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Article

Elastase Inhibitor Cyclotheonellazole A: Total Synthesis and In Vivo Biological Evaluation for Acute Lung Injury

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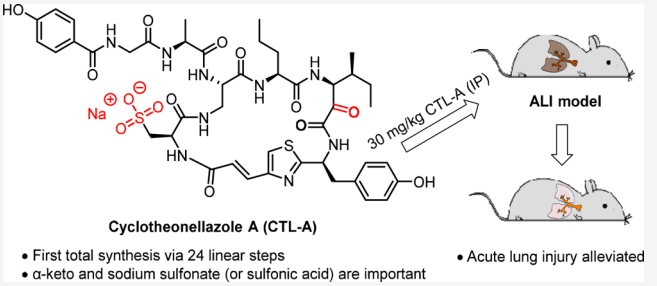
ABSTRACT: Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is one of the most common complications in COVID-19. Elastase has been recognized as an important target to prevent ALI/ARDS in the patient of COVID-19. Cyclotheonellazole A (CTL-A) is a natural macrocyclic peptide reported to be a potent elastase inhibitor. Herein, we completed the first total synthesis of CTL-A in 24 linear steps. The key reactions include three-component MAC reactions and two late-stage oxidations. We also provided seven CTL-A analogues and elucidated preliminary structure−activity relationships. The *in vivo* ALI mouse model further suggested that CTL-A alleviated acute lung injury with reductions in lung edema and pathological deterioration, which is better than sivelestat, one approved elastase inhibitor. The activity of CTL-A against elastase, along with its cellular safety and well-established synthetic route, warrants further investigation of CTL-A as a candidate against COVID-19 pathogeneses.



INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has become a life-threatening pandemic worldwide.[1−3] The occurrence of acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is the major contributor to poor prognosis and low survival rates in critically ill patients.[4−6] The high mortality rates (25% for COVID-19 patients with ALI/ARDS) and lack of effective treatments have provoked the global academic community and pharmaceutical industry to develop various therapeutic strategies.[7] Currently, vaccination is the most prevalent method to fight SARS-CoV-2. However, emergence of several SARS-CoV-2 variants resulted in reduced susceptibility to vaccine-induced immunity.[8−10] Development of effective drug candidates to combat COVID-19 is still in high demand.

Elastase is known as a serine protease that is mainly expressed in the pancreas and neutrophils. In physiological conditions, elastase helps remove bacteria, cleans up damaged tissue, and promotes tissue regeneration. However, in pathological conditions, excessive expression of elastase can damage vascular composition, induce inflammation, and promote virus or bacteria infection.[11] Once SARS-CoV-2 infects the host cell, systemic immune overactivation (Figure 1) is especially noteworthy in severely ill patients. At the first stage of immune response, elastase released from neutrophils plays a key role.[12,13] Its pathogenesis has been proposed to involve (1) activation of epithelial Na^+ transport, which causes hypertension and dehydration of the fluid lung airways, resulting in inefficient mucociliary clearance,[14] and (2) dysfunction of the lung permeability barrier and release of pro-inflammatory cytokines, causing severe ALI/ARDS in COVID-19 patients.[15,16] Elastase is therefore considered to be an important target for the prevention of ALI/ARDS during SARS-CoV-2 infection.[17] Sivelestat, a specific elastase inhibitor with an IC_{50} of 44 nM, is currently the only approved elastase inhibitor for the treatment of neutrophil-induced damage in high-risk COVID-19 patients.[18] However, the efficacy of sivelestat has not been convincingly demonstrated through multicenter clinical trials.[18−20]

An ideal inhibitor scaffold should have the key quality: be structural amenable for chemical modification to match its interactions with a target protein and to improve its pharmacokinetic profiles. Beyond all doubt, natural products still serve as the most advantageous resources for isolation, identification, and modification into a promising inhibitor with potency and specificity.

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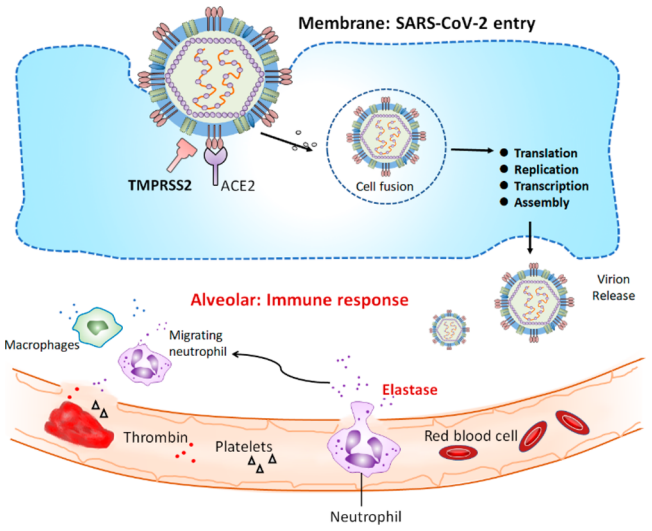
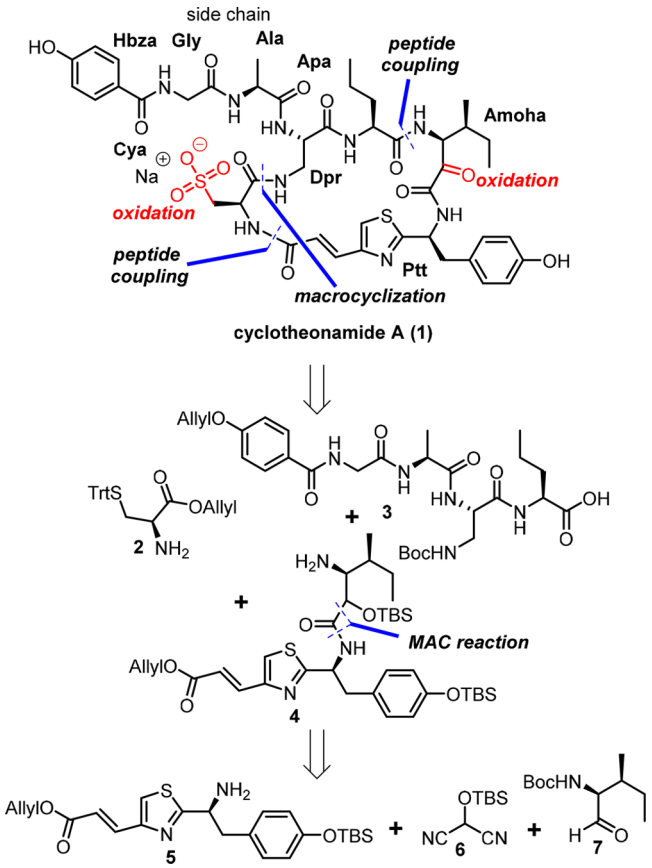


Figure 1. Schematic diagram of the mechanism of SARS-CoV-2 entry and virus-induced immune response. Release of the virus from host cells activates neutrophils and macrophages and increases circulation of inflammatory cytokines. Activated neutrophils produce large amounts of elastase, which induce membrane damage and epithelial injury.

Cyclotheonellazole A (CTL-A, Scheme 1) is a unique natural macrocyclic peptide that was first discovered in the sponge *Theonella affinis swinhoei* by Carmeli et al.[21] Although the majority of thiazole-containing cyclopeptides from marine resources exhibit anticancer activity,[22] CTL-A was reported to exhibit a significant IC_{50} value of 0.034 nM against elastase. The structure of CTL-A is composed of eight acid units, six of which are non-proteinogenic amino acids. Unique moieties such as α-keto-β-amino acid (3-amino-4-methyl-2-oxohexanoic acid [Amoha]), diaminopropionic acid (Dpr), sodium sulfonate, and vinylogous thiazole (4-propenoyl-2-tyrosylthiazole [PTT]) are present within the peptide sequence.

Scheme 1. Retrosynthetic Analysis of CTL-A



Carmeli speculated that the CTL-A–elastase complex exhibits a tetrahedral transition state involving the Ser195-OH and the α-keto group of CTL-A.[21] However, the specific mode of action of CTL-A and its therapeutic potential for the treatment of COVID-19 patients remain unknown. Systematic structural variations of CTLs to ensure the identification of key structural features accounting for their high affinity on elastase can assist in the development of more druggable analogues. Nevertheless, the scarcity of CTL-A from natural resources poses an obstacle that has deterred further investigation of CTL-A as a potential lead compound. All of these factors have spurred our attention toward the chemical synthesis of CTL-A.

RESULTS

Chemical Synthesis of CTL-A. The retrosynthetic route of CTL-A is shown in Scheme 1. Considering the high polarity of the sulfonic acid intermediate and difficulty in separating it from the reaction mixture, we introduced the sulfonic acid group in the penultimate step via direct oxidation of the thioether. The highly electrophilic carbonyl functionality in the α-keto-β-amino carboxamide was first masked as a protected alcohol and then unveiled by deprotection and oxidation. The macrocyclization site was selected to lie between the Dpr N-terminus and the Cya C-terminus because (1) it represents the position with the least steric hindrance; (2) the Dpr N-terminus is a common ring cyclization site in the biogenic synthesis of cyclopeptides;[23] and (3) embedding turn-inducing elements (such as the Amoha moiety in the middle of the linear precursor) can help ring cyclization. Backward, labile cysteine could be attached to an advanced linear peptide obtained from the coupling of **3** and **4**. To prepare **4**, a mild, three-component masked acyl cyanide (MAC) procedure developed by Nemoto et al.[24] and elegantly used in the synthesis of cyclotheonamide C by Aitken et al.[25] was employed.

Synthesis commenced with the construction of building blocks **3** and **4**. Hbza-Gly **11** was first prepared using four reaction sequences (Scheme 2A). The Dpr moiety was constructed following a modified Hofmann rearrangement reaction (Scheme 2B).[26] Treatment of compound **13** with iodobenzene diacetate (PIDA) under mild conditions triggered oxidative decarbonylation, which gave rise to **14**. Continuous operations on protection groups and couplings delivered pentapeptide **19** (precursor of **3**) on a multigram scale without any detectable epimerization.

Next, we proceeded to synthesize building block **4** (Scheme 2C). The amine and phenol groups of L-tyrosine **20** were first successively protected with Boc and TBS, respectively. The carboxylic acid was then ammoniated and consequently converted to thioamide **22** in satisfactory yield by employing Lawesson's reagent. Ethyl bromopyruvate **23** was chosen for the annulation reaction to construct the thiazole. This annulation proceeded cleanly at room temperature in the presence of TFAA as a dehydrant. By employing a combination of $CaCl_2/NaBH_4$, **25** was obtained in excellent yield. Alcohol **25** then underwent Dess-Martin periodinane

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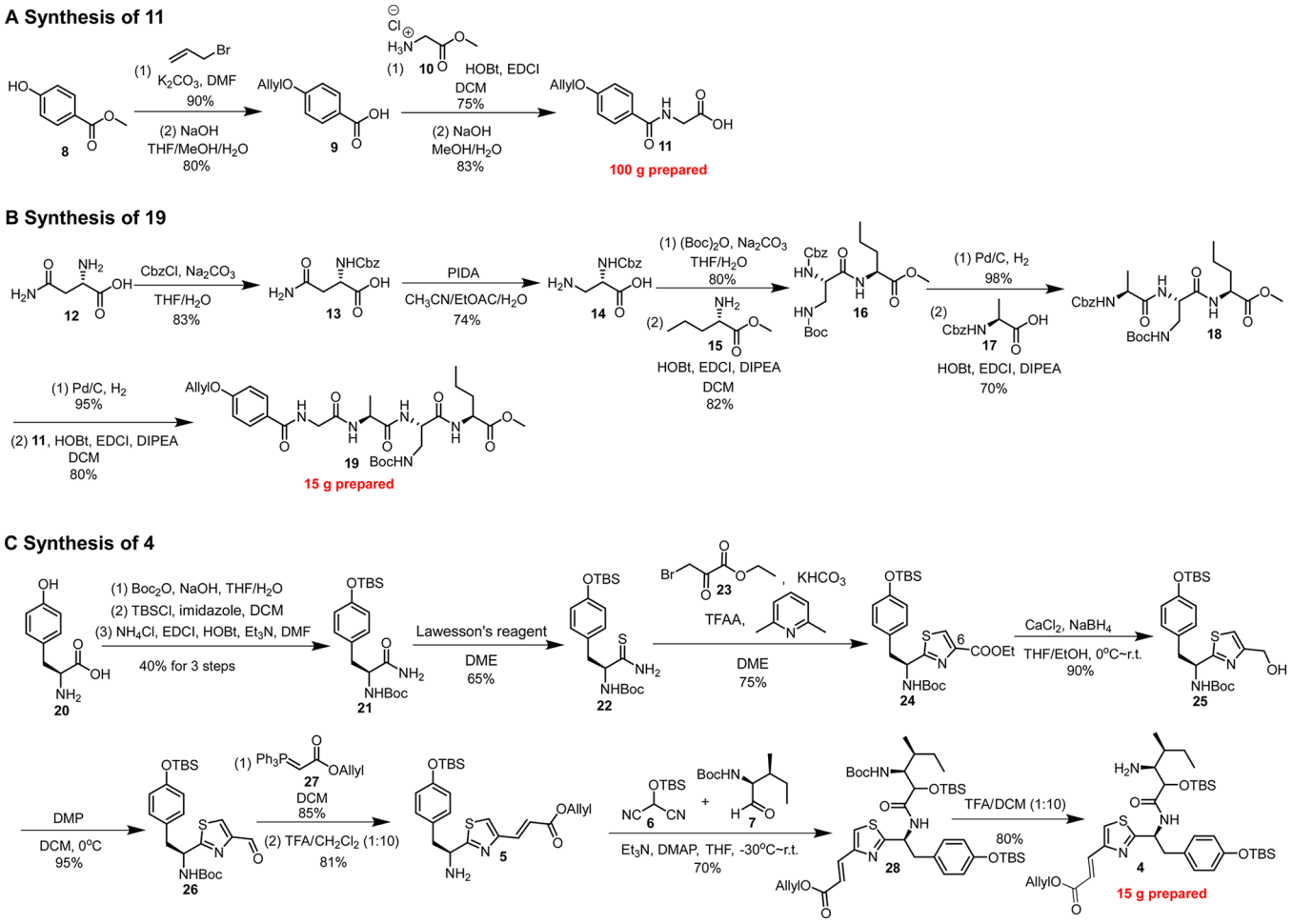
Scheme 2. Synthesis of 11, 19, and 4



oxidation to provide aldehyde **26**, onto which the vinylogous ester was installed in the *E* configuration via the Wittig reaction. Free amine **5** was accessible by TFA-mediated Boc cleavage. With the key aldehyde **7** (for the synthesis of **7**, see the Experimental Section) and amine **5** in hand, we turned our attention to the one-pot MAC reaction. The conditions were screened, and 1.3 equiv of Et_3N/2.0 equiv of DMAP were applied as the optimal conditions to provide **28** in 70% yield (dr = 6:1 according to HPLC data). Subsequent Boc deprotection was performed using TFA. The overall synthesis of **4** was achieved in 11 linear steps on a multigram scale.

With building blocks **19** and **4** in hand, we embarked on tackling their assembly (Scheme 3A). Hydrolysis of **19** delivered **3**, which was subjected to HATU coupling with **4** to produce the advanced linear heptapeptide **29** in satisfactory yield. Considering the nucleophilic and labile nature of mercaptan group within the cysteine moiety, we decided to introduce a Trt group for temporary protection. With the concern that Boc deprotection with TFA might lead to acid-labile Trt degradation, the Boc group of **29** was replaced in advance with a Fmoc group to yield **30**. Simultaneous deprotection of both allyl groups in **30** was achieved. Next, the unmasked carboxylic acid was coupled with the Trt-protected Cys moiety **2** to furnish **31** with all of the acid units connected in the appropriate sequence. Sequential deprotection of the allyl and Fmoc groups delivered **32** with exposed carboxylic acid and amine groups, which was ready for macrocyclization. This ring closure reaction proceeded smoothly in the presence of HOBt and EDCI to produce 3.6 g of macrocyclic compound **33** (for the structure, see the Supporting Information).

With the end of the synthesis now in sight, it was time to liberate the α-keto and sulfonate functionalities. Simultaneous deprotection of both TBS groups with TBAF generated **34** with a secondary α-hydroxy group; oxidation of this α-hydroxy group was pursued, but disappointingly, it was proven to be problematic due to decomposition of the macroscaffold when using common oxidants. We speculated that the phenols were vulnerable during this transformation, thus we reinstalled TBS groups on the phenols to obtain compound **35** on a 1.5 g scale.

