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Total Synthesis and Biological Evaluation of Tubulysin Analogues

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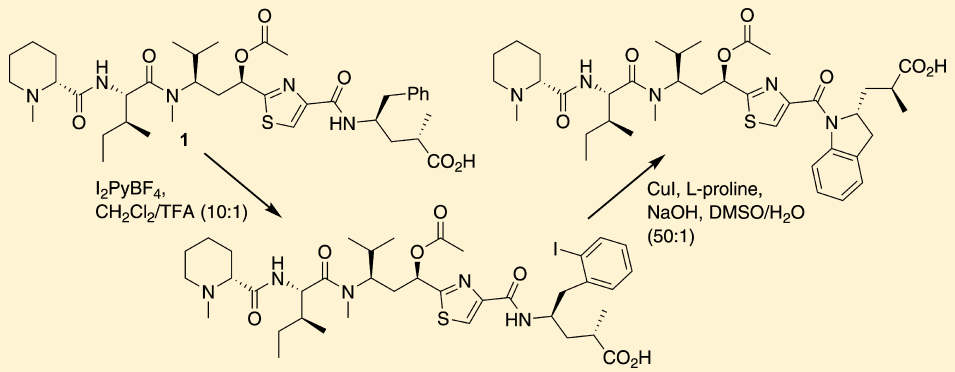
Supporting Information



ABSTRACT: We report a second-generation synthesis of the exceedingly potent antimitotic agent N^{14}-desacetoxytubulysin H (**1**) as well as the preparation of nine analogues of this lead structure. Highlights of our synthetic efforts include an efficient late-stage functionalization that allows for the preparation of new side-chain- and backbone-modified analogues. We also discovered C-terminal modifications that preserve the exquisite biological activity of acid **1** and offer the opportunity for effective conjugation to cell type-targeting moieties. All analogues had antiproliferative activities in the high picomolar to low nanomolar range and caused apoptosis and mitotic arrest as measured in a high content nuclear morphology assay. The ten synthetic agents described herein spanned a range of almost 4 orders of magnitude in biological activity and illustrate the continued potential to discover extraordinarily potent antiproliferative compounds based on natural product leads.

INTRODUCTION

Tubulysins are a family of natural tetrapeptides with attractive prospects in anticancer drug development due to their exquisitely potent antiproliferative and antiangiogenic properties (Figure 1).[1,2] Tubulysins can perturb microtubule polymerization and induce apoptosis of various cancer cell lines at subnanomolar concentrations.[3]

The remarkable ability of tubulysins to inhibit the growth of cancer cells, including those with multidrug resistance,[4] exceeds paclitaxel, epothilones, and vinblastine by 10−2000 fold.[5] Besides inhibition of cell proliferation, tubulysins show strong angiogenesis effects,[6] display antivascular properties in vitro and in vivo,[7] and therefore represent promising novel agents for the treatment of metastatic and invasive tumors, in particular in combination with drug-targeting strategies.[8,9] While tubulysins have previously been isolated in low quantities (0.5−5 mg/L) from the myxobacterial strains *Archangium gephyra* and *Angiococcus disciformis*,[1] substantial efforts have also been directed toward the total synthesis of these natural products and their analogues.[3,10]

Shortly after the elucidation of the structure and configuration of tubulysin D by degradation studies,[11] the preparation of two subunits was reported.[12,13] Subsequently, several preparations of stereoisomers of tubulysins U and V were published,[14−16] including a total synthesis of tubulysins U and V.[17] Two total syntheses of tubulysin D, which contains the acid- and base-labile *N,O*-acetal functionality, were reported in 2006 and 2010.[16,18] Tubulysin B has also received considerable attention, and a total synthesis was first reported in 2009,[19] followed by a more efficient synthesis which also allowed the preparation of folate conjugates for tumor site-directed drug delivery.[20]

In 2009, pretubulysin D was isolated in minor amounts from cultures of *A. disciformis*.[21] This putative tubulysin biosynthesis precursor lacks the *N,O*-acetal and 11-OAc groups. Its structure was elucidated by a combination of mass spectrometry, total synthesis, and NMR.[22] While pretubulysin D is ca. 10-fold less

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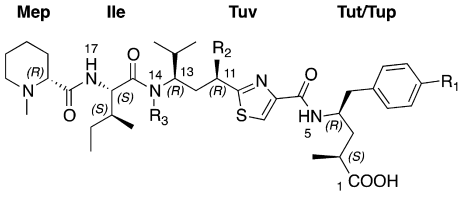

Natural tubulysins

Tubulysin	R_1	R_2	R_3
A	OH	OAc	$CH_2OC(O)CH_2CH(CH_3)_2$
B	OH	OAc	$CH_2OC(O)CH_2CH_2CH_3$
C	OH	OAc	$CH_2OC(O)CH_2CH_3$
D	H	OAc	$CH_2OC(O)CH_2CH(CH_3)_2$
E	H	OAc	$CH_2OC(O)CH_2CH_2CH_3$
F	H	OAc	$CH_2OC(O)CH_2CH_3$
G	OH	OAc	$CH_2OC(O)CH{=}C(CH_3)_2$
H	H	OAc	$CH_2OC(O)CH_3$
I	OH	OAc	$CH_2OC(O)CH_3$
U	H	OAc	H
V	H	OAc	H
W	OH	OAc	$CH_2OC(O)CH_2CH_2CH_3$
X	OH	OAc	H
Z	OH	OAc	H
Pretubulysin A	OH	H	Me
Pretubulysin D	H	H	Me
Desacetyl tubulysin D	H	OH	$CH_2OC(O)CH_2CH(CH_3)_2$
Desacetyl tubulysin E	H	OH	$CH_2OC(O)CH_2CH_2CH_3$

Synthetic tubulysins

N^{14}-Desacetoxytubulysin H (1)	H	OAc	Me
TUB-OMOM	H	OCH_2OCH_3	Me
KEMTUB10	F	OAc	Bn

Figure 1. Structures of select natural and synthetic tubulysins.

active than tubulysins A and D, it is still effective at nano- to picomolar concentrations against cancer cell lines and retains the high tubulin-degrading activity of some of the more complex tubulysins. Other pretubulysins from *A. disciformis* An d48 and *Cystobacter* SBCb004 strains demonstrate, in fact, that structural complexity can be reduced without a dramatic drop in potency.[22] Pretubulysin D showed strong antiangiogenic and antivascular properties in vitro and in vivo, which bodes well for the treatment of metastatic cancer.[6−8] Pretubulysins and their structural analogues might prove to be more appealing for tumor therapy since preclinical studies have shown that the more potent tubulysins are generally too toxic for use as chemotherapeutics and possess a very narrow therapeutic window.[20,23,24] Accordingly, drug-targeting approaches offer a more attractive treatment option. Tubulysins can be conjugated to polymers, small ligands, or antibodies in order to increase their tumor specificity. For example, tubulysin A was covalently attached to a linear β-cyclodextrin-based polymer through a disulfide linker (CDP-TubA), and this conjugate showed a 100-fold increase in the maximum tolerated dose (MTD) in nude mice and an increased efficacy in two human cancer xenografts, whereas tubulysin A at its MTD was completely inactive.[23] The synthetic tubulysin analogue TUB-OMOM was conjugated to trastuzumab (a humanized anti-HER2 monoclonal antibody), and the resulting construct was shown to have dose-dependent antitumor effects, including complete in vivo tumor eradication in trastuzumab-sensitive tumors.[25] Finally, encouraging results were obtained in animal experiments with EC0305, a small molecule folate−tubulysin conjugate, taking advantage of the high expression of the folate receptor in many cancer types.[20,24,26] In contrast, natural tubulysin B alone again proved inactive when administered at doses near or greater than its MTD. EC0305 also displayed superior antitumor activity compared to a folate−desacetylvinblastine monohydrazide conjugate (EC145, currently in phase II clinical trials).[27]

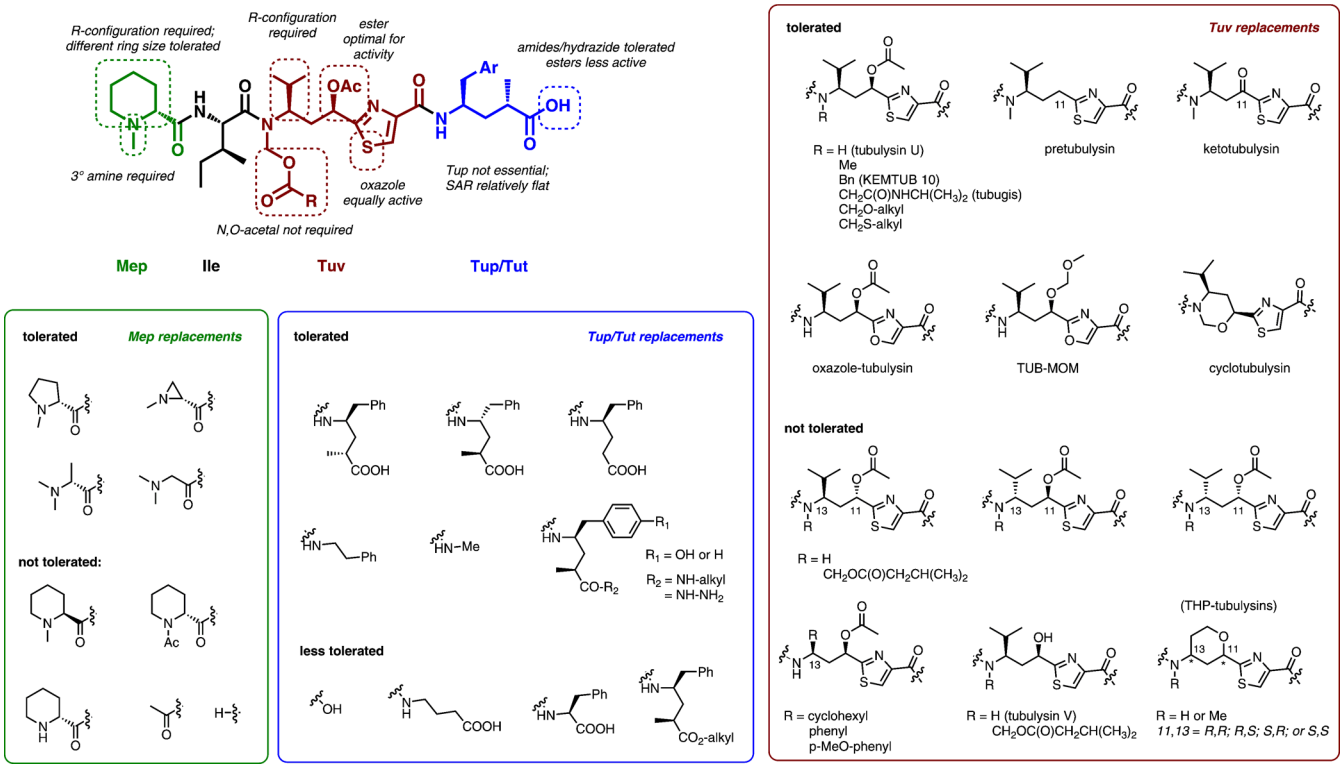

Figure 2. Summary of key SAR features of tubulysins.

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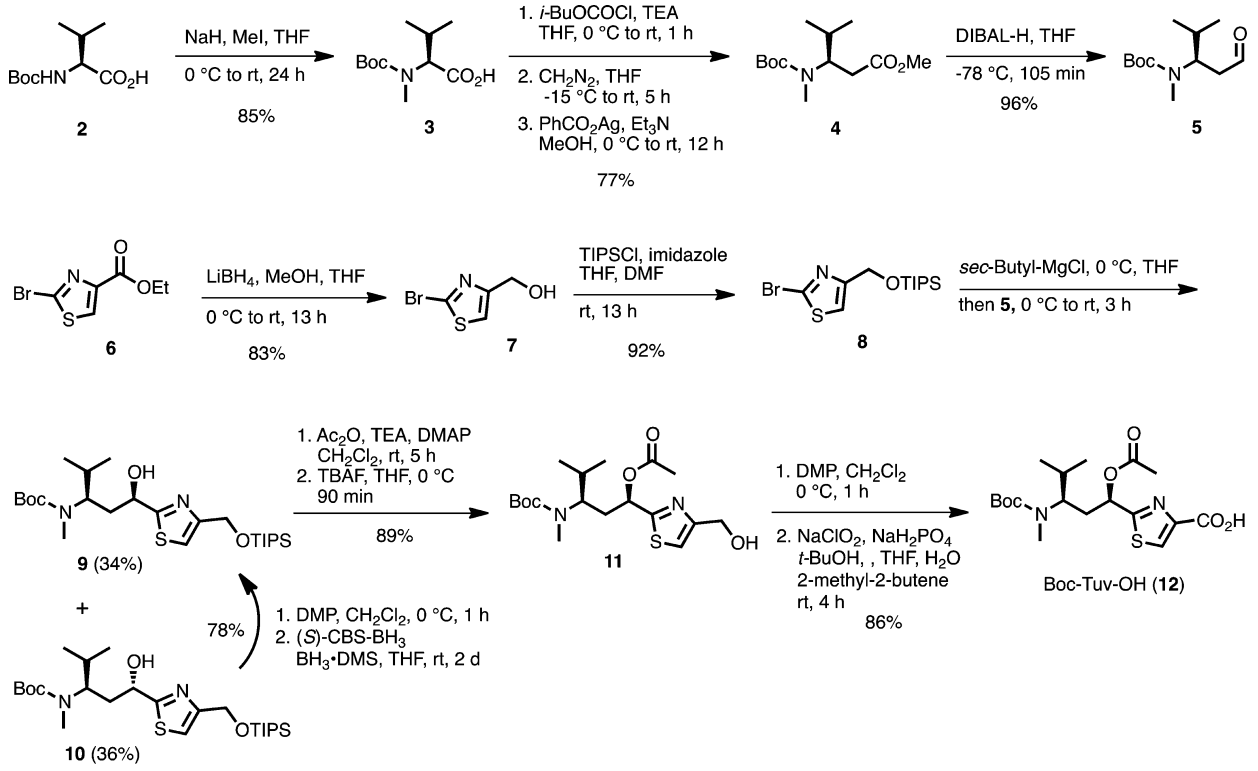
Scheme 1. Preparation of Boc-Tuv-OH (12) Using a Grignard Exchange Reaction with Bromide 8



EC1456, a second-generation folate−tubulysin B conjugate bearing a highly hydrophilic linker, is being evaluated in a phase I study in patients with advanced solid tumors.[28−30]

As part of our investigations of natural products targeting tubulin,[31−33] our group reported in 2007 the synthesis of N^{14}-desacetoxytubulysin H (**1**),[34,35] a simplified analogue that was later also synthesized by Patterson et al.[36] N^{14}-Desacetoxytubulysin H (**1**) possesses all of the structural features of tubulysin H, except its labile *N,O*-acetal functionality, and maintains high cytotoxicity in vitro. Several other non-natural tubulysin analogues have been prepared recently, reflecting an unabated interest in this drug class.[37] SAR studies have mainly focused on iterative replacements of the four amino acid fragments (*N*-methyl-D-pipecolic acid (Mep), L-isoleucine (Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup)/tubutyrosine (Tut)) (Figure 2).

