DME (13.4 mL) and cooled at −20 °C. A preformed mixture of trifluoroacetic anhydride (3.0 mL, 21.4 mmol) and 2,6-lutidine (5.6 mL, 48.15 mmol) was added dropwise to the stirring solution. After 2.5 h at the same temperature, the mixture was diluted with CH_2Cl_2 (250 mL), washed with 1 N HCl (250 mL) and saturated aq $NaHCO_3$ (300 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hexanes/*t*BuOMe, 1:1). The title product was obtained as a yellowish oil (1.91 g, 99%). 95% ee; H_2O (0.045% TFA):MeCN (0.036% TFA), 50% MeCN (t_R = 10.30 min); detected at 254 nm. $[\alpha]_D$ −15.4 (c = 1.00, CH_2Cl_2). IR (KBr) 3327, 2982, 2933, 1715, 1237, 1212 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.42 (t, J = 7.6 Hz, 3 H), 3.34 (d, J = 6.4 Hz, 2 H), 4.44 (q, J = 7.6 Hz, 2 H), 4.54 (d, J = 5.6 Hz, 2 H), 5.19 (d, J = 10.4 Hz, 1 H), 5.26 (d, J = 16.8 Hz, 1 H), 5.30−5.38 (m, 1 H), 5.51 (bs, 1 H),

5.77−5.94 (m, 1 H), 7.07−7.12 (m, 2 H), 7.19−7.29 (m, 3 H), 8.04 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.7 (q), 43.9 (t), 56.6 (d), 63.8 (t), 68.3 (t), 120.2 (t), 129.5 (d), 129.6 (d), 131.0 (d), 131.7 (d), 134.8 (d), 138.3 (s), 149.7 (s), 157.8 (s), 163.6 (s)174.5 (s) ppm. HRMS m/z calcd for $C_{18}H_{21}O_4N_2S$ (M + H) 361.1217, found 361.1218.

*(S)-2-(1-(Allyloxycarbonylamino)-2-phenylethyl)thiazole-4-carboxylic Acid (**11**).* Aqueous 2 N LiOH (5 mL, 10.18 mmol) was added to a stirring solution of **10** (1.84 g, 5.09 mmol) in THF (57 mL). The mixture was stirred at rt under air. After 15 h, EtOAc (250 mL) and H_2O (250 mL) were added and the layers were separated. Starting material was recovered from the organic fraction (257 mg, 14%). Aqueous 2N HCl was added to the aqueous layer until it reached a pH of 2−3, and it was extracted with EtOAc (3 × 250 mL). The title product was obtained as a white solid (1.46 g, 86%). The crude product was used in following reactions without further purification or characterization.

General Method for Solid-Phase Peptide Synthesis. The Rink-Amide Chem-Matrix resin (loading = 0.52 mmol/g) was swollen in MeOH, then in DMF, and finally in CH_2Cl_2. Fmoc-AA-OH (3 equiv), preactivated by vigorous shaking for 4 min with DIPCDI (3.3 equiv) and Oxyma Pure (3.3 equiv) in DMF, was poured onto the resin and the resulting mixture was gently shaken for 1 h. The resin was then washed with DMF and CH_2Cl_2 (5× each). The *N*-terminus was deprotected using 20% piperidine in DMF (treatments of 2 × 1 min, then 2 × 5 min). The resin was then washed with CH_2Cl_2 and DMF (5× each). Loading onto the resin (0.249 mmol/g, 48%) was determined through measuring dibenzofulvene absorbance at 290 nm of cleavage solutions and washings.

Elongation of the peptide proceeded as follows; the number of equivalents refers to the original functionalization, not the loading. Fmoc-AA-OH (2.3 equiv) was preactivated by vigorous shaking for 4 min in the presence of DIPCDI (2.5 equiv) and Oxyma Pure (2.5 equiv) in DMF and was then poured onto the resin. The resulting mixture was gently shaken for 1.5 h. Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the desired peptide. The peptide was cleaved from the resin by treatment with 95% TFA in CH_2Cl_2 (4 × 20 min) at rt followed by filtration and collection of the filtrate. Next, washing of the resin with CH_2Cl_2 (×6) was performed. Most TFA was removed under vacuum, and the resulting concentrated solution was poured into cold Et_2O. Centrifugation and pouring off the solvent yielded the desired peptide as TFA salt. Purity was determined by HPLC (linear gradient: 0−100% MeCN in H_2O over 8 min; flow rate = 1.0 mL/min).

*H-Sec(Ph)-Ala-Pro-NH$_2$ (**12**).* Tripeptide **12** was prepared according to the general method for solid-phase peptide synthesis, starting from 1.0 g of resin. The title product was obtained as a pale powder (131 mg, quant based on calculated loading of the resin). HPLC purity: 100%; H_2O (0.045% TFA):MeCN (0.036% TFA), 0−100% MeCN (t_R = 3.91 min); detected at 254 nm; mp (Et_2O) decomposes above 125 °C. $[\alpha]_D$ −22.2 (c = 0.50, CH_2Cl_2). IR (KBr) 1675, 1630, 1207, 1130 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.26 (d, J = 7.2 Hz, 3 H), 1.78−2.11 (m, 4 H), 3.23−3.37 (m, 2 H), 3.55−3.64 (m, 2 H), 4.08−4.16 (m, 1 H), 4.25 (dd, J = 8.4, 3.6 Hz, 1 H), 4.47−4.58 (m, 1 H), 6.94 (bs, 2 H), 7.29−7.36 (m, 3 H), 7.51−7.57 (m, 2 H), 7.58−7.62 (m, 1 H), 8.51 (bs, 3 H), 8.85 (d, J = 7.6 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 18.1 (q), 25.3 (t), 28.1 (t), 30.3 (t), 47.5 (d), 47.6 (t), 52.7 (d), 60.5 (d), 128.2 (d), 130.0 (s), 130.2 (d), 132.9 (d), 167.3 (s), 170.4 (s), 174.4 (s) ppm. HRMS m/z calcd for $C_{17}H_{25}N_4O_3Se$ (M + H) 413.1086, found 413.1087