Considering the difficulty obtaining **35**, we investigated the following oxidations using a simpler model substrate **39**. We surveyed several conditions, some of which are shown in Scheme 3B (for details, see Table S1 in the Supporting Information). Interestingly, Swern oxidation with oxalyl chloride/DMSO furnished **40** in 52% yield. Encouraged by this, we turned our emphasis on different types of DMSO-mediated oxidations[27] and eventually identified DMSO/EDCI with $CHCl_2COOH$ as an additive in toluene as the optimal conditions. Next, we anticipated cleavage of the Trt group and oxidation of the resulting mercaptan could be achieved in one pot. Different conditions for this transformation were screened (Scheme 3B).[28,29] Fortunately, the thioether oxidized

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Scheme 3. (A) Synthesis of CTL-A, (B) Model Reactions for Two Late-Stage Oxidations, and (C) Structure of Analogue 42

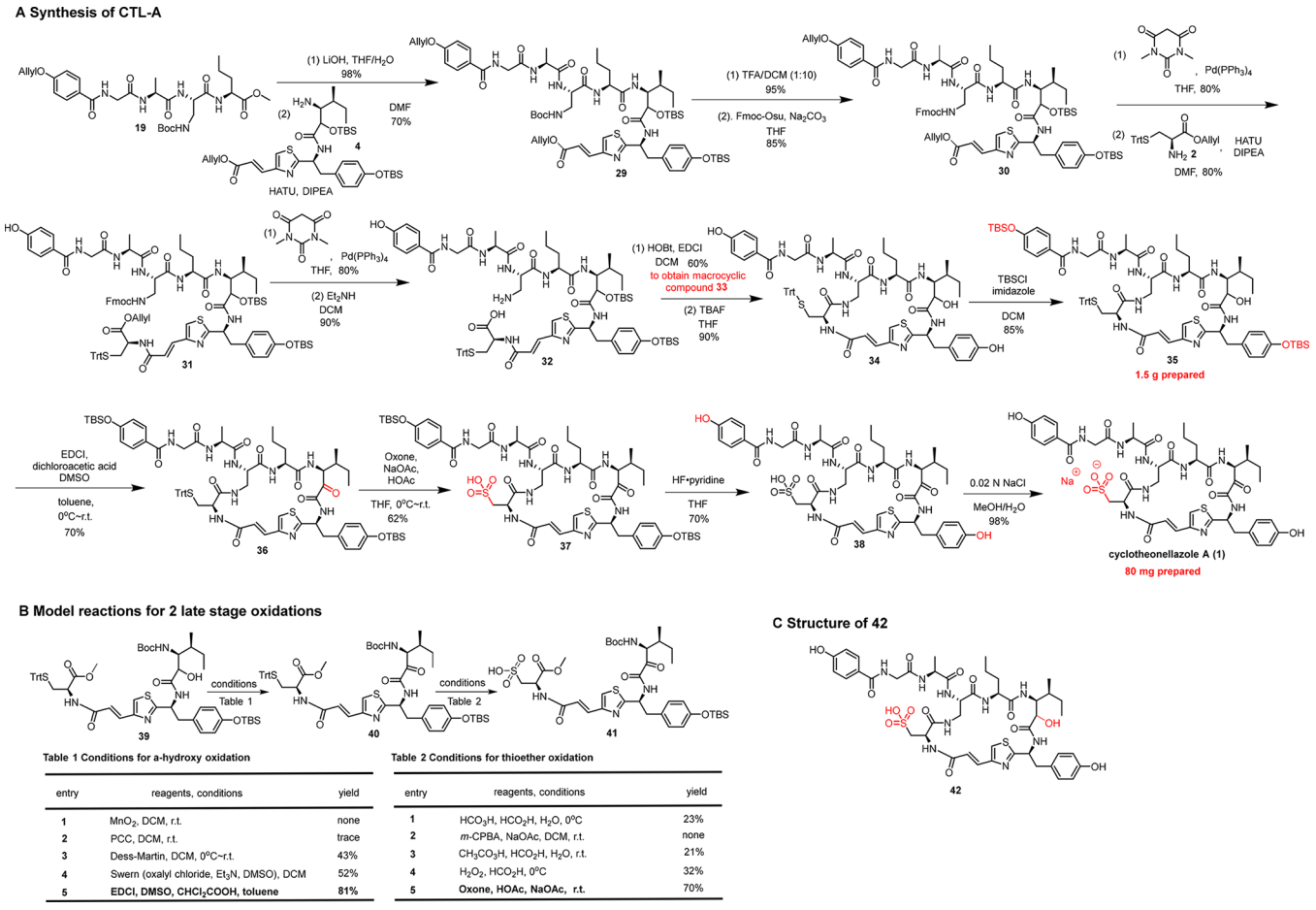

Table 1 Conditions for a-hydroxy oxidation

entry	reagents, conditions	yield
1	MnO_2, DCM, r.t.	none
2	PCC, DCM, r.t.	trace
3	Dess-Martin, DCM, 0°C~r.t.	43%
4	Swern (oxalyl chloride, Et_3N, DMSO), DCM	52%
5	**EDCI, DMSO, $CHCl_2COOH$, toluene**	**81%**

Table 2 Conditions for thioether oxidation

entry	reagents, conditions	yield
1	HCO_3H, HCO_2H, H_2O, 0°C	23%
2	*m*-CPBA, NaOAc, DCM, r.t.	none
3	CH_3CO_3H, HCO_2H, H_2O, r.t.	21%
4	H_2O_2, HCO_2H, 0°C	32%
5	**Oxone, HOAc, NaOAc, r.t.**	70%

smoothly within a few hours in oxone/HOAc/NaOAc to produce sulfonic acid **41** in 70% yield.

We then proceeded to apply the optimal conditions for oxidation to substrate **35**. By utilizing the EDCI/DMSO/$CHCl_2COOH$ conditions, only a slight decrease in the yield was observed compared with that in the model reaction. In sharp contrast, Swern oxidation with oxalyl chloride/DMSO provided only trace amounts of desired compound **36**. Forwardly, sulfonic acid **37** was prepared also in an acceptable yield under oxone/HOAc/NaOAc conditions. Next, we moved on to the penultimate step; conditions were evaluated for the removal of TBS. After screening, HF·pyridine was found to generate the desired sulfonic acid **38** in 70% yield. The last step was supposed to be simple; however, sodium salinization using sodium bases (e.g., NaOH or $NaHCO_3$) was unsuccessful with excess salinization of phenol groups. We finally opted for 0.02 N NaCl in MeOH/H_2O for the sodium−hydrogen exchange. A few minutes after initiation of the reaction, removal of MeOH under vacuum followed by lyophilization provided compound **1**. According to the data comparison, synthetic material **1** and its sulfonic acid **38** both matched the ^1H and ^{13}C NMR spectra, MS profile, and optical rotation with samples[21] isolated from natural sources. Therefore, we can confidently conclude that our synthetic target is identical to naturally occurring CTL-A.

Anticipating that small structural changes could have an impact on biological activities, we also generated analogue **42** (Scheme 3C) for structure−activity relationship (SAR) analysis.

Characterization of Biological Activity. To validate the biological properties of the CTL-A scaffold toward elastase, we conducted further biological tests.

Preliminary SAR Study of Compounds 1, 33−38, and 42 against Elastase. First, the inhibitory effects of CTL-A and seven analogues **33**−**38** and **42** were evaluated using enzyme activity assays. We chose sivelestat or MeOSu-AAPV-CMK to benchmark the synthetic compounds. The results showed that the IC_{50} values of CTL-A against porcine pancreatic elastase (PPE) and human neutrophil elastase (HNE) were 0.114 ± 0.002 and 0.321 ± 0.003 μM, respectively, whereas sivelestat showed IC_{50} values of 2.96 ± 0.128 and 0.704 ± 0.132 μM, respectively. These results indicate that CTL-A is more potent than sivelestat against elastase (Figures 2A and S1A). The inhibitory activity of precursor **38** containing a sulfonic acid group was comparable to that of CTL-A (Figure 2B). Conversely, thioethers **33**−**36** were essentially inactive at concentrations up to 1.25 μM, which indicated that the oxidized state of the sulfur atom is of vital importance. By switching the α-keto group to an α-hydroxy group, the diminished activity of compound **42** revealed the impact of the α-keto group. Consequently as expected, with an α-keto group, compound **37** exhibited moderate inhibitory activity.

To further validate the interaction of CTL-A with HNE, we performed a docking study using the crystal structure of HNE in complex with cyclotheonamide A (PDB ID: 1TYN). The docking study (Figures 2C and S2) indicated that one phenolic hydroxyl group of CTL-A forms close hydrogen bonding with

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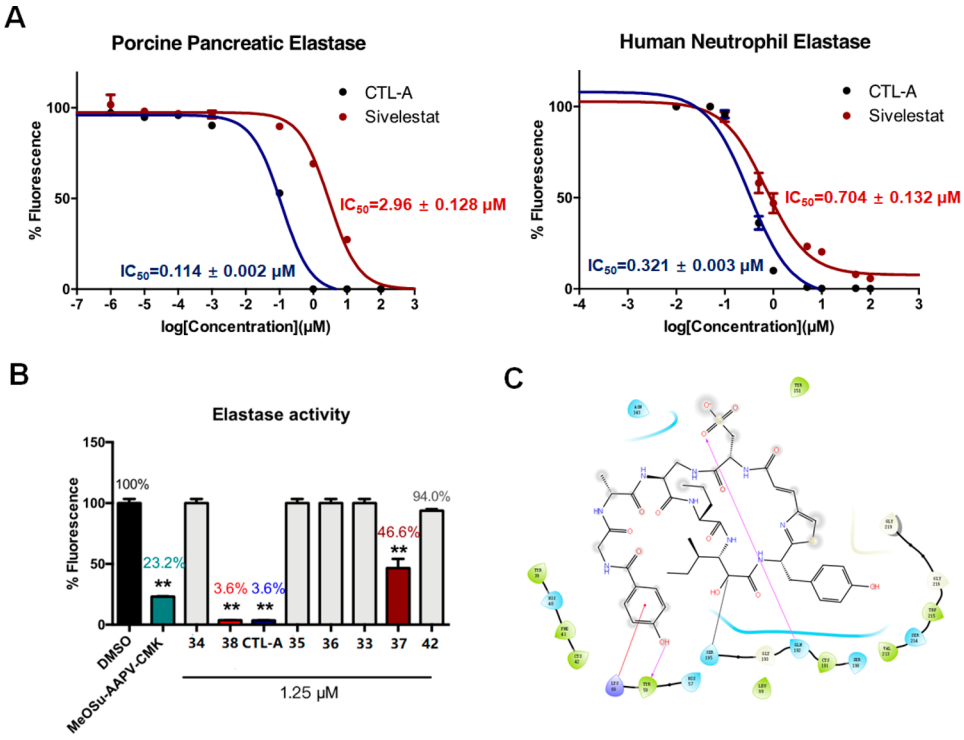

Figure 2. Biological activities of CTL-A and related compounds. (A) Inhibition of elastase by CTL-A identified from fluorescence measurements. Elastase activities were determined using an EnzCHek elastase assay kit. Elastin substrate at 25 μg/mL, PPE at 0.4 U/mL, HNE at 0.2 U/mL, and increasing amounts of CTL-A or sivelestat were incubated for 40 min at room temperature. (B) Inhibition of elastase activity by MeOSu-AAPV-CMK, CTL-A, and seven analogues. Elastin and elastase were incubated with 2.5 μM of MeOSu-AAPV-CMK or 1.25 μM of the compounds. (C) Representative image modeled for the docking of HNE and CTL-A.

the carboxyl group of Tyr59; the distance is 2.5 Å. Obviously, a π−cation interaction is formed between the benzene ring and Lys60 with a distance of 3.7 Å. At the same time, we noticed that a hydrogen bond is formed between the negatively charged sulfonic acid and Gln192 (the distance is 3.15 Å), suggesting that the sulfonate group might contribute to HNE binding. This finding is consistent with the literature, which reported that negatively charged groups are important for HNE inhibition.[30,31] Other COVID-19-related proteases including TMPRSS2, 3CL^{pro}, cathepsin L, and trypsin were also tested. The results indicated slight activity decreases of these proteases when treated with high concentrations of CTL-A (Figure S1B).

Cytotoxicity of CTL-A against Normal Cells. The activity of CTL-A in mammalian cells was characterized by viability experiments in a human lung epithelial cell line (BEAS-2B) and an ACE2 overexpression cell line (ACE2^{+}-293T). The data showed that CTL-A does not display any cytotoxicity at concentrations up to 100 μM (Figure S1C), indicating that CTL-A is safe for normal lung cells.

Bleomycin-Induced Acute Lung Injury Mouse Model. We further tested the therapeutic effects of CTL-A in a bleomycin (BLM)-induced acute lung injury mouse model.[32,33] Compared to the normal group (sham operation), all ALI mice displayed a loss of body weight (Figure 3A). The wet/dry ratios of the left lungs were measured to quantify the effect of CTL-A on ALI-induced lung edema. The wet/dry ratio of lungs from mice treated with CTL-A (4.91, p = 0.15) was less than that of the ALI group (5.38, Figure 3B). The right lung sections were randomized and blindly assessed for damage to the lung architecture. Pathological alterations in the lungs of the ALI group were characterized by the destruction of alveolar structure and deterioration of histopathology, whereas CTL-A and sivelestat relieved these changes (Figure 3E). The administration of CTL-A or sivelestat at a dosage of 30 mg/kg/day significantly decreased inflammatory cell infiltration (neutral granulocytes and monocytes), and the thickness of the alveolar wall was 1−2 times less than that of the ALI group (Figure 3C,E). During this investigation, two mice died in each of the ALI and sivelestat groups, and one died in the CTL-A group (Figure 3D).

CONCLUSIONS AND DISCUSSIONS

The emergence of SARS-CoV-2 has led to a worldwide health crisis. COVID-19 symptoms can persist for months in patients, and the virus triggers multiple complicated mechanisms to damage the lungs and respiratory system among others.

Elastase has been recognized as an important target to prevent ALI/ARDS in the patient of COVID-19. Under pathological conditions, excessive expression of elastase can damage vascular composition and induce an inflammatory cytokine storm. We confirmed that CTL-A exhibited an inhibition activity against elastase superior to that of sivelestat, though the potential of CTL-A we obtained has a significant discrepancy from the report of Carmeli's work (0.034 nM against elastase).[21] The discrepancy possibly has arisen from the different source of enzyme, the concentration of enzyme and substrate used, or the different incubation time. In addition, the cellular safety and experiments with an ALI model further confirmed the potential therapeutic benefits of CTL-A.

Although CTL-A has a complicated structure and contains unique moieties that make it challenging to synthesize, we

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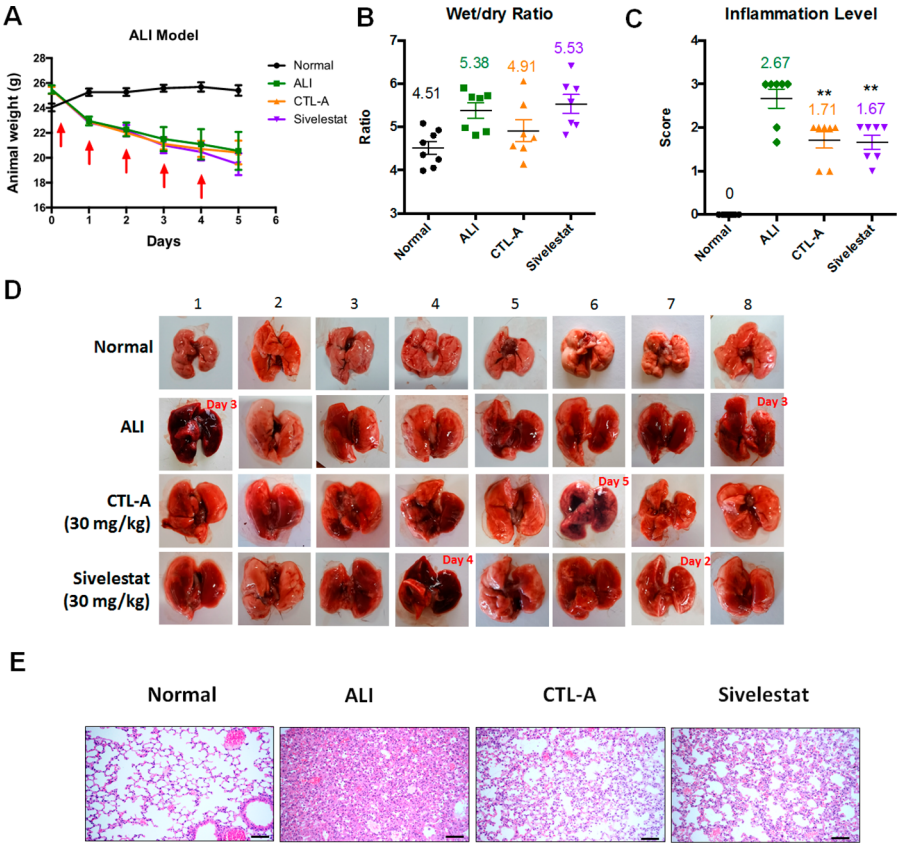

Figure 3. Effects of CTL-A in an acute lung injury mouse model. (A) Body weight of animals. Experimental mice were separated into four groups: normal (vehicle treatment), ALI (4 mg/kg of BLM, vehicle treatment), CTL-A (4 mg/kg of BLM, 30 mg/kg of CTL-A treatment), and sivelestat (4 mg/kg of BLM, 30 mg/kg of sivelestat treatment). (B) Left lung wet/dry ratios of experimental animals (dead animals were not included). (C) Lung injury scores (inflammation level) of the four groups (dead animals were not included). Lung injury scores standard are listed in Table S2 in the Supporting Information. (D) Photographs of lungs from experimental animals showing the morphology. Death day of dead animals in different groups are shown in red. (E) Representative images (randomly picked) from HE-stained sections of the lungs with 200× magnification, scale bar = 50 μm. Data are representative of 8 mice; $^{**}p < 0.01$.

accomplished its first total synthesis in 49 total steps and 24 linear steps. Structural complexity requires judicious choices at the level of individual building block synthesis, as well as peptide assembly sequences. Each of the devised building blocks can be prepared in multigram quantities. The key reaction includes the MAC reaction to construct α-keto-β-amino carboxamide. A model substrate was employed to identify the optimal conditions for the two late-stage oxidations. Seven previously inaccessible analogues together with CTL-A revealed that α-keto and sulfonic acid functionalities are necessary to inhibit elastase activity and illuminated how minute structural differences can result in activity loss. This observation was partially supported by a model docking study.