Replacement of the Mep residue with other tertiary amine-containig acids exhibited little change in biological activity (Figure 2).[36,38−42] In contrast, the (*R*)-configuration proved to be important at this site.[39,40] Moreover, demethylating Mep, acylating, or deleting this residue was also detrimental to activity.[36,38−40]

While the SAR of the Ile residue has been relatively neglected, extensive modifications were directed toward modifications of the Tuv residue. Replacement of the labile *N,O*-acetal at the N-14 position with H- or Me-groups left the biological activity substantially unaltered.[21,35,36] The *N,O*-acetal was also successfully simplified using an *N*-alkylamide in tubugis[43] or an *N*-benzyl group in KEMTUB10.[44] Cyclotubulysin, an analogue containing a cyclic *N,O*-acetal with the C-11 alcohol, showed activity similar to that of tubulysin U.[16] *N,S*-thioacetals demonstrated antiproliferative activities comparable to tubulysins A and B.[45] In contrast, replacing the isopropyl group with a cyclohexyl, phenyl, or *p*-methoxyphenyl erased activity (IC_{50} > 1000 nM).[46] Substitution of the acetyl group of tubulysin U with a methoxymethyl or methyl ether reduced activity more modestly by a factor of 5 or 60, respectively.[46] The configuration at C-11 and, particularly, at C-13 proved essential for cytotoxic potency. An evaluation of all four stereoisomers of tubuvaline showed that only the natural compound (i.e., (11*R*,13*S*)-tubulysin D) had a subnanomolar IC_{50} in a selection of cell lines.[16,47] A C-11 epimer was 2−3 orders of magnitude less active, and a C-13 epimer as well as a dual epimer at C-11 and C-13 dropped by 3−5 orders and >6 orders of magnitude, respectively. A similar pattern was observed for the C-13 and C-11 epimers of tubulysin U and tubulysin V[15,38] and rigidified, tetrahydropyran-containing tubulysins.[48,49] Deacetylation of tubuvaline decreased activity by 1000-fold, but a replacement of the acetate in Tuv with a ketone apparently conserved activity.[15] As a general trend, increased lipophilicity in the Tuv residue correlated with more potent antiproliferative activity, perhaps due to a higher cellular uptake of the more lipophilic analogues.[2] An oxazole replacement of the thiazole in tubulysin U only reduced cytotoxicity ca. 2-fold, but substitutions with phenyl or triazole rings were 2−4 orders of magnitude less potent than the parent tubulysin D.[50−52]

The C-terminal tubutyrosine (Tut) and tubuphenylalanine (Tup) residues are, in general, more readily modified than other residues, and Tup-containing analogues were slightly more potent than tubulysins with Tut residues.[2] Tubulysins B and U and their C-2 methyl epimers have almost identical biological activities.[19,38] Inversion of configuration at the benzylated C-4 position in tubulysin U resulted in a 10-fold less potent compound,[38] and 2 orders of magnitude were lost when both benzyl and methyl groups were removed.[36] C-Terminal amides and hydrazides were almost as active as the parent acids, and these functionalizations have therefore been

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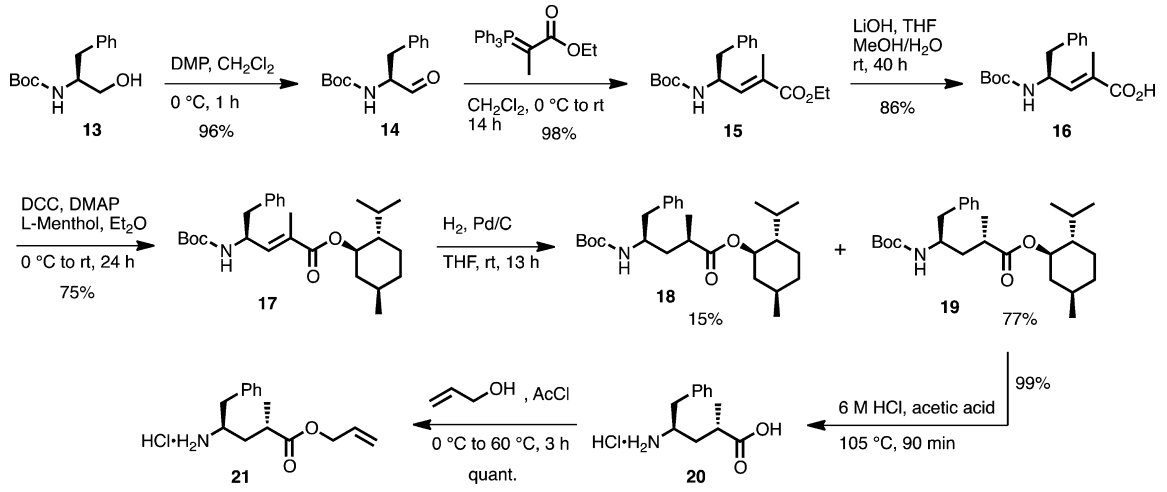
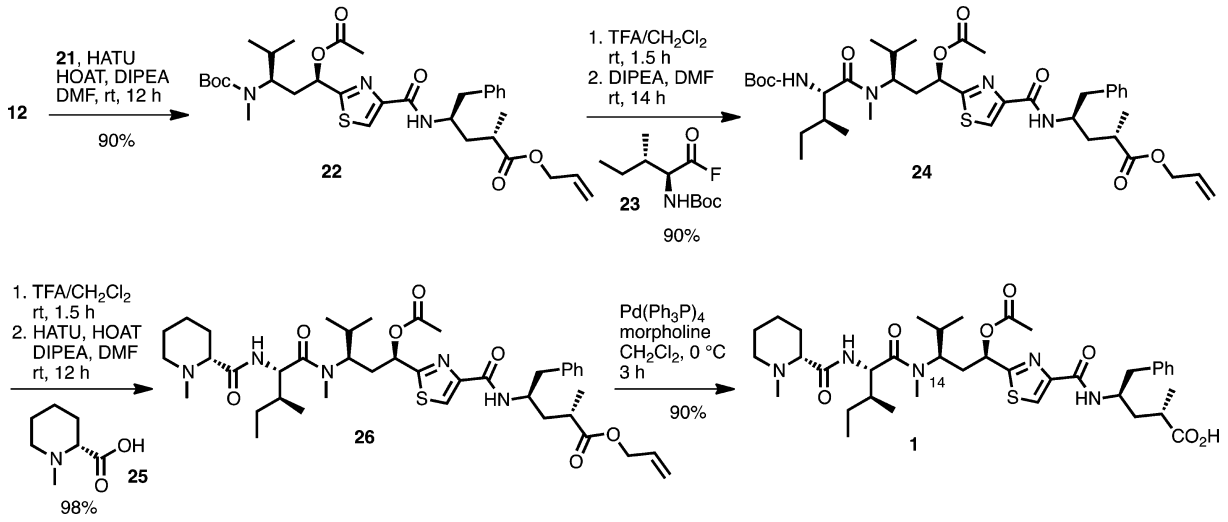
Scheme 2. Preparation of Tubuphenylalanine Ester 21



Scheme 3. N-Terminal Chain Extensions To Give N^{14}-Desacetoxytubulysin H (1)



used to prepare tubulysin conjugates.[23,24,36] Ester prodrugs proved to be similarly potent in vivo,[20] but, not surprisingly, were generally less active in vitro.[53,54] Some truncated versions of Tup/Tut residues in tubulysin D retained sub- or low-nanomolar IC_{50} values.[36] Similarly, the C-2 desmethyl pretubulysin was 10-fold less active than pretubulysin.[21] An analogue of pretubulysin with a C-terminal L-Phe in place of the Tup residue was also less active.[41]

Recent results support the merits of using synthetic tubulysins in targeted anticancer therapies; for example, KEMTUB10, featuring a *p*-fluorophenyl residue and an N-14 benzyl tubuvaline, demonstrated picomolar potency in breast cancer cells,[44] and exceptional potency was also reported for Tb14, a novel propellane-containing tubulysin.[55] We now report a second-generation synthesis of N^{14}-desacetoxytubulysin H (**1**), the preparation of a C-terminal hydrazide and a methyl ester of **1**, as well as late-stage functionalizations of the Tup residue and the synthesis of other non-natural analogues of tubulysins that may serve as warheads in antibody–drug conjugates for applications in cancer chemotherapy.

RESULTS AND DISCUSSION

Our first-generation total synthesis provided N^{14}-desacetoxytubulysin H (**1**) in 20 steps and 2.1% overall yield.[34] In order to improve the overall conversion, we developed a modified second-generation synthesis of **1** (Schemes 1-3). For the preparation of the tubuvaline fragment (Scheme 1), Boc-Val-OH (**2**) was *N*-methylated and subjected to an Arndt–Eistert chain extension followed by reduction with DIBAL-H to give aldehyde **5** in five steps. The commercially available thiazole ester **6** was reduced, silylated with TIPS-Cl, and condensed with aldehyde **5** after using Knochel's conditions[56] on bromide **8** for a Grignard exchange reaction. Tubuvaline alcohols **9** and **10** were obtained as a 1:1 mixture of diastereomers, and the undesired diastereomer **10** was converted in 78% yield to the desired **9** by oxidation with Dess–Martin periodinane and selective reduction with (*S*)-2-methyl-CBS-oxazaborolidine.[57] Acetylation of **9** and desilylation of the primary alcohol followed by a two-step oxidation to the acid completed the preparation of Boc-Tuv-OH (**12**) in excellent yield.

For the synthesis of tubuphenylalanine ester **21**, we oxidized phenylalaninol **13** and subjected the resulting aldehyde **14** to a Wittig chain extension to give alkenoate **15** (Scheme 2).[21,38,46] Saponification and coupling with L-menthol provided ester **17**,

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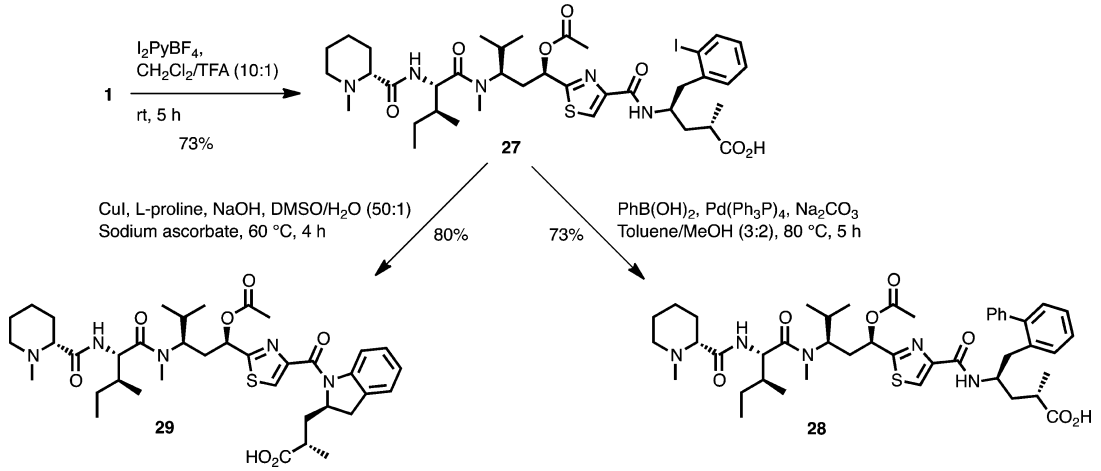
Scheme 4. Late-Stage Side Chain (28) and Backbone (29) Modifications of 1 Based on a Highly Selective Barluenga Iodination of the Tup Residue



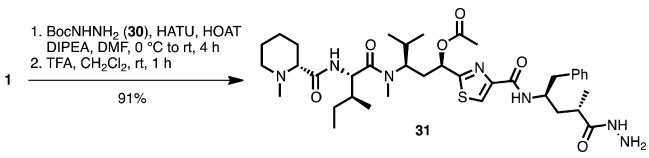
which provided a 5:1 ratio in favor of the desired diastereomer **19** on hydrogenation with Pd/C in THF. In contrast, in EtOAc or MeOH, only a 2:1 ratio was achieved.[21,38,41,46] Hydrolysis of **19** in a 5:1 mixture of 6 M HCl and acetic acid at 105 °C cleanly provided acid **20**, whereas the use of 6 M HCl at 130−140 °C[38,46] in the absence of acetic acid was not reproducible in our hands.

A series of sequential amide couplings completed the synthesis of N^{14}-desacetoxytubulysin H (**1**, Scheme 3). The use of the HATU/HOAT coupling reagent provided Tuv-Tup dipeptide **22** in excellent yield,[38] but acid fluoride **23** proved to be superior for the coupling of the sterically hindered α-branched secondary amine derived from **22**.[59] HATU/HOAT was used again in the acylation with Mep-OH **25** to give allyl ester **26**. C-Terminal ester deprotection with $Pd[PPh_3]_4$ and morpholine afforded N^{14}-desacetoxytubulysin H (**1**) in 20 steps and 16% overall yield for the longest linear sequence starting with **2**.

This modular and high-yielding route was well suited for iterative analogue preparations and SAR studies, but we were also interested in exploring late-stage functionalizations of the peptide backbone and amino acid side chains. After failed attempts to prepare a phenylalanine boronate via a recently reported Ir-catalyzed C−H activation,[60] we turned our attention to the selective iodination conditions reported by Barluenga et al.[61] We were pleased to observe complete consumption of **1** after 5 h at room temperature in the presence of I_2PyBF_4 in CH_2Cl_2 and TFA (Scheme 4). Analysis of the crude reaction mixture showed that the main product was iodotubulysin **27**, which was isolated in 73% yield after RP-HPLC purification. A Suzuki coupling of **27** proceeded under standard conditions with phenyl boronate and yielded 72% of the novel *o*-biphenyl tubulysin **28**. Alternatively, Ullmann-type coupling conditions effected an intramolecular amidation to give indoline **29** in 80% yield.[62] 2D ^{1}H NMR analysis of **29** and comparison to literature data confirmed the structural assignment.[63] In addition to the opportunity to broaden the chemical diversity of tubulysins with these late-stage transformations, we suggest that this strategy for combined backbone and side-chain modifications could be applied to other peptides with aromatic residues.

Other modifications of the terminal residue in **1** took advantage of available synthetic intermediates and traditional functional group interconversions (Scheme 5). The hydrazide of tubulysin B was reported to be active,[24] and therefore, we

Scheme 5. Synthesis of Hydrazide 31 from Tubulysin 1 and Boc-hydrazine (30)



prepared the corresponding analogue **31** by coupling of **1** and hydrazide **30** followed by cleavage of the Boc group with TFA. Previously published methods for the formation of related hydrazides were low yielding due to multiple side reactions, as discussed by Vlahov et al.[64]

The synthesis of enoate analogues **35** and **37** started from the unsaturated Tup building block **15** (Scheme 6).[12] Hydrolysis of **15** followed by conversion of the acid to the allyl ester gave **32**, which was deprotected with TFA and then coupled with the mixed anhydride of segment **12** to afford dipeptide **33**. Attachment of the Ile-residue was effectively achieved by coupling of **33** with Fmoc-Ile-F,[58] and the resulting protected tripeptide **34** was converted to analogue **35** as well as the N-terminal proline derivative **37**. Deprotection of the Fmoc group in **34** and coupling with Mep-OPFP or the proline active ester **36**, followed by deprotection of the allyl ester provided **35** and **37**, respectively. These analogues share the C-terminal 2-methyl α,β-unsaturated acid moiety with the potent microtubule-targeting hemiasterlins.[65]

The Ile-Tuv segment has previously been modified but still offers opportunities for structural simplification with minimal loss of activity, as demonstrated for pretubulysin. We were particularly interested in comparing the deacetoxylated analogue **49** (cLogP 4.0), containing an alkene at the C(2)−C(3) Tup position with the corresponding Val-analogue **50** (cLogP 3.5), since these combined substitutions would decrease lipophilicity and simplify the synthesis.

The known imine **40** was prepared according to a literature protocol from amide **38** and aldehyde **39**,[66] and trimethylsilyl acetylide addition in the presence of trimethylaluminum[67] provided **41** in 17:1 dr and 94% yield (Scheme 7).

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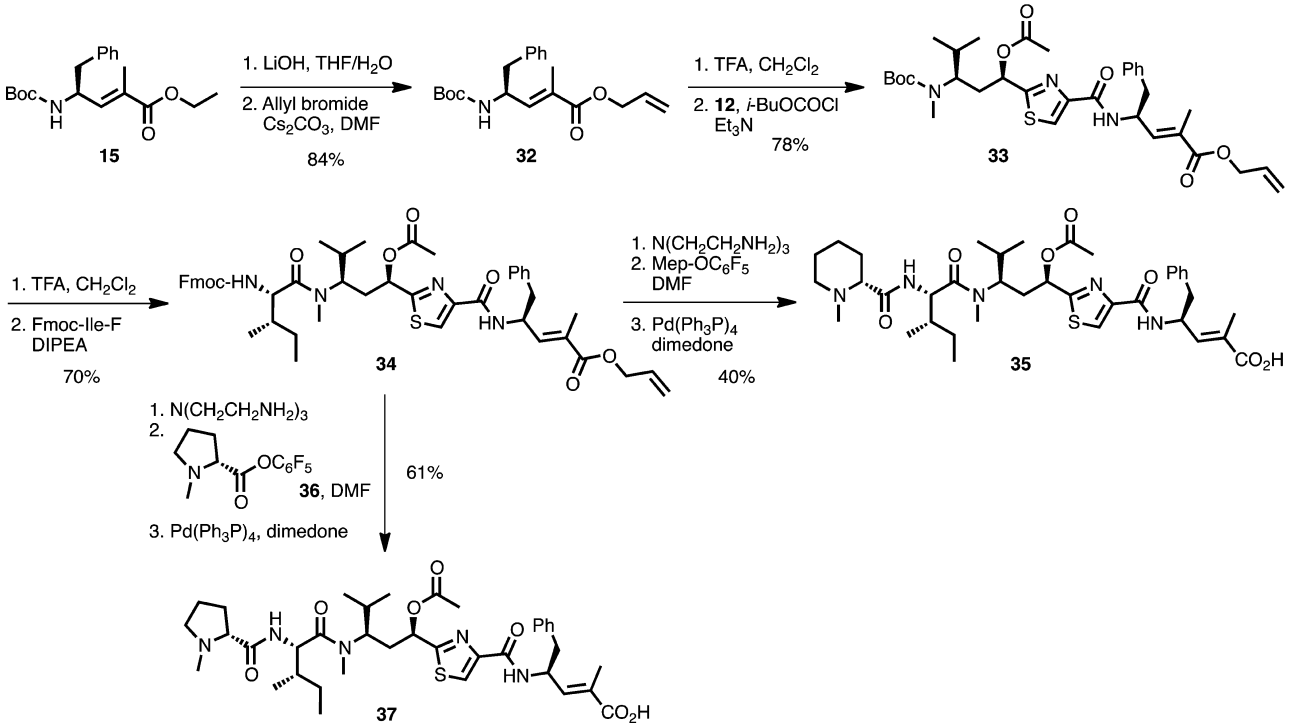
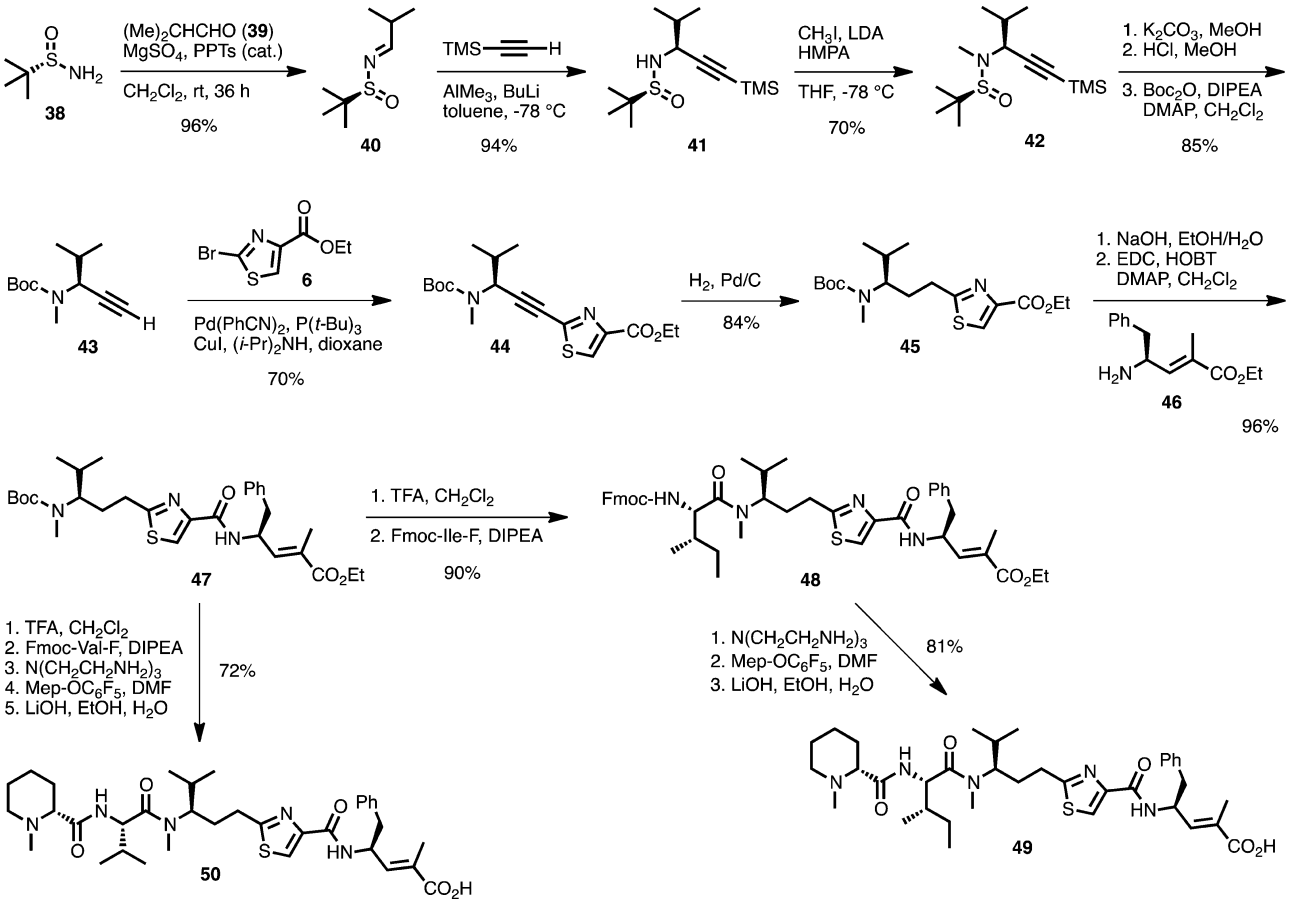
Scheme 6. Preparation of C- and N-Terminal Analogues 35 and 37 from Synthetic Intermediates 12 and 15 Obtained en Route to 1