*H-Sec(Ph)-Ala-Pro-Sec(Ph)-NH$_2$ (**13**).* Tetrapeptide **13** was prepared according to the general method for solid-phase peptide synthesis, starting from 1.0 g of resin. The title product was obtained as a white powder (187 mg, quant based on calculated loading of the resin). HPLC purity: 100%; H_2O (0.045% TFA):MeCN (0.036% TFA), 0−100% MeCN (t_R= 5.21 min); detected at 254 nm; mp (Et_2O) 87−90 °C. $[\alpha]_D$ −26.8 (c = 1.00, CH_2Cl_2). IR (KBr) 1662, 1201, 1130 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.28 (d, J = 7.2 Hz, 3 H), 1.78−2.15 (m, 4 H), 3.12−3.40 (m, 4 H), 3.50−3.69 (m, 2 H), 4.07−4.16 (m, 1 H), 4.27−4.35 (m, 1 H), 4.36−4.45 (m, 1 H), 4.52−4.63 (m, 1 H), 7.23−7.39 (m, 8 H), 7.49−7.59 (m, 4 H), 8.01−8.09 (m, 1 H), 8.51 (bs, 3 H), 8.90−8.97 (m, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 18.1 (q), 25.4 (t), 28.1 (t), 29.8 (t), 29.9 (t), 47.6 (d), 47.8 (t), 52.7 (d), 53.4 (d), 61.1 (d), 127.6 (d), 128.2 (d), 129.9 (s), 130.2 (d), 130.2 (d), 131.2 (s), 132.4 (d), 132.9 (d), 167.3 (s), 171.1 (s), 172.2 (s); 172.8 (s) ppm. HRMS m/z calcd for $C_{26}H_{34}N_5O_4Se_2$ (M + H) 640.0936, found 640.0951.

*Protected Open Macrocycle Thiazole Analogue (**17**).* A mixture of EDC·HCl (69 mg, 0.359 mmol), HOAt (49 mg, 0.359 mmol), and DIPEA (60 μL, 0.359 mmol) was added to a solution of **15**[18] (310 mg, 0.299 mmol) and **11** (119 mg, 0.359 mmol) in dry DMF (6 mL) cooled in an ice bath. The resulting solution was stirred at 0 °C for 2.5 h, then was allowed to reach rt and stirred for another 2 h. The reaction mixture was diluted with EtOAc (150 mL), washed with saturated aq NH_4Cl (100 mL), $NaHCO_3$ (100 mL), and H_2O (100 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/EtOAc, 2:8 to EtOAc). The title compound was obtained as a white solid (343 mg, 85%), mp (EtOAc) 135−138 °C. $[\alpha]_D$ +9.6 (c = 0.33, CH_2Cl_2). IR (KBr) 3440, 2922, 1719, 1643, 1510, 1424, 1239 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.44 (t, J = 7.0 Hz, 3 H), 1.98−2.32 (m, 4 H), 2.36−2.60 (m, 3 H), 2.69−3.41 (m, 6 H), 3.46−4.06 (m, 2 H), 4.26−4.62 (m, 6 H), 4.72−4.84 (m, 2 H), 4.91−5.42 (m, 8 H), 5.43−5.50 (m, 1 H), 5.58 (bs, 1 H), 5.68−6.10 (m, 4 H), 6.62−6.94 (m, 2 H), 6.98−7.25 (m, 7 H), 7.41 (s, 1 H), 7.81 (bs, 1 H), 8.02−8.08 (m, 1 H), 8.11−8.15 (m, 1 H), 8.25 (d, J = 8.2 Hz, 1 H), 8.28−8.33 (m, 1 H), 8.37 (d, J = 8.2 Hz, 1 H), 8.59−8.68 (m, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.0 (q), 14.7 (q), 24.8 (t), 32.2 (t), 37.5 (t), 38.9 (t), 40.6 (t), 47.8 (t), 48.1 (d), 53.1 (d), 54.2 (d), 59.2 (d), 62.0 (t), 66.2 (t), 66.2 (t), 69.0 (t), 115.2 (d), 115.4 (d), 117.8 (t), 118.3 (t), 119.0 (d), 119.2 (t), 121.8 (d), 123.0 (s), 124.1 (d), 127.2 (d), 128.5 (d), 128.9 (d), 129.7 (s), 129.9 (d), 130.2 (s), 130.6 (d), 130.6 (d), 130.8 (s), 132.1 (d), 132.9 (d), 133.6 (d), 136.6 (s), 140.3 (d), 146.8 (s), 149.0 (s), 149.1 (s), 149.3 (s), 149.6 (s), 151.3 (s), 151.4 (s), 154.1 (s), 154.3 (s), 154.6 (s), 155.9 (s), 157.5 (s), 157.9 (s), 161.2 (s), 161.6 (s), 161.8 (s), 161.9 (s), 162.1(s), 169.1 (s), 172.4 (s), 173.3 (s) ppm. HRMS m/z calcd for $C_{64}H_{61}O_{12}N_{12}S_5$ (M + H) 1349.3130, found 1349.3196.

*Thiazole Analogue Ethyl Ester (**19**).* A solution of $Pd(PPh_3)_4$ (64 mg, 0.055 mmol) in dry CH_2Cl_2 (7 mL) was added to a stirring solution of **17** (745 mg, 0.552 mmol) in dry CH_2Cl_2 (30 mL). $PhSiH_3$ (340 μL, 2.760 mmol) was subsequently added. The resulting mixture was stirred at rt. After 7 h, more $Pd(PPh_3)_4$ (32 mg, 0.028 mmol) in dry CH_2Cl_2 (3.5 mL) was added and the reaction mixture stirred for another 2 h. Upon consumption of all starting material, volatiles were evaporated under reduced pressure. The flask was filled with N_2 and the crude dissolved in dry DMF (550 mL). Addition of EDC·HCl (131 mg, 0.662 mmol) and HOAt (90 mg, 0.662 mmol) followed. After 3 days, a further amount of EDC·HCl (131 mg, 0.662 mmol) and HOAt (90 mg, 0.662 mmol) were added. After three more days, all starting material was consumed and the DMF volume was reduced to approximately 100 mL under reduced pressure. H_2O (250 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 250 mL). Combined organics were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 95:5). The title product was obtained as a white solid (392 mg, 61%). HPLC: 30−80% MeCN (t_R = 7.70 min); mp (CH_2Cl_2) decomposes above 120 °C. $[\alpha]_D$ +117.8 (c = 1.00, CH_2Cl_2). IR (KBr) 3383, 3118, 2924, 2852, 1781, 1728, 1655, 1546, 1497, 1211, 1168 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.65 (d, J = 16.8, 1 H), 1.44 (t, J = 7.1 Hz, 3 H), 1.97−2.08 (m, 1 H), 2.10−2.22 (m, 1 H), 2.26−2.40 (m, 2 H), 2.45−2.58 (m, 1 H), 2.83 (s, 3 H), 3.09 (dd, J = 14.0, 4.8 Hz, 1 H), 3.18 (dd, J = 14.0, 3.0 Hz, 1 H), 3.35 (d, J = 5.2 Hz, 2 H), 3.95−4.05 (m, 2 H), 4.47 (q, J = 7.1 Hz, 2 H), 5.02−5.10 (m, 1 H), 5.29−5.44 (m, 3 H), 5.60 −5.67 (m, 1 H), 6.60 (d, J = 8.4 Hz, 2 H), 6.82−6.97 (m, 4 H), 7.18−7.24 (m, 3 H), 7.57 (s, 1 H), 7.73 (d, J = 10.0 Hz, 1 H), 7.86 (s, 1 H), 7.93 (s, 1 H), 8.09 (s, 1 H), 8.27−8.33 (m, 2 H), 8.41 (d, J = 8.0 Hz, 1 H), 8.62 (d, J = 7.2 Hz, 1 H), 8.70 (d, J = 9.6 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.4 (q), 14.7 (q), 25.8 (t), 34.2 (t), 37.2 (t), 38.5 (t), 43.5 (t), 48.2