As the epidemic continues, SARS-CoV-2 is constantly evolving to various subtypes. Among these subtypes, D614G genotype is highly infectious and accounted for the repeated outbreaks in countries and districts. The high infectivity of the D614G genotype was proposed to stem from a mutant elastase cleavage site in the S protein.[34] Thus, considering the potent inhibition activity of CTL-A against elastase, CTL-A might be effective for COVID-19 patients with D614G genotype infection.

Collectively, by initiating synthesis of CTL-A on a large scale, it should be possible, at least at the outset, to scale up the production of any synthetic CTL analogue for further lead optimization and preclinical development. However, there are important challenges to overcome, among which is the modification of CTL-A to tune its activities against elastase and other COVID-19-involved proteases. Computational tools can further provide important assistance and insight into the future medicinal design of CTL-A. Meanwhile, elastase has previously been studied in detail, yielding high-resolution crystal structures and known inhibitors. These inhibitors, together with some newly reported natural products, are also worthy of future study to combat COVID-19.

EXPERIMENTAL SECTION

Chemistry. Reagents and solvents were purchased at the highest commercial quality and used without purification. Reactions were monitored by thin layer chromatography (TLC) carried out on silica gel plates using UV light as the visualizing agent and aqueous phosphomolybdic acid or basic aqueous potassium permanganate as the developing agent. ^{1}H NMR, ^{13}C NMR, and 2D NMR were recorded on Bruker AV 400 MHz and calibrated by using internal references and solvent signals for $CHCl_3$ (δH = 7.26 ppm, δC = 77.16 ppm) and DMSO-d_6 (δH = 2.50 ppm, δC = 39.52 ppm). ^{1}H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants and integration. IR spectra were recorded on a Bruker Tensor 27 instrument. High-resolution mass spectra (HRMS) were detected with a Varian 7.0T FTMS. Optical rotations were recorded on an Insmark IP 120 digital polarimeter.

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All compounds are >95% pure by HPLC analysis. HPLC trace is included in the Supporting Information for a compound which has *in vivo* data described in the article.

For clarity and conciseness, several intermediates obtained during total synthesis of CTL-A were omitted. Please find the structures of these compounds (compounds **S1**−**S23** and **S33**) in page S1 of the Supporting Information.

*Methyl 4-(allyloxy)benzoate (**S1**).* To a stirred solution of compound **8** (100.0 g, 657.5 mmol) and K_2CO_3 (118.0 g, 854.7 mmol) in DMF (700 mL) was added allyl bromide (95.0 g, 789.0 mmol) slowly at room temperature (rt). The resulting mixture was stirred for 5 h at rt, diluted with EA (500 mL), washed with H_2O (1000 mL), and then separated. The aqueous phase was extracted with EA (500 mL × 2). The combined organic phases were washed with brine (700 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (10% EA in PE) to obtain product **S1** (113.7 g, 90%) as a colorless gum: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.13−5.99 (m, 1H), 5.43 (dd, J = 17.3, 1.1 Hz, 1H), 5.31 (dd, J = 7.1, 5.8 Hz, 1H), 4.59 (d, J = 5.3 Hz, 2H), 3.88 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 162.4, 132.7, 131.7, 122.8, 118.3, 114.4, 69.0, 52.0.

*4-(Allyloxy)benzoic acid (**9**).* To a stirred solution of compound **S1** (10.0 g, 52.0 mmol) in THF/MeOH/H_2O (60 mL/20 mL/20 mL) was added NaOH (6.20 g, 156 mmol) at rt. The resulting mixture was stirred overnight at rt and then diluted with H_2O (100 mL) and concentrated under reduced pressure. The remained aqueous phase was extracted with PE/EA (v/v, 3:1, 60 mL × 2). Then to the resulting aqueous phase was added HCl (1 N) until the pH value reached 1−2 and then was extracted with EA (100 mL × 3). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to obtain product **9** (7.40 g, 80%) as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 12.64 (s, 1H), 7.88 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 6.13−5.98 (m, 1H), 5.41 (d, J = 17.3 Hz, 1H), 5.28 (d, J = 10.5 Hz, 1H), 4.64 (d, J = 5.2 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 167.0, 161.8, 133.2, 131.4, 123.1, 117.9, 114.5, 68.4.

*Methyl (4-(Allyloxy)benzoyl)glycinate (**S2**).* To a stirred mixture of compound **9** (17.5 g, 98.20 mmol) and compound **10** (13.6 g, 108.2 mmol) in DCM (300 mL) were added HOBt (17.2 g, 127.7 mmol) and EDCI (24.5 g, 127.7 mmol) at rt. After being stirred for 5 min, to the resulting mixture was added DIPEA solution dropwise (19.0 g, 147.3 mmol in 50 mL DCM) and stirred overnight at rt. The reaction was quenched with H_2O (500 mL) and separated. The aqueous phase was extracted with DCM (300 mL × 2). The combined organic phases were washed with brine (300 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2% MeOH in DCM) to obtain compound **S2** (18.4 g, 75%) as a white solid: ν_{max} (KBr) 3291, 1753, 1638, 1608, 1556, 1511, 1260, 1205, 1174, 1122, 1002, 845, 772 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 6.61 (s, 1H), 6.10−5.98 (m, 1H), 5.42 (dq, J = 17.2, 1.3 Hz, 1H), 5.31 (dq, J = 10.5, 1.3 Hz, 1H), 4.58 (dt, J = 5.3, 1.5 Hz, 2H), 4.23 (d, J = 5.1 Hz, 2H), 3.79 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 167.2, 161.2, 132.5, 128.9, 125.8, 117.8, 114.2, 68.6, 52.1, 41.5; HRMS (ESI) calcd for $C_{13}H_{15}NO_4$ $[M + H]^+$ 250.1074, found 250.1074.

*(4-(Allyloxy)benzoyl)glycine (**11**).* To a stirred solution of compound **S2** (22.8 g, 91.5 mmol) in MeOH/H_2O (210 mL/70 mL) was added NaOH (7.30 g, 183 mmol) at rt. The reaction mixture was stirred for 5 h and then diluted with H_2O (400 mL) and concentrated carefully under reduced pressure to remove MeOH. The resulting mixture was extracted with PE/EA (v/v, 1:1, 100 mL × 2). The aqueous phase was cooled to 0 °C, and HCl (1 N) was until the pH value reached 1−2 and then extracted with EA (200 mL × 3). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to obtain **11** (17.9 g, 83%) as a white solid: ν_{max} (KBr) 3296, 1731, 1633, 1612, 1544, 1506, 1246, 1184, 995, 943, 846, 773 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 12.55 (s, 1H), 8.70 (t, J = 5.8 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H), 6.05 (m, 1H), 5.41 (dd, J = 17.3, 1.4 Hz, 1H), 5.28 (dd, J = 10.5, 1.4 Hz, 1H), 4.64 (d, J = 5.2 Hz, 2H), 3.90 (d, J = 5.8 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 171.5, 166.0, 160.6, 133.4, 129.1, 126.2 117.8, 114.2, 68.3, 41.2; HRMS (ESI) calcd for $C_{12}H_{13}NO_4$ $[M + H]^+$ 236.0917, found 236.0918.

*((Benzyloxy)carbonyl)-L-asparagine (**13**).* To a stirred mixture of compound **12** (30.0 g, 227.1 mmol) in THF/H_2O (300 mL/300 mL) was added Na_2CO_3 (48.1 g, 454.2 mmol) at rt. The resulting mixture was stirred for 20 min and then cooled to 0 °C. A solution of CbzCl (46.5 g, 272.5 mmol) in THF (100 mL) was added dropwise to the reaction mixture over a period of 30 min. Then, the resulting mixture was stirred overnight at rt, diluted with H_2O (300 mL), and concentrated carefully under reduced pressure to remove THF. The resulting residue was extracted with PE/EA (v/v, 3:1, 400 mL × 2). The aqueous phase was cooled to 0 °C, and HCl (1 N) was added until the pH value reached 1−2 and then filtered. The filtered cake was washed with H_2O (200 mL × 2), collected, and dried under vacuum to give product **13** (54.5 g, 90%) as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 7.48 (d, J = 8.3 Hz, 1H), 7.40−7.28 (m, 5H), 6.94 (s, 1H), 5.02 (s, 2H), 4.33 (m, 1H), 2.54 (dd, J = 15.5, 5.3 Hz, 1H), 2.43 (dd, J = 15.4, 7.9 Hz, 1H).

*(S)-3-Amino-2-(((benzyloxy)carbonyl)amino)propanoic Acid (**14**).* To a stirred suspension of compound **13** (110 g, 413 mmol) in CH_3CN/EA/H_2O (600 mL/600 mL/300 mL) was added iodobenzene diacetate (PIDA, 160 g, 496 mmol) at 0 °C. The resulting mixture was stirred overnight at rt and filtered, and the filtered cake was washed with EA (200 mL × 3), collected, and dried under vacuum to give compound **14** (68.0 g, 70%) as a white solid: ^{1}H NMR (400 MHz, DMSO+TFA) δ 8.07 (s, 2H), 7.76 (d, J = 8.6 Hz, 1H), 7.46−7.28 (m, 5H), 5.07 (s, 1H), 4.37−4.24 (m, 1H), 3.24 (m, 1H), 3.04 (m, 1H); ^{13}C NMR (101 MHz, DMSO+TFA) δ 170.9, 156.3, 136.7, 128.5, 128.0, 127.9, 65.9, 51.9.

*(S)-2-(((Benzyloxy)carbonyl)amino)-3-((tert-butoxycarbonyl)-amino)propanoic Acid (**S3**).* To a stirred mixture of compound **14** (55.0 g, 230.7 mmol) in THF/H_2O (800 mL/400 mL) was added Na_2CO_3 (36.7 g, 346.0 mmol) at 0 °C. The resulting mixture was stirred for 20 min at 0 °C followed by addition of $(Boc)_2O$ (55.4 g, 253.7 mmol). The reaction mixture was stirred overnight at rt, diluted with H_2O (500 mL), concentrated under reduced pressure to remove THF, and extracted with PE (300 mL × 2). The aqueous phase was cooled to 0 °C, and HCl (1 N) was added until the pH value reached 1−2 and extracted with EA (500 mL × 3), The combined organic phases were washed with brine (600 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to obtain **S3** (62.5 g, 80%) as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 12.69 (s, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.33 (m, 5H), 6.84 (t, J = 5.4 Hz, 1H), 5.03 (s, 2H), 4.07 (m, 1H), 3.32−3.22 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, DMSO) δ 172.1, 156.0, 155.7, 137.0, 128.4, 127.9, 127.8, 78.1, 65.6, 54.2, 41.2, 28.2.

*Methyl (S)-2-((S)-2-(((Benzyloxy)carbonyl)amino)-3-((tert-butoxycarbonyl)amino)propanamido)pentanoate (**16**).* To a stirred mixture of compound **S3** (40.0 g, 118.3 mmol) and compound **15** (21.7 g, 130.1 mmol) in DCM (500 mL) were added HOBt (20.8 g, 153.8 mmol) and EDCI (29.5 g, 153.8 mmol) at 0 °C. After being stirred for 5 min, the resulting mixture was added DIPEA (34.9 g, 270.1 mmol) and stirred overnight at rt. The reaction was quenched with H_2O (500 mL) and separated. The aqueous phase was extracted with DCM (300 mL × 2). The combined organic phases were washed with brine (500 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−5% MeOH in DCM) to obtain compound **16** (43.7 g, 82%) as a white solid: $[\alpha]_D^{20}$ = +4.8 (c = 1.0, MeOH); ν_{max} (KBr) 3332, 2961, 2934, 1687, 1657, 1539, 1269, 1248, 1167, 1027, 747, 698, 653 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.26 (m, 5H), 6.41 (s, 1H), 5.51 (s, 1H), 5.11 (s, 2H), 4.53 (td, J = 7.9, 5.4 Hz, 1H), 4.38 (d, J = 5.1 Hz, 1H), 3.70 (s, 3H), 3.49 (d, J = 4.5 Hz, 2H), 1.77 (m, 1H), 1.64 (m, 1H), 1.41 (s, 9H), 1.31 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 170.3, 157.0, 156.6, 136.2, 128.5, 128.1, 128.0, 79.8, 67.0, 55.9, 52.3,

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52.2, 42.6, 34.0, 28.3 18.6, 13.6; HRMS (ESI) calcd for $C_{22}H_{33}N_3O_7$ $[M + Na]^+$ 474.2211, found 474.2215.

*Methyl (S)-2-((S)-2-Amino-3-((tert-butoxycarbonyl)amino)-propanamido)pentanoate (**S4**).* Into a 500 mL round-bottom flask were added compound **16** (21.7 g, 48.1 mmol), MeOH (220 mL), and Pd/C (1.70 g). The flask was degassed and filled with hydrogen three times. The resulting mixture was stirred for 3 h at rt and then filtered through a pad of Celite. The filtered cake was washed with MeOH (50 mL), and the filtrates were collected and concentrated under reduced pressure to give compound **S4** (14.9 g, 98%) as a white solid: $[\alpha]_D^{20}$ = +5.0 (*c* = 1.0, MeOH); ν_{max} (KBr) 3505, 3484, 2965, 1694, 1524, 1275, 1253, 1170, 578 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 6.9 Hz, 1H), 5.43 (s, 1H), 4.45 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.67 (s, 3H), 3.59 (s, 1H), 3.40 (s, 2H), 3.17 (s, 2H), 1.74 (m, 1H), 1.64 (m, 1H), 1.36 (s, 9H), 1.29 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 172.6, 157.1, 79.7, 55.4, 52.3, 52.0, 44.3, 34.0, 28.67−28.01 (m), 18.7, 13.6; HRMS (ESI) calcd for $C_{14}H_{27}N_3O_5$ $[M + H]^+$ 318.2023, found 318.2026.

*Methyl (5S,8S,11S)-8-(((tert-Butoxycarbonyl)amino)methyl)-5-methyl-3,6,9-trioxo-1-phenyl-11-propyl-2-oxa-4,7,10-triazadodecan-12-oate (**18**).* To a stirred mixture of **S4** (15.0 g, 47.3 mmol) and **17** (10.5 g, 47.3 mmol) in DCM (150 mL) were added HOBt (9.60 g, 70.9 mmol), EDCI (13.6 g, 70.9 mmol), and DIPEA (12.2 g, 94.5 mmol) at 0 °C. The resulting mixture was stirred overnight at rt. Then the mixture was diluted with DCM (100 mL), washed with H_2O (200 mL), and separated. The aqueous phase was extracted with DCM (100 mL × 2). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−3% MeOH in DCM) to obtain product **18** (17.2 g, 70%) as a white solid: $[\alpha]_D^{20}$ = −0.62 (*c* = 1.0, MeOH); ν_{max} (KBr) 3305, 2962, 1695, 1650, 1536, 1254, 1169, 697 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 8.13 (d, *J* = 7.5 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 6.9 Hz, 1H), 7.42−7.26 (m, 5H), 6.54 (d, *J* = 6.4 Hz, 1H), 5.02 (q, *J* = 12.6 Hz, 2H), 4.33 (d, *J* = 6.3 Hz, 1H), 4.23 (d, *J* = 6.8 Hz, 1H), 4.09−3.96 (m, 1H), 3.61 (s, 3H), 3.29 (m, 1H), 3.19 (m, 1H), 1.62 (m, 2H), 1.37 (s, 9H), 1.29 (m, 2H), 1.21 (d, *J* = 7.1 Hz, 3H), 0.85 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.8, 172.8, 170.2, 156.3, 156.1, 137.4, 128.8, 128.3, 128.2, 78.5, 65.9, 53.4, 52.3, 52.2, 50.8, 42.2, 33.3, 28.6, 18.9, 18.3, 13.9; HRMS (ESI) calcd for $C_{25}H_{38}N_4O_8$ $[M + Na]^+$ 545.2587, found 545.2585.