Scheme 7. Total Syntheses of Tubulysins 49 and 50 Using the Structurally Simplified Tuv-Tup Analogue 47 as a Common Intermediate



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Chromatographic purification removed the minor diastereomer of **41**, and the desired absolute configuration was confirmed by an X-ray crystallographic analysis. *N*-Methylation of **41**, cleavage of the TMS group in the resulting **42**, and removal of the chiral auxiliary followed by Boc protection afforded alkyne **43**. A Sonogashira coupling of **43** and bromothiazole **6** gave enantiomerically pure **44** (ee > 99.9% by chiral HPLC analysis). Hydrogenation of **44** yielded the deacetoxylated Tuv analogue **45**. Saponification of the ethyl ester and coupling with amine **46**, obtained by protective group removal from **15**, led to key intermediate **47** in 10 linear steps and 30% overall yield from *tert*-butanesulfinamide **38**. Removal of the N-terminal protective group in **47** and coupling with Fmoc-Ile fluoride led to tripeptide **48**, which was converted to the desired tubulysin analogue **49** in three steps and high yield. An analogous deprotection and coupling sequence using Fmoc-protected valine acylfluoride, followed by condensation with *N*-methyl pipecolic acid pentafluorophenyl ester, generated tubulysin **50**. The total number of synthetic transformations (18) from commercially available materials required for the preparation of **49** and **50** compared very well to the 27 total steps required for the synthesis of **1**, but of course, the utility of these simplified analogues had yet to be determined by biological testing.

BIOLOGICAL EVALUATION OF TUBULYSINS

In the biological study of tubulysins, we used paclitaxel, vincristine, and monomethyl auristatins D (MMAD) and E (MMAE) as standards due to their related mechanism of action and potency range (Table 1).[68] The effects of these compounds and tubulysin analogues on cell death and mitotic arrest were benchmarked as described earlier.[69,70] Briefly, HeLa human cervical carcinoma cells (10000 per well) were plated in collagen-1 coated 384-well microplates and treated with vehicle (DMSO) or 10 2-fold concentration gradients of test agents within 4–6 h of seeding. Cells were incubated for 24 h at 37 °C, fixed with formaldehyde, and labeled with 10 μg/mL of Hoechst 33342. Plates were analyzed on an ArrayScan high content reader (Thermo Fisher Cellomics) for cell numbers and nuclear condensation. Cell growth inhibition was assessed by fitting of the resulting sigmoidal cell density curves to a four-parameter logistic equation, followed by extrapolating the concentration of drugs that caused 50% cell loss (IC_{50}). Nuclear condensation curves, which measure apoptosis and/or mitotic arrest, were based on the percentage of cells with condensed nuclei. Because the shape of these curves was often not sigmoidal, we determined minimum detectable effective concentrations (MDEC) as previously described.[69]

Table 1. Antiproliferative Activities and Nuclear Condensation Effects of Tubulysin Analogues and Reference Compounds in HeLa Cells

	cell density IC_{50} (nM)			nuclear condensation MDEC (nM)		
compd	mean	SD	*n*	mean	SD	*n*
paclitaxel	1.21	0.26	10	0.29	0.14	6
vincristine	15.7	7.2	9	5.11	4.44	9
MMAD	0.14	0.12	3	0.04	0.02	3
MMAE	0.32	0.14	3	0.19	0.11	3
1	0.23	0.12	3	0.11	0.03	3
26	7.5	4.0	3	0.63	0.21	3
27	0.24	0.12	3	0.12	0.08	2
28	1.11	0.64	2	0.42	0.23	2
29	18.3	2.8	2	8.12	6.34	2
31	0.99	1.2	3	0.10	0.05	3
35	81.3	55.2	4	12.6	2.0	4
37	309	145	3	39.5	24.7	3
49	379	62	3	215	128	3
50	1,466	657	3	482	204	3

As expected, the highly potent MMAD and MMAE demonstrated subnanomolar IC_{50} values and MDECs of 40 and 190 pM, respectively (Table 1). Paclitaxel was still very potent with a IC_{50} of 1.2 nM and an MDEC of 290 pM, whereas vincristine was 10–20 times less potent (IC_{50} 15.7 nM, MDEC 5.1 nM). Compound **1** was the most potent of all analogues prepared and averaged an IC_{50} of 0.23 nM and a MDEC of 110 pM, approximately in between MMAD and MMAE, and significantly more potent than paclitaxel and vincristine. Not surprisingly, the allyl ester of **1**, compound **26**, lost about a factor of 30 in IC_{50} potency in HeLa cells (7.5 nM), but interestingly, the nuclear condensation assay showed an MDEC of 630 pM for allyl ester **26**, i.e., only ca. 6-fold higher than acid **1**. The most surprising biological response, however, was found for hydrazide **31**, which, while ca. 9-fold less potent than **1** in the growth inhibition assay (IC_{50} 0.99 nM), was equivalent to **1** in the nuclear condensation assay (MDEC 100 pM). These results bode well for optimizing the physicochemical properties of tubulysin acids and enhance their cell-type specificity through C-terminal conjugation to antibodies or small molecule receptor or enzyme ligands.

Our discovery of the ability of Barluenga's iodination protocol to selectively *ortho*-functionalize the Tup residue in **1** also allowed us to test the antiproliferative profile of novel tubulysin analogues, such as iodide **27**, biphenyl **28**, and indoline **29**. Perhaps not surprisingly, given the relatively minor structural modification, iodobenzene **27** was equipotent to the parent compound **1** in both growth inhibition and nuclear condensation assays, and therefore, **27** or an isotopically labeled variant could become useful as a reference in target identification and PK/PD studies. For compound **28** and, in particular, the backbone modified **29**, both IC_{50} and MDEC values dropped off significantly by factors of 4–5 (for **28** vs **1**) and 80 (for **29** vs **1**). The *o*-phenyl substitution in **28** is likely to interfere sterically with tubulin binding; the drop in activity is in qualitative agreement with a model positioning the Tup side chain into a hydrophobic binding pocket near Q15 on β1-tubulin.[68] For indoline **29**, this interaction is similarly, but more severely impacted, and the conformational constraint potentially disrupts hydrogen bonds of the Tuv amide carbonyl group with the main chain NH of Tyr224 and Gly225 at the *N*-terminus of helix H7.[68] Finally, the dehydroanalogue of **1**, the α,β-unsaturated acid **35**, the corresponding *N*-methyl proline analogue **37**, and the synthetically streamlined deacetoxylated compounds **49** and **50** showed steep drop-offs in potency, with the α,β-unsaturated acid **35** being the most potent derivative (IC_{50} 81.3 nM, MDEC 12.6 nM) and the valine deacetoxy enoate **50** falling into the low micromolar range (IC_{50} 1.5 μM, MDEC 0.48 μM). The differential cytotoxicity range of these derivatives could make them attractive candidates to test for synergistic activities with signaling pathway inhibitors or other mechanism-based anticancer agents, as well as for cancer cell type specificities.

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CONCLUSION

In summary, we devised a high-yielding second-generation route for the synthesis of N^{14}-desacetoxytubulysin H (**1**) and introduced efficient late-stage functionalizations that allow the preparation of novel side-chain- and backbone-modified analogues. We further expanded on the SAR of tubulysins and discovered C-terminal modifications that preserve the exquisite biological activity of acid **1** and offer the opportunity for conjugation to biomolecules and receptor ligands to improve target selectivity. We also discovered moderately active analogues that might prove useful in synergistic combination cancer drug treatment regimens. Overall, our 10 biologically characterized synthetic analogues span a range of almost 4 orders of magnitude in biological potency, illustrating the diverse biological response that can be achieved in structure−activity investigations of tubulysins and natural products in general.[71]

EXPERIMENTAL SECTION

General Information. All reactions involving moisture-sensitive reagents were conducted in oven-dried glassware under a nitrogen or argon atmosphere. Analytical thin-layer chromatography (TLC) was preformed on SiO_2 60 F-254 plates. Visualization was accomplished by UV irradiation at 254 nm or by staining with any one of the following reagents: iodine, 5% phosphomolybdic acid hydrate in ethanol, ninhydrin (0.3% w/v in glacial acetic acid/*n*-butyl alcohol 3:97), Vaughn's reagent (4.8 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 0.2 g of $Ce(SO_4)_2{\cdot}4H_2O$ in 10 mL of concd H_2SO_4 and 90 mL of H_2O), or *p*-anisaldehyde (7.5 mL of *p*-anisaldehyde, 25 mL of concd H_2SO_4, and 7.5 mL of acetic acid in 675 mL of 95% ethanol). Flash chromatography was performed using SiO_2 60 (particle size 0.040−0.055 mm, 230−400 mesh). Hydrogenation reactions were performed on an H-cube. Melting points were obtained on a capillary melting point apparatus fitted with a digital thermometer and are not corrected. Specific rotations of chiral compounds were obtained at the designated concentration and temperature using a 1 dm cell. Infrared spectra were collected with neat material (oil or solid) on a FT-IR spectrometer, and ATR correction was performed on the spectra obtained. Proton and carbon NMR spectra were obtained on 300, 400, 500, and 600 MHz NMR spectrometers. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to residual solvent. ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). LC−MS analysis was performed using an analytical C18 column (100 × 4.6 mm, 3.5 μm, 0.4 mL/min). HRMS analyses used an Orbitrap quadrupole instrument in positive (+) and negative (−) mass detection mode.

Synthetic Procedures and Spectroscopic Data. *(S)-2-((tert-Butoxycarbonyl)methylamino)-3-methylbutanoic Acid (**3**).*[72] Sodium hydride (1.24 g, 46.0 mmol) was added in small portions over 45 min to a stirred solution of Boc-Val-OH (**2**, 1.00 g, 4.60 mmol) and methyl iodide (2.8 mL, 46 mmol) in THF (30 mL) at 0 °C. The reaction mixture was stirred at rt for 24 h under a nitrogen atmosphere and then quenched with water (15 mL). Ethyl acetate (15 mL) was added, and the mixture was concentrated under vacuum, diluted with water (100 mL), and washed with diethyl ether (2x). The aqueous solution was acidified to pH 3 with solid citric acid and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated to afford **3** (0.91 g, 85%) as a pale yellow oil which crystallized on standing: $[\alpha]_D^{20}$ −106 (*c* 0.6 in EtOH); ^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$) δ 4.00−3.97 (m, 1 H), 2.91 (s, 1.5 H), 2.90 (s, 1.5 H), 2.40−2.20 (m, 1 H), 1.50 (s, 9 H), 1.06 (d, *J* = 6.6 Hz, 3 H), 0.95 (d, *J* = 6.6 Hz, 1.5 H), 0.93 (d, *J* = 6.5 Hz, 1.5 H).

*(R)-Methyl 3-((tert-butoxycarbonyl)methylamino)-4-methylpentanoate (**4**).*[34] A solution of **3** (0.91 g, 3.9 mmol) in THF (60 mL) was treated at 0 °C with triethylamine (0.61 mL, 4.3 mmol) and isobutyl chloroformate (0.58 mL, 4.3 mmol). The reaction mixture was stirred at rt for 1 h, cooled to 0 °C, and filtered through a small plug of Celite. The plug was washed with cold THF. The solution was concentrated to afford (*S*)-2-((*tert*-butoxycarbonyl)methylamino)-3-methylbutanoic (isobutyl carbonic) anhydride **3a** as a yellow oil that was used in the next step without further purification: ^{1}H NMR (mixture of rotamers, 300 MHz, $CDCl_3$) δ 4.57 (d, *J* = 10.1 Hz, 1 H), 4.08 (d, *J* = 6.7 Hz, 2 H), 2.93 (s, 1.5 H), 2.87 (s, 1.5 H), 2.38−2.25 (m, 1 H), 2.12−1.99 (m, 1 H), 1.49 (s, 9 H), 1.09 (d, *J* = 6.6 Hz, 3 H), 1.10−0.95 (m, 10 H).

Aqueous NaOH (15%, 6 mL) was added dropwise to a suspension of Diazald (4.0 g, 19 mmol) in EtOH (20 mL). The formed diazomethane was transferred and bubbled with a flow of N_2 into a solution of (*S*)-2-((*tert*-butoxycarbonyl)methylamino)-3-methylbutanoic (isobutyl carbonic) anhydride **3a** (1.3 g, 3.9 mmol) in THF (30 mL) at −10 °C. After the complete addition of NaOH into the Diazald solution, the reaction flask was kept under nitrogen and allowed to sit without stirring at rt for 5 h. The reaction mixture was quenched with 0.5 M AcOH in Et_2O (excess), diluted with satd $NaHCO_3$ and Et_2O, and extracted with Et_2O (2×). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography through a small plug of SiO_2 (hexanes/EtOAc 90:10−85:15) to afford (*S*)-*tert*-butyl (1-diazo-4-methyl-2-oxopentan-3-yl)methylcarbamate **3b** (0.87 g, 87%) as a yellow oil: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 5.47−5.35 (m, 1 H), 4.32−4.08 (m, 1 H), 2.74 (s, 3 H), 2.33−2.22 (m, 1 H), 1.49 (s, 9 H), 0.96 (d, *J* = 6.5 Hz, 3 H), 0.87 (d, *J* = 6.4 Hz, 3 H).

Silver benzoate (0.093 g, 0.41 mmol) in Et_3N (4.3 mL) was added dropwise to a stirred solution of (*S*)-*tert*-butyl (1-diazo-4-methyl-2-oxopentan-3-yl)methylcarbamate **3b** (0.87 g 3.4 mmol) in MeOH (34 mL) at 0 °C under nitrogen in a flask protected from light. The reaction mixture was stirred at 0 °C for 1 h (bubbles were observed), 10 h at rt, then concentrated. The crude black residue was purified by chromatography on SiO_2 (hexanes/EtOAc 90:10 to 85:15) to afford **4** (0.79 g, 89%) as a colorless oil: ^{1}H NMR (mixture of rotamers, 300 MHz, $CDCl_3$) δ 4.07 (td, *J* = 10.4, 4.3 Hz, 0.5 H), 3.98−3.91 (m, 0.5 H), 3.67 (s, 1.6 H), 3.66 (s, 1.4 H), 2.76 (s, 1.6 H), 2.72 (s, 1.4 H), 2.67−2.45 (m, 2 H), 1.88−1.76 (m, 1 H), 1.48 (s, 5 H), 1.46 (s, 4 H), 0.95 (d, *J* = 6.6 Hz, 3 H), 0.89 (d, *J* = 6.6 Hz, 3 H); ^{13}CNMR (mixture of rotamers, 76 MHz, $CDCl_3$) δ 172.3, 172.1, 155.8, 79.5, 79.1, 60.3, 59.4, 51.6, 36.4, 36.0, 31.0, 30.5, 30.1, 28.4, 20.1, 20.0, 19.7, 19.6.