(d), 48.5 (t), 51.0 (d), 53.3 (d), 61.7 (d), 62.0 (t), 114.3 (d), 117.2 (d), 119.2 (d), 122.7 (d), 122.8 (d), 122.9 (s); 124.7 (d), 125.5 (s), 127.4 (d), 128.6 (d), 130.2 (d), 130.3 (s), 130.6 (d), 131.6 (d), 135.6 (s), 138.8 (d), 148.6 (s), 148.9 (s), 148.9 (s), 149.2 (s), 151.0 (s), 151.9 (s), 154.5 (s), 154.6 (s), 156.0 (s), 156.3 (s), 160.0 (s), 160.3 (s), 160.7 (s), 161.6 (s), 163.3 (s), 169.0 (s), 169.2 (s), 171.0 (s), 172.4 (s), 173.1 (s); 173.8 (s) ppm. HRMS *m/z* calcd for $C_{54}H_{47}O_9N_{12}S_5$ (M + H) 1167.2187, found 1167.2190.

*Thiazole Analogue Carboxylic Acid (**21**).* Aqueous 3 N LiOH (340 μL, 1.028 mmol) was added to a stirring solution of **19** (200 mg, 0.171 mmol) in THF (1.7 mL). The resulting mixture was stirred at rt under air. After 17 h, the reaction was diluted with THF/CH_2Cl_2 (25 mL, 1:1) and 2 M HCl (25 mL) were added. Layers were separated, and the aqueous phase was extracted with THF/CH_2Cl_2 (2 × 25 mL, 1:1). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The title product was obtained as a white solid (193 mg, 99%). HPLC: 50−100% MeCN (t_R = 2.68 min); mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +119.5 (*c* = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3385, 2917, 2847, 1649, 1549, 1489, 1425, 1201 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.15−1.42 (m, 1 H), 1.66−2.28 (m, 5 H), 2.81 (s, 3 H), 3.17−3.85 (m, 6 H), 5.38−5.54 (m, 1 H), 5.63−5.73 (m, 1 H), 5.76−5.85 (m, 1 H), 5.88−6.07 (m, 1 H), 7.04−7.13 (m, 2 H), 7.15−7.22 (m, 5 H), 7.39 (d, *J* = 8.4 Hz, 2 H), 7.64 (bs, 1 H), 7.98 (bs, 1 H), 8.04 (s, 1 H), 8.22 (s, 1 H), 8.28 (s, 1 H), 8.32 (d, *J* = 8.0 Hz, 1 H), 8.37 (s, 1 H), 8.38−8.42 (m, 1 H), 8.52 (d, *J* = 8.0 Hz, 1 H), 8.76 (s, 1 H), 9.08 (d, *J* = 7.2 Hz, 1 H), 9.46 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.9 (q), 25.2 (t), 33.6 (t), 37.7 (t), 39.0 (t), 43.5 (t), 48.0 (t), 49.5 (d), 52.2 (d), 53.6 (d), 61.3 (d), 115.6 (d), 116.6 (d), 118.9 (d), 122.9 (d), 123.0 (d), 124.8 (d), 126.4 (s), 127.3 (d), 128.7 (d), 130.2 (d), 131.0 (s), 131.5 (d), 136.7 (d), 139.3 (d), 149.3 (s), 149.3 (s), 151.3 (s), 151.6 (s), 152.2 (s), 154.0 (s), 154.9 (s), 156.0 (s), 158.1 (s), 160.1 (s), 160.5 (s), 161.3 (s), 162.8 (s), 164.0 (s), 168.4 (s), 169.2 (s), 171.7 (s), 173.1 (s), 175.0 (s), 175.1 (s) ppm. HRMS *m/z* calcd for $C_{52}H_{43}O_9N_{12}S_5$ (M + H) 1139.1874, found 1139.1888.

*Thiazole Amide Analogue (**23**).* A mixture of NH_4HCO_3 (10 mg, 132.0 μmol), EDC·HCl (10 mg, 52.7 μmol), HOAt (7 mg, 52.7 μmol), and DIPEA (24 μL, 132.0 μmol) was added to a stirring solution of **21** (50 mg, 43.9 μmol) in dry DMF (0.9 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 28 h, CH_2Cl_2 (25 mL) was added and the resulting solution was washed with saturated aq NH_4Cl (25 mL) and saturated aq $NaHCO_3$ (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 99:1 to 96:4). The title product was obtained as a white solid (34 mg, 68%). HPLC: 50−100% MeCN (t_R = 2.60 min); mp (CH_2Cl_2) decomposes above 180 °C. $[\alpha]_D$ +114.4 (*c* = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3385, 2917, 2847, 1649, 1534, 1489, 1419 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.92−2.27 (m, 5 H), 2.39−2.51 (m, 1 H), 2.78 (s, 3 H), 2.82−2.97 (m, 2 H), 3.10−3.26 (m, 2 H), 3.71−3.92 (m, 2 H), 5.03−5.13 (m, 1 H), 5.30−5.42 (m, 2 H), 5.63−5.72 (m, 1 H), 6.68 (d, *J* = 8.4 Hz, 2 H), 6.83 (bs, 1 H), 7.12 (d, *J* = 8.4 Hz, 2 H), 7.17−7.38 (6 H), 7.74−7.81 (m, 2 H), 8.00 (d, *J* = 9.2 Hz, 1 H), 8.10 (bs, 1 H), 8.13 (s, 1 H), 8.23 (s, 1 H), 8.37 (s, 1 H), 8.50 (d, *J* = 8.2 Hz, 1 H), 8.52 (s, 1 H), 8.56 (d, *J* = 8.2 Hz, 1 H), 8.70−8.80 (m, 2 H), 9.25 (s, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 12.6 (q), 25.5 (t), 33.6 (t), 38.0 (t), 39.0 (t), 42.6 (t), 48.2 (t), 49.4 (d), 52.7 (d), 53.4 (d), 60.4 (d), 116.1 (d), 117.2 (d), 119.6 (d); 123.2 (d), 123.3 (s), 124.5 (d), 125.8 (d), 127.4 (d), 127.7 (s), 128.3 (d), 129.2 (d), 130.4 (d), 131.3 (d), 137.4 (s), 140.9 (d), 148.5 (s), 149.8 (s), 151.8 (s), 152.1 (s), 152.7 (s), 154.0 (s), 154.6 (s), 156.6 (s), 157.0 (s), 160.0 (s), 161.1 (s), 161.7 (s), 162.0 (s), 163.0 (s), 167.6 (s), 170.3 (s), 171.2 (s), 172.6 (s), 174.5 (s), 175.3 (s) ppm. HRMS *m/z* calcd for $C_{52}H_{44}O_8N_{13}S_5$ (M + H) 1138.2034, found 1138.2043.