*Methyl (S)-2-((S)-2-((S)-2-Aminopropanamido)-3-((tert-butoxycarbonyl)amino)propanamido)pentanoate (**S5**).* Into a 500 mL round-bottom flask were added compound **18** (19.1 g, 36.6 mmol), MeOH (200 mL), and Pd/C (1.90 g). The flask was degassed and filled with hydrogen three times. The resulting mixture was stirred for 4 h at rt and then filtered through a pad of Celite. The filtered cake was washed with MeOH (50 mL), and the filtrates were collected and concentrated to give compound **S5** (13.5 g, 95%) as a white solid, which was used directly for the next step without further purification.

*Methyl (6S,9S,12S)-1-(4-(Allyloxy)phenyl)-9-(((tert-butoxycarbonyl)amino)methyl)-6-methyl-1,4,7,10-tetraoxo-12-propyl-2,5,8,11-tetraazatridecan-13-oate (**19**).* To a stirred mixture of **11** (9.60 g, 40.8 mmol) and **S5** (15.8 g, 40.8 mmol) in DCM (150 mL) were added HOBt (7.10 g, 53.1 mmol), EDCI (10.1 g, 53.1 mmol), and DIPEA (7.90 g, 61.2 mmol) at 0 °C. The resulting mixture was stirred overnight at rt, diluted with DCM (100 mL), washed with H_2O (200 mL), and separated. The aqueous phase was extracted with DCM (100 mL × 2). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−3% MeOH in DCM) to give product **19** (19.6 g, 80%) as a white solid: $[\alpha]_D^{20}$ = +31.9 (*c* = 0.75, $CHCl_3$/MeOH = 2:1); ν_{max} (KBr) 3306, 2933, 1692, 1643, 1544, 1508, 1301, 1230, 846, 557 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 8.68 (t, *J* = 5.7 Hz, 1H), 8.23 (d, *J* = 7.0 Hz, 1H), 7.97 (d, *J* = 7.1 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 8.9 Hz, 2H), 7.02 (d, *J* = 8.9 Hz, 2H), 6.63 (t, *J* = 5.9 Hz, 1H), 6.10−5.98 (m, 1H), 5.40 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.27 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.64 (dt, *J* = 5.3, 1.6 Hz, 2H), 4.36−4.29 (m, 1H), 4.28−4.24 (m, 1H), 4.10 (q, *J* = 7.4 Hz, 1H), 3.95−3.80 (m, 2H), 3.59 (s, 3H), 3.38−3.33 (m, 1H), 3.21 (m, 1H), 1.56 (m, 2H), 1.36 (s, 9H), 1.24 (d, *J* = 7.1 Hz, 3H), 1.20 (m, 1H), 0.79 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.3, 172.0, 169.7, 169.3, 166.3, 160.7, 155.6, 133.3, 129.2, 126.1, 117.6, 114.2, 78.0, 68.3, 52.9, 51.8, 51.7, 48.6, 42.9, 41.6, 32.7, 28.2, 18.4, 17.8, 13.4; HRMS (ESI) calcd for $C_{29}H_{43}N_5O_9$ $[M + Na]^+$ 628.2953, found 628.2958.

*Allyl 2-Bromoacetate (**S7**).* To a stirred solution of allyl alcohol (16.1 g, 277 mmol) in DCM (160 mL) was added pyridine (22.0 g, 277 mmol) at rt. The solution was cooled to 0 °C followed by addition of a solution of 2-bromoacetyl bromide **S6** (56.0 g, 277 mmol in 100 mL of DCM). The reaction mixture was stirred for 1 h at 0 °C and 3 h at rt. Next, the reaction was quenched with cold H_2O (500 mL) and extracted with DCM (300 mL × 3). The combined organic phases were washed with brine (500 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (PE/EA, 15:1) to give compound **S7** (39.7 g, 80%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 5.93 (m, 1H), 5.38 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.29 (dd, *J* = 10.4, 1.1 Hz, 1H), 4.67 (d, *J* = 5.8 Hz, 2H), 3.87 (s, 2H).

*(2-(Allyloxy)-2-oxoethyl)triphenylphosphonium Chloride (**S8**).* To a stirred solution of triphenylphosphine (57.1 g, 217.9 mmol) in THF (400 mL) was added a solution of **S7** (39.0 g, 217.9 mmol in 100 mL of THF) at rt. The resulting mixture was stirred overnight at rt and then filtered. The filtered cake was washed with THF (50 mL × 3), collected, and dried under reduced pressure to give **S8** (95.0 g, 99%) as a white solid, which was used directly for the next step.

*Allyl 2-(Triphenyl-λ^5-phosphaneylidene)acetate (**27**).* To a stirred suspension of **S8** (20.0 g, 45.3 mmol) in H_2O (200 mL) was added a solution of NaOH (2.0 g, 49.85 mmol in 50 mL of H_2O) at 0 °C. The resulting mixture was stirred for 30 min at 0 °C, diluted with H_2O (200 mL), and extracted with EA (100 mL × 3). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give **27** (16.0 g, 98%) as a colorless gum, which was used directly for the next step without further purification.

*2-Acetylmalononitrile (**S10**).* To a stirred of suspension of NaH (24.2 g, 60 wt %, 605.1 mmol) in THF (200 mL) at −10 °C was added a solution of malononitrile **S9** (20.0 g, 302.6 mmol in 100 mL of THF) over a period of 1.5 h (during addition, the temperature maintained below 0 °C). The resulting mixture was stirred for 30 min at −5 °C followed by addition of a solution of acetyl chloride (23.8 g, 302.6 mmol in 100 mL of THF) over a period of 3 h (temperature maintained below 0 °C). After addition, the reaction mixture was stirred for 5 h at −5 °C, quenched carefully at −5 °C with cold water (100 mL), diluted with H_2O (400 mL), and extracted with PE (250 mL × 2). To the aqueous phase was added HCl (1 N) until the pH value reached 1−2 and was extracted with EA (160 mL × 5). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give product **S10** (22.9 g, 70%) as a light yellow solid: ^1H NMR (400 MHz, D_2O) δ 2.20 (s, 3H); ^{13}C NMR (101 MHz, D_2O) δ 192.1, 117.6, 115.5, 58.1, 21.4.

*2-((tert-Butyldimethylsilyl)oxy)malononitrile (**6**).* To a stirred of solution of **S10** (32.0 g, 296 mmol) in HOAc/H_2O (300 mL/450 mL) was added *m*-chloroperbenzoic acid (76.6 g, 444 mmol) at rt. The resulting mixture was stirred for 2 h at rt and filtered. The filtrate was collected and concentrated under vacuum to give a crude product, which was dissolved in DMF (270 mL) at 0 °C followed by addition of TBSCl (66.9 g, 444 mmol) in one portion and a solution of imidazole (30.2 g, 444 mmol in 130 mL of DMF) dropwise (the temperature maintained below 5 °C). After addition, the resulting mixture was stirred for 30 min at 0 °C and 30 min at rt. The reaction mixture was then quenched with H_2O (500 mL) and extracted with EA (200 mL × 3). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated to give a crude product, which was purified by silica gel column chromatography (3−5% EA in PE) to give compound **6** (29.0 g, 50% for two steps) as a light yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ

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5.33 (s, 1H), 0.94 (s, 9H), 0.28 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 112.5, 51.0, 25.3, 18.2, −5.2.

*tert-Butyl ((2S,3S)-1-(Methoxy(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamate (**S12**).* To a stirred solution of Boc-L-isoleucine **S11** (50.0 g, 216.2 mmol) in DCM (500 mL) were added HOBt (38.0 g, 281.0 mmol) and EDCI (53.9 g, 281.0 mmol) at rt. The mixture was stirred for 20 min followed by addition of *N*,*O*-dimethylhydroxylamine hydrochloride (25.3 g, 259.4 mmol) and *N*-methyl morpholine (32.8 g, 324.3 mmol). The resulting mixture was stirred overnight at rt, quenched with H_2O (1000 mL), and extracted with EA (500 mL × 3). The combined organic phases were washed with brine (1000 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (15−25% EA in PE) to obtain compound **S12** (50.4 g, 85%) as a light yellow sticky oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.08 (d, J = 9.0 Hz, 1H), 4.59 (s, 1H), 3.75 (s, 3H), 3.19 (s, 3H), 1.75−1.62 (m, 1H), 1.58−1.48 (m, 1H), 1.40 (s, 9H), 1.16−1.02 (m, 1H), 0.89 (d, J = 6.9 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 155.8, 79.5, 61.6, 54.3, 38.2, 32.0, 28.5, 24.4, 15.6, 11.4.

*tert-Butyl ((2S,3S)-3-Methyl-1-oxopentan-2-yl)carbamate (**7**).* To a stirred solution of **S12** (20.0 g, 72.9 mmol) in anhydrous THF (300 mL) was added $LiAlH_4$ (3.0 g, 80.2 mmol) in portions (temperature maintained below −5 °C). After addition, the reaction mixture was stirred for 1 h at −10 °C, quenched carefully with 3 mL of H_2O (temperature maintained below 0 °C), diluted with H_2O (500 mL), and filtered through a pad of Celite. The filtrate was extracted with EA (200 mL × 3). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (10−15% EA in PE) to obtain compound **7** (12.6 g, 80%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 5.12 (s, 1H), 4.27 (s, 1H), 2.01 (m, 1H), 1.48 (m, 1H), 1.41 (s, 9H), 1.24 (m, 1H), 0.96 (d, J = 6.9 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.7, 155.8, 80.0, 64.3, 36.5, 28.4, 25.4, 15.8, 12.0.

*(S)-2-((tert-Butoxycarbonyl)amino)-3-(4-((tert-butyldimethylsilyl)oxy)phenyl)propanoic acid (**S14**).* To a stirred solution of **S13** (200 g, 711 mmol) in DCM (1500 mL) was added imidazole (194 g, 2.84 mol) at rt. The mixture was stirred for 20 min followed by addition of TBSCl (193 g, 1.28 mol). The resulting mixture was stirred overnight at rt and quenched with H_2O (2000 mL). The aqueous phase was extracted with DCM (1500 mL × 2). The combined organic phases were washed with brine (2000 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−3% MeOH in DCM) to obtain compound **S14** (196.9 g, 70%) as a light yellow gum, which was used directly for the next step.

*tert-Butyl (S)-(1-Amino-3-(4-((tert-butyldimethylsilyl)oxy)-phenyl)-1-oxopropan-2-yl)carbamate (**21**).* To a stirred solution of **S14** (105 g, 265.4 mmol) in DMF (1000 mL) were added Et_3N (80.5 g, 797.0 mmol), HOBt (53.8 g, 398.2 mmol), and EDCI (76.4 g, 398.5 mmol) at rt. The mixture was stirred for 20 min followed by addition of NH_4Cl (28.4 g, 560.8 mmol). The resulting mixture was stirred overnight at rt, quenched with H_2O (2000 mL), and extracted with EA (1000 mL × 3). The combined organic phases were washed with brine (1500 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−5% MeOH in DCM) to obtain compound **21** (73.5 g, 70%) as a white solid: $[\alpha]_D^{20}$ = +8.2 (c = 1.0, $CHCl_3$); ν_{max} (KBr) 3394, 3349, 2957, 2931, 1680, 1511, 1255, 1169, 917, 838, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.3 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 5.72 (s, 1H), 5.34 (s, 1H), 5.02 (s, 1H), 4.29 (s, 1H), 3.07−2.91 (m, 2H), 1.42 (s, 9H), 0.97 (s, 9H), 0.18 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 155.6, 154.7, 130.4, 129.4, 120.3, 80.2, 55.6, 37.8, 28.4, 25.8, 18.3, −4.3; HRMS (ESI) calcd for $C_{20}H_{34}N_2O_4Si$ [M + Na]$^+$ 417.2180, found 417.2183.

*tert-Butyl (S)-(1-Amino-3-(4-((tert-butyldimethylsilyl)oxy)-phenyl)-1-thioxopropan-2-yl)carbamate (**22**).* To a stirred solution of **21** (80.0 g, 202.7 mmol) in DME (1000 mL) was added Lawesson's reagent (41.2 g, 101.9 mmol) at rt. The mixture was stirred for 6 h at rt, quenched with cold water (1000 mL), and extracted with EA (800 mL × 3). The combined organic phases were washed with brine (1000 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−3% MeOH in DCM) to give compound **22** (54.0 g, 65%) as light yellow solid: $[\alpha]_D^{20}$ = +37.1 (c = 1.0, $CHCl_3$); ν_{max} (KBr) 3303, 3194, 2957, 2931,1659, 1511, 1256, 1168, 917, 840, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.18 (s, 1H), 7.10 (d, J = 8.4 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 4.53 (q, J = 7.1 Hz, 1H), 3.16−3.02 (m, 2H), 1.41 (s, 9H), 0.97 (s, 9H), 0.18 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.6, 155.6, 154.6, 130.3, 129.2, 120.1, 80.3, 61.1, 41.2, 28.3, 25.7, 18.2, −4.40; HRMS (ESI) calcd for $C_{20}H_{34}N_2O_3SSi$ [M + Na]$^+$ 433.1952, found 433.1955.

*Ethyl (S)-2-(1-((tert-butoxycarbonyl)amino)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazole-4-carboxylate (**24**).* A mixture of compound **22** (50.0 g, 121.8 mmol) and $KHCO_3$ (60.9 g, 609.2 mmol) in DME (1000 mL) was stirred for 15 min at rt followed by addition of compound **23** (47.3 g, 243.9 mmol). The resulting mixture was stirred for 3 h at rt followed by dropwise addition of a solution of trifluoroacetic anhydride (76.8 g, 365.7 mmol) and 2,6-dimethylpyridine (78.4 g, 731.3 mmol) in DME (500 mL) (the temperature maintained at 5−15 °C). After addition, the resulting mixture was stirred for 5 h at rt, quenched with H_2O (1500 mL), and extracted with EA (1000 mL × 3). The combined organic phases were washed with brine (1000 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (10−15% EA in PE) to give compound **24** (46.3 g, 75%) as a light brown solid: $[\alpha]_D^{20}$ = −6.2 (c = 1.0, $CHCl_3$); ν_{max} (KBr) 3352, 2931, 1721, 1691, 1512, 1255, 1239, 1175, 922, 840, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 6.93 (d, J = 8.2 Hz, 2H), 6.72 (d, J = 8.2 Hz, 2H), 5.25 (s, 2H), 4.43 (q, J = 7.1 Hz, 2H), 3.24 (s, 2H), 1.41 (t, J = 7.2 Hz, 3H), 1.40 (s, 9H), 0.96 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 161.3, 155.0, 154.6, 147.2, 130.4, 128.9, 127.1, 120.2, 80.1, 61.4, 54.1, 40.7, 28.2, 25.6, 18.1, 14.3, −4.5; HRMS (ESI) calcd for $C_{25}H_{38}N_2O_5SSi$ [M + Na]$^+$ 529.2163, found 529.2168.

*tert-Butyl (S)-(2-(4-((tert-Butyldimethylsilyl)oxy)phenyl)-1-(4-(hydroxymethyl)thiazol-2-yl)ethyl)carbamate (**25**).* To a stirred suspension of $CaCl_2$ (10.0 g, 90.8 mmol) in THF (120 mL) was added $NaBH_4$ (6.87 g, 181.6 mmol) at 0 °C. A solution of compound **24** (23.0 g, 45.4 mmol) in THF/EtOH (120 mL/120 mL) was added dropwise to the mixture (temperature maintained below 5 °C). After addition, the resulting mixture was stirred for 4 h at rt. Then the reaction mixture was cooled to 0 °C followed by dropwise addition of 230 mL of saturated aqueous NH_4Cl (temperature maintained below 10 °C). After that, the mixture was diluted with H_2O (200 mL), extracted with EA (200 mL × 3), washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (25−30% EA in PE) to give compound **25** (19.0 g, 90%): $[\alpha]_D^{20}$ = −4.3 (c = 1.0, $CHCl_3$); ν_{max} (KBr) 3350, 2931, 1687, 1512, 1255, 1165, 1058, 918, 839, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (s, 1H), 6.91 (d, J = 8.3 Hz, 2H), 6.70 (d, J = 8.4 Hz, 2H), 5.44 (d, J = 7.6 Hz, 1H), 5.17 (d, J = 6.6 Hz, 1H), 4.73 (s, 2H), 3.65 (s, 1H), 3.17 (dd, J = 13.8, 6.5 Hz, 2H), 1.38 (s, 9H), 0.95 (s, 9H), 0.15 (s, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 156.5, 155.1, 154.6, 130.5, 129.2, 120.2, 114.5, 80.1, 60.8, 54.0, 41.2, 28.4, 25.8, 18.3, −4.4; HRMS (ESI) calcd for $C_{23}H_{36}N_2O_4SSi$ [M + Na]$^+$ 487.2057, found 487.2063.