*(R)-tert-Butyl Methyl(4-methyl-1-oxopentan-3-yl)carbamate (**5**).*[34] A solution of **4** (0.6 g, 2.3 mmol) in dry Et_2O (30 mL) was treated at −78 °C dropwise over 30 min with a 1 M solution of DIBALH in hexane (3.0 mL, 3.0 mmol). The reaction mixture was stirred at −78 °C for 105 min, quenched at −78 °C with H_2O (0.1 mL), 15% NaOH in H_2O (0.1 mL), and water (0.4 mL), slowly warmed to 0 °C, dried (Na_2SO_4), and filtered through a small pad of Celite. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc 85:15−80:20) to afford **5** (0.51 g, 96%) as a colorless oil that was used immediately without further purification: ^{1}H NMR (characteristic signals, mixture of rotamers, 400 MHz, $CDCl_3$) δ 9.68 (t, *J* = 4.4 Hz, 1 H), 4.34 (td, *J* = 10.6, 4.1 Hz, 0.5 H), 4.11 (td, *J* = 9.1, 3.9 Hz, 0.5 H), 2.79−2.46 (m, 5 H), 1.85−1.78 (m, 1 H), 1.49 (s, 4 H), 1.46 (s, 5 H), 0.99−0.96 (m, 3 H), 0.92 (d, *J* = 6.6 Hz, 3 H).

*2-Bromothiazol-4-yl)methanol (**7**).*[34] To an ice-cooled solution of ethyl 2-bromothiazole-4-carboxylate **6** (2.5 g, 10.5 mmol) in anhydrous THF (25 mL) was added dropwise lithium borohydride (4.0 M solution in THF, 4.0 mL, 15.9 mmol) over 20 min. A solution of methanol (0.64 mL, 15.9 mmol) in anhydrous THF (2 mL) was then added over 30 min, and the mixture was stirred for 4 h, allowing the temperature to rise to rt. The reaction mixture was diluted with ethyl acetate, washed with 1 M $KHSO_4$, satd $NaHCO_3$, and brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes 3:7) to afford **7** (1.3 g, 83%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (s, 1 H), 4.77 (d, *J* = 4.8 Hz, 2 H), 2.37 (t, *J* = 4.8 Hz, 1 H).

*2-Bromo-4-((((triisopropylsilyl)oxy)methyl)thiazole (**8**).*[73] A solution of triisopropylchlorosilane (2.0 mL, 9.3 mmol) in anhydrous THF

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(8 mL) was added over 20 min to a solution of **7** (1.5 g, 7.7 mmol) and imidazole (0.63 g, 9.3 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred overnight, diluted with 1 M $KHSO_4$, and extracted with Et_2O (3×). The combined organic layers were washed with 1 M $KHSO_4$, satd $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc 95:05) to afford **8** (2.5 g, 92%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (t, J = 1.4 Hz, 1 H), 4.85 (d, J = 1.4 Hz, 2 H), 1.14−1.05 (m, 3 H), 1.02−0.99 (m, 18 H).

*tert-Butyl ((1R,3R)-1-Hydroxy-4-methyl-1-(4-(((triisopropylsilyl)-oxy)methyl)thiazol-2-yl)pentan-3-yl)methylcarbamate (**9**) and tert-Butyl ((1S,3R)-1-Hydroxy-4-methyl-1-(4-(((triisopropylsilyl)oxy)-methyl)thiazol-2-yl)pentan-3-yl)methylcarbamate (**10**).* A 2 M solution of *s*-BuMgCl in THF (3.7 mL, 7.5 mmol) was added to a solution of bromide **8** (2.6 g, 7.5 mmol) in THF (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 15 min, kept under vacuum (gentle bubbling) at 0 °C for 5 min, stirred at 0 °C for 15 min, kept under vacuum at 0 °C for 5 min, and stirred at 0 °C for other 5 min. A solution of aldehyde **5** (0.90 g, 3.9 mmol) in dry THF (4 mL) was added dropwise, and the reaction mixture was stirred at 0 °C for 30 min, warmed to rt and stirred for 3 h, quenched with satd $NaHCO_3$, and extracted with EtOAC (3×). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc 9:1 then 85:15 then 8:2) to afford **9** (0.66 g, 34%) and epimer **10** (0.70 g, 36%). For the conversion of **10** to **9**, a solution of **10** (0.4 g, 0.80 mmol) in CH_2Cl_2 (10 mL) was treated at 0 °C with Dess−Martin periodinane (DMP, 0.37 g, 0.87 mmol), stirred for 45 min at 0 °C, and then diluted with CH_2Cl_2, 1 M sodium thiosulfate, and satd $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2×), and the combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified through a small plug of SiO_2 (hexanes/EtOAc, 8:2) to afford a colorless oil, which was cooled to 0 °C and treated with a mixture of BH_3−DMS complex (0.8 mL, 1.6 mmol) and (*S*)-CBS−BH_3 complex (0.17 g, 0.80 mmol) in dry THF (6 mL). The reaction mixture was stirred for 24 at rt. Additional BH_3−DMS complex (4.2 mL, 8.4 mmol) was added, and stirring was continued at rt for 3 days. The mixture was quenched by dropwise, very slow addition of MeOH (2 mL) at 0 °C, followed by water (2 mL) and ethanolamine (0.5 mL). The solution was stirred at rt for 1 h, concentrated, diluted with $NaHCO_3$, and extracted with EtOAc (3×). The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 90:10 to 85:15) to afford additional **9** (0.31 g, 78%) as a colorless oil.

9: R_f 0.5 (hexanes/EtOAc, 8:2); $[\alpha]_D^{20}$ −11.2 (c 1.0, CH_2Cl_2); IR 3386, 2957, 2942, 2864, 1689, 1663, 1462, 1137, 1100, 880, 682 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 1 H), 4.98−4.95 (m, 3 H), 4.69 (d, J = 10.8 Hz, 1 H), 3.98 (td, J = 11.3, 2.7 Hz, 1 H), 2.78−2.74 (m, 3 H), 2.08−1.91 (m, 2 H), 1.80−1.71 (m, 1 H), 1.49 (s, 9 H), 1.23−1.15 (m, 3 H), 0.98−0.91 (m, 18 H), 0.97 (d, J = 6.5 Hz, 3 H), 0.92 (d, J = 6.5 Hz, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 158.5, 157.1, 112.9, 80.6, 69.2, 63.0, 57.9, 38.0, 29.9, 28.6, 28.5, 28.3, 20.3 (2C), 18.1, 12.1; HRMS (ESI+) m/z calcd for $C_{25}H_{49}O_4N_2SSi$ 501.3177, found 501.3176.

10: R_f 0.25 (hexanes/EtOAc, 8:2); ^{1}H NMR (mixture of rotamers, 300 MHz, $CDCl_3$) δ 7.11 (s, 0.3), 7.04 (s, 0.7 H), 4.95−4.83 (m, 3.3 H), 4.69 (d, J = 8.3 Hz, 0.7 H), 3.77 (td, J = 10.4, 3.4 Hz, 0.8 H), 3.67−3.58 (m, 0.2 H), 2.50 (s, 0.7 H), 2.32−2.11 (m, 4 H), 1.72−1.60 (m, 1 H), 1.34 (s, 2 H), 1.32 (s, 7 H), 1.14−1.05 (m, 4 H), 1.03−1.01 (m, 18 H), 0.93 (d, J = 6.6 Hz, 2.3 H), 0.88 (d, J = 6.6 Hz, 0.7 H), 0.76 (d, J = 6.5 Hz, 3 H); HRMS (ESI+) m/z calcd for $C_{25}H_{49}O_4N_2SSi$ 501.3177, found 501.3173.

*(1R,3R)-3-((tert-Butoxycarbonyl)methylamino)-1-(4-(hydroxymethyl)thiazol-2-yl)-4-methylpentyl acetate (**11**).*[34] Ac_2O (0.52 mL, 5.6 mmol) and DMAP (0.17 g, 1.4 mmol) were added to a solution of alcohol **9** (1.4 g, 2.8 mmol) and Et_3N (0.77 mL, 5.6 mmol) in CH_2Cl_2 (28 mL). The reaction mixture was stirred at rt for 5 h, diluted with EtOAc, washed with 1 M $NaHSO_4$, satd $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 9:1) to afford (1*R*,3*R*)-3-((*tert*-butoxycarbonyl)methylamino)-4-methyl-1-(4-(((triisopropylsilyl)oxy)methyl)thiazol-2-yl)pentyl acetate **9a**[55] (1.42 g, 94%) as a colorless oil: $[\alpha]_D^{20}$ +10.6 (c 1.0, CH_2Cl_2); IR 2957, 2938, 2864, 1753, 1689, 1462, 1365, 1219, 1134, 880, 682 cm^{-1}; ^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$) δ 7.21 (s, 1 H), 5.93 (dd, J = 9.9, 2.7 Hz, 0.3 H), 5.86 (dd, J = 11.7, 2.8 Hz, 0.7 H), 4.98 (d, J = 1.1 Hz, 1.3 H), 4.97 (d, J = 1.0 Hz, 0.7 H), 4.14−4.07 (m, 0.7 H), 3.89−3.67 (s, 0.3), 2.72 (s, 3 H), 2.41−2.29 (m, 1 H), 2.19 (s, 3 H), 2.10−2.03 (m, 1 H), 1.76−1.67 (m, 1 H), 1.46 (s, 9 H), 1.24−1.16 (m, 3 H), 1.12−1.08 (m, 18 H), 1.00 (d, J = 6.5 Hz, 2.3 H), 0.99 (d, J = 6.4 Hz, 0.7 H), 0.88 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$) δ 170.2, 169.2, 157.4, 156.3, 128.5, 127.9, 113.5, 113.4, 79.6, 79.3, 70.1, 69.2, 62.8, 62.7, 56.4, 34.9, 30.4, 28.5, 28.4, 28.1, 21.1, 21.0, 20.0, 19.6, 18.0, 17.9, 12.0; HRMS (EI) m/z calcd for $C_{27}H_{51}O_5N_2SSi$ 543.3282, found 543.3281.

A 1 M solution of TBAF in THF (7.3 mL, 7.3 mmol) was added to a solution of **9a** (1.6 g, 2.9 mmol) in THF (20 mL) at 0 °C. The reaction mixture was stirred for 90 min at 0 °C, quenched with satd $NaHCO_3$, and extracted with EtOAc (3×). The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc 4:6−3:7) to afford **11** as a colorless oil (1.07 g, 95%): $[\alpha]_D^{20}$ +14.7 (c 1.0, CH_2Cl_2); IR 3438, 2964, 2931, 2875, 1749, 1685, 1473, 1447, 1365, 1219, 1152, 1130, 1040, 772 cm^{-1}; ^{1}H NMR (mixture of rotamers, 300 MHz, $CDCl_3$) δ 7.16 (s, 0.3 H), 7.15 (s, 0.7 H), 5.92 (dd, J = 9.0, 3.8 Hz, 0.3 H), 5.84 (dd, J = 11.6, 2.8 Hz, 0.7 H), 4.74 (s, 2 H), 4.08 (td, J = 11.1, 3.0 Hz, 0.7 H), 3.83−3.72 (m, 0.3), 3.08 (s, 1 H), 2.70 (s, 2 H), 2.64 (s, 1 H), 2.41−2.24 (m, J = 3.7 Hz, 1 H), 2.15 (s, 3 H), 2.08−1.99 (m, J = 2.8 Hz, 1 H), 1.75−1.63 (m, 1 H), 1.45 (s, 9 H), 1.33−1.23 (m, 1 H), 0.98 (d, J = 6.6 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$) δ 170.9, 170.2, 169.9, 169.6, 156.6, 156.5, 156.3 (2C), 115.0, 114.7, 79.7, 79.3, 70.6, 69.4, 60.9, 60.8, 56.4, 53.9, 35.0, 30.8 (2C), 30.4, 28.5, 28.4, 28.1 (2C), 20.9, 20.8, 20.3, 20.0, 19.8, 19.6, 14.1; HRMS (ESI+) m/z calcd for $C_{18}H_{31}O_5N_2S^+$ 387.1948, found 387.1943.

*2-((1R,3R)-1-Acetoxy-3-((tert-butoxycarbonyl)methylamino)-4-methylpentyl)thiazole-4-carboxylic Acid (**12**).*[34] A solution of **11** (0.54 g, 1.4 mmol) and Dess−Martin periodinane (DMP, 0.68 g, 1.6 mmol) in CH_2Cl_2 (25 mL) was stirred at 0 °C for 1 h and then diluted with CH_2Cl_2 (100 mL), 1 M $Na_2S_2O_3$ (30 mL) and $NaHCO_3$ (30 mL). The organic phase was washed with satd $NaHCO_3$, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (EtOAc/hexanes 3:7−4:6) to afford (1*R*,3*R*)-3-((*tert*-butoxycarbonyl)methylamino)-1-(4-formylthiazol-2-yl)-4-methylpentyl acetate **11a** (0.51 g, 95%) as a colorless oil that was used immediately in the next step: IR 3102, 2068, 1749, 1685, 1480, 1387, 1365, 1216, 1152, 1130, 1044 cm^{-1}; ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 10.03 (s, 0.3 H), 10.02 (s, 0.7 H), 8.16 (s, 0.3 H), 8.15 (s, 0.7 H), 5.97 (dd, J = 9.9, 2.8 Hz, 0.3 H), 5.89 (dd, J = 11.6, 2.9 Hz, 0.7 H), 4.13−4.08 (m, 0.7 H), 3.82 (br s, 0.3 H), 2.73 (s, 3 H), 2.41−2.34 (m, 1 H), 2.19 (s, 3 H), 2.18−2.12 (m, 1 H), 1.78−1.64 (m, 2 H), 1.46 (s, 9 H), 1.01−0.99 (m, 3 H), 0.89 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (mixture or rotamers, 101 MHz, CD_2Cl_2) δ 184.7, 184.6, 172.0, 171.0, 170.2, 169.6, 156.3, 156.1, 154.8, 127.7, 127.3, 79.7, 79.4, 70.1, 69.4, 56.4, 34.6, 30.7, 30.4, 29.7, 28.43, 28.37, 28.1, 21.0, 20.8, 20.2, 20.0, 19.8, 19.6; HRMS (ESI+) m/z calcd for $C_{18}H_{28}O_5N_2SNa^+$ 407.1611, found 407.1610.

A solution of aldehyde **11a** (0.49 g, 1.3 mmol) in *tert*-butyl alcohol (20 mL) was treated with a solution of 2-methyl-2-butene in THF (2 M, 5.1 mL, 10.2 mmol), followed by the dropwise addition of a mixture of sodium chlorite (0.69 g, 7.6 mmol) and sodium dihydrogen phosphate monohydrate (2.3 g, 19 mmol) in water (10 mL). The reaction mixture was stirred at room temperature for 4 h, diluted with 0.5 M $NaHSO_4$ (50 mL), and extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried (Na_2SO_4), concentrated under vacuum, and purified by chromatography on SiO_2 (CH_2Cl_2/MeOH 97:3 then CH_2Cl_2/MeOH/AcOH 95:5:0.5) to obtain **12** (0.48 g, 94%) as a colorless oil: IR 3468, 3121, 2968, 2938, 2882, 1738, 1685, 1480, 1447, 1368, 1216, 1160, 1044 cm^{-1}; ^{1}H

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NMR (mixture of rotamers, 400 MHz, $CDCl_3$) δ 9.18 (s, 1 H), 8.25 (s, 0.3 H), 8.23 (s, 0.7 H), 5.96 (dd, J = 9.2, 3.1 Hz, 0.3), 5.88 (dd, J = 11.4, 2.8 Hz, 0.7 H), 4.11−4.05 (m, 0.7 H), 3.76 (br s, 0.3 H), 2.69 (s, 2.1 H), 2.67 (s, 0.9 H), 2.37−2.29 (m, J = 3.5 Hz, 1 H), 2.18−2.10 (m, 4 H), 1.73−1.65 (m, 1 H), 1.43 (s, 9 H), 1.22−1.15 (m, 1 H), 0.96 (d, J = 6.5 Hz, 3 H), 0.88−0.84 (m, 4 H); ^{13}C NMR (mixture of rotamers, 101 MHz; $CDCl_3$) δ 171.7, 170.5, 170.3, 169.6, 164.1, 163.9, 156.4, 146.6, 146.4, 129.0, 80.1, 79.6, 70.3, 69.4, 56.3, 34.6, 31.8, 30.3, 29.0, 28.4, 28.3, 28.1, 22.6, 21.0, 20.8, 20.2, 19.9, 19.7, 19.5, 14.1. HRMS (ESI-) m/z calcd for $C_{18}H_{27}O_6N_2S$ 399.1595, found 399.1605.

*(S)-tert-Butyl (1-Oxo-3-phenylpropan-2-yl)carbamate (**14**).*[74] A solution of phenylalaninol **13** (2.5 g, 10 mmol) in CH_2Cl_2 (100 mL) was treated with Dess−Martin periodinane (4.6 g, 11 mmol), stirred at rt for 1 h, and diluted with 1 M $Na_2S_2O_3$ (30 mL) and $NaHCO_3$ (30 mL). The organic phase was separated and washed with $NaHCO_3$, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (EtOAc/hexanes 5:5−6:4) to afford **14** (2.4 g, 96%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 1 H), 7.37−7.28 (m, 3 H), 7.20−7.17 (m, 2 H), 5.04 (s, 1 H), 4.44 (d, J = 6.4 Hz, 1 H), 3.13 (d, J = 6.4 Hz, 2 H), 1.45 (s, 9 H).