*Allyl trans-4-(tert-butoxycarbonylamino)cyclohexanoate (**S1**).* Dry DMF (23 mL) was added to a flask charged with 4-*trans*-(*tert*-butoxycarbonylamino)cyclohexanoic acid (1.00 g, 4.11 mmol) and $NaHCO_3$ (2.49 g, 29.59 mmol). The mixture was stirred at rt under inert atmosphere. After 10 min, allyl bromide (7.5 mL, 86.31 mmol) was added and the mixture stirred for another 24 h at rt. The reaction mixture was then diluted with EtOAc (250 mL), washed with H_2O (250 mL) and brine (2 × 100 mL), dried (Na_2SO_4), and then concentrated in vacuo. The desired product was obtained as a colorless oil (1.13 g, 97%). IR (film) 3378, 2976, 2936, 2862, 1733, 1713m 1519, 1173 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.05−1.17 (m, 2 H), 1.43 (s, 9 H), 1.47−1.63 (m, 2 H), 1.98−2.12 (m, 4 H), 2.25 (tt, *J* = 12.0, 3.6 Hz, 1 H), 3.41 (bs, 1 H), 4.37 (bs, 1 H), 4.56 (dt, *J* = 5.6, 1.2 Hz, 2 H), 5.20−5.33 (m, 2 H), 5.84−5.96 (m, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.2 (t), 28.8 (q), 32.9 (t), 42.8 (d), 49.3 (d), 65.3 (t), 79.6 (s), 118.4 (t), 132.6 (d), 155.5 (s), 175.4 (s) ppm. HRMS *m/z* calcd for $C_{15}H_{26}O_4N$ (M + H) 284.1856, found 2841858.

*Allyl trans-4-Aminocyclohexanoate Hydrochloride (**24**).* A solution of 4 M HCl in 1,4-dioxane (18 mL, 70.2 mmol) was added to a stirring solution of **S1** (996 mg, 3.51 mmol) in dry 1,4-dioxane (18 mL). After 4 h, the reaction mixture was diluted with CH_2Cl_2 and then concentrated in vacuo. The title product was obtained as a white solid (747 mg, 97%). The crude product was used in following reactions without further purification or characterization.

*Thiazole Cyclohexanoic Acid Analogue (**25**).* **24** (14 mg, 65.8 μmol), EDC·HCl (10 mg, 52.7 μmol), HOAt (7 mg, 52.7 μmol), and DIPEA (12 μL, 65.8 μmol) were added to a stirring solution of **21** (50 mg, 43.9 μmol) in dry DMF (0.9 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 5 h, CH_2Cl_2 (25 mL) was added and the resulting solution was washed with saturated aq NH_4Cl (25 mL) and saturated aq $NaHCO_3$ (25 mL), dried (Na_2SO_4), and then concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 96:4). The condensation product was obtained as a white solid (36 mg, 63%). A solution of $Pd(PPh_3)_4$ (3 mg, 2.8 μmol) in dry CH_2Cl_2 (1.9 mL) and $PhSiH_3$ (17 μL, 0.138 mmol) was added to a flask charged with the condensation product (36 mg, 27.6 μmol). The resulting solution was stirred at rt for 2 h. Volatiles were removed under reduced pressure and the crude product purified by preparative reverse-phase column (C18, 30− 70% MeCN in H_2O). The title product was obtained as a white solid (21 mg, 39% for two steps). HPLC: 30−80% MeCN (t_R = 6.26 min); mp (CH_2Cl_2) decomposes above 140 °C. $[\alpha]_D$ +92.7 (*c* = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3385, 2917, 2841, 1656, 1534, 1495, 1419, 1194 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.18−2.05 (m, 8 H), 2.12−2.36 (m, 5 H), 2.42−2.52 (m, 1 H), 2.79 (s, 3 H), 3.19−3.62 (m, 5 H), 3.66−3.84 (m, 2 H), 4.33−4.46 (m, 1 H), 5.38−5.46 (m, 1 H), 5.63−5.72 (m, 1 H), 5.74−5.83 (m, 1 H), 5.98−6.06 (m, 1 H), 7.04−7.10 (m, 2 H), 7.16−7.22 (m, 5 H), 7.38 (d, *J* = 8.4 Hz, 2 H), 7.64 (bs, 1 H), 7.95−8.03 (m, 3 H), 8.21 (s, 1 H), 8.23−8.28 (m, 2 H), 8.36 (s, 1 H), 8.39 (d, *J* = 10.0 Hz, 1 H), 8.66 (s, 1 H), 8.71−8.76 (d, *J* = 8.8 Hz, 1 H), 9.08 (d, *J* = 6.8 Hz, 1 H), 9.46 (d, *J* = 8.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.8 (q), 25.2 (t); 28.9 (t), 32.4 (t), 33.7 (t), 37.7 (t); 39.1 (t), 43.2 (d), 43.5 (t), 48.0 (t), 48.6 (d), 49.5 (d), 52.2 (d), 53.6 (d), 61.3 (d), 115.6 (d), 116.6 (d), 118.7 (d), 122.9 (d), 123.0 (d), 124.8 (d), 126.4 (s), 127.0 (s), 127.3 (d), 128.7 (d), 130.2 (d), 131.0 (s), 131.5 (d), 136.7 (d), 139.1 (d), 149.2 (s), 149.4 (s), 151.4 (s), 152.2 (s), 153.1 (s), 153.9 (s), 154.8 (s), 156.0 (s), 158.1 (s), 160.1 (s), 160.5 (s), 160.6 (s), 161.3 (s), 162.8 (s), 168.0 (s), 169.2 (s), 171.7 (s), 173.1 (s), 175.1 (s), 175.1 (s), 177.8 (s) ppm. HRMS *m/z* calcd for $C_{59}H_{54}O_{10}N_{13}S_5$ (M + H) 1264.2715, found 1264.2741.