*tert-Butyl (S)-(2-(4-((tert-Butyldimethylsilyl)oxy)phenyl)-1-(4-formylthiazol-2-yl)ethyl)carbamate (**26**).* To a stirred solution of compound **25** (20.0 g, 43.0 mmol) in DCM (200 mL) was added Dess-Martin oxidant (22.0 g, 51.7 mmol) slowly at 0 °C. The resulting mixture was stirred for 2 h at 0 °C, quenched with H_2O (400 mL), and separated. The aqueous phase was extracted with DCM (150 mL × 3). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under

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reduced pressure to give a crude product, which was purified by silica gel column chromatography (5% EA in PE) to give compound **26** (18.9 g, 95%): $[\alpha]_D^{20}$ = −5.5 (*c* = 1.0, $CHCl_3$); ν_{max} (KBr) 3346, 2958, 1707, 1512, 1255, 1168, 918, 840, 781 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.01 (s, 1H), 8.05 (s, 1H), 6.93 (d, *J* = 8.2 Hz, 2H), 6.73 (d, *J* = 8.2 Hz, 2H), 5.24 (s, 1H), 3.23 (s, 2H), 1.41 (s, 9H), 0.96 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.6, 174.2, 155.1, 154.9, 154.8, 130.4, 128.7, 128.1, 120.3, 80.4, 54.1, 40.8, 28.3, 25.7, 18.3, −4.4; HRMS (ESI) calcd for $C_{23}H_{34}N_2O_4SSi$ $[M + Na]^+$ 485.1906, found 485.1906.

*Allyl (S,E)-3-(2-(1-((tert-Butoxycarbonyl)amino)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazol-4-yl)acrylate (**S15**).* To a stirred solution of compound **26** (10.0 g, 21.6 mmol) in DCM (100 mL) was added a solution of compound **27** (8.60 g, 23.8 mmol in 50 mL of DCM) at rt. The resulting solution was stirred for 2 h at rt. The solvent was removed under reduced pressure to give a crude product, which was purified by silica gel column chromatography (5% EA in PE) to obtain compound **S15** (10.0 g, 85%) as a colorless gum: $[\alpha]_D^{20}$ = −8.9 (*c* = 1.0, $CHCl_3$) ν_{max} (KBr) 3358, 2957, 2931,1715, 1640, 1511, 1366, 1267, 1164, 916, 840, 781, 689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 15.5 Hz, 1H), 7.29 (s, 1H), 6.93 (d, *J* = 8.3 Hz, 2H), 6.81 (d, *J* = 15.5 Hz, 1H), 6.72 (d, *J* = 8.2 Hz, 2H), 5.99 (m, 1H), 5.38 (d, *J* = 17.2 Hz, 1H), 5.27 (d, *J* = 10.4 Hz, 1H), 5.23 (m, 1H), 4.72 (d, *J* = 5.6 Hz, 2H), 3.22 (s, 2H), 1.42 (s, 9H), 0.96 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 166.9, 155.1, 154.7, 151.6, 136.7, 132.3, 130.5, 129.0, 121.5, 120.4, 120.2, 118.3, 80.2, 65.3, 54.1, 40.9, 28.4, 25.8, 18.3, −4.4; HRMS (ESI) calcd for $C_{28}H_{40}N_2O_5SSi$ $[M + H]^+$ 545.2500, found 545.2503.

*Allyl (S,E)-3-(2-(1-Amino-2-(4-((tert-butyldimethylsilyl)oxy)-phenyl)ethyl)thiazol-4-yl)acrylate (**5**).* To a stirred solution of compound **S15** (20.0 g, 36.7 mmol) in DCM (200 mL) was added trifluoroacetic acid (20.0 mL) slowly at 0 °C. The resulting mixture was stirred for 5 h at rt, diluted with DCM (100 mL), washed carefully with cold saturated aqueous $NaHCO_3$ (400 mL), and separated. The aqueous phase was extracted with DCM (150 mL × 2). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (PE/EA, 3:1 to 2:1) to obtain compound **5** (13.2 g, 51%) as a light yellow oil: $[\alpha]_D^{20}$ = +4.8 (*c* = 1.0, CH_3OH); ν_{max} (KBr) 2955, 2930, 2858, 1714, 1639, 1510, 1267, 1159, 916, 840, 781 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 15.5 Hz, 1H), 7.35 (s, 1H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 15.5 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 2H), 5.99 (ddd, *J* = 22.8, 10.8, 5.6 Hz, 1H), 5.37 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.26 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.71 (dt, *J* = 5.6, 1.3 Hz, 2H), 4.46 (dd, *J* = 9.0, 4.4 Hz, 1H), 3.29 (dd, *J* = 13.7, 4.4 Hz, 1H), 2.83 (dd, *J* = 13.7, 9.0 Hz, 1H), 0.98 (s, 9H), 0.19 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.8, 167.0, 154.7, 151.7, 137.1, 132.4, 130.5, 130.0, 121.8, 120.3, 120.1, 118.2, 65.2, 55.6, 44.0, 25.8, 18.3, −4.3; HRMS (MALDI) calcd for $C_{23}H_{32}N_2O_3SSi$ $[M + H]^+$ 445.1976, found 445.1979.

*Allyl (E)-3-(2-((1S)-1-((3S,4S)-3-((tert-Butoxycarbonyl)amino)-2-((tert-butyldimethylsilyl)oxy)-4-methylhexanamido)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazol-4-yl)acrylate (**28**).* To a stirred solution of compound **7** (4.70 g, 21.9 mmol) in dry THF (40 mL) under argon atmosphere was added a solution of compound **6** (4.30 g, 21.9 mmol in 10 mL of THF) at −30 °C followed by addition of Et_3N (2.20 g, 21.9 mmol in 10 mL of THF). The resulting mixture was stirred for 30 min at −30 °C followed by subsequent addition of compound **5** (7.50 g, 16.9 mmol in 40 mL of THF) and DMAP (4.10 g, 33.7 mmol). The resulting mixture was stirred for 8 h at −30 °C. Then the reaction vessel was transferred into a Dewar flask (ice-salt bath) and stirred overnight, and the temperature was allowed to warm slowly to rt. After compound **5** disappeared, as monitored by TLC analysis, the reaction mixture was diluted with EA (100 mL), washed with H_2O (200 mL), and separated. The aqueous phase was extracted with EA (100 mL × 2). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (5−10% EA in PE) to obtain compound **28** (9.50 g, 70%, dr = 6:1) as a light yellow gum: $[\alpha]_D^{20}$ = +14.1 (*c* = 0.5, $CHCl_3$); ν_{max} (KBr) 3414, 2956, 2929, 2858, 1716, 1638, 1617, 1510, 1489, 1262, 1164, 918, 840 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 15.5 Hz, 1H), 7.28 (s, 1H), 6.93 (d, *J* = 7.5 Hz, 2H), 6.79 (d, *J* = 15.5 Hz, 1H), 6.71 (d, *J* = 7.4 Hz, 2H), 5.98 (m, 1H), 5.46 (d, *J* = 7.1 Hz, 1H), 5.46 (d, *J* = 7.1 Hz, 1H), 5.38 (d, *J* = 17.2 Hz, 1H), 5.27 (d, *J* = 10.4 Hz, 1H), 5.10 (d, *J* = 10.4 Hz, 1H), 4.72 (d, *J* = 5.2 Hz, 3H), 4.26 (d, *J* = 2.8 Hz, 1H), 3.69 (t, *J* = 9.2 Hz, 1H), 3.28 (dd, *J* = 13.7, 5.9 Hz, 1H), 3.07 (dd, *J* = 13.5, 8.1 Hz, 1H), 1.46 (m, 3H), 1.29 (s, 9H), 1.09−1.02 (m, 1H), 0.99 (s, 9H), 0.96 (s, 9H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.74 (t, *J* = 7.2 Hz, 3H), 0.18 (s, 3H), 0.16 (s, 6H), 0.09 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.2, 166.8, 155.5, 154.80, 151.3, 136.4, 132.3, 130.5, 128.8, 121.6, 120.7, 120.3, 118.2, 78.9, 74.2, 65.3, 58.0, 52.6, 41.7, 35.7, 28.3, 26.0, 25.8, 25.3, 18.3, 18.1, 16.9, 11.1, −4.3, −4.5−5.1; HRMS (MALDI) calcd for $C_{41}H_{67}N_3O_7SSi_2$ $[M + Na]^+$ 824.4130, found 824.4136.

*Allyl (E)-3-(2-((1S)-1-((3S,4S)-3-Amino-2-((tert-butyldimethylsilyl)oxy)-4-methylhexanamido)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazol-4-yl)acrylate (**4**).* To a stirred solution of compound **28** (8.00 g, 9.97 mmol) in DCM (80 mL) was added trifluoroacetic acid (8.0 mL) slowly at 0 °C. The resulting mixture was stirred for 6 h at rt, diluted with DCM (80 mL), washed carefully with cold saturated aqueous $NaHCO_3$ (200 mL), and separated. The aqueous phase was extracted with DCM (100 mL × 2). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2% MeOH in DCM) to obtain compound **4** (5.60 g, 80%) as a light yellow sticky oil: $[\alpha]_D^{20}$ = +0.12 (*c* = 1.0, CH_3OH) ν_{max} (KBr) 3417, 2957, 2931, 2858, 1717, 1510, 1265, 1161, 917, 838, 780 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 8.40 (d, *J* = 8.3 Hz, 1H), 8.04 (s, 1H), 7.65 (d, *J* = 15.6 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 2H), 6.71 (d, *J* = 8.1 Hz, 2H), 6.67 (d, *J* = 15.6 Hz, 1H), 6.05−5.90 (m, 1H), 5.41 (m, 1H), 5.34 (dd, *J* = 17.3, 1.8 Hz, 1H), 5.23 (dd, *J* = 10.5, 1.7 Hz, 1H), 4.67 (d, *J* = 5.3 Hz, 2H), 4.09 (d, *J* = 2.8 Hz, 1H), 3.29 (dd, *J* = 14.2, 5.0 Hz, 1H), 3.08 (dd, *J* = 14.1, 10.0 Hz, 1H), 2.32 (dd, *J* = 7.6, 2.8 Hz, 1H), 1.56 (m, 1H), 1.26 (m, 1H), 1.10 (m, 1H), 0.92 (s, 9H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.82 (s, 9H), 0.76 (t, *J* = 7.3 Hz, 3H), 0.15 (s, 6H), −0.03 (s, 3H), −0.12 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 173.2, 172.5, 165.8, 153.7, 150.2, 137.1, 132.7, 130.1, 130.0, 124.2, 119.5, 119.0, 117.6, 74.2, 64.5, 58.8, 54.9, 52.1, 35.9, 25.7, 25.5, 24.4, 17.9, 16.30 10.8, −4.6, −4.8, −5.3; HRMS (ESI) calcd for $C_{36}H_{59}N_3O_5SSi_2$ $[M + Na]^+$ 724.3606, found 724.3610.

*(S)-Trityl-L-cysteine (**S17**).* A mixture of L-cysteine hydrochloride **S16** (6.50 g, 41.2 mmol) and trityl chloride (15.0 g, 53.6 mmol) in DMF (25 mL) was stirred overnight at a rt followed by addition of sodium acetate (aqueous, 10 wt %, 180 mL). The precipitate was filtered, washed with distilled water, stirred in acetone (50 mL) for 30 min at 50 °C, and cooled to rt. The resulting mixture was filtered again, and the filtered cake was washed with acetone (20 mL) and diethyl ether (20 mL) and dried in vacuum to give **S17** (14.5 g, 97%) as a white powder, which was used directly for the next step without further purification.

*N-(((9H-Fluoren-9-yl)methoxy)carbonyl)-(S)-trityl-L-cysteine (**S18**).* To a stirred suspension of compound **S17** (14.5 g, 39.9 mmol) in dioxane/H_2O (100 mL/50 mL) was added $NaHCO_3$ (3.4 g, 40.5 mmol) at rt. The resulting mixture was stirred for 20 min at rt followed by addition of Fmoc-Osu (13.5 g, 40.0 mmol). The resulting mixture was stirred overnight at rt, diluted with H_2O (300 mL), evaporated under reduced pressure, and separated. The aqueous phase was cooled to 0 °C, and HCl (1 N) was added until the pH value reached 2−3, extracted with EA (150 mL × 3), and separated. The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give compound **S18** (20.0 g, 85%) as a yellow gum, which was used directly for the next step without further purification.

*Allyl N-(((9H-Fluoren-9-yl)methoxy)carbonyl)-(S)-trityl-L-cysteinate (**S19**).* To a stirred solution of compound **S18** (20.0 g, 34.2

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mmol) in DMF (200 mL) was added K_2CO_3 (4.70 g, 34.2 mmol) at rt. The resulting mixture was stirred for 20 min at rt followed by addition of allyl bromide (13.5 g, 40.0 mmol) and and then stirred for 2 h at rt. Next, the reaction mixture was quenched with H_2O (400 mL), extracted with EA (100 mL × 3), and separated. The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (15% EA in PE) to obtain compound **S19** (17.1 g, 80%) as a light yellow gum: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, J = 7.4, 3.6 Hz, 2H), 7.54−7.49 (m, 2H), 7.30 (m, 8H), 7.24−7.07 (m, 14H), 5.77 (m, 1H), 5.26−5.11 (m, 3H), 4.52 (d, J = 4.6 Hz, 2H), 4.27 (m, 3H), 4.14 (t, J = 7.0 Hz, 1H), 2.58 (qd, J = 12.5, 5.5 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 155.7, 144.4, 144.0, 143.9, 141.4, 131.5, 129.6, 128.1, 127.8, 127.2, 127.0, 125.27, 125.23, 120.1, 118.9, 67.3, 67.2, 66.4, 53.1, 47.2, 34.2.

*Allyl (S)-Trityl-L-cysteinate (**2**).* To a stirred solution of **S19** (10.0 g, 16.0 mmol) in THF (100 mL) was added piperidine (10 mL) at rt. The reaction mixture was stirred for 2 h at rt, washed with H_2O (300 mL), extracted with EA (100 mL × 3), and separated. The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (25% EA in PE) to obtain compound **2** (5.50 g, 85%) as a light yellow gum: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46−7.40 (m, 6H), 7.30−7.23 (m, 6H), 7.22−7.16 (m, 3H), 5.85 (m, 1H), 5.25 (dd, J = 15.7, 1.4 Hz, 1H), 5.22−5.18 (m, 1H), 4.54 (d, J = 5.7 Hz, 2H), 3.22 (m, 1H), 2.58 (dd, J = 12.4, 4.8 Hz, 1H), 2.49 (dd, J = 12.4, 7.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5, 144.6, 131.8, 129.6, 128.0, 126.8, 118.5, 66.9, 65.7, 53.9, 37.0.

*(6S,9S,12S)-1-(4-(Allyloxy)phenyl)-9-(((tert-butoxycarbonyl)-amino)methyl)-12-ethyl-6-methyl-1,4,7,10-tetraoxo-2,5,8,11-tetraazatridecan-13-oic acid (**3**).* To a stirred solution of compound **19** (19.3 g, 31.9 mmol) in THF/H_2O (150 mL/50 mL) was added LiOH·H_2O (2.00 g, 47.8 mmol) at rt. The resulting solution was stirred for 3 h at rt, diluted with H_2O (200 mL), evaporated under reduced pressure, and separated. To the aqueous phase was added HCl (1 N) until the pH value reached 1−2 and then extracted with EA (150 mL × 3). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give compound **3** (18.5 g, 98%) as a white solid: $[\alpha]_D^{20}$ = −35.6 (c = 0.25, $CHCl_3$); ν_{max} (KBr) 3427, 3314, 1641, 1543, 1506, 1254, 1168 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 12.60 (s, 1H), 8.68 (t, 1H), 8.21 (d, J = 7.0 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.84 (d, 1H), 7.02 (d, J = 8.3 Hz, 2H), 6.63 (t, J = 6.2 Hz, 1H), 6.05 (m, 1H), 5.40 (d, J = 17.3 Hz, 1H), 5.28 (d, J = 10.5 Hz, 1H), 4.64 (d, J = 5.1 Hz, 2H), 4.39−4.21 (m, 2H), 4.06 (q, J = 7.2 Hz, 1H), 3.94−3.78 (m, 2H), 3.36 (m, 1H), 3.20 (m, 1H), 1.68−1.47 (m, 2H), 1.36 (s, 9H), 1.25 (m, 2H), 1.23 (d, J = 7.1 Hz, 3H), 0.80 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 173.5, 155.6, 133.4, 129.2, 117.7, 114.2, 78.0, 68.3, 53.0, 51.8, 48.5, 42.9, 41.7, 32.9, 28.2, 18.5, 18.0, 13.5; HRMS (MALDI) calcd for $C_{28}H_{41}N_5O_9$ $[M + Na]^+$ 614.2796, found 614.2801.