*(S,E)-Ethyl 4-((tert-Butoxycarbonyl)amino)-2-methyl-5-phenylpent-2-enoate (**15**).*[17,41] (1-(Ethoxycarbonyl)ethylidene)-triphenylphosphorane (5.86 g, 16.2 mmol) was added as solid in a single portion to a solution of **14** (2.88 g, 11.6 mmol) in CH_2Cl_2 (70 mL) at 0 °C. The reaction mixture was stirred at rt for 14 h, concentrated to 10−15 mL, and poured into a plug of SiO_2 moistened with EtOAc/hexanes, 4:6, and eluted with EtOAc/hexanes, 5:5, to afford **15** (3.8 g, 98%) as a colorless solid: ^{1}H NMR (400 MHz. $CDCl_3$) δ 7.32−7.31 (m, 2 H), 7.24 (t, J = 7.2 Hz, 1 H), 7.20−7.18 (m, 2 H), 6.54 (dq, J = 9.1, 1.2 Hz, 1 H), 4.68−4.61 (m, 2 H), 4.20 (q, J = 7.1 Hz, 2 H), 2.94 (dd, J = 13.3, 5.6 Hz, 1 H), 2.80 (dd, J = 13.4, 7.1 Hz, 1 H), 1.72 (d, J = 0.7 Hz, 3 H), 1.43 (s, 9 H), 1.30 (t, J = 7.1 Hz, 3 H).

*(S,E)-4-((tert-Butoxycarbonyl)amino)-2-methyl-5-phenylpent-2-enoic Acid (**16**).*[74] A solution of ester **15** (3.8 g, 11 mmol) in THF/H_2O/MeOH, 1:0.5:1 (25 mL) was treated with 4 M LiOH (11 mL), stirred at rt for 40 h, and concentrated. The aqueous layer was washed with Et_2O (2×), acidified to pH 2−3 with 1 M $KHSO_4$, and extracted with EtOAc (2×). The combined organic layers were washed with brine and concentrated to afford **16** (3.0 g, 86%) as a colorless solid: $[\alpha]_D^{20}$ +44.0 (c 1, $CHCl_3$, lit.[75] $[\alpha]_D^{20}$ +44.9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.25 (s, 1 H), 7.35−7.19 (m, 5 H), 6.68 (d, J = 8.7 Hz, 1 H), 4.67 (s, 2 H), 2.97 (dd, J = 13.1, 5.4 Hz, 1 H), 2.81 (dd, J = 13.3, 7.1 Hz, 1 H), 1.71 (s, 3 H), 1.44 (s, 9 H).

*(S,E)-(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl 4-((tert-Butoxycarbonyl)amino)-2-methyl-5-phenylpent-2-enoate (**17**).*[74] Dicyclohexylcarbodiimide (DCC, 0.45 g, 2.2 mmol) was added to a solution of **16** (0.60 g, 1.8 g), menthol (0.42 g, 2.7 mmol), and DMAP (20 mg, 0.15 mmol) in Et_2O (30 mL) at 0 °C. The reaction mixture was stirred at rt for 24 h, cooled to 0 °C, filtered through a small plug of Celite, and concentrated. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc 95:5−92.5:7.5) to afford **17** (0.6 g, 75%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33−7.25 (m, 4 H), 7.19−7.16 (m, 2 H), 6.49 (dd, J = 9.1, 0.9 Hz, 1 H), 4.77−4.51 (m, 3 H), 3.01−2.90 (m, 1 H), 2.89−2.77 (m, 1 H), 2.07−2.01 (m, 1 H), 1.93−1.83 (m, J = 2.7 Hz, 1 H), 1.74−1.69 (m, 5 H), 1.44−1.40 (m, 11 H), 1.34−1.29 (m, 1 H), 1.12−1.00 (m, 2 H), 0.95−0.89 (m, 8 H), 0.79 (d, J = 6.9 Hz, 3 H).

*(2R,4R)-(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl 4-((tert-Butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (**18**) and (2S,4R)-(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl 4-((tert-Butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (**19**).*[74] A solution of **17** (2.6 g, 0.90 mmol) in THF (100 mL) was treated with Pd/C (0.293 mg, 0.29 mmol), stirred under an atmosphere of H_2 overnight at rt, filtered on Celite, and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/Et_2O 85:15) to afford **18** (0.39 g, 15%) and **19** (2.0 g, 77%) as well as a mixed fraction (0.15 g, 6%).

18: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, J = 7.4 Hz, 2 H), 7.24 (d, J = 7.2 Hz, 1 H), 7.20 (t, J = 7.1 Hz, 2 H), 4.67 (td, J = 10.8, 4.2 Hz, 1 H), 4.40 (d, J = 8.7 Hz, 1 H), 3.92−3.92 (m, 1 H), 2.86−2.75 (m, 2 H), 2.49 (q, J = 6.2 Hz, 1 H), 1.98 (d, J = 11.6 Hz, 1 H), 1.87−1.67 (m, 4 H), 1.57−1.36 (m, 13 H), 1.16 (d, J = 7.0 Hz, 3 H), 1.11−1.02 (m, 1 H), 0.99−0.87 (m, 9 H), 0.77 (d, J = 6.9 Hz, 3 H).

19: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 7.0 Hz, 2 H), 7.26−7.23 (m, 1 H), 7.21 (t, J = 6.4 Hz, 2 H), 4.70 (td, J = 10.9, 4.3 Hz, 1 H), 4.39−4.37 (m, 1 H), 3.90 (br s, 1 H), 2.87−2.75 (m, 2 H), 2.65−2.54 (m, 1 H), 2.02 (d, J = 11.9 Hz, 1 H), 1.93−1.83 (m, 2 H), 1.73−1.67 (m, 2 H), 1.51−1.37 (m, 14 H), 1.17 (t, J = 7.4 Hz, 3 H), 1.12−1.02 (m, 2 H), 0.98−0.84 (m, 9 H), 0.77 (d, J = 7.0 Hz, 3 H).

*(2S,4R)-4-Amino-2-methyl-5-phenylpentanoic Acid Hydrochloride (**20**).*[74] A suspension of **19** (1.33 g, 2.98 mmol) in 6 M HCl (35 mL) and AcOH (10.5 mL) was heated at reflux (110 °C) for 1.5 h, diluted with H_2O, washed with Et_2O, and concentrated to afford **20** (0.72 g, 99%) as a colorless crystalline solid: ^{1}H NMR (300 MHz, D_2O) δ 7.38−7.24 (m, 5 H), 3.54 (qt, J = 6.9 Hz, 1 H), 2.98 (dd, J = 14.2, 6.5 Hz, 1 H), 2.85 (dd, J = 14.2, 7.8 Hz, 1 H), 2.61 (app sxt, J = 7.1 Hz, 1 H), 1.96 (ddd, J = 14.7, 8.5, 6.1 Hz, 1 H), 1.71−1.62 (m, 1 H), 1.11 (d, J = 7.0 Hz, 3 H).

*(2S,4R)-Allyl 4-amino-2-methyl-5-phenylpentanoate Hydrochloride (**21**).*[34] Acetyl chloride (2.4 mL, 34 mmol) was slowly added to iced-cold allyl alcohol (12 mL). The reaction mixture was stirred for 5 min at 0 °C, treated with acid **20** (0.36 g, 1.2 mmol), stirred at 60 °C for 3 h, and concentrated. The residue was dried under vacuum to afford the desired **21** (0.42 g, quant) as a wax: ^{1}H NMR (300 MHz, CD_3OD:$CDCl_3$, 1:1) δ 7.39−7.22 (m, 5 H), 5.94−5.80 (m, 1 H), 5.32−5.22 (m, 2 H), 4.56 (d, J = 5.8 Hz, 2 H), 3.68−3.58 (m, 1 H), 3.52−3.41 (m, 1 H), 2.99 (dd, J = 13.9, 6.5 Hz, 1 H), 2.88 (dd, J = 14.0, 7.7 Hz, 1 H), 2.73−2.66 (m, J = 5.3 Hz, 1 H), 2.06−1.98 (m, 1 H), 1.67 (ddd, J = 14.3, 8.4, 5.5 Hz, 1 H), 1.21 (d, J = 7.0 Hz, 3 H).

*(2S,4R)-Allyl 4-(2-((1R,3R)-1-Acetoxy-3-((tert-butoxycarbonyl)-methylamino)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate (**22**).*[34] Diisopropylethylamine (0.87 mL, 5.0 mmol) was added to a solution of **12** (0.5 g, 1.2 mmol), HATU (0.62 g, 1.6 mmol), and HOAT (0.23 g, 1.6 mmol) in DMF (10 mL) at 0 °C. After 30 min at 0 °C, the reaction mixture was treated with **21** (0.42 g, 1.5 mmol), stirred at 0 °C for 1 h and at rt for 14 h, diluted with EtOAc and 1 M $KHSO_4$, washed with $KHSO_4$, satd $NaHCO_3$ (2×), and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc 8:2−6:4) to afford **22** (0.71 g, 90%) as a colorless oil: $[\alpha]_D^{20}$ +9.3 (c 1.0, CH_2Cl_2); IR 3270, 3121, 3087, 2968, 2934, 2875, 1745, 1724, 1674, 1644, 1219, 1148, 1037 cm^{-1}; ^{1}H NMR (mixture of rotamers 6:4, 400 MHz, CD_2Cl_2) δ 7.97 (s, 0.6 H), 7.97 (s, 0.4 H), 7.30−7.19 (m, 5.4 H), 7.11 (d, J = 9.4 Hz, 0.6 H), 5.92−5.85 (m, 1.4 H), 5.77 (dd, J = 11.4, 2.9 Hz, 0.6 H), 5.27 (dt, J = 17.2, 1.4 Hz, 1 H), 5.18 (dq, J = 10.5, 1.2 Hz, 1 H), 4.56−4.47 (m, 2 H), 4.40−4.33 (m, 1 H), 4.05 (td, J = 11.1, 2.7 Hz, 0.6 H), 3.81 (bs, 0.4 H), 2.94−2.84 (m, 2 H), 2.70 (s, 1.8 H) 2.68−2.57 (m, 2.2 H), 2.33−2.19 (m, 1 H), 2.15 (s, 1.2 H), 2.12 (s, 1.8 H), 2.06−1.98 (m, 2 H), 1.76−1.58 (m, 2 H), 1.43 (s, 5.4 H), 1.43 (s, 3.6 H), 1.16 (d, J = 7.1 Hz, 3 H), 0.99 (d, J = 6.9 Hz, 1.7 H), 0.98 (d, J = 6.9 Hz, 1.3 H), 0.89−0.86 (m, 3 H); ^{13}C NMR (mixture or rotamers, 101 MHz, CD_2Cl_2) δ 175.5, 170.64, 170.44, 170.0, 169.5, 160.1, 156.0, 150.21, 150.04, 138.09, 137.93, 132.6, 129.50, 129.44, 128.29, 128.25, 126.40, 126.34, 123.07, 122.93, 117.3, 79.4, 79.0, 70.7, 69.4, 65.0, 56.5, 48.49, 48.35, 41.43, 41.32, 38.06, 37.93, 36.5, 35.6, 35.0, 30.6, 30.4, 29.7, 28.18, 28.12, 20.88, 20.70, 20.0, 19.8, 19.59, 19.45, 17.70, 17.58; HRMS (ESI+) m/z calcd for $C_{33}H_{47}O_7N_3SNa$ 652.3027, found 652.3024.

*tert-Butyl ((2S,3S)-1-Fluoro-3-methyl-1-oxopentan-2-yl)-carbamate (**23**).*[76] A solution of DAST (0.77 mL, 4.8 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to a solution of Boc-Ile-OH (0.93 g, 4.0 mmol) and pyridine (0.33 mL, 4.0 mmol) in dry CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 45 min, diluted with CH_2Cl_2, washed with ice-cold water, dried (Na_2SO_4), filtered, concentrated. The crude residue (0.87 g, 93%) was used in the next step without purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (d, J = 7.6 Hz, 1 H), 4.45 (dd, J = 8.3, 4.7 Hz, 1 H), 1.99−1.94 (m, 1 H), 1.52−1.48 (m, 10 H), 1.32−1.16 (m, 1 H), 1.05 (t, J = 6.9 Hz, 3 H), 0.98 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162 (d, J_{C-F}

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= 373 Hz), 155.3, 80.7, 57.1 (d, J_{C-F} = 56.6 Hz), 37.1, 28.2, 25.1, 15.5, 11.5.

*(2S,4R)-Allyl 4-(2-((6S,9R,11R)-6-((S)-sec-Butyl)-9-isopropyl-2,2,8-trimethyl-4,7,13-trioxo-3,12-dioxa-5,8-diazatetradecan-11-yl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate (**24**).* A solution of TFA (3 mL) in CH_2Cl_2 (3 mL) was added to a solution of **22** (0.445 g, 0.709 mmol) in CH_2Cl_2 (7 mL). The reaction mixture was stirred at rt for 90 min and then concentrated. The resulting oil was diluted with Et_2O and concentrated three times to afford (2*S*,4*R*)-allyl 4-(2-((1*R*,3*R*)-1-acetoxy-4-methyl-3-(methylamino)pentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate 2,2,2-trifluoroacetate as a colorless film which was used in the next step without further purification: HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{40}O_5N_3S$ 530.2683, found 530.2679. Diisopropylethylamine (0.52 mL, 0.13 mmol) was added to a solution of (2*S*,4*R*)-allyl 4-(2-((1*R*,3*R*)-1-acetoxy-4-methyl-3-(methylamino)pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate 2,2,2-trifluoroacetate (0.48 g, 0.75 mmol), and **23** (0.53 g, 2.0 mmol) in DMF (8 mL) at rt. The reaction mixture was stirred for 14 h and then diluted with EtOAc and 0.5 M $KHSO_4$. The organic layer was washed with satd $NaHCO_3$ (2×) and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc 65:35−6:4) to afford **24** (0.501 g, 90%) as a colorless foam: $[\alpha]_D^{20}$ −3.7 (*c* 1.0, CH_2Cl_2); IR 3389, 3303, 2964, 2931, 2875, 1733, 1704, 1637, 1492, 1219, 1167, 1044, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1 H), 7.30−7.27 (m, 2 H), 7.23−7.20 (m, 3 H), 7.13 (d, *J* = 9.3 Hz, 1 H), 5.87 (ddt, *J* = 17.2, 10.5, 5.6 Hz, 1 H), 5.64 (dd, *J* = 11.4, 2.3 Hz, 1 H), 5.27 (dq, *J* = 17.2, 1.5 Hz, 1 H), 5.19 (dq, *J* = 10.5, 1.3 Hz, 1 H), 5.14 (d, *J* = 9.8 Hz, 1 H), 4.58−4.50 (m, 3 H), 4.46−4.39 (m, 2 H), 3.01 (s, 3 H), 2.97 (dd, *J* = 13.7, 5.8 Hz, 1 H), 2.89 (dd, *J* = 13.8, 6.7 Hz, 1 H), 2.68−2.61 (m, 1 H), 2.32 (ddd, *J* = 14.9, 11.6, 3.1 Hz, 1 H), 2.17 (s, 3 H), 2.08−2.01 (m, 2 H), 1.80−1.61 (m, 4 H), 1.45−1.41 (m, 10H), 1.19 (d, *J* = 7.1 Hz, 3 H), 1.15−1.09 (m, 1 H), 1.04 (d, *J* = 6.5 Hz, 3 H), 0.97 (d, *J* = 6.7 Hz, 3 H), 0.91 (t, *J* = 7.4 Hz, 3 H), 0.85 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (mixture of rotamers, 126 MHz, $CDCl_3$) δ 175.7, 174.3, 170.04, 170.00, 160.3, 156.0, 150.0, 137.5, 132.3, 129.6, 128.4, 126.5, 123.4, 117.9, 79.5, 69.5, 65.1, 55,6, 55.1, 48.4, 41.0, 37.6, 37.0, 36.6, 34.7, 30.0, 29.5, 28.3, 24.1, 20.8, 20.1, 19.5, 17.7, 16.0, 11.1; HRMS (ESI+) *m*/*z* calcd for $C_{39}H_{59}O_8N_4S$ 743.4048, found 743.4049.

*(R)-1-Methylpiperidine-2-carboxylic Acid (**25**).*[14] A suspension of 10% Pd/C (0.20 g, 0.19 mmol), 37% aqueous formaldehyde (2.9 mL, 39 mmol), and D-pipecolic acid (1.0 g, 7.7 mmol) in MeOH (10 mL) was stirred for 24 h under a hydrogen atmosphere, filtered through Celite, and concentrated to afford **25** as an off-white solid (1.0 g, 90%): ^1H NMR (300 MHz, D_2O) δ 3.44−3.36 (m, 2 H), 2.96 (td, *J* = 12.6, 2.9 Hz, 1 H), 2.78 (s, 3 H), 2.16−2.11 (m, 1 H), 1.88−1.77 (m, 2 H), 1.71−1.57 (m, 2 H), 1.53−1.45 (m, 1 H).

*(2S,4R)-Allyl 4-(2-((1R,3R)-1-Acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate (**26**).*[34] A solution of TFA (4 mL) in CH_2Cl_2 (4 mL) was added to a solution of **24** (0.66 g, 0.88 mmol) in CH_2Cl_2 (8 mL). The reaction mixture was stirred at rt for 60 min and then concentrated. The resulting oil was diluted with CH_2Cl_2 and concentrated three times to afford (2*S*,4*R*)-allyl 4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*S*)-2-amino-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate 2,2,2-trifluoroacetate as a colorless film that was used in the next step without further purification: HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{51}O_6N_4S$ 643.3524, found 643.3525.