*Thiazole Tripeptide Analogue (**26**).* **12** (25 mg, 0.047 mmol), EDC·HCl (9 mg, 0.047 mmol), HOAt (6 mg, 0.047 mmol), and DIPEA (8 μL, 0.047 mmol) were added to a stirring solution of **21** (45 mg, 0.039 mmol) in dry DMF (0.8 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 5 h, CH_2Cl_2 (15 mL) was added and the resulting solution was washed with saturated aq NH_4Cl (10 mL), saturated aq $NaHCO_3$ (10 mL), and brine (10 mL), dried (Na_2SO_4), and then concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10). The condensation product was obtained as white solid (32 mg, 53%). Then 5.5 M *t*BuOOH in decane (38 μL, 0.209 mmol) was added to a solution of the condensation product (32 mg, 20.9 μmol) in dry CH_2Cl_2 (7 mL), and the resulting solution was stirred at

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rt. Then 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added twice more after 5 and 22 h after the first addition. Upon consumption of the starting material after 48 h, CH_2Cl_2 (5 mL) and a mixture of saturated aq $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (3 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10). The title product was obtained as a white solid (21 mg, 73%). HPLC: 40−60% MeCN (t_R = 5.11 min); mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +42.0 (*c* = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3340, 2911, 2847, 1681, 1643, 1515, 1201, 1124 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.14−1.31 (m, 2 H), 1.59 (d, *J* = 6.8 Hz, 3 H), 1.64−2.25 (m, 7 H), 2.80 (s, 3 H), 3.19−3.31 (m, 2 H), 3.36 (dd, *J* = 14.6, 4.6 Hz, 1 H), 3.47 (dd, *J* = 16.6, 3.0 Hz, 1 H), 3.56 (dd, *J* = 13.4, 4.2 Hz, 1 H), 3.64−3.94 (m, 4 H), 4.91 (dd, *J* = 8.2, 3.4 Hz, 1 H), 5.10−5.25 (m, 1 H), 5.38−5.47 (m, 1 H), 5.37−5.72 (m, 1 H), 5.76−5.83 (m, 1 H), 5.97−6.04 (m, 1 H), 6.13 (s, 1 H), 7.03−7.11 (m, 3 H), 7.13−7.22 (m, 5 H), 7.38 (d, *J* = 8.0 Hz, 2 H), 7.64 (bs, 1 H), 7.89−8.00 (m, 2 H), 8.03 (s, 1 H), 8.15−8.30 (m, 5 H), 8.33−8.42 (m, 2 H), 8.63 (s, 1 H), 9.08 (d, *J* = 6.8 Hz, 1 H), 9.45 (d, *J* = 8.4 Hz, 1 H), 9.70 (d, *J* = 7.2 Hz, 1 H), 10.64 (s, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.8 (q), 17.3 (q), 25.2 (t), 29.4 (t), 30.0 (t), 33.7 (t), 37.6 (t), 39.0 (t), 43.5 (t), 47.4 (t), 48.0 (t), 48.5 (d), 49.5 (d), 52.2 (d), 53.6 (d), 60.5 (d), 61.3 (d), 103.2 (t), 115.6 (d), 116.6 (d), 118.8 (d), 123.0 (s), 123.3 (d), 123.5 (d), 124.9 (d), 126.4 (s), 127.3 (d), 128.0 (d), 128.7 (d), 130.2 (d), 130.9 (s), 131.5 (d), 136.7 (s), 139.4 (d), 149.2 (s), 149.3 (s), 150.8 (s), 152.0 (s), 152.1 (s), 154.0 (s), 154.7 (s), 155.9 (s), 158.1 (s), 159.7 (s), 160.1 (s), 160.4 (s), 161.2 (s), 162.7 (s), 164.7 (s), 168.3 (s), 169.2 (s), 171.7 (s), 171.8 (s), 173.1(s), 174.5 (s), 175.0 (s), 175.1 (s) ppm. HRMS *m*/*z* calcd for $C_{63}H_{59}O_{11}N_{16}S_5$ (M + H) 1375.3147, found 1375.3247.

Thiazoline Tripeptide Analogue (27). Trimethyltin hydroxide (47 mg, 0.256 mmol) was added to a solution of **20**[18] (50 mg, 42.8 μmol) in dry 1,2-dichloroethane (0.85 mL), and the reaction mixture was then stirred at 60 °C. After 15 h, the mixture was diluted in CH_2Cl_2 (40 mL), washed with 6% HCl (30 mL), dried (Na_2SO_3), and concentrated in vacuo. EDC·HCl (10 mg, 51.4 μmol), HOAt (7 mg, 51.4 μmol), and DIPEA (9 μL, 51.4 μmol) were added to a stirring solution of the crude carboxylic acid (**22**)[18] and **12** (27 mg, 51.4 μmol) in DMF (0.9 mL) at 0 °C. After 17 h, the mixture was diluted in CH_2Cl_2 (20 mL), washed with 1 M $NaHCO_3$ (20 mL), dried (Na_2SO_3), and concentrated in vacuo with the aid of toluene to remove DMF traces. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 90:10). The condensation product was obtained as a white solid (47 mg, 72%). Then 5.5 M *t*BuOOH in decane (56 μL, 0.306 mmol) was added to a solution of the condensation product (47 mg, 30.6 μmol) in dry CH_2Cl_2 (10 mL), and the resulting solution was stirred at rt. Then 5.5 M *t*BuOOH in decane (70 μL, 0.384 mmol) was added twice more after 5 and 22 h after the first addition. Upon consumption of the starting material after 46 h, CH_2Cl_2 (10 mL) and a mixture of saturated aq $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (2 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product purified by preparative reverse-phase column (C18, 40−50% MeCN in H_2O). The title product was obtained as a white solid (37 mg, 88%). HPLC: 40−60% MeCN (t_R = 5.23 min); mp (CH_2Cl_2) decomposes above 130 °C. $[\alpha]_D$ +3.2 (*c* = 0.5, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3327, 2917, 2841, 1649, 1502, 1457 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ = 1.23−1.32 (m, 2 H), 1.38 (d, *J* = 6.6 Hz, 3 H), 1.81−2.34 (m, 7 H), 2.39−2.50 (m, 1 H), 2.74 (s, 3 H), 2.79−3.06 (m, 2 H), 3.09−3.45 (m, 4 H), 3.48−3.72 (m, 2 H), 4.26−4.31 (m, 1 H), 4.72−4.89 (m, 2 H), 4.90−5.11 (m, 2 H), 5.27−5.50 (m, 2 H), 5.97 (s, 1 H), 6.48−6.68 (m, 3 H), 6.83−7.15 (m, 3H), 7.16−7.46 (m, 5 H), 7.62−7.77 (m, 1 H), 7.86−8.08 (m, 1 H), 8.31−8.75 (m, 4 H), 8.76−8.87 (m, 4 H), 8.98 (d, *J* = 9.0 Hz, 1 H), 9.13 (d, *J* = 8.4 Hz, 1 H), 10.04−10.11 (m, 2 H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ = 12.5 (q), 17.4 (q), 25.4 (t), 25.6 (t), 29.9 (t), 30.1 (t), 33.5 (t), 37.1 (t), 37.4 (t), 39.1 (t), 39.1 (t), 47.5 (t), 48.3 (d), 49.3 (d), 53.2 (d), 54.9 (d), 60.3 (d), 60.6 (d), 78.2 (d), 104.3 (t), 115.9 (d), 117.4 (d), 119.1 (d), 123.3 (d), 124.8 (d), 127.5 (d), 129.2 (d), 129.6 (d), 130.2 (d), 130.5 (s), 131.4 (d), 134.5 (s), 138.1 (s), 141.8 (d), 148.8 (s), 150.2 (s), 150.8 (s), 151.5 (s), 152.5 (s), 153.8 (s), 154.6 (s), 156.9 (s), 157.2 (s), 158.9 (s), 159.5 (s), 160.2 (s), 163.7 (s), 168.4 (s), 170.1 (s), 171.0 (s), 172.3 (s), 174.0 (s), 174.4 (s), 175.3 (s), 176.0 (s) ppm. HRMS *m*/*z* calcd for $C_{63}H_{61}O_{11}N_{16}S_5$ (M + H) 1377.3304, found 1377.3327.