*Allyl (E)-3-(2-((6S,9S,12S,15S,19S)-1-(4-(Allyloxy)phenyl)-9-(((tert-butoxycarbonyl)amino)methyl)-15-((S)-sec-butyl)-16-((tert-butyldimethylsilyl)oxy)-20-(4-((tert-butyldimethylsilyl)oxy)phenyl)-6-methyl-1,4,7,10,13,17-hexaoxo-12-propyl-2,5,8,11,14,18-hexaazaicosan-19-yl)thiazol-4-yl)acrylate (**29**).* To a stirred solution of compound **3** (5.10 g, 8.62 mmol) and compound **4** (6.10 g, 8.62 mmol) in DMF/DCM (50 mL/50 mL) was added HATU (4.30 g, 11.2 mmol) at 0 °C followed by addition of DIPEA (1.70 g, 12.9 mmol). The resulting mixture was stirred overnight at rt, quenched with H_2O (200 mL), extracted with ethyl acetate (100 mL × 3), and separated. The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2% MeOH in DCM) to give compound **29** (7.70 g, 70%) as a white solid: $[\alpha]_D^{20}$ = −13.1 (c = 1.0, CH_3OH); ν_{max} (KBr) 3360, 3294, 2959, 2931, 2860, 1721, 1679, 1659, 1539, 1510, 1256, 1166, 918, 840, 780 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 8.62 (t, J = 5.6 Hz 1H), 8.17 (d, J = 6.8 Hz, 1H), 8.13 (d, J = 7.7 Hz, 1H), 8.01 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 7.8 Hz, 1H), 7.61 (d, J = 15.6 Hz, 1H), 7.21 (d, J = 9.7 Hz, 1H), 7.01 (d, J = 8.3 Hz, 4H), 6.69 (d, J = 8.3 Hz, 2H), 6.66 (m, 1H), 6.61 (d, J = 15.6 Hz, 1H), 6.11−5.91 (m, 2H), 5.44−5.20 (m, 5H), 4.67 (d, J = 5.3 Hz, 2H), 4.63 (d, J = 5.1 Hz, 2H), 4.32−4.23 (m, 2H), 4.18 (m, 1H), 4.14 (d, J = 3.4 Hz, 1H), 3.86 (d, J = 5.0 Hz, 2H), 3.85−3.79 (m, 1H), 3.25−3.09 (m, 4H), 1.48 (m, 2H), 1.34 (m, 2H), 1.34 (s, 9H), 1.21 (d, J = 7.1 Hz, 4H), 1.12 (m, 2H), 0.97 (m, 1H), 0.91 (s, 9H), 0.87 (s, 9H), 0.84 (d, J = 6.6 Hz, 3H), 0.69 (t, J = 7.1 Hz, 6H), 0.13 (s, 6H), 0.02 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.2, 171.5, 171.1, 170.9, 169.5, 169.2, 166.2, 165.8, 160.6, 155.6, 153.7, 150.0, 137.0, 133.3, 132.7, 130.3, 129.8, 129.2, 126.2, 124.1, 119.6, 119.0, 117.62, 117.57, 114.1, 77.9, 72.5, 68.3, 64.5, 55.9, 53.3, 52.26, 52.23, 48.5, 42.8, 41.6, 34.5, 33.5, 28.1, 25.8, 25.5, 24.4, 18.3, 17.91, 17.86, 17.78, 16.2, 13.6, 10.7, −4.6, −5.0, −5.1; HRMS (ESI) calcd for $C_{64}H_{98}N_8O_{13}SSi_2$ $[M + Na]^+$ 1297.6405, found 1297.6409.

*Allyl (E)-3-(2-((6S,9S,12S,15S,19S)-1-(4-(Allyloxy)phenyl)-9-(aminomethyl)-15-((S)-sec-butyl)-16-((tert-butyldimethylsilyl)oxy)-20-(4-((tert-butyldimethylsilyl)oxy)phenyl)-6-methyl-1,4,7,10,13,17-hexaoxo-12-propyl-2,5,8,11,14,18-hexaazaicosan-19-yl)thiazol-4-yl)acrylate (**S20**).* To a stirred solution of compound **29** (9.80 g, 7.68 mmol) in DCM (100 mL) was added trifluoroacetic acid (10 mL) slowly at 0 °C. After addition, the resulting mixture was stirred for 6 h at rt, diluted with DCM (100 mL), and washed carefully with cold saturated aqueous $NaHCO_3$ (200 mL). The aqueous phase was extracted with DCM (100 mL × 2). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give compound **S20** (8.50 g, 95%) as a light yellow sticky oil, which was used directly for the next step without further purification.

*Allyl (E)-3-(2-((6S,9S,12S,15S,19S)-9-((((((9H-Fluoren-9-yl)-methoxy)carbonyl)amino)methyl)-1-(4-(allyloxy)phenyl)-15-((S)-sec-butyl)-16-((tert-butyldimethylsilyl)oxy)-20-(4-((tert-butyldimethylsilyl)oxy)phenyl)-6-methyl-1,4,7,10,13,17-hexaoxo-12-propyl-2,5,8,11,14,18-hexaazaicosan-19-yl)thiazol-4-yl)acrylate (**30**).* To a stirred solution of compound **S20** (8.50 g, 9.97 mmol) in THF (100 mL) was added Na_2CO_3 (1.10 g, 10.9 mmol) at rt, followed by addition of Fmoc-Osu (3.70 g, 10.9 mmol). The resulting mixture was stirred for 5 h at rt, diluted with EA (200 mL), washed with H_2O (500 mL), and separated. The aqueous phase was extracted with EA (100 mL × 2). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (1−3% MeOH in DCM) to give compound **30** (5.60 g, 80%) as a white solid: $[\alpha]_D^{20}$ = −10.0 (c = 1.0, CH_3OH); ν_{max} (KBr) 3299, 2957, 2929, 2857, 1637, 1509, 1255, 1160, 918, 841, 781 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 8.62 (t, J = 5.5 Hz, 1H), 8.21 (d, J = 7.1 Hz, 1H), 8.12 (d, J = 7.4 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.99 (s, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 15.6 Hz, 1H), 7.42−7.33 (m, 3H), 7.32−7.23 (m, 3H), 6.98 (m, 4H), 6.66 (d, J = 8.3 Hz, 2H), 6.60 (d, J = 15.6 Hz, 1H), 6.09−5.90 (m, 2H), 5.43−5.20 (m, 5H), 4.66 (d, J = 5.3 Hz, 2H), 4.61 (d, J = 5.2 Hz, 2H), 4.39−4.13 (m, 7H), 3.94−3.80 (m, 3H), 3.34 (m, 1H), 3.15 (m, 2H), 1.50 (m, 2H), 1.38 (m, 2H), 1.22 (d, J = 7.1 Hz, 3H), 1.13 (m, 2H), 0.99 (m, 1H), 0.90 (s, 9H), 0.86 (s, 9H), 0.86 (t, J = 7.2 Hz, 3H), 0.69 (d, J = 6.9 Hz, 3H), 0.69 (t, J = 7.3 Hz, 3H), 0.12 (s, 6H), 0.02 (s, 3H), −0.06 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.2, 171.4, 171.1, 169.6, 169.3, 166.2, 165.8, 160.6, 156.3, 153.7, 150.1, 143.83, 143.79, 140.7, 137.0, 133.3, 132.7, 130.2, 129.7, 129.2, 127.6, 127.1, 126.2, 125.32, 125.26, 124.1, 120.0, 119.5, 119.0, 117.59, 117.56, 114.1, 73.5, 68.3, 65.9, 64.5, 56.0, 53.2, 52.4, 52.2, 48.5, 46.6, 42.9, 42.0, 34.5, 33.5, 25.7, 25.5, 24.5, 18.5, 17.84, 17.77, 16.2, 13.6, 10.7, −4.6, −5.0, −5.1; HRMS (ESI) calcd for $C_{74}H_{100}N_8O_{13}SSi_2$ $[M + H]^+$ 1397.6742, found 1397.6748.

(E)-3-(2-((6S,9S,12S,15S,19S)-9-((((((9H-Fluoren-9-yl)methoxy)-carbonyl)amino)methyl)-15-((S)-sec-butyl)-16-((tert-butyldimethylsilyl)oxy)-20-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1-(4-hydroxyphenyl)-6-methyl-1,4,7,10,13,17-hexaoxo-12-propyl-

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*2,5,8,11,14,18-hexaazaicosan-19-yl)thiazol-4-yl)acrylic acid (**S21**).* Into a 250 mL round-bottom flask were added compound **30** (8.00 g, 5.72 mmol), 1,3-dimethylbarbituric acid (2.20 g, 14.3 mmol), THF (100 mL), and $Pd(PPh_3)_4$ (661 mg, 0.570 mmol). The flask was degassed and filled with argon three times. The resulting mixture was stirred for 3 h at rt, diluted with EA (100 mL), washed with H_2O (200 mL), and separated. The aqueous phase was extracted with EA (100 mL × 2). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (5−10% MeOH in DCM) to give compound **S21** (6.00 g, 80%) as a light yellow foam solid: $[\alpha]_D^{20}$ = −15.4 (*c* = 1.0, CH_3OH); ν_{max} (KBr) 3319, 2958, 2931, 1668, 1510, 1256, 1026, 839 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 8.56 (t, *J* = 5.6 Hz, 1H), 8.24 (d, *J* = 6.9 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.08 (d, *J* = 7.9 Hz, 1H), 7.88(s, 1H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.66 (dd, *J* = 7.4, 2.6 Hz, 2H), 7.45 (d, *J* = 15.9 Hz, 1H), 7.39 (m, 3H), 7.29 (m, 3H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 6.53 (d, *J* = 15.6 Hz, 1H), 5.31 (d, *J* = 7.3 Hz, 1H), 4.41−4.11 (m, 8H), 3.84 (m, 3H), 3.14 (m, 2H), 1.47 (m, 2H), 1.36 (m, 2H), 1.22 (d, *J* = 3.0 Hz, 3H), 1.17 (m, 1H), 1.12 (m, 1H), 0.97 (m, 1H), 0.90 (s, 9H), 0.84 (s, 9H), 0.84 (t, *J* = 7.2 Hz, 3H), 0.69 (d, *J* = 6.9 Hz, 3H), 0.69 (t, *J* = 7.3 Hz, 3H), 0.11 (s, 6H), 0.01 (s, 3H), −0.08 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.3, 171.3, 171.2, 171.1, 169.6, 169.4, 166.5, 160.5 156.3, 153.7, 150.7, 143.87, 143.8, 140.7, 135.3, 130.3, 129.9, 129.3, 127.7, 127.1, 125.4, 125.3, 124.5, 122.6, 120.1, 119.6, 114.9, 73.6, 65.9, 56.08, 56.05, 53.2, 52.5, 52.2, 48.5, 46.6, 42.9, 42.0, 34.6, 33.5, 25.8, 25.6, 24.4, 18.5, 17.9, 17.8, 16.2, 13.7, 10.8, −4.6, −5.0, −5.1; HRMS (ESI) calcd for $C_{68}H_{92}N_8O_{13}SSi_2$ $[M + H]^+$ 1317.6116, found 1317.6118.

*Allyl N-((E)-3-(2-((1S)-1-((3S,4S)-3-((S)-2-((S)-3-(((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-2-((S)-2-(2-(4-hydroxybenzamido)-acetamido)propanamido)propanamido)pentanamido)-2-((tert-butyldimethylsilyl)oxy)-4-methylhexanamido)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazol-4-yl)acryloyl)-(S)-trityl-L-cysteinate (**31**).* To a stirred mixture of compound **S21** (5.30 g, 3.90 mmol) and HATU (1.90 g, 5.07 mmol) in DMF (50 mL) was added DIPEA (756 mg, 5.86 mmol) at 0 °C, and the resulting mixture was stirred for 5 min followed by addition of compound **2** (1.89 g, 4.68 mmol in 6 mL of DCM). The reaction solution was stirred overnight at rt, diluted with EA (100 mL), washed with H_2O (100 mL), and separated. The aqueous phase was extracted with EA (100 mL × 2). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−5% MeOH in DCM) to give compound **31** (5.30 g, 80%) as a white solid: $[\alpha]_D^{20}$ = −12.3 (*c* = 1.0, CH_3OH); ν_{max} (KBr) 3308, 2957, 2929, 2856, 1665, 1510, 1256, 840, 759, 701, 676 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 10.05 (s, 1H), 8.76 (d, *J* = 7.8 Hz, 1H), 8.56 (t, *J* = 6.3 Hz, 1H), 8.26 (d, *J* = 6.9 Hz, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 2H), 7.84 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 3.4 Hz, 1H), 7.66 (d, *J* = 3.3 Hz, 1H), 7.52−7.13 (m, 20H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.89 (d, *J* = 15.3 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 8.0 Hz, 2H), 5.88−5.76 (m, 1H), 5.38 (d, *J* = 7.4 Hz, 1H), 5.20 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.16 (dd, *J* = 10.5, 1.6 Hz, 1H), 4.59−4.44 (m, 2H), 4.38 (d, *J* = 7.0 Hz, 1H), 4.33−4.10 (m, 7H), 3.89 (m, 3H), 3.24 (m, 2H), 2.66 (t, *J* = 10.7 Hz, 1H), 2.47 (m, 1H), 1.62 (m, 1H),1.45 (m, 2H), 1.25 (d, *J* = 7.0 Hz, 3H), 1.06 (m, 1H), 0.89 (s, 9H), 0.83 (s, 9H), 0.74 (d, *J* = 7.4 Hz, 3H), 0.71 (s, 2H), 0.10 (s, 6H), −0.00 (s, 3H), −0.10 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.8, 172.3, 171.7, 171.6, 170.3, 170.1, 169.9, 167.0, 165.5, 160.8, 156.8, 154.1, 151.4, 144.6, 144.3, 144.2, 141.1, 133.0, 132.6, 130.6, 130.5, 129.8, 129.5, 128.6, 128.1, 127.5, 127.3, 125.8, 125.7, 125.0, 123.3, 122.5, 120.5, 120.1, 117.9, 115.3, 74.1, 66.9, 66.3, 65.5, 56.6, 53.7, 53.0, 52.6, 52.2, 49.0, 47.1, 43.4, 42.5, 35.1, 34.0, 33.3, 26.2, 26.0, 24.8, 19.0, 18.3, 18.3, 16.7, 14.1, 11.3, −4.1, −4.5, −4.7; HRMS (ESI) calcd for $C_{93}H_{115}N_9O_{14}S_2Si_2$ $[M + Na]^+$ 1725.7469, found 1725.7462.

*N-((E)-3-(2-((1S)-1-((3S,4S)-3-((S)-2-((S)-3-((((9H-Fluoren-9-yl)-methoxy)carbonyl)amino)-2-((S)-2-(2-(4-hydroxybenzamido)-acetamido)propanamido)propanamido)pentanamido)-2-((tert-butyldimethylsilyl)oxy)-4-methylhexanamido)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazol-4-yl)acryloyl)-(S)-trityl-L-cysteine (**S22**).* Into a 100 mL round-bottom flask were added compound **31** (2.70 g, 1.59 mmol), 1,3-dimethylbarbituric acid (619 mg, 3.96 mmol), THF (40 mL), and $Pd(PPh_3)_4$ (183 mg, 0.160 mmol). The flask was degassed and filled with argon three times. The resulting mixture was stirred for 2 h at rt, diluted with EA (50 mL), washed with H_2O (100 mL), and separated. The aqueous phase was extracted with EA (50 mL × 2), The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (5−10% MeOH in DCM) to give compound **S22** (2.09 g, 80%) as a light yellow solid: $[\alpha]_D^{20}$ = −13.4 (*c* = 1.0, MeOH); ν_{max} (KBr) 3358, 2973, 1756, 1701, 1682, 1665, 1624, 1523, 1363, 1206, 1167 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 10.05 (s, 1H), 8.56 (m, 2H), 8.25 (s, 1H), 8.18 (s, 2H), 7.91−7.82 (m, 4H), 7.73 (d, *J* = 8.2 Hz, 2H), 7.66 (m, 2H), 7.41−7.23 (m, 24H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.90 (d, *J* = 15.7 Hz, 1H), 6.78 (d, *J* = 8.3 Hz, 2H), 6.67 (d, *J* = 8.1 Hz, 2H), 5.36 (m, 1H), 4.35−4.10 (m, 9H), 3.86 (m, 3H), 3.21 (m, 2H), 2.56 (m, 1H), 2.41 (m, 1H), 1.58 (m, 1H), 1.43 (m, 3H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.16 (m, 1H), 0.88 (s, 9H), 0.82 (s, 9H), 0.82 (t, *J* = 7.2 Hz, 3H), 0.70 (d, *J* = 7.1 Hz, 3H), 0.70 (t, *J* = 7.1 Hz, 3H), 0.09 (s, 6H), −0.02 (s, 3H), −0.12 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 172.4, 171.8, 171.3, 169.7, 169.5, 166.6, 164.9, 160.5, 156.4, 153.7, 151.0, 144.3, 143.9, 143.8, 140.7, 132.2, 130.3, 130.1, 129.4, 129.2, 128.1, 127.7, 127.2, 126.8, 125.4, 124.5, 123.4, 121.9, 120.1, 119.6, 114.9, 73.7, 66.1, 65.9, 56.2, 53.3, 52.6, 52.2, 51.8, 48.6, 46.7, 42.9, 42.0, 34.7, 33.5, 25.8, 25.6, 24.3, 18.6, 17.91, 17.86, 16.3, 13.7, 10.9, −4.5, −4.9, −5.1; HRMS (ESI) calcd for $C_{90}H_{111}N_9O_{14}S_2Si_2$ $[M + H]^+$ 1662.7303, found 1662.7303.