Diisopropylethylamine (0.70 mL, 4.0 mmol) was added to a solution of **25** (0.19 g, 1.3 mmol), HATU (0.51 g, 1.3 mmol), and HOAT (0.18 g, 1.3 mmol) in DMF (5 mL) at 0 °C. After 30 min at 0 °C, the reaction mixture was treated with to (2*S*,4*R*)-allyl 4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*S*)-2-amino-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate 2,2,2-trifluoroacetate (0.67 g, 0.89 mmol), stirred at 0 °C for 30 min and at rt for 5 h, diluted with EtOAc, washed with satd $NaHCO_3$ (2×) and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH 100:2−100:8) to afford **26** (0.67 g, 98%) as a colorless oil: $[\alpha]_D^{20}$ +20.6 (*c* 0.5, CH_2Cl_2); IR 3386, 3270, 3076, 2934, 2893, 2856, 1733, 1641, 1540, 1495, 1372, 1219, 843, 738 cm^{-1}; ^1H NMR (601 MHz, MeOD) δ 8.10 (s, 1 H), 7.28−7.24 (m, 4 H), 7.20−7.18 (m, 1 H), 5.88 (ddt, *J* = 17.2, 10.5, 5.6 Hz, 1 H), 5.72 (dd, *J* = 11.2, 2.4 Hz, 1 H), 5.26 (dq, *J* = 17.2, 1.6 Hz, 1 H), 5.17 (dq, *J* = 10.5, 1.4 Hz, 1 H), 4.77 (d, *J* = 8.1 Hz, 1 H), 4.55−4.47 (m, 3 H), 4.41−4.37 (m, *J* = 3.6 Hz, 1 H), 3.13 (s, 3 H), 3.01−2.98 (m, 1 H), 2.94 (dd, *J* = 13.7, 7.1 Hz, 1 H), 2.88 (dd, *J* = 13.6, 6.8 Hz, 1 H), 2.69 (d, *J* = 10.5 Hz, 1 H), 2.66−2.63 (m, 1 H), 2.39 (ddd, *J* = 14.9, 11.5, 3.1 Hz, 1 H), 2.29−2.24 (m, 4 H), 2.22−2.15 (m, 4 H), 2.01 (ddd, *J* = 14.0, 10.0, 3.8 Hz, 1 H), 1.91−1.74 (m, 5 H), 1.70−1.55 (m, 5 H), 1.38−1.28 (m, 2 H), 1.22−1.17 (m, 4 H), 1.05 (d, *J* = 6.6 Hz, 3 H), 1.00 (d, *J* = 6.8 Hz, 3 H), 0.94 (t, *J* = 7.4 Hz, 3 H), 0.83 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (151 MHz, MeOD) δ 176.0, 173.7, 170.4, 170.3, 161.3, 149.4, 138.0, 132.3, 129.1, 128.0, 126.1, 123.8, 116.8, 69.8, 68.9, 64.8, 55.2, 53.5, 48.9, 43.2, 41.0, 37.5, 37.4, 36.5, 36.3, 34.3, 30.1, 29.6, 29.0, 24.6, 24.1, 22.7, 19.4, 19.1, 18.9, 16.7, 15.0, 9.8; HRMS (ESI+) *m*/*z* calcd for $C_{41}H_{62}O_7N_5S$ 768.4364, found 768.4364.

*(2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic Acid Trifluoroacetate (**1**).* Morpholine (0.068 mL, 0.78 mmol) was added to a solution of **26** (0.148 g, 0.20 mmol) in CH_2Cl_2 (6 mL) at 0 °C under an atmosphere of N_2, and then $Pd(Ph_3P)_4$ (15 mg, 0.015 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred for 3 h at 0 °C and purified by chromatography on SiO_2 (the reaction mixture was directly charged onto the column, prepacked with CH_2Cl_2/MeOH, 99:1, using CH_2Cl_2/MeOH, 99:1 to 90:1 as the eluent) to afford **1** as a pale yellow film that was further purified by RP chromatography (12-g C18 cartridge, 0.1% TFA/H_2O/CH_3CN 85:15−0:60). Lyophilization afforded **1** (0.148 g, 90%) as a colorless fluffy solid: ^1H NMR (500 MHz, MeOD) δ 8.10 (s, 1 H, Tuv thiazole C*H*), 7.27−7.24 (m, 4 H, Tup *o*-, *o*′-, *m*-, and *m*′-phenyl C*H*s), 7.20−7.17 (m, 1 H, Tup *p*-phenyl C*H*), 5.74 (d, *J* = 11.0, 1 H Tuv C*H*OAc), 4.74 (d, *J* = 7.5, 1 H, Ile C*H*-NH), 4.42−4.38 (m, 2 H, Tuv C*H*-N, Tup C*H*-NH), 3.77 (dd, *J* = 12.2, 2.1 Hz, 1 H, Mep C*H*-N), 3.49 (d, *J* = 12.7 Hz, 1 H, Mep C*H*2-N a), 3.14 (s, 3 H, Tuv N-C*H*3), 3.09 (td, *J* = 12.9, 2.1 Hz, 1 H, Mep C*H*2-N b), 2.95−2.89 (m, 2 H Tup C*H*2-Ph), 2.76 (s, 3 H, Mep C*H*3), 2.59−2.54 (m, 1 H, Tup *α* C*H*), 2.41 (ddd, *J* = 14.7, 11.6, 2.9 Hz, 1 H, Tuv C*H*2 a), 2.35−2.30 (m, 1 H, Tuv C*H*2 b), 2.19−2.17 (m, 4 H, Tuv COC*H*3, Mep *β* C*H*2 a), 2.02 (ddd, *J* = 13.9, 9.9, 4.0 Hz, 1 H, Tup *β* C*H*2 a), 1.97−1.87 (m, 4 H, Tuv C*H*-CH3, Ile C*H*-CH3, Mep *γ* C*H*2 a, Mep *δ* C*H*2 a), 1.84−1.75 (m, 2 H, Mep *β* C*H*2 b, Mep *δ* C*H*2 b), 1.69 (ddd, *J* = 14.2, 10.2, 4.1 Hz, 1 H, Tup *β* C*H*2 b), 1.65−1.58 (m, 2 H, Mep *γ* C*H*2 b, Ile C*H*2 a), 1.24−1.19 (m, 4 H, Ile C*H*2 b, Tup C*H*3), 1.06 (d, *J* = 6.6 Hz, 3 H, Tuv CH-C*H*3 a), 1.04 (d, *J* = 6.8 Hz, 3 H, Ile CH-C*H*3), 0.96 (t, *J* = 7.4 Hz, 3 H, Ile CH2-C*H*3), 0.87 (d, *J* = 6.6 Hz, 3 H, Tuv CH-C*H*3 b); ^{13}C NMR (101 MHz, MeOD) δ 178.5 (Tup *C*O2H), 173.2 (Ile *C*O-N), 170.37 (Tuv thiazole C^2), 170.18 (Tuv *C*OCH3), 167.7 (Mep *C*O-NH), 161.4 (Tuv *C*O-NH), 149.4 (Tuv thiazole C^4, 138.1 (Tup phenyl C^1), 129.1 (Tup m,*m*′-phenyl *C*Hs), 127.9 (Tup o,*o*′-phenyl *C*Hs), 126.0 (Tup *p*-phenyl *C*H), 123.7 (Tuv thiazole *C*H), 69.8 (Tuv *C*H-OAc), 66.8 (Mep *C*H−N), 56.9 (Tuv *C*H-N), 54.8 (Mep CH2−N), 54.6 (Ile *C*H-NH), 49.3 (Tup *C*H-NH), 41.5 (Mep *C*H3), 40.8 (Tup CH2-Ph), 37.7 (Tup *β* CH2), 36.4 (Tup *α* CH), 36.0 (Ile CH-CH3), 34.2 (Tuv *C*H2), 29.5 (Tuv CH-*C*H3, Tuv N-*C*H3), 28.9 (Mep *β* *C*H2), 23.8 (Ile *C*H2), 22.6 (Mep *δ* *C*H2), 21.0 (Mep *γ* CH2), 19.4 (Tuv COCH3), 19.1 (Tuv CH-*C*H3 a), 18.9 (Tuv CH-CH3 b), 17.1 (Tup *C*H3), 14.8 (Ile CH-*C*H3), 9.9 (Ile CH2-*C*H3); HRMS (ESI+) *m*/*z* calcd for $C_{38}H_{58}O_7N_5S$ 728.4051, found 728.4058.

*(2S,4R)-4-(2-((1R,3R)-1-Acetoxy-4-methyl-3-((2S,3S)-3-methyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)-thiazole-4-carboxamido)-5-(2-iodophenyl)-2-methylpentanoic Acid 2,2,2-Trifluoroacetate (**27**).* A solution of **1** (7.0 mg, 0.0083 mmol) in CH_2Cl_2 (0.6 mL) and TFA (0.06 mL) was treated with IPy_2BF_4 (5.6 mg, 0.014 mmol), and the maroon reaction mixture was stirred for 3 h at rt, concentrated, dissolved in acetonitrile/water (1:1), and purified immediately (less than 5 min after the sample was redissolved) by RP-HPLC (water +0.1% TFA/acetonitrile +0.1% TFA, 70:30 to 35:60).

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Lyophilization afforded **27** (5.9 mg, 73%) as a colorless fluffy solid: ^{1}H NMR (mixture of rotamers, 400 MHz, MeOD) δ, 8.05 (s, 1 H, Tuv thiazole C*H*), 7.82 (d, J = 7.9 Hz, 1 H, Tup phenyl C*H* ortho to I and meta to CH_2), 7.32 (d, J = 7.4, 1 H, Tup phenyl C*H* meta to I and ortho to CH_2), 7.24 (t, J = 7.4 Hz, 1 H, Tup phenyl C*H* para to I and meta to CH_2), 6.91 (t, J = 7.6 Hz, 1 H, Tup phenyl C*H* meta to I and para to CH_2), 5.77−5.74 (m, 1 H, Tuv C*H*-OAc), 4.75 (d, J = 7.5 Hz, 1 H, Ile C*H*-NH), 4.58−4.51 (m, 1 H, Tup C*H*-NH), 4.39 (bs, 1 H, Tuv CH-N), 3.75−3.72 (m, 1 H, Mep C*H*-N), 3.51−3.47 (m, 1 H, Mep ε C*H*2-N a), 3.16−3.02 (m, 6 H, Tuv C*H*3, Mep ε C*H*2-N b, Tup δ C*H*2), 2.75 (s, 3 H, Mep C*H*3), 2.61 (br s, 1 H, Tup α C*H*), 2.42−2.37 (m, 2 H, Tuv C*H*2), 2.18−2.09 (m, 5 H, OCOC*H*3, Tup β C*H*2 a, Mep β C*H*2 a), 1.96−1.90 (m, 4 H, Tuv C*H*-CH3, Ile C*H*-CH3, Mep γ C*H*2 a, Mep δ C*H*2 a), 1.86−1.77 (m, 3 H, Mep β C*H*2 b, Mep γ C*H*2 b, Tup β C*H*2 b), 1.66−1.60 (m, 2 H, Mep δ C*H*2 b, Ile C*H*2 a), 1.25 (m, 4 H, Tup C*H*3, Ile C*H*2 b), 1.06 (m, 6 H, Tuv CH-C*H*3 a, Ile CH-C*H*3), 0.97 (t, J = 7.3 Hz, 3 H, Ile CH2-C*H*3), 0.88 (d, J = 6.6 Hz, 3 H, Tuv CH-C*H*3 b); ^{13}C NMR (101 MHz, MeOD) δ 178.4, 173.2, 170.4, 170.1, 167.7, 161.0, 149.0, 141.1, 139.2, 130.4, 128.0, 127.8, 123.9, 100.4, 70.0, 66.8, 57.1, 54.9, 54.6, 48.6, 45.2, 41.5, 38.2, 36.5, 36.0, 34.2, 29.5, 29.4, 28.9, 23.9, 22.6, 21.0, 19.4, 19.1, 18.9, 17.2, 14.9, 9.9; HRMS (ESI+) m/z calcd for $C_{38}H_{57}O_7N_5SI$ 854.3018, found 854.3003.

*(2S,4R)-5-([1,1′-Biphenyl]-2-yl)-4-(2-((1R,3R)-1-acetoxy-4-methyl-3-((2S,3S)-3-methyl-2-((R)-1-methylpiperidine-2-carboxamido)-pentanamido)pentyl)thiazole-4-carboxamido)-2-methylpentanoic Acid 2,2,2-Trifluoroacetate (**28**).* Deoxygenated toluene (0.2 mL) was added under argon to a mixture of **27** (4.5 mg, 0.0046 mmol), $PhB(OH)_2$ (1.1 mg, 0.0093 mmol), and Na_2CO_3 (2.0 mg, 0.019 mmol) in a MW vial. Then $Pd(PPh_3)_4$ (0.1 mL of a toluene solution, containing 0.25 mg of Pd, 0.00023 mmol) was added, followed by MeOH (0.2 mL). The reaction flask was sealed, and the mixture was stirred at 80 °C for 5 h. After being cooled to room temperature, the reaction mixture was filtered through a 0.45 μm HPLC filter and concentrated. The residue was redissolved in acetonitrile and water and purified by RP-HPLC (C-18, water + 0.1% TFA/acetonitrile + 0.1% TFA 35:75−60:40) to obtain powdery **28** (3.1 mg, 73%): ^{1}H NMR (400 MHz, MeOD) δ 8.04 (s, 1 H Tuv thiazole C*H*), 7.45−7.41 (m, 2 H Tup biphenyl C*H*s), 7.38−7.32 (m, 4 H, Tup biphenyl C*H*s), 7.23−7.21 (m, 2 H, Tup biphenyl C*H*s), 7.16−7.14 (m, 1 H, Tup biphenyl C*H*), 5.73 (d, J = 11.0 Hz, 1 H, Tuv C*H*-OAc), 4.74 (d, J = 7.5 Hz, 1 H, Ile C*H*-NH), 4.39 (bs, 1 H, Tuv C*H*-N), 4.28−4.21 (m, 1 H, Tup C*H*-NH), 3.77 (dd, J = 12.2, 2.8 Hz, 1 H, Mep C*H*-N), 3.49 (d, J = 12.2 Hz, 1 H, Mep C*H*2-N a), 3.14−3.05 (m, 4 H, Tuv N-C*H*3, Mep C*H*2-N b), 3.01 (dd, J = 13.9, 5.4 Hz, 1 H, Tup C*H*2-biphenyl a), 2.83−2.74 (m, 4 H, Tup C*H*2-biphenyl b, Mep N-C*H*3), 2.46−2.28 (m, 3 H, Tuv C*H*2 a, Tuv C*H*2 b, Tup α C*H*), 2.19−2.17 (m, 4 H, Tuv COC*H*3, Mep β C*H*2 a), 1.96−1.90 (m, 4 H, Ile C*H*-CH3, Tuv C*H*-CH3, Mep δ C*H*2 a, Mep γ C*H*2 a), 1.84−1.77 (m, 3 H, Tup β C*H*2 a, Mep β C*H*2 b, Mep δ C*H*2 b), 1.64−1.60 (m, 2 H, Mep γ C*H*2 b, Ile C*H*2 a), 1.51 (ddd, J = 14.0, 9.1, 4.9 Hz, 1 H, Tup β C*H*2 b), 1.25−1.19 (m, 1 H, Ile C*H*2 b), 1.07 (m, 9 H, Tup C*H*3, Tuv CH-C*H*3 a, Ile CH-C*H*3), 0.96 (t, J = 7.4 Hz, 3 H, Ile CH2-C*H*3), 0.87 (d, J = 6.5 Hz, 3 H, Tuv CH-C*H*3 b); ^{13}C NMR (101 MHz, MeOD) δ 178.4, 173.2, 170.4, 170.0, 167.7, 161.1, 149.4, 142.6, 141.8, 135.6, 130.0, 129.7, 129.1, 127.9, 126.8, 126.6, 125.9, 123.6, 69.7, 66.8, 56.1, 54.8, 54.6, 49.0, 41.5, 38.1, 37.7, 36.2, 36.0, 34.1, 29.5, 28.9, 23.8, 22.6, 21.0, 19.4, 19.2, 19.0, 16.7, 14.9, 9.9; HRMS (ESI+) m/z calcd for $C_{44}H_{62}O_7N_5S$ 804.4364, found 804.4385.

*(S)-3-((R)-1-(2-((1R,3R)-1-Acetoxy-4-methyl-3-((2S,3S)-3-methyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)-thiazole-4-carbonyl)indolin-2-yl)-2-methylpropanoic Acid 2,2,2-Trifluoroacetate (**29**).* A solution of CuI (0.00089 mmol, 0.17 mg) and proline (0.00096 mmol, 0.11 mg) in deoxygenated DMSO (0.2 mL) was added to a solution of **27** (0.0044 mmol, 4.3 mg) in deoxygenated DMSO (0.5 mL), followed by addition of NaOH (0.010 mmol, 0.43 mg) and sodium ascorbate (0.00096 mmol, 0.19 mg) in H_2O (0.010 mL). The reaction flask was sealed and heated at 60 °C for 5 h. After being cooled to room temperature, the mixture was diluted with water (2 mL) and purified by RP-HPLC (C-18, water + 0.1% TFA/acetonitrile + 0.1% TFA 35:75−60:40). Lyophilization afforded **29** (3.0 mg, 80%): ^{1}H NMR (600 MHz, MeOD) δ 8.20 (bs, 2 H, Tuv thiazole C*H*, Tup phenyl C*H* ortho to N and meta to CH2), 7.32 (d, J = 7.3 Hz, 1 H, Tup phenyl C*H* ortho to CH2 and meta to N), 7.22 (bs, 1 H, Tup phenyl C*H* meta to N and para to CH2), 7.13 (t, J = 5.7 Hz, 1 H, Tup phenyl C*H* para to N and meta to CH2), 5.76 (d, J = 10.5 Hz, 1 H, Tuv C*H*OAc), 5.34 (bs, 1 H, Tup C*H*-N), 4.73 (d, J = 7.4 Hz, 1 H, Ile CH-NH), 4.39 (bs, 1 H, Tuv C*H*-N), 3.77 (dd, J = 12.2, 2.5 Hz, 1 H, Mep C*H*-N), 3.48 (d, J = 12 Hz, 1 H, Mep C*H*2-N a), 3.46−3.42 (m, 1 H, Tup C*H*2-Ar a), 3.13−3.07 (m, 4 H, Tuv N-C*H*3, Mep C*H*2-N b), 2.91 (d, J = 15.7 Hz, 1 H, Tup C*H*2-Ar b), 2.76 (s, 3 H, Mep N-C*H*3), 2.46−2.43 (m, 2 H, Tup α C*H*, Tuv C*H*2 a), 2.35−2.29 (m, 1 H, Tuv C*H*2 b), 2.18−2.15 (m, 4 H, Tuv COC*H*3, Mep β C*H*2 a), 1.94−1.91 (m, 5 H, Ile C*H*-CH3, Tuv C*H*-CH3, Mep γ C*H*2 a, Mep δ C*H*2 a, Tup β C*H*2 a), 1.84−1.75 (m, 2 H, Mep β C*H*2 b, Mep δ C*H*2 b), 1.68−1.59 (m, 3 H, Tup β C*H*2 b, Mep γ C*H*2 b, Ile C*H*2 a), 1.24−1.19 (m, 1 H, Ile C*H*2 b), 1.05−1.03 (m, 9 H, Tuv CH-C*H*3 a, Tup C*H*3, Ile CH-C*H*3), 0.95 (t, J = 7.4 Hz, 3 H, Ile CH2-C*H*3), 0.86 (d, J = 6.5 Hz, 3 H, Tuv CH-C*H*3 b); HRMS (ESI+) m/z calcd for $C_{38}H_{56}O_7N_5S$ 726.3895, found 726.3892.