Thiazole Tetrapeptide Analogue (28). A mixture of **13** (35 mg, 0.047 mmol), EDC·HCl (9 mg, 0.047 mmol), HOAt (6 mg, 0.047 mmol), and DIPEA (8 μL, 0.047 mmol) was added to a stirring solution of **21** (45 mg, 0.039 mmol) in dry DMF (0.8 mL) and cooled in an ice bath. Next, the reaction mixture was allowed to reach rt. After 4 h, CH_2Cl_2 (15 mL) was added and the resulting solution was washed with saturated aq NH_4Cl (10 mL), saturated aq $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4), and then concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10). The condensation product was obtained as white solid (36 mg, 52%). Then 5.5 M *t*BuOOH in decane (56 μL, 0.306 mmol) was added to a solution of the condensation product (36 mg, 20.4 μmol) in dry CH_2Cl_2 (7 mL), and the resulting solution was stirred at rt. Then 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added twice more after 5 and 22 h after the first addition. Upon consumption of the starting material after 47 h, CH_2Cl_2 (5 mL) and a mixture of saturated aq $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (3 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10). The title product was obtained as a white solid (19 mg, 64%). HPLC: 40−60% MeCN (t_R= 5.93 min); mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +63.7 (*c* = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3334, 2917, 2847, 1643, 1515, 1419 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.67 (d, *J* = 6.8 Hz, 3 H), 1.70−2.02 (m, 7 H), 2.14−2.26 (m, 2 H), 2.80 (s, 3 H), 3.23−3.32 (m, 2 H), 3.37 (dd, *J* = 14.6, 4.6 Hz, 1 H), 3.48 (dd, *J* = 16.4, 3.2 Hz, 1 H), 3.56 (dd, *J* = 13.2, 4.0 Hz, 1 H), 3.65−3.84 (m, 3 H), 3.85−3.94 (m, 1 H), 4.94−5.00 (m, 1 H), 5.15−5.25 (m, 1 H), 5.40−5.47 (m, 1 H), 5.64−5.74 (m, 1 H), 5.77−5.85 (m, 1 H), 5.96 (s, 1 H), 5.97−6.05 (m, 1 H), 6.15 (s, 1 H), 6.85 (s, 1 H), 7.04−7.12 (m, 3 H), 7.14−7.22 (m, 5 H), 7.38 (d, *J* = 8.4 Hz, 2 H), 7.63 (bs, 1 H), 7.97 (bs, 1 H), 8.03 (s, 1 H), 8.21 (s, 1 H), 8.24 (s, 1 H), 8.27 (d, *J* = 8.0 Hz, 1 H), 8.30 (d, *J* = 8.0 Hz, 1 H), 8.37 (s, 1 H), 8.49 (bs, 1 H), 8.63 (s, 1 H), 8.86−8.94 (m, 1 H), 9.09 (d, *J* = 6.8 Hz, 1 H), 9.46 (d, *J* = 8.8 Hz, 1 H), 9.77 (d, *J* = 6.8 Hz, 1 H), 9.85 (s, 1 H), 10.63 (s, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.8 (q), 17.5 (q), 25.3 (t), 25.3 (t), 28.8 (t), 33.7 (t), 37.7 (t), 39.1 (t), 43.5 (t), 47.5 (t), 48.1 (t), 48.5 (d), 49.5 (d), 52.2 (d), 53.6 (d), 61.3 (d), 61.7 (d), 102.8 (t), 103.3 (t), 115.6 (d), 116.6 (d), 118.9 (d), 123.0 (s), 123.4 (d), 123.6 (d), 124.0 (s), 124.9 (d), 126.4 (s), 127.3 (d), 128.0 (d), 128.8 (d), 130.2 (d), 130.9 (s), 131.6 (d), 135.0 (s), 136.7 (s), 139.4 (d), 149.2 (s), 149.3 (s), 149.3 (s), 150.8 (s), 152.0 (s), 152.1 (s), 154.0 (s); 154.8 (s), 155.9 (s), 158.1 (s), 159.7 (s), 160.1 (s), 160.5 (s), 161.2 (s), 162.8 (s), 164.8 (s), 166.8 (s), 168.4 (s), 169.2 (s), 171.0 (s), 171.8 (s), 172.9 (s), 173.1 (s), 175.0 (s), 175.2 (s) ppm. HRMS *m*/*z* calcd for $C_{66}H_{62}O_{12}N_{17}S_5$ (M + H) 1444.3362, found 1444.3399.

Thiazoline Tetrapeptide Analogue (29). Trimethyltin hydroxide (47 mg, 0.256 mmol) was added to a solution of **20**[18] (50 mg, 42.8 μmol) in dry 1,2-dichloroethane (0.85 mL), and the reaction mixture was then stirred at 60 °C. After 15 h, the mixture was diluted in CH_2Cl_2 (40 mL), washed with 6% HCl (30 mL), dried (Na_2SO_3), and concentrated in vacuo. EDC·HCl (10 mg, 51.4 μmol), HOAt (7 mg, 51.4 μmol), and DIPEA (9 μL, 51.4 μmol) were added to a stirring solution of the crude **22**[18] and **13** (39 mg, 51.4 μmol) in DMF (0.9 mL) at 0 °C. After 17 h, the mixture was diluted in CH_2Cl_2 (20 mL), washed with 1 M $NaHCO_3$ (20 mL), dried (Na_2SO_3), and then concentrated in vacuo with the aid of toluene to remove DMF traces. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10). The condensation product was obtained as a white solid (45 mg, 60%). Then 5.5 M *t*BuOOH in