*N-((E)-3-(2-((1S)-1-((3S,4S)-3-((S)-2-((S)-3-Amino-2-((S)-2-(2-(4-hydroxybenzamido)acetamido)propanamido)propanamido)-pentanamido)-2-((tert-butyldimethylsilyl)oxy)-4-methylhexanamido)-2-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethyl)thiazol-4-yl)-acryloyl)-(S)-trityl-L-cysteine (**32**).* To a stirred solution of compound **S22** (2.30 g, 1.38 mmol) in DCM (30 mL) was added diethylamine (6.0 mL) at rt. The resulting solution was stirred for 3 h at rt. The volatiles were removed under reduced pressure to give a residue, which was subsequently dissolved in DCM (30 mL). To the resulting solution was added DIPEA (1.0 mL) and concentrated under reduced pressure to give a light yellowed solid. The above operation was repeated three times to obtain final product **32** (2.50 g, crude) as a light yellow solid, which was used directly for the next step without further purification.

*N-(2-(((2S)-1-(((1^{2}Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-5-((tert-butyldimethylsilyl)oxy)-2-(4-((tert-butyldimethylsilyl)oxy)-benzyl)-4,8,11,15,18-pentaoxo-9-propyl-16-((tritylthio)methyl)-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-12-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-4-hydroxybenzamide (**33**).* To a stirred solution of compound **32** (4.00 g, 2.78 mmol) in DCM (2.60 L) was added HOBt (3.75 g, 27.8 mmol) at 0 °C. The resulting mixture was stirred for 10 min followed by addition of EDCI (10.6 g, 55.5 mmol). The resulting mixture was stirred overnight (temperature was allowed to warm slowly to rt), washed with cold water (1.50 L), and separated. The aqueous phase was extracted with DCM (800 mL × 2). The combined organic phases were washed with brine (1000 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (2−10% MeOH in DCM) to give product **33** (2.37 g, 60%) as a light yellow solid: $[\alpha]_D^{20}$ = +30.2 (*c* = 1.0, CH_3OH); ν_{max} (KBr) 3409, 3299, 2957, 2930, 2857, 1656, 1609, 1510, 1262, 840, 700 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 9.98 (s, 1H), 8.45 (t, *J* = 5.8 Hz 1H), 8.39 (s, 1H), 8.17 (m, 2H), 8.08 (d, *J* = 8.2 Hz 1H), 8.00 (d, *J* = 7.5 Hz 1H), 7.88 (s, 1H), 7.76 (d, *J* = 9.8 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.39−7.24 (m, 18H), 7.17 (d, *J* = 8.4 Hz, 3H), 6.79 (d, *J* = 8.5 Hz, 3H), 6.73 (d, *J* = 8.3 Hz, 2H), 6.41 (s, 1H), 5.76 (m, 1H), 4.38−4.23 (m, 5H), 4.01 (d, *J* = 8.2 Hz, 1H), 3.83

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(dd, J = 14.8, 8.7 Hz, 2H), 3.57 (m, 1H), 3.11 (dd, J = 24.0, 16.9 Hz, 4H), 2.65 (d, J = 11.8 Hz, 2H), 1.56 (s, 4H), 1.14 (d, J = 7.1 Hz, 3H), 0.94 (s, 9H), 0.94 (t, J = 7.3 Hz, 3H), 0.82 (s, 9H), 0.67 (t, J = 7.1 Hz, 3H), 0.55 (d, J = 6.5 Hz, 3H), 0.16 (s, 6H), 0.00 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 174.6, 172.4, 171.9, 170.5, 169.4, 168.8, 166.3, 164.7, 160.3, 153.8, 149.7, 144.5, 132.1, 130.1, 129.9, 129.3, 129.2, 128.1, 126.9, 124.7, 124.4, 123.0, 119.7, 114.9, 74.9, 66.4, 57.9, 54.1, 53.0, 51.4, 50.7, 47.8, 42.6, 40.8, 40.5, 35.9, 35.1, 33.0, 31.4, 29.1, 25.9, 25.6, 22.4, 22.2, 19.4, 18.5, 18.0, 17.83, 15.6, 13.8, 12.0, −4.5, −5.0, −5.2; HRMS (ESI) calcd for $C_{75}H_{99}N_9O_{11}S_2Si_2$ [M + Na]$^+$ 1444.6336, found 1444.6340.

*N-(2-(((2S)-1-(((1^{2}Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-5-hydroxy-2-(4-hydroxybenzyl)-4,8,11,15,18-pentaoxo-9-propyl-16-((tritylthio)methyl)-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-12-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-4-hydroxybenzamide (**34**).* To a stirred solution of **33** (500 mg, 0.350 mmol) in THF (6.0 mL) was added TBAF (0.880 mL, 0.880 mmol, 1 M in THF) at 0 °C. The resulting mixture was stirred for 2 h at rt, diluted with EA (20 mL) and H_2O (20 mL, containing 1 mL of HCl (1 N)), and separated. The aqueous phase was extracted with EA (20 mL × 3). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give crude product, which was purified by silica gel column chromatography (3−10% MeOH in DCM) to obtain **34** (378 mg, 90%) as a white solid: $[\alpha]_D^{20}$ = +11.2 (c = 1.0, CH_3OH); ν_{max} (KBr) 3291, 2961, 2929, 1652, 1514, 1273, 1236, 744, 701 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 9.99 (s, 1H), 9.23 (s, 1H), 8.47 (t, J = 5.8 Hz, 1H), 8.42 (s, 1H), 8.31 (d, J = 9.4 Hz, 1H), 8.21 (d, J = 9.1 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H), 7.84 (s, 1H), 7.72 (d, J = 8.6 Hz, 2H), 7.40−7.15 (m, 17H), 7.09 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 6.60 (s, 1H), 5.87 (d, J = 5.1 Hz, 1H), 5.46 (dd, J = 15.4, 9.4 Hz, 1H), 4.43−4.17 (m, 4H), 4.10 (m, 1H), 3.94 (dd, J = 10.0, 5.3 Hz, 1H), 3.83 (qd, J = 16.3, 5.3 Hz, 2H), 3.62 (m, 1H), 3.20−3.12 (m, 1H), 3.11−2.95 (m, 3H), 2.61 (d, J = 9.6 Hz, 2H), 1.56 (m, 2H), 1.43 (m, 2H), 1.15 (d, J = 7.0 Hz, 3H), 0.94 (t, J = 7.3 Hz, 3H), 0.68 (t, J = 7.3 Hz, 3H), 0.55 (d, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 173.8, 173.2, 171.8, 170.3, 169.3, 168.8, 166.2, 164.6, 160.3, 156.1, 149.7, 144.5, 132.1, 130.4, 129.2, 128.1, 127.4, 127.0, 124.7, 124.2, 122.8, 115.1, 114.8, 71.6, 66.4, 57.5, 54.4, 53.3, 52.3, 50.8, 47.8, 42.6, 40.6, 35.3, 32.9, 23.1, 19.2, 18.8, 18.4, 15.7, 14.0, 13.5, 11.9; HRMS (ESI) calcd for $C_{63}H_{71}N_9O_{11}S_2$ [M + Na]$^+$ 1216.4607, found 1216.4610.

*N-(2-(((2S)-1-(((12Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-2-(4-((tert-butyldimethylsilyl)oxy)benzyl)-5-hydroxy-4,8,11,15,18-pentaoxo-9-propyl-16-((tritylthio)methyl)-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-12-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-4-((tert-butyldimethylsilyl)oxy)benzamide (**35**).* To a stirred mixture of **34** (500 mg, 0.420 mmol) and imidazole (570 mg, 8.37 mmol) in DCM (4 mL) was added TBSCl (631 mg, 4.19 mmol) at 0 °C. The resulting mixture was stirred for 3 h at rt, diluted with EA (20 mL) and H_2O (20 mL), and separated. The aqueous phase was extracted with EA (20 mL × 2). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give crude product, which was purified by silica gel column chromatography (2−5% MeOH in DCM) to obtain **35** (506 mg, 85%) as a white solid: $[\alpha]_D^{20}$ = −10.3 (c = 1.0, CH_3OH); ν_{max} (KBr) 3405, 2958, 2928, 2856, 1652, 1607, 1509, 1264, 912 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 8.62 (t, J = 5.4 Hz, 1H), 8.44 (s, 1H), 8.31 (d, J = 9.3 Hz, 1H), 8.25 (d, J = 9.4 Hz, 1H), 8.09 (d, J = 7.6 Hz, 1H), 8.05 (d, J = 7.4 Hz, 1H), 7.83 (s, 1H), 7.81 (d, J = 8.6 Hz, 2H), 7.41−7.23 (m, 21H), 7.18 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.3 Hz, 2H), 6.63 (s, 1H), 5.89 (s, 1H), 5.56−5.45 (m, 1H), 4.45−4.18 (m, 4H), 4.10 (m, 1H), 3.97 (m, 1H), 3.93−3.78 (m, 2H), 3.64 (m, 1H), 3.20−2.99 (m, 3H), 2.63 (d, J = 9.7 Hz, 2H), 1.56 (s, 3H), 1.44 (s, 3H), 1.17 (d, J = 7.0 Hz, 3H), 1.17 (m, 1H), 0.95 (s, 9H), 0.93 (s, 9H), 0.93 (t, J = 7.3 Hz, 3H), 0.69 (t, J = 7.0 Hz, 3H), 0.57 (d, J = 5.8 Hz, 3H), 0.20 (s, 6H), 0.16 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 173.9, 173.2, 171.9, 171.8, 170.4, 169.4, 168.7, 166.1, 164.7, 157.9, 153.8, 149.7, 144.5, 132.1, 130.6, 130.2, 129.3, 129.2, 128.1, 127.2, 127.0, 124.3, 122.8, 119.6, 119.5, 71.6, 66.4, 57.5, 54.4, 53.3, 52.1, 50.8, 47.9, 42.6, 40.5, 35.3, 32.9, 25.59, 25.53, 22.3, 18.8, 18.4, 18.0, 17.94, 15.8, 14.0, 11.9, −4.48, −4.53; HRMS (ESI calcd for $C_{75}H_{99}N_9O_{11}S_2Si_2$ [M + Na]$^+$ 1444.6336, found 1444.6340.

*N-(2-(((S)-1-(((1^{2}Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-2-(4-((tert-butyldimethylsilyl)oxy)benzyl)-4,5,8,11,15,18-hexaoxo-9-propyl-16-((tritylthio)methyl)-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-12-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-4-((tert-butyldimethylsilyl)oxy)benzamide (**36**).* To a stirred mixture of **35** (300 mg, 0.210 mmol) and EDCI (404 mg, 2.11 mmol) in DMSO/toluene (1 mL/1 mL) under argon protection was added a solution of dicholoacetic acid (135 mg, 1.05 mmol in 0.5 mL of toluene) at 0 °C. The reaction mixture was stirred for 3 h at rt, diluted with EA (20 mL) and H_2O (20 mL), and separated. The aqueous phase was extracted with EA (20 mL × 3). The combined organic phases were washed with brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to obtain crude product, which was purified by silica gel column chromatography (3−5% MeOH in DCM) to obtain **36** (210 mg, 70%) as a white solid: $[\alpha]_D^{20}$ = −5.7 (c = 1.0, MeOH); ν_{max} (KBr) 3402, 2958, 2930, 2857, 1652, 1508, 1262, 1026 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 9.30 (d, J = 8.7 Hz, 1H), 8.59 (t, J = 5.7 Hz, 1H), 8.52 (d, J = 8.2 Hz, 1H), 8.16 (d, J = 3.7 Hz, 1H), 8.03 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 10.2 Hz, 1H), 7.86 (s, 1H), 7.80 (d, J = 8.6 Hz, 2H), 7.40 (d, J = 15.0 Hz, 1H), 7.34−7.21 (m, 17H), 7.06 (d, J = 15.1 Hz, 1H), 7.02 (m, 1H), 6.91 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 5.42 (m, 1H), 5.23 (dd, J = 8.3, 2.9 Hz, 1H), 4.51−4.42 (m, 1H), 4.41−4.25 (m, 4H), 3.93−3.77 (m, 2H), 3.55 (m, 1H), 3.13 (m, 2H), 3.04−2.96 (m, 1H), 2.67 (dd, J = 15.8, 10.8 Hz, 2H), 2.18 (m, 1H), 1.63−1.55 (m, 2H), 1.53−1.44 (m, 2H), 1.16 (d, J = 7.0 Hz, 3H), 0.95 (s, 9H), 0.94 (s, 9H), 0.94 (t, J = 7.2 Hz, 3H), 0.83 (d, J = 7.0 Hz, 3H), 0.71 (t, J = 7.4 Hz, 3H), 0.21 (s, 6H), 0.17 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 197.5, 173.9, 171.8, 170.2, 170.0, 169.2, 168.6, 166.0, 164.7, 164.2, 157.9, 153.9, 149.5, 144.5, 132.3, 130.5, 129.9, 129.22, 129.17, 129.07, 128.0, 127.2, 126.9, 124.0, 122.9, 119.6, 119.4, 66.5, 61.0, 53.9, 53.3, 52.9, 50.6, 47.8, 42.6, 40.5, 36.5, 35.5, 33.3, 25.54, 25.48, 23.1, 18.7, 18.3, 17.93, 17.9, 15.7, 13.9, 11.7, −4.56, −4.59; HRMS (ESI) calcd for $C_{75}H_{97}N_9O_{11}S_2Si_2$ [M + Na]$^+$ 1442.6180, found 1442.6185.

*((1^{2}Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-12-((S)-2-(2-(4-((tert-butyldimethylsilyl)oxy)benzamido)acetamido)-propanamido)-2-(4-((tert-butyldimethylsilyl)oxy)benzyl)-4,5,8,11,15,18-hexaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-16-yl)methanesulfonic acid (**37**).* To a stirred solution of **36** (170 mg, 0.120 mmol) in HOAc/THF (1.5 mL/4.5 mL) at 0 °C was added sodium acetate (98 mg, 1.12 mmol). The resulting mixture was stirred for 10 min followed by addition of oxone (184 mg, 0.299 mmol) at 0 °C and then stirred for 10 h (temperature was allowed to warm slowly to rt). The reaction mixture was diluted with EA (20 mL), washed with cold water (20 mL, containing 1.0 mL of HCl (1 N)), and separated. The aqueous phase was extracted with EA (20 mL × 5). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified on prep-TLC developed by DCM/CH_3OH = 5:1 to obtain product **37** (91.0 mg, 62%) as a white solid: $[\alpha]_D^{20}$ = −19.6 (c = 1.0, CH_3OH); ν_{max} (KBr) 3435, 2959, 2931, 1644, 1510, 1261, 914 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ 9.41 (d, J = 8.0 Hz, 1H), 8.62 (d, J = 7.2 Hz, 1H), 8.58 (t, J = 6.0 Hz, 1H), 8.29 (d, J = 9.3 Hz, 1H), 8.16 (d, J = 9.2 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 7.5 Hz, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 15.1 Hz, 1H), 7.27 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 6.78 (d, J = 8.4 Hz 2H), 6.77 (d, J = 15.1 Hz, 1H), 5.26 (m, 1H), 5.04 (dd, J = 9.4, 3.6 Hz, 1H), 4.75 (s, 1H), 4.35 (m, 1H), 4.33 (m, 1H), 4.05 (m, 1H), 3.91 (dd, J = 16.4, 5.9 Hz, 1H), 3.82 (dd, J = 16.5, 5.6 Hz, 1H), 3.76 (m, 1H), 3.25 (m, 1H), 3.17 (dd, J = 14.5, 9.5 Hz, 1H), 3.09 (dd, J = 14.0, 5.9 Hz, 1H), 2.71 (dd, J = 13.8, 5.6 Hz 1H), 2.39 (m, 1H), 2.33 (m, 1H), 1.66 (m, 1H), 1.54 (m, 1H), 1.33 (m, 1H), 1.17 (d, J = 7.0 Hz, 3H), 1.14 (m, 1H), 1.10 (m, 1H), 0.95 (s, 9H), 0.94 (s, 9H), 0.94 (t, J = 7.2 Hz, 3H), 0.78 (d, J = 6.9 Hz, 3H), 0.72 (t, J = 7.4 Hz, 3H), 0.21 (s, 6H), 0.18 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 197.9, 174.8, 171.3, 171.1, 170.6,

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170.4, 168.8, 168.6 166.2, 166.0, 164.8, 163.5, 160.2, 157.9, 153.9, 149.5, 132.5, 130.6, 130.2, 129.3, 127.2, 124.7, 123.4, 123.1, 119.7, 119.5, 114.8, 59.9, 54.3, 54.2, 50.9, 50.5, 49.3, 47.8, 42.6, 40.8, 36.8, 32.1, 25.8, 25.6, 25.5, 23.3, 19.7, 18.4, 17.99, 17.97, 16.1, 13.8, 11.8, −3.2, −4.50, −4.54; HRMS (ESI) calcd for $C_{56}H_{83}N_9O_{14}S_2Si_2$ $[M + H]^+$ 1226.5112, found 1226.5112.