*(1R,3R)-3-((2S,3S)-N,3-Dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-1-(4-(((2R,4S)-5-hydrazinyl-4-methyl-5-oxo-1-phenylpentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentyl Acetate Trifluoroacetate (**31**).* Diisopropylethylamine (0.138 mL, 0.79 mmol) was added to a solution of **1** (0.148 g, 0.18 mmol), HATU (0.082 g, 0.21 mmol), and HOAT (0.029 g, 1.2 mmol) in DMF (2 mL) at 0 °C. After 30 min at 0 °C, Boc-hydrazine (**30**, 0.035 g, 0.26 mmol) was added, and the reaction mixture was stirred at 0 °C for 4 h, diluted with EtOAc and satd $NaHCO_3$, washed with satd $NaHCO_3$ (2×) and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH 99:1−9:1) to afford *tert*-butyl 2-((2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*S*)-*N*,3-dimethyl-2-((*R*)-1-methylpiperidine-2-carboxamido)-pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoyl)hydrazinecarboxylate **30a** (0.135 g, 91%) as a colorless glass: ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 9.65 (bs, 1 H), 8.04 (s, 1 H), 7.32 (d, J = 9.6 Hz, 2 H), 7.28−7.22 (m, 6 H), 6.55 (bs, 1 H), 5.67 (dd, J = 11.2, 2.6 Hz, 1 H), 4.78 (t, J = 8.4 Hz, 2 H), 4.54 (br s, 1 H), 3.07 (s, 3 H), 3.01−2.91 (m, 3 H), 2.36−2.32 (m, 5 H), 2.20 (s, 3 H), 2.07−1.99 (m, 2 H), 1.88−1.61 (m, 14 H), 1.51−1.48 (m, 13 H), 1.20−1.17 (m, 1 H), 1.14 (d, J = 6.8 Hz, 3 H), 1.06 (d, J = 6.6 Hz, 3 H), 1.00 (d, J = 6.7 Hz, 3 H), 0.94 (t, J = 7.4 Hz, 3 H), 0.83 (d, J = 6.6 Hz, 3 H); HRMS (ESI+) m/z calcd for $C_{43}H_{68}O_8N_7S$ 842.4845, found 842.4847. A solution of TFA (1 mL) in CH_2Cl_2 (1 mL) was added to a solution of **30a** (0.107 g, 0.13 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at rt for 60 min and then concentrated. The resulting oil was diluted with CH_2Cl_2 and concentrated three times, diluted with water, and lyophilized to afford colorless fluffy **31** (0.120 g, quant): ^{1}H NMR (500 MHz, MeOD) δ 8.12 (s, 1 H, Tuv thiazole C*H*), 7.26−7.17 (m, 5 H, Tup phenyl C*H*s), 5.72 (dd, J = 11.3, 1.7 Hz, 1 H, Tuv C*H*-OAc), 4.75 (d, J = 7.4 Hz, 1 H, Ile C*H*-NH), 4.50 (br t, J = 9.6 Hz, 1 H, Tup C*H*-NH), 4.29−4.27 (m, 1 H, Tuv C*H*-N), 3.77 (dd, J = 12.1, 2.0 Hz, 1 H, Mep C*H*-N), 3.50 (d, J = 12.5 Hz, 1 H, Mep C*H*2-N a), 3.15−3.07 (m, 4 H, Tuv N-C*H*3, Mep C*H*2-N b), 2.91−2.90 (m, 2 H, Tup C*H*2-Ph), 2.77 (s, 3 H, Mep C*H*3), 2.53 (bs, 1 H, Tup α C*H*), 2.40 (ddd, J = 14.7, 11.8, 2.7 Hz, 1 H, Tuv C*H*2 a), 2.28−2.19 (m, 2 H, Tuv C*H*2 b, Mep β C*H*2 a), 2.17 (s, 3 H, Tuv COC*H*3), 2.09 (t, J = 12.1 Hz, 1 H, Tup β C*H*2 a), 1.98−1.85 (m, 4 H, Ile C*H*-CH3, Tuv C*H*-CH3, Mep γ C*H*2 a, Mep δ C*H*2 a), 1.83−1.75 (m, 2 H, Tup β C*H*2 b, Mep δ C*H*2 b), 1.71−1.60 (m, 3 H, Tup β C*H*2 b, Mep γ C*H*2 b, Ile C*H*2 a), 1.26−1.17 (m, 4 H, Ile C*H*2 b, Tup C*H*3), 1.06 (d, J = 6.9 Hz, 3 H, Tuv CH-C*H*3 a), 1.04 (d, J = 7.0 Hz, 3 H, Ile CH-C*H*3), 0.97 (t, J = 7.4 Hz, 3 H, Ile CH2-C*H*3), 0.86 (d, J = 6.6 Hz, 3 H, Tuv CH-C*H*3 b); ^{13}C NMR (101 MHz, MeOD) δ 175.6, 173.2, 170.53, 170.35, 167.7, 161.8, 149.3, 137.9, 129.0, 127.9, 126.1, 124.1, 69.8, 66.8, 54.9, 54.6, 49.1, 41.5, 41.2, 38.3, 36.0, 35.4, 34.3, 29.5, 28.9, 23.9, 22.6, 21.0, 19.4, 19.1, 18.9, 17.6, 14.9, 9.9; HRMS (ESI+) m/z calcd for $C_{38}H_{60}O_6N_7S$ 742.4320, found 742.4296.

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*(S,E)-Allyl 4-(tert-butoxycarbonylamino)-2-methyl-5-phenylpent-2-enoate (**32**).* A solution of **15** (0.36 g, 1.1 mmol) and LiOH (1.0 *N*, 4.0 mL, 4.0 mmol) in THF (8 mL) was heated at 60 °C for 8 h. The reaction mixture was quenched by addition of 1 N HCl (5.0 mL, 5.0 mmol), and the aqueous layer was extracted with ethyl acetate (25 mL × 3). The combined organic layers were dried (Na_2SO_4) and concentrated to give crude acid as a colorless solid. After addition of anhydrous DMF (3 mL), cesium carbonate (0.98 g, 3.0 mmol), and allyl bromide (2.0 mL, 23.1 mmol), the mixture was stirred at room temperature for 24 h, diluted with ethyl acetate, washed with saturated sodium bicarbonate and brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:5) to give **32** (0.32 g, 84% for two steps) as a colorless solid: $[\alpha]_D^{23}$ +34.4 (*c* 3.83, CH_2Cl_2); Mp 56.5−58.0 °C; IR (cm^{-1}) 3366, 2977, 2931, 1712, 1515, 1366, 1250, 1169; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.34 (m, 2 H), 7.28 (t, 1 H, *J* = 7.2 Hz), 7.24 (d, 2 H, *J* = 7.2 Hz), 6.66 (d, 1 H, *J* = 9.0 Hz), 6.03−5.97 (m, 1 H), 5.38 (dd, 1 H, *J* = 16.5, 1.5 Hz), 5.30 (dd, 1 H, *J* = 10.5, 0.9 Hz), 4.97 (bs, 1 H), 4.76−4.73 (m, 1 H), 4.72−4.70 (m, 2 H), 3.03−2.99 (m, 1 H), 2.84 (dd, 1 H, *J* = 12.3, 7.5 Hz), 1.77 (s, 3 H), 1.48 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 167.2, 154.9, 140.6, 136.6, 132.1, 129.4, 128.9, 128.2, 126.5, 117.7, 79.3, 65.2, 50.0, 41.0, 28.2, 12.4; HRMS (ESI) *m*/*z* calcd for $C_{20}H_{27}NO_4Na$ (M + Na) 368.1838, found 368.1823.

*(S,E)-Allyl 4-(2-((1R,3R)-1-Acetoxy-3-(tert-butoxycarbonylmethylamino)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoate (**33**).* A solution of **32** (74 mg, 0.21 mmol) in CH_2Cl_2 (1.0 mL) and trifluoroacetic acid (0.4 mL, 4.2 mmol) was stirred at room temperature for 2 h. The solution was partitioned between ethyl acetate and water, and the organic layer was washed with saturated sodium bicarbonate, dried (Na_2SO_4), and concentrated to give crude amine (52 mg, 99%) as a colorless oil: MS (ESI) *m*/*z* 268 ($[M + Na]^+$), 246 ($[M + H]^+$). To a solution of **12** (52 mg, 0.13 mmol) and triethylamine (0.05 mL, 0.38 mmol) in anhydrous THF (1 mL) cooled to −20 °C was added dropwise isobutyl chloroformate (0.03 mL, 0.22 mmol), and the resulting colorless suspension was stirred for 20 min. A solution of the crude amine (52 mg, 0.21 mmol) in anhydrous THF (1 mL) was then added via cannula, and the mixture was stirred overnight, allowing the temperature to gradually rise to room temperature. The reaction mixture was diluted with ethyl acetate, washed with brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:2) to give **33** (64 mg, 78% for two steps) as a colorless oil: $[\alpha]_D^{23}$ +22.7 (*c* 0.71, CH_2Cl_2); IR (cm^{-1}) 3367, 2971, 2930, 1753, 1713, 1689, 1538, 1390, 1224; ^1H NMR (2:1 mixture of rotamers, 600 MHz, $CDCl_3$) for major rotamer δ 8.03 (s, 1 H), 7.38 (d, 1 H, *J* = 8.4 Hz), 7.30−7.26 (m, 2 H), 7.23−7.21 (m, 3 H), 6.68 (dd, 1 H, *J* = 9.0, 1.5 Hz), 5.97−5.90 (m, 1 H), 5.81 (dd, 1 H, *J* = 11.4 Hz, 3.0 Hz), 5.33−5.29 (m, 1 H), 5.25−5.22 (m, 1 H), 5.19−5.13 (m, 1 H), 4.67−4.59 (m, 2 H), 4.12−4.05 (m, 1 H), 3.11 (dd, 1 H, *J* = 13.2, 6.0 Hz), 2.93 (dd, 1 H, *J* = 13.2, 8.4 Hz), 2.72 (s, 3 H), 2.32 (ddd, 1 H, *J* = 15.2, 11.7, 4.2 Hz), 2.26−2.21 (m, 1 H), 2.16 (s, 3 H), 2.05−2.00 (m, 1 H), 1.77 (d, 3 H, *J* = 1.2 Hz), 1.45 (s, 9 H), 1.02 (d, 3 H, *J* = 6.6 Hz), 0.89 (d, 3 H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 170.2, 167.3, 160.1, 156.2, 149.5, 139.4, 136.5, 132.2, 130.2, 129.5, 128.5, 126.8, 123.7, 118.0, 79.4, 69.1, 65.4, 56.3, 48.7, 40.8, 34.8, 30.4, 28.3, 20.8, 20.0, 19.6, 12.7; Characteristic peaks of minor rotamer: ^1H NMR ($CDCl_3$, 600 MHz) δ 8.03 (s, 1 H), 7.62 (d, 1 H, *J* = 8.4 Hz), 6.72 (d, 1 H, *J* = 10.2 Hz), 2.65 (s, 3 H), 2.17 (s, 3 H), 1.76 (d, 3 H, *J* = 1.2 Hz), 0.99 (d, 3 H, *J* = 6.6 Hz), 0.88 (d, 3 H, *J* = 5.4 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.4, 167.3, 160.0, 149.7, 139.5, 136.6, 132.2, 130.0, 129.5, 128.4, 126.7, 123.5, 117.9, 79.8, 70.7, 65.4, 48.8, 40.8, 28.4, 21.0, 20.3, 19.7; HRMS (ESI) calcd for $C_{33}H_{45}N_3O_7NaS$ (M + Na) 650.2876, found 650.2906.

*(S,E)-Allyl 4-(2-((5S,8R,10R)-5-((S)-sec-Butyl)-1-(9H-fluoren-9-yl)-8-isopropyl-7-methyl-3,6,12-trioxo-2,11-dioxa-4,7-diazatridecan-10-yl)thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoate (**34**).* To a solution of **33** (49 mg, 78 μmol) in dichloromethane (1 mL) was added trifluoroacetic acid (0.4 mL, 5.2 mmol), and the reaction mixture was stirred at room temperature for 4 h. The solution was partitioned between ethyl acetate and water, and the organic layer was washed with saturated sodium bicarbonate, dried (Na_2SO_4), and concentrated to give the crude amine as a colorless oil: MS (ESI) *m*/*z* 528 ($[M + H]^+$). To a solution of the amine in anhydrous DMF (0.3 mL) were added *N*,*N*-diisopropylethylamine (0.03 mL, 0.17 mmol) and Fmoc-Ile-F (60 mg, 0.17 mmol), and the mixture was stirred at room temperature for 18 h. The solution was diluted with ethyl acetate, washed with saturated sodium bicarbonate and brine, dried (Na_2SO_4), concentrated under vacuum, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:2) to give **34** (47 mg, 70%) as a colorless oil: $[\alpha]_D^{23}$ +13.7 (*c* 0.60, CH_2Cl_2); ^1H NMR ($CDCl_3$, 600 MHz) δ 8.05 (s, 1 H), 7.77 (d, 2 H, *J* = 7.5 Hz), 7.59 (dd, 2 H, *J* = 7.5, 3.5 Hz), 7.42−7.28 (m, 6 H), 7.25−7.22 (m, 3 H), 6.71 (dd, 1 H, *J* = 9.5, 1.0 Hz), 5.97−5.90 (m, 1 H), 5.65 (dd, 1 H, *J* = 11.5, 2.0 Hz), 5.47 (d, 1 H, *J* = 9.5 Hz), 5.32 (dd, 1 H, *J* = 17.2, 1.2 Hz), 5.24 (d, 1 H, *J* = 10.5 Hz), 5.21−5.15 (m, 1 H), 4.68−4.60 (m, 2 H), 4.56 (dd, 2 H, *J* = 9.5, 7.0 Hz), 4.42−4.31 (m, 2 H), 4.22 (t, 1 H, *J* = 7.2 Hz), 3.12 (dd, 1 H, *J* = 13.5, 5.5 Hz), 3.01 (s, 3 H), 2.94 (dd, 1 H, *J* = 13.5, 7.5 Hz), 2.36 (ddd, 1 H, *J* = 14.6, 11.6, 2.9 Hz), 2.19 (s, 3 H), 2.11−2.05 (m, 2 H), 1.83−1.73 (m, 6 H), 1.65−1.62 (m, 1 H), 1.05 (d, 3 H, *J* = 6.5 Hz), 1.01 (d, 3 H, *J* = 6.5 Hz), 0.94 (t, 3 H, *J* = 7.2 Hz), 0.83 (d, 3 H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6, 170.1, 170.0, 167.3, 160.0, 156.3, 149.6, 143.9, 143.7, 141.25, 141.22, 139.4, 136.4, 132.2, 130.2, 129.6, 128.5, 127.7, 127.0, 126.8, 125.10, 125.07, 123.8, 119.9, 118.0, 69.3, 67.0, 65.4, 55.7, 48.6, 47.1, 40.9, 37.3, 34.4, 29.9, 23.8, 20.8, 20.1, 19.5, 16.0, 12.8, 11.2; HRMS (ESI) *m*/*z* calcd for $C_{49}H_{58}N_4O_8NaS$ (M + Na) 885.3873, found 885.3911.