decane (70 μL, 0.384 mmol) was added to a solution of the condensation product (45 mg, 25.6 μmol) in dry CH_2Cl_2 (8.5 mL), and the resulting solution was stirred at rt. Then 5.5 M *t*BuOOH in decane (70 μL, 0.384 mmol) was added twice more after 5 and 22 h after the first addition. Upon consumption of the starting material after 45 h, CH_2Cl_2 (10 mL) and a mixture of saturated aq $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (2 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by preparative reverse-phase column (C18, 40−50% MeCN in H_2O). The title product was obtained as a white solid (34 mg, 92%). HPLC: 40−60% MeCN (t_R = 5.47 min); mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +12.4 (*c* = 0.50, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3430, 2917, 2841, 1739, 1649, 1444, 1041 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.58−1.70 (m, 2 H), 1.88−2.06 (m, 4 H), 2.08−2.41 8m, 6 H), 2.52−2.66 (m, 2 H), 3.11−3.20 (m, 3 H), 3.36−3.43 (m, 1 H), 3.53−3.58 (m, 1 H), 3.73−3.78 (m, 1 H), 3.96−4.17 (m, 5 H), 4.22−4.30 (m, 1 H), 5.31−5.46 (m, 2 H), 5.50−5.66 (m, 2 H), 5.73−5.80 (m, 2 H), 6.23−6.28 (m, 1 H), 6.33 (s, 1 H), 6.52 (s, 1 H), 7.22 (s, 1 H), 7.40−7.58 (m, 8 H), 7.64−7.77 (m, 2 H), 7.97 (bs, 1 H), 8.09 (d, *J* = 9.0 Hz, 1 H), 8.27 (bs, 1 H), 8.35 (s, 1 H), 8.52−8.62 (m, 4 H), 8.86 (bs, 1 H), 8.99 (s, 1 H), 9.12 (d, *J* = 6.6 Hz, 1 H), 9.29 (bs, 1 H), 9.70 (d, *J* = 9.0 Hz, 1 H), 10.12 (d, *J* = 6.6 Hz, 1 H), 10.23 (d, *J* = 6.6 Hz, 1 H),10.94−11.00 (m, 1 H) ppm. ^{13}C NMR (150 MHz, pyridine-d_5) δ = 11.8 (q), 17.5 (q), 25.2 (t), 25.5 (t), 28.8 (t), 30.0 (t), 34.3 (t), 36.2 (t), 36.8 (t), 38.4 (t), 39.9 (t), 47.5 (t), 48.5 (d), 49.2 (d), 52.3 (d), 54.1 (d), 61.7 (d), 61.9 (d), 78.8 (d), 102.8 (t), 103.3 (t), 116.2 (d), 116.8 (d), 118.6 (d), 123.0 (d), 123.1 (d), 125.8 (s), 127.3 (d), 127.9 (d), 128.7 (d), 130.2 (s), 130.2 (d), 131.0 (s), 131.7 (d), 136.9 (s), 139.2 (d), 148.9 (s), 150.8 (s), 150.9 (s), 152.0 (s), 153.9 (s), 154.7 (s), 155.9 (s), 157.9 (s), 159.7 (s), 160.5 (s), 161.2 (s), 162.7 (s), 164.8 (s), 166.8 (s), 168.3 (s), 169.1 (s), 171.0 (s), 171.2 (s), 172.6 (s), 173.0 (s), 173.2 (s), 174.8 (s), 175.1 (s) ppm. HRMS *m*/*z* calcd for $C_{66}H_{64}O_{12}N_{17}S_5$ (M + H) 1446.3518, found 1446.3603.

*Thiazole Pentapeptide Analogue (**30**).* A mixture of **14**[18] (36 mg, 42.1 μmol), EDC·HCl (8 mg, 42.1 μmol), HOAt (6 mg, 42.1 μmol), and DIPEA (8 μL, 42.1 μmol) was added to a stirring solution of **21** (40 mg, 35.1 μmol) in dry DMF (0.7 mL) and cooled in an ice bath. The reaction mixture was allowed to reach rt. After 4 h, CH_2Cl_2 (25 mL) was added and the resulting solution was washed with saturated aq NH_4Cl (25 mL), saturated aq $NaHCO_3$ (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10). The condensation product was obtained as white solid (49 mg, 70%). Then 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added to a solution of the condensation product (47 mg, 23.7 μmol) in dry CH_2Cl_2 (8 mL), and the resulting solution was stirred at rt. Then 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added twice more after 5 and 26 h after the first addition. Upon consumption of the starting material after 32 h, CH_2Cl_2 (5 mL) and a mixture of saturated aq $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (4 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 99:1 to 90:10). The title product was obtained as a white solid (26 mg, 72%). HPLC: 40−60% MeCN (t_R = 5.67 min); mp (CH_2Cl_2) decomposes above 120 °C. $[\alpha]_D$ +20.6 (*c* = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3443, 2917, 2853, 1649, 1489, 1419 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.24−1.31 (m, 1 H), 1.40 (d, *J* = 6.8 Hz, 3 H), 1.85−2.26 (m, 8 H), 2.40−2.51 (m, 1 H), 2.78 (s, 3 H), 2.83−2.97 (m, 2 H), 3.09−3.27 (m, 2 H), 3.34−3.44 (m, 1 H), 3.62−3.91 (m, 3 H), 4.52−4.60 (m, 1 H), 4.71−4.82 (m, 1 H), 5.02−5.13 (m, 1 H), 5.30−5.43 (m, 2 H), 5.58 (s, 1 H), 5.62−5.72 (m, 2 H), 5.90 (s, 1 H), 5.98 (s, 1 H), 6.14 (s, 1 H), 6.56 (s, 1 H), 6.68 (d, *J* = 8.6 Hz, 2 H), 6.83 (bs, 1 H), 7.11 (d, *J* = 8.6 Hz, 2 H), 7.20 (d, *J* = 6.8 Hz, 2 H), 7.24−7.39 (m, 4 H), 7.57 (bs, 1 H), 7.76 (s, 1 H), 7.96−8.04 (m, 2 H), 8.13 (s, 1 H), 8.23 (s, 1 H), 8.32−8.40 (m, 2 H), 8.58 (d, *J* = 8.4 Hz, 1 H), 8.71 (s, 1 H), 8.76 (d, *J* = 7.6 Hz, 2 H), 8.85 (d, *J* = 6.4 Hz, 1 H), 9.13 (s, 1 H), 9.52 (s, 1 H), 10.09 (s, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 12.6 (q), 17.3 (q), 25.5 (t), 25.6 (t), 29.9 (t), 29.9 (t), 33.5 (t), 37.9 (t), 42.6 (t), 47.7 (t), 48.3 (t), 48.3 (d), 49.4 (d), 52.7 (d), 53.4 (d), 60.4 (d), 61.0 (d), 104.3 (t), 105.3 (t), 107.9 (t), 116.1 (d), 117.2 (d), 119.3 (d), 123.4 (d), 123.5 (s), 124.5 (d), 125.8 (d), 127.4 (s), 127.7 (d), 129.1 (s), 129.2 (d), 129.6 (d), 130.1 (s); 130.4 (d); 131.3 (d), 134.4 (s), 135.6 (s), 137.3 (s), 137.4 (s), 141.3 (d), 148.5 (s), 149.8 (s), 151.2 (s), 151.5 (s), 152.2 (s), 153.9 (s), 154.7 (s), 156.5 (s), 157.0 (s), 159.5 (s), 160.0 (s), 161.1 (s), 161.8 (s), 162.0 (s); 163.2 (s), 163.8 (s), 166.0 (s), 168.4 (s), 170.3 (s), 171.2 (s), 171.5 (s), 172.2 (s), 172.6 (s), 174.6 (s), 175.3 (s) ppm. HRMS *m*/*z* calcd for $C_{69}H_{65}O_{13}N_{18}S_5$ (M + H) 1513.3577, found 1513.3673.