*((1²Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-12-((S)-2-(2-(4-hydroxybenzamido)acetamido)propanamido)-2-(4-hydroxybenzyl)-4,5,8,11,15,18-hexaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-16-yl)methanesulfonic acid (**38**).* To a stirred solution of **37** (64.0 mg, 0.05 mmol) in THF (1 mL) at 0 °C was added a solution of HF·pyridine (68 wt %, 0.2 mL in 1 mL of THF) slowly. The mixture was stirred for 4 h (temperature was allowed to warm slowly to rt) and then diluted with H_2O (6 mL). The volatiles were removed carefully under reduced pressure, and the residue was lyophilized at −70 °C to give crude product, which was purified on prep-TLC developed by CH_2Cl_2/CH_3OH = 5:1 to obtain product **38** (37.0 mg, 70%): $[\alpha]_D^{24}$ = −22.1 (*c* = 1.0, CH_3OH); ν_{max} (KBr) 3437, 1644, 1514, 1210, 1043, 994, 632 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 9.40 (d, *J* = 8.0 Hz, 1H), 9.30 (s, 1H), 8.61 (d, *J* = 7.1 Hz, 1H), 8.48 (t, *J* = 5.8 Hz, 1H), 8.33 (d, *J* = 9.4 Hz, 1H), 8.16 (d, *J* = 9.3 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 7.88 (brs, 1H), 7.86 (s, 1H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 15.0 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.7 Hz,2H), 6.77 (d, *J* = 15.2 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 2H), 5.23 (m, 1H), 5.05 (dd, *J* = 9.4, 3.5 Hz, 1H), 4.77 (m, 1H), 4.34 (m, 1H), 4.33 (m, 1H), 4.05 (m, 1H), 3.89 (dd, *J* = 16.5, 5.8 Hz, 1H), 3.81 (dd, *J* = 16.4, 5.7 Hz, 1H), 3.75 (m, 1H), 3.28 (m, 1H), 3.10 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.02 (dd, *J* = 13.7, 5.7 Hz, 1H), 2.72 (dd, *J* = 13.6, 5.4 Hz, 1H), 2.49 (m,1H), 2.40 (m,1H), 2.30 (m, 1H), 1.65 (m, 1H), 1.54 (m, 1H), 1.33 (m, 1H), 1.18 (d, *J* = 7.0 Hz, 3H), 1.14 (m, 1H), 1.08 (m, 1H), 0.93 (t, *J* = 7.2 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H), 0.73 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 197.9, 174.8, 171.4, 171.1, 170.7, 170.6, 168.8, 166.3, 164.9, 163.7, 160.3, 156.2, 149.5, 132.5, 130.4, 129.3, 127.4, 124.7, 123.4, 123.1, 115.2, 114.8, 60.1, 54.4, 54.3, 50.9, 50.5, 49.3, 47.8, 42.6, 40.8, 39.0, 36.9, 32.1, 23.3, 19.7, 18.4, 16.1, 13.8, 11.8; HRMS (ESI) calcd for $C_{44}H_{55}N_9O_{14}S_2$ $[M + H]^+$ 998.3383, found 998.3383.

*((1²Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-12-((S)-2-(2-(4-hydroxybenzamido)acetamido)propanamido)-2-(4-hydroxybenzyl)-4,5,8,11,15,18-hexaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-16-yl)methanesulfonate sodium (Cyclotheonellazole A (**1**)).* To a stirred solution of **38** (25 mg, 0.025 mmol) in CH_3OH/H_2O (1 mL/1 mL) at 0 °C was added NaCl solution (0.02 M, 1.1 mL, 0.0225 mmol). The excess CH_3OH was evaporated carefully under reduced pressure, and the residue was lyophilized at −70 °C to obtain cyclotheonellazole A (25 mg, 98%) as a white powder: $[\alpha]_D^{20}$ = −22.0 (*c* = 1.0, CH_3OH); ν_{max} (KBr) 3292, 1655, 1515, 1240, 1222, 1173, 1041, 1026, 632 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 10.01 (s, 1H), 9.44 (d, *J* = 7.9 Hz, 1H), 9.32 (s, 1H), 8.62 (d, *J* = 7.0 Hz, 1H), 8.50 (t, *J* = 5.8 Hz, 1H), 8.36 (d, *J* = 9.4 Hz, 1H), 8.16 (d, *J* = 9.3 Hz, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.87 (s, 1H), 7.87 (brs, 1H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 15.1 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 15.2 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 2H), 5.22 (m, 1H), 5.05 (dd, *J* = 9.5, 3.4 Hz, 1H), 4.75 (m, 1H), 4.33 (m, 1H), 4.32 (m, 1H), 4.04 (m, 1H), 3.90 (dd, *J* = 16.5, 6.0 Hz, 1H), 3.79 (dd, *J* = 16.5, 5.6 Hz, 1H), 3.74 (m, 1H), 3.27 (m, 1H), 3.10 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.03 (dd, *J* = 13.7, 5.7 Hz, 1H), 2.72 (dd, *J* = 13.5, 5.3 Hz, 1H), 2.49 (m, 1H), 2.42 (m, 1H), 2.30 (m, 1H), 1.63 (m, 1H), 1.56 (m, 1H), 1.33 (m, 1H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.15 (m, 1H), 1.08 (m, 1H), 0.93 (t, *J* = 7.2 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H), 0.73 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 198.0, 174.8, 171.4, 171.1, 170.67, 170.57, 168.8, 166.3, 164.9, 163.7, 160.3, 156.2, 149.5, 132.6, 130.4, 129.3, 127.3, 124.6, 123.3, 123.2, 115.2, 114.8, 60.1, 54.4, 54.3, 50.9, 50.5, 49.3, 47.8, 42.6, 40.8, 39.2, 36.9, 32.2, 23.3, 19.7, 18.4, 16.1, 13.8, 11.8; HRMS (ESI) calcd for $C_{44}H_{54}N_9NaO_{14}S_2$ $[M + Na]^+$ 1042.3022, found 1042.3021.

*((1²Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-12-((S)-2-(2-(4-((tert-butyldimethylsilyl)oxy)benzamido)acetamido)-propanamido)-2-(4-((tert-butyldimethylsilyl)oxy)benzyl)-5-hydroxy-4,8,11,15,18-pentaoxo-9-propyl-3,7,10,14,17-pentaaza-1-(2,4)-thiazolacycloicosaphan-19-en-16-yl)methanesulfonic acid (**S23**).* To a stirred solution of **35** (66 mg, 0.05 mmol) in acetic acid/THF (0.60 mL/2.0 mL) at 0 °C was added sodium acetate (38 mg, 0.46 mmol). The resulting mixture was stirred for 10 min followed by addition of oxone (71 mg, 0.12 mmol) at 0 °C. The resulting mixture stirred for 10 h (temperature was allowed to warm slowly to rt), diluted with EA (20 mL), washed with cold water (20 mL, containing 1.0 mL of HCl (1 N)), and separated. The aqueous phase was extracted withEA (20 mL × 5). The combined organic phase were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product, which was purified on prep-TLC developed by CH_2Cl_2/CH_3OH = 5:1 to obtain product **S23** (50 mg, 87%) as a white solid.

*((1²Z,2S,6S,9S,12S,16R,19E)-6-((S)-sec-Butyl)-5-hydroxy-12-((S)-2-(2-(4-hydroxybenzamido)acetamido)propanamido)-2-(4-hydroxybenzyl)-4,8,11,15,18-pentaoxo-9-propyl-3,7,10,14,17-pentaaza-1(2,4)-thiazolacycloicosaphan-19-en-16-yl)methanesulfonic acid (**42**).* To a stirred solution of **S23** (50 mg, 0.04 mmol) in THF (1 mL) at 0 °C was a solution of HF·pyridine (68 wt %, 0.2 mL in 1 mL of THF) slowly. The mixture was stirred for 3 h (temperature was allowed to warm slowly to rt) and diluted with H_2O (6 mL). The volatiles were removed carefully under reduced pressure, and the residue was lyophilized at −70 °C to give crude product, which was purified on prep-TLC developed by CH_2Cl_2/CH_3OH = 5:1 to obtain product **42** (20.0 mg, 50%): $[\alpha]_D^{24}$ = −43.4 (*c* = 0.5, CH_3OH); ^1H NMR (400 MHz, DMSO) δ 10.00 (s, 1H), 9.24 (s, 1H), 8.65 (d, *J* = 7.9 Hz, 1H), 8.48 (t, *J* = 5.9 Hz, 1H), 8.44−8.37 (m, 2H), 8.03 (d, *J* = 7.9 Hz, 1H), 7.97 (d, *J* = 7.5 Hz, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.79 (s, 1H), 7.79 (brs, 1H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 15.1 Hz, 1H), 7.25 (d, *J* = 15.2 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.2 Hz, 2H), 5.73 (d, *J* = 5.1 Hz, 1H), 5.35 (m, 1H), 4.76 (m, 1H), 4.34 (m, 1H), 4.20 (m, 1H), 4.14 (m, 1H), 4.03 (m, 1H), 3.95−3.71 (m, 4H), 3.25 (d, *J* = 13.5 Hz, 1H), 3.09 (dd, *J* = 13.8, 9.5 Hz, 1H), 2.97 (dd, *J* = 13.8, 5.7 Hz, 1H), 2.75 (dd, *J* = 13.7, 5.4 Hz, 1H), 2.12 (m, 1H), 1.70 (m, 1H), 1.53−1.41 (m, 4H), 1.39−1.31 (m, 1H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.68 (t, *J* = 7.3 Hz, 3H), 0.48 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 174.6, 173.8, 172.3, 171.33, 171.28, 171.0, 168.8, 166.2, 164.8, 160.2, 156.0, 150.2, 131.6, 130.4, 129.7, 129.3, 127.8, 124.7, 124.6, 122.2, 115.1, 114.8, 70.7, 56.7, 54.5, 52.7, 51.1, 50.9, 49.8, 47.8, 42.5, 41.0, 35.4, 32.1, 22.1, 19.4, 18.4, 16.9, 13.9, 12.0.

Elastase Activity Assay. Elastase activities were determined by EnzCHek elastase assay kit (Invitrogen, E-12056). DQ elastin substrate at 25 μg/mL, porcine pancreatic elastase (Invitrogen, E-12056) at 0.4 U/mL, or human neutrophil elastase (Enzo, BML-SE284−0100) at 0.2 U/mL was mixed with an increasing amount of CTL-A or sivelestat (Bidepharm, BD151226) in a 96-well microplate and incubated for 40 min at rt in the dark. Fluorescence was measured by a fluorescence multiwell plate reader (Tecan) with excitation at 485 nm and emission detection at 535 nm. Each experiment was repeated three times. The statistical significance of differences was determined using a paired Student's *t* test of each experiment compared against appropriate controls.

Cytotoxicity Analysis. Human ACE2 overexpressed ACE2-293T stable cells were purchased from Genewiz. Lung epithelial cell line BEAS-2B were originally obtained from ATCC. Briefly, 1 × 10^4 cells were seeded in a 96-well plate. After cell adherence (about 12 h), cells were treated with different concentrations of compounds for 24 h. Then 10 μL/well of the CCK-8 (Biosharp) was added into a 96-well plate, and an OD at 450 nm was measured using a microplate reader (Biorad) after incubation for 2 h at 37 °C. The percentage of each concentration accounted for was presented as cell viability. The IC_{50} value was calculated using SPSS.

Acute Lung Injury Mouse Model and Drug Treatment. All applicable institutional and/or national guidelines (including Declaration of Helsinki) for the care and use of animals were followed. All procedures were carried out with the approval by the Experimental Animal Ethics Committee of Nankai University (No. 2021-SYDWLL-000374).

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Eight-week-old male C57BL/6J mice were purchased from Vital River Laboratories and kept in the Animal Resources Center of Nankai University. Thirty-two mice were weighed and randomly divided into four groups (n = 8 in each group). The animal model of bleomycin sulfate (BLM)-induced acute lung injury (ALI) was used as previously described.[35] Briefly, mice were first injected with 6% chloral hydrate at 0.5 mL/100 g for anesthesia. Then the neck skin of anesthetic mice was cut with scissors; the neck tissue was peeled with tweezers, and the trachea was exposed. BLM (4 mg/kg) as sulfate salt dissolved in 0.1 mL of normal saline was injected to mice via intratracheal instillation to induce ALI. Mice in the control group received equal volumes of intratracheal normal saline instead of BLM. Successful ALI mice were randomly separated to three groups, which are the ALI group, the CTL-A group, and the sivelestat group. For the CTL-A group and sivelestate group, 40 mg/mL CTL-A or sivelestat was dissolved in 10% DMSO + 90% saline and given via intraperitoneal injection with 30 mg/kg dosage continuously for up to 5 days. The control group and ALI model group were applied to 10% DMSO + 90% saline. On the sixth day, mice were sacrificed and lungs were excised and analyzed by hematoxylin/eosin (HE) and wet/dry ratio analysis.

Histological Analysis and Wet/Dry Ratio Determination. The right lung from mice was subjected to HE staining, and left lungs were subjected to wet/dry ratio analysis.

For HE staining, the right lungs from the mice were washed with ice-cold oxygenated saline and then fixed in 10% neutral-buffered formalin at 4 °C before processing for paraffin embedding. Blocks were sectioned with 4 μm thickness, mounted on slides, and then deparaffinized and rehydrated by successive incubations in xylene, 100% ethanol, 95% ethanol, 75% ethanol, and PBS. Finally, sections were stained with hematoxylin and eosin using standard procedures. Three staining pictures were prepared from each lung, and the inflammatory score was evaluated by an experienced histologist from the Department of Pathology at Shandong Xinbo Pharmaceutical R&D under blinded conditions (blinded to the mode of injury and clinical outcome). The severity of alveolitis was graded using the following criteria, which were previously reported:[35] none (0), no alveolitis; mild (1), alveolitis less than 20% of the lung; moderate (2), alveolitis involving 20%−50% of the lung; severe (3), a diffuse alveolitis involving more than 50% of the lung.

The left lungs from separate groups of mice were weighed immediately after dissection and then desiccated in an oven at 78 °C for 48 h until a stable dry weigh was achieved. The were then dry-reweighted as a dry weight for calculation of the wet/dry weight ratio.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01583.

Organic synthesis experiments; biological experiments; docking study; NMR comparison between cyclotheonellazole A (from sponge) and compound **38**; NMR comparison between cyclotheonellazole A (from sponge) and cyclotheonellazole A (synthetic); NMR spectra (PDF)

Human neutrophil elastase (PDB ID: 1TYN) (PDB, PDB)

Docking between CTL-A and HNE; TMPRSS2 (PDB ID: 7MEQ) (PDB)

Docking between CTL-A and TMPRSS2 (PDB)

Molecular formula strings (CSV)

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Notes

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■ ABBREVIATIONS

3CL^{pro}, coronavirus main proteinase; ACE2, angiotensin converting enzyme 2; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CTL-A, cyclotheonellazole A; DMP, Dess-Martin periodinane; DMAP, 4-dimethylaminopyridine; EDCI, 1-ethyl-3(3-dimethylpropylamine) carbodiimide; HATU, 2-(7-Azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate; HOBT, 1-hydroxybenzotriazole; HNE, human neutrophil elastase; MAC, masked acyl cyanide; PIDA, iodobenzene diacetate; PPE, porcine pancreatic elastase; TBAF, tetrabutylammonium fluoride; TFAA, trifluoroacetic anhydride; TMPRSS2, transmembrane serine protease 2

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