*(S,E)-4-(2-((1R,3R)-1-Acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoic Acid 2,2,2-Trifluoroacetate (**35**).* To a solution of the tripeptide **34** (18 mg, 0.021 mmol) in dichloromethane (1 mL) was added tris(2-aminoethyl)amine (0.10 mL, 0.67 mmol). The reaction mixture was stirred at room temperature for 6 h until TLC analysis showed a complete consumption of starting material. The mixture was then diluted in EtOAc, washed with a mixture of saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated to give the crude amine which was used without further purification. To a solution of *N*-methyl-D-pipecolic acid (15 mg, 0.10 mmol) and pentafluorophenol (33 mg, 0.18 mmol) in anhydrous DMF (0.5 mL) was added DCC (26 mg, 0.13 mmol), and the solution was stirred at room temperature for 18 h. The mixture was filtered through a 4 μm microfilter unit to give a clear solution, which was used without further purification to dissolve the amine prepared above. The reaction mixture was stirred at room temperature for 24 h and then directly purified by chromatography on SiO_2 (MeOH/CH_2Cl_2 5:95) to give the slightly impure corresponding allyl ester (16 mg) as a colorless oil. A solution of the allyl ester (16 mg, 0.021 mmol) and dimedone (17 mg, 0.12 mmol) in anhydrous THF (0.4 mL) was treated with $Pd(PPh_3)_4$ (9.0 mg, 0.013 mmol), and the mixture was degassed by the freeze−pump−thaw method. The solution was stirred at room temperature under an Ar atmosphere for 8 h and purified by chromatography on SiO_2 (MeOH/CH_2Cl_2 1:9) to give the crude product as a colorless oil. Further purification by semipreparative HPLC (C-18, MeOH/0.1% TFA in water; MeOH gradient from 70% to 99% over 20 min; 2 mL/min; UV detection at 254 nm) afforded the trifluoroacetic acid salt of **35** (7 mg, 40% over three steps) as a colorless oil: t_R 14.6 min; $[\alpha]_D^{23}$ +5.7 (*c* 0.44, MeOH); IR (cm^{-1}) 3262, 2966, 2936, 2879, 1671, 1544, 1240, 1202, 1180, 1132; ^1H NMR (CD_3OD, 600 MHz) δ 8.14 (s, 1 H, Tuv thiazole CH), 7.27−7.25 (m, 4 H, Tup *o*-, *o*′-, *m*-, and *m*′-phenyl CHs), 7.21−7.19 (m, 1 H, Tup *p*-phenyl CH), 6.78 (dd, 1 H, *J* = 9.3, 1.5 Hz, Tup CH = C), 5.71 (d, 1 H, *J* = 10.8 Hz, Tuv CH-OAc), 5.12 (q, 1 H, *J* = 8.2 Hz, Tup CH-NH), 4.71 (d, 1 H, *J* = 7.8 Hz, Ile CH-NH), 4.50 (br s, 1 H, Tuv CH-N), 3.76 (dd, 1 H, *J* = 12.6, 2.7 Hz, Mep CH-N), 3.48−3.46 (m, 1 H, Mep CH2-N a), 3.12−3.05 (m, 5 H, Tuv N-CH3, Mep CH2-N b, Tup CH2-Ph a), 2.94 (dd, 1 H, *J* = 13.2, 7.8 Hz, Tup CH2-Ph b), 2.72 (s, 3 H, Mep CH3), 2.61−2.32 (m, 2 H, Tuv CH2 a, Tuv CH2 b), 2.16−2.13 (m, 4 H, Tuv COCH3, Mep β CH2 a), 1.94−1.88 (m, 4 H, Ile CH-CH3, Tuv CH-CH3, Mep δ CH2 a, Mep γ CH2 a), 1.83−1.76 (m, 2 H, Mep β CH2 b, Mep δ CH2 b), 1.67 (d, 3 H, *J* = 1.2 Hz, Tup CH3), 1.62−1.58 (m, 2 H, Mep γ CH2 b, Ile CH2 a), 1.30−1.11 (m, 1 H, Ile CH2 b), 1.04−1.01 (m, 6 H, Tuv

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CH-C*H3* a, Ile CH-C*H3*), 0.94 (t, 3 H, *J* = 7.2 Hz, Ile CH2-C*H3*), 0.85 (d, 3 H, *J* = 6.6 Hz, Tuv CH-C*H3* b); ^{13}C NMR (CD_3OD, 150 MHz; contains traces of EtOAc) δ 174.6, 171.8, 171.7, 171.1, 169.2, 162.4, 150.7, 140.9, 138.6, 131.2, 130.6, 129.5, 127.7, 125.6, 71.2, 68.2, 56.2, 56.1, 50.9, 49.6, 43.0, 41.6, 37.4, 35.5, 30.9, 30.8, 30.3, 25.2, 24.0, 22.4, 20.9, 20.5, 20.4, 16.2, 12.8, 11.3; HRMS (ESI) *m*/*z* calcd for $C_{38}H_{56}N_5O_7S$ (M + H) 726.3900, found 726.3900.

*(S,E)-4-(2-((1R,3R)-1-Acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpyrrolidine-2-carboxamido)pentanamido)-4-methylpentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoic Acid 2,2,2-Trifluoroacetate (**37**).* To a solution of *N*-methyl-D-proline (15 mg, 0.12 mmol) and pentafluorophenol (26 mg, 0.14 mmol) in anhydrous DMF (0.5 mL) was added DCC (30 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 18 h and filtered through a 0.45 μm membrane to give a clear solution of **36** that was used without further purification in the next step. To a solution of the tripeptide **34** (17 mg, 0.020 mmol) in dichloromethane (1 mL) was added tris(2-aminoethyl)amine (0.1 mL, 0.67 mmol), and the mixture was stirred at room temperature for 6 h until TLC analysis showed a complete consumption of the starting material. The mixture was then diluted with EtOAc (40 mL), washed with a mixture of saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4), and evaporated to give a colorless oily amine (16 mg) that was used without further purification. A solution of **36** in anhydrous DMF (0.6 mL) was added to the amine (16 mg, 0.020 mmol), and the mixture was stirred at room temperature for 24 h and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:3 to 1:1; followed by MeOH:CH_2Cl_2, 5:95) to give a colorless oil (16 mg, 0.02 mmol) that was treated with a solution of dimedone (17 mg, 0.12 mmol) in anhydrous THF (0.4 mL) and $Pd(PPh_3)_4$ (6 mg, 0.005 mmol). The reaction mixture was degassed by the freeze−pump−thaw method, stirred at room temperature under an Ar atmosphere for 8 h, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:1; then CH_2Cl_2/MeOH, 95:5 to 90:10) to give a colorless oil that was further purified by semipreparative HPLC (C-18 column, methanol/0.1% TFA in water; methanol gradient from 50% to 99% over 40 min; 2 mL/min; UV detection at 254 nm) to provide the trifluoroacetic acid salt of **37** (10 mg, 61% for three steps) as a colorless oil: R_f 26.7 min (C-18 column, 250 × 10 mm; methanol/0.1% TFA in water; methanol gradient from 50% to 99% over 40 min; 2 mL/min; UV detection at 254 nm); $[\alpha]_D^{23}$ +7.7 (*c* 0.43, MeOH); IR (cm^{-1}) 3278, 3062, 2968, 2942, 2880, 1747, 1674, 1642, 1544, 1202, 1134; ^{1}H NMR (CD_3OD, 600 MHz) δ 8.14 (s, 1 H, Tuv thiazole C*H*), 7.28−7.25 (m, 4 H, Tup *o*-, *o*′-, *m*-, and *m*′-phenyl C*H*s), 7.22−7.19 (m, 1 H, Tup *p*-phenyl C*H*), 6.78 (dd, 1 H, *J* = 9.9, 1.5 Hz, Tup C*H* = C), 5.73−5.71 (m, 1 H, Tuv C*H*-OAc), 5.12 (q, 1 H, *J* = 7.8 Hz, Tup C*H*-NH), 4.75 (d, 1 H, *J* = 7.8 Hz, Ile C*H*-NH), 4.45 (br s, 1 H, Tuv C*H*-N) 4.08 (t, 1 H, *J* = 9.3 Hz, Mep C*H*-N), 3.72−3.68 (m, 1 H, *N*-Me-Pro C*H2*-N a), 3.22−3.18 (m, 1 H, *N*-Me-Pro C*H2*-N b), 3.14 (s, 3 H, Tuv N-C*H3*), 3.11 (dd, 1 H, *J* = 12.9, 6.9 Hz, Tup C*H2*-Ph a), 2.94 (dd, 1 H, *J* = 13.8, 8.4 Hz, Tup C*H2*-Ph b), 2.85 (s, 3 H, *N*-Me-Pro N-C*H3*), 2.64−2.59 (m, 1 H, *N*-Me-Pro C*H2* a), 2.43−2.38 (m, 2 H, Tuv C*H2* a, Tuv C*H2* b), 2.24−2.22 (m, 1 H, *N*-Me-Pro C*H2* b), 2.15 (s, 3 H, Tuv COC*H3*), 2.08−2.02 (m, 2 H, *N*-Me-Pro C*H2* c, *N*-Me-Pro C*H2* d), 1.95−1.91 (m, 2 H, Tuv C*H*-CH3, Ile C*H*-CH3), 1.67 (d, 3 H, *J* = 1.2 Hz, Tup C*H3*), 1.63−1.59 (m, 1 H, Ile C*H2* a), 1.32−1.14 (m, 1 H, Ile C*H2* b), 1.04 (d, 3 H, *J* = 6.6 Hz, Tuv CH-C*H3* a), 1.01 (d, 3 H, *J* = 7.2 Hz, Ile CH-C*H3*), 0.94 (t, 3 H, *J* = 7.2 Hz, Ile CH2-C*H3*), 0.86 (d, 3 H, *J* = 6.6 Hz, Tuv CH-C*H3* b); ^{13}C NMR (CD_3OD, 150 MHz) δ 174.6, 171.9, 171.7, 171.1, 168.4, 162.3, 150.6, 140.9, 138.6, 131.2, 130.6, 129.4, 127.7, 125.6, 71.2, 70.1, 57.2, 56.2, 50.9, 49.8, 49.6, 41.6, 40.7, 37.4, 35.5, 31.0, 30.7, 25.3, 23.7, 20.8, 20.5, 20.3, 16.2, 12.8, 11.3; HRMS (ESI+) *m*/*z* calcd for $C_{37}H_{54}N_5O_7S$ ($M + H^+$) 712.3744, found 712.3738.

*(S,E)-2-Methyl-N-(2-methylpropylidene)propane-2-sulfinamide (**40**).* According to the protocol developed by Ellman and co-workers,[66] condensation of **38** (4.70 g, 38.8 mmol) with freshly distilled **39** (13.0 mL, 140 mmol) afforded **40** (6.55 g, 96%) as a colorless oil: $[\alpha]_D^{23}$ +278.4 (*c* 1.12, CH_2Cl_2). Chiral HPLC analysis (Chiralcel OD, 250 × 4.6 mm; isocratic elution with 90% isopropanol in hexanes, 0.5 mL/min; detection at 254 nm) gave a single peak at 7.84 min, and the *ee* was determined to be >99% (analysis of a racemic sample showed the enantiomer peak at 8.60 min).

*(S)-2-Methyl-N-((S)-4-methyl-1-(trimethylsilyl)pent-1-yn-3-yl)-propane-2-sulfinamide (**41**).*[77] To a solution of trimethylsilylacetylene (9.86 g, 98.4 mmol) in anhydrous toluene (160 mL) cooled in a dry ice/acetone bath was added a solution of *n*-BuLi in hexanes (1.30 M, 54.0 mL, 70.2 mmol) dropwise over 20 min. The reaction mixture was stirred for 30 min before a cooled solution (−78 °C) of **40** (6.78 g, 38.7 mmol) and trimethylaluminum (2.0 M solution in toluene, 23.0 mL, 46.0 mmol) in toluene (40 mL) was cannulated dropwise (40 min) into the solution. The mixture was allowed to gradually warm to room temperature overnight, cooled in an ice bath, and slowly quenched with water (30 mL) (*CAUTION*: gas-forming and exothermic). The solution was partitioned between ethyl acetate (50 mL) and hydrochloric acid (1 N, 150 mL). The aqueous phase was extracted with ethyl acetate (60 mL × 2), and the combined organic layers were washed with saturated sodium bicarbonate and brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes 1:10−1:5) to give **41** (10.0 g, 94%) as a colorless oil: $[\alpha]_D^{23}$ +33.4 (*c* 1.10, CH_2Cl_2); R_f 0.26 (EtOAc/hexanes, 1:3); IR (cm^{-1}) 3208, 2960, 2901, 2173, 1468, 1249, 1058, 840; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.91 (t, 1 H, *J* = 5.4 Hz), 3.28 (d, 1 H, *J* = 5.4 Hz), 1.94−1.7 (m, 1 H), 1.21 (s, 9 H), 0.98 (d, 3 H, *J* = 6.6 Hz), 0.96 (d, 3 H, *J* = 6.6 Hz), 0.15 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 103.7, 90.2, 56.1, 53.8, 33.3, 22.5, 18.9, 17.1, −0.2; HRMS (ESI+) calcd for $C_{13}H_{27}NOSSiNa$ ($[M + Na]^+$) 296.1475, found 296.1472. Diastereomeric excess (de) was determined to be 98.7% based on ^{1}H NMR integration of the NH doublet (δ 3.28, *J* = 6.0 Hz for the major diastereomer; δ 3.22, *J* = 6.6 Hz for the minor diastereomer).

*(S)-N,2-Dimethyl-N-((S)-4-methyl-1-(trimethylsilyl)pent-1-yn-3-yl)propane-2-sulfinamide (**42**).* To a solution of diisopropylamine (5.0 mL, 35.0 mmol) in anhydrous THF (50 mL) cooled at −78 °C was added dropwise butyllithium (1.27 M solution in hexanes, 20.0 mL, 25.4 mmol), and the resulting yellow mixture was stirred for 15 min before it was transferred via cannula over 15 min to a solution of **41** (5.23 g, 19.1 mmol) and HMPA (3.80 mL, 21.6 mmol) in anhydrous THF (150 mL) cooled in a dry ice/acetone bath. A red solution was generated, which was stirred for 10 min before iodomethane (5.0 mL, 79.5 mmol) was added dropwise. The reaction mixture turned yellow upon the addition of iodomethane and was stirred at −78 °C for 4 h and then quenched with water (20 mL). Most of the THF was evaporated under reduced pressure. The residue was partitioned between ethyl acetate (80 mL) and hydrochloric acid (1 N, 20 mL), and the organic phase was washed with saturated sodium bicarbonate, sodium thiosulfate (1 M) and brine, dried (Na_2SO_4), concentrated, and chromatographed on SiO_2 (EtOAc/hexanes, 1:7) to give **42** (3.85 g, 70%) as a yellow oil: R_f 0.40 (EtOAc/hexanes, 1:3); $[\alpha]_D^{23}$ −136.7 (*c* 1.44, CH_2Cl_2); IR (cm^{-1}) 2960, 2872, 2166, 1467, 1249, 1084, 1022, 840, 759; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.66 (d, 1 H, *J* = 10.2 Hz), 2.63 (s, 3 H), 1.93−1.85 (m, 1 H), 1.20 (s, 9 H), 1.06 (d, 3 H, *J* = 7.2 Hz), 0.94 (d, 3 H, *J* = 6.6 Hz), 0.16 (s, 9 H); ^{13}C NMR (CD_3OD, 150 MHz) δ 104.7, 91.8, 61.8, 60.7, 32.8, 28.7, 23.7, 20.4, 20.1, −0.1; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{29}NOSSiNa$ (M + Na) 310.1637, found 310.1616.

*(S)-tert-Butyl Methyl(4-methylpent-1-yn-3-yl)carbamate (**43**).* To a solution of **42** (2.0 g, 6.96 mmol) in MeOH (40 mL) was added potassium carbonate (2.0 g, 14.47 mmol), and the mixture was stirred at room temperature overnight. The suspension was filtered, and the filtrate was evaporated, redissolved in water (20 mL), and extracted with ethyl acetate (60 mL). The organic phase was washed with brine, dried (Na_2SO_4), and concentrated to give the desilylated product (1.67 g, 100%) as a pale yellow oil that was dissolved in methanol (40 mL) and treated with hydrogen chloride in methanol (1.25 M, 12.0 mL, 15.0 mmol). The reaction mixture was stirred at room temperature for 3 h. TLC analysis (EtOAc/hexanes, 1:3) showed only a baseline spot. The solvent was evaporated under reduced pressure to give the corresponding amine hydrochloride as a colorless solid that was used without further purification. To a solution of the amine hydrochloride in anhydrous dichloromethane (30 mL) were

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added diisopropylethylamine (3.5 mL, 21 mmol), DMAP (0.10 g, 0.81 mmol), and di-*tert*-butyl dicarbonate (2.30 g, 10.22 mmol), and the mixture was stirred at room temperature for 24 h, diluted in dichloromethane, washed with saturated sodium bicarbonate and brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:20) to give **43** (1.26 g, 85% for three steps) as a colorless oil: $[\alpha]_D^{23}$ −61.2 (*c* 1.57, CH_2Cl_2); ^{1}H NMR analysis at room temperature showed a 1.2:1 mixture of rotamers. Major rotamer: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.63 (d, 1 H, J = 7.8 Hz), 2.81 (s, 3 H), 2.28 (bs, 1 H), 1.93−1.80 (m, 1 H), 1.44 (s, 9 H), 1.04 (d, 3 H, J = 6.9 Hz), 0.85 (d, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.5, 81.6, 79.7, 72.4, 53.7, 31.7, 29.5, 28.3, 19.5, 18.5; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.41 (d, 1 H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.0, 79.9, 72.7, 54.9, 29.2; IR (film, cm^{-1}) 3310, 3249, 2968, 2930, 2874, 1695, 1388, 1309, 1152; HRMS (ESI) *m*/*z* calcd for $C_9H_{14}NO_2$ (M-C_3H_7) 168.1024, found 168.1023.

(S)-Ethyl 2-(3-(tert-Butoxycarbonylmethylamino)-4-methylpent-1-ynyl)thiazole-4-carboxylate (44). A mixture of **43** (0.482 g, 2.28 mmol), **6** (0.632 g, 2.85 mmol), copper(I) iodide (14 mg, 0.07 mmol), and bis(benzonitrile)palladium(II) chloride (48 mg, 0.12 mmol) in anhydrous acetonitrile (2.0 mL) was degassed with nitrogen by the freeze−pump−thaw method. *N*,*N*-Diisopropylethylamine (0.57 mL, 3.41 mmol) was then added followed by tri-*tert*-butylphosphine (1.0 M solution in toluene