*Thz-Dha-Ala-Pro-Dha-Dha-NH$_2$ (**31**).* The title peptide could be obtained by two different methods: (A) on-resin oxidation/elimination, (B) oxidation/elimination after cleavage.

Method A. Prepared according to the general method for solid-phase peptide synthesis, starting from 1.06 g of resin. Prior to cleavage from the resin, an overnight treatment with 3 M *t*BuOOH in isooctane (6 mL, 18 mmol, 32 equiv) in CH_2Cl_2 (3 mL) at rt was performed. After cleavage, the crude product was further purified by silica flash column chromatography (EtOAc/THF, 9:1 to 8:2). The title product was obtained as a white solid (102 mg, 36% based on functionalization of the resin).

Method B. First, 3 M *t*BuOOH in isooctane (3.5 mL, 10.5 mmol, 34 equiv) was added to a solution of **32** (304 mg, 0.311 mmol) in dry CH_2Cl_2 (9 mL). The resulting mixture was stirred at rt for 7 h . The crude product was purified by silica flash column chromatography (EtOAc/THF, 9:1 to 8:2). The title product was obtained as a white solid (112 mg, 72%). HPLC: 50−100% MeCN (t_R = 1.18 min); mp (EtOAc) decomposes above 100 °C. $[\alpha]_D$ −85.9 (*c* = 1.00, CH_2Cl_2). IR (KBr) 3334, 2981, 2911, 2873, 1630, 1515 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ = 1.56 (d, *J* = 6.8 Hz, 3 H), 2.12−2.30 (m, 4 H), 3.77−3.85 (m, 1 H), 3.88−3.96 (m, 1 H), 4.62−4.69 (m, 1 H), 4.90 (q, *J* = 3.0, 1 H), 5.61 (bs, 1 H), 5.68 (bs, 1 H), 5.72 (bs, 1 H),6.24 (bs, 1 H), 6.31 (d, *J* = 1.2 Hz, 1 H), 6.40 (d, *J* = 1.2 Hz, 1 H), 6.68 (d, *J* = 1.6 Hz, 1 H), 6.96 (bs, 1 H), 7.66 (d, *J* = 6.4 Hz, 1 H), 8.37 (d, *J* = 2.0 Hz, 1 H), 8.79 (bs, 1 H), 8.94 (bs, 1 H), 9.10 (d, *J* = 2.0 Hz, 1 H), 10.05 (bs, 1 H) ppm. ^{13}C NMR (100 MHz, acetone-d_6) δ = 16.9 (q), 25.2 (t), 28.6 (t), 47.6 (t), 48.1 (d), 61.3 (d), 102.0 (t), 104.5 (t), 105.0 (t), 124.7 (d), 134.4 (s), 134.4 (s), 136.0 (s), 151.2 (s), 154.8 (d), 159.5 (s), 162.6 (s), 163.5 (s), 165.7 (s), 171.3 (s), 172.4 (s) ppm. HRMS *m*/*z* calcd for $C_{21}H_{26}O_6N_7S$ (M + H) 504.1660, found 504.1663.

*Thz-Sec(Ph)-Ala-Pro-Sec(Ph)-Sec(Ph)-NH$_2$ (**32**).* Compound **32** was prepared according to the general method for solid-phase peptide synthesis, starting from 1.35 g of resin. The crude product was further purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5). The title product was obtained as a pale solid (330 mg, 47% based on functionalization of the resin). HPLC: 0−100% MeCN (t_R = 7.29 min); mp (CH_2Cl_2) 185−188 °C. $[\alpha]_D$ −116.6 (*c* = 0.33, CH_2Cl_2). IR (KBr) 3436, 3295, 2924, 1668, 1623, 1041 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.26 (d, *J* = 6.8 Hz, 3 H), 1.77−2.12 (m, 4 H), 3.08−3.19 (m, 2 H), 3.24−3.50 (m, 4 H), 3.54−3.68 (m, 2 H), 4.28−4.47 (m, 3 H), 4.56−4.65 (m, 1 H), 4.76−4.86 (m, 1 H), 7.21−7.36 (m, 9 H), 7.46−7.56 (m, 6 H), 8.15 (d, *J* = 8.4 Hz, 1 H), 8.22 (d, *J* = 7.2 Hz, 1 H), 8.39 (d, *J* = 2.0 Hz, 1 H), 8.48−8.58 (m, 2 H), 9.22 (d, *J* = 2.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 17.9 (q), 25.4 (t), 29.4 (t), 29.6 (t), 29.9 (t), 30.4 (t), 47.5(d), 47.7 (t), 53.4 (d); 53.8 (d), 54.1 (d), 60.9 (d), 125.7 (d), 127.6 (d), 127.6 (d), 127.8 (d), 130.0 (d), 130.2 (d), 130.2 (d), 130.7 (s), 130.8 (s), 131.0 (s), 132.4 (d), 132.7 (d), 132.7 (d), 150.1 (s), 155.9 (d), 161.0 (s), 170.0 (s), 170.8 (s), 171.5 (s), 172.4 (s), 172.7 (s) ppm. HRMS *m*/*z* calcd for $C_{39}H_{44}N_7O_6SSe_3$ (M + H) 998.0392, found 998.0454.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra images of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

TFAA, trifluoroacetic anhydride; DME, dimethoxyethane; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOAt, 1-hydroxy-7-azabenzotriazole; DIPEA, diisopropylethylamine; TFA, trifluoroacetic acid; MIC, minimal inhibitory concentration; BLD, below limit of detection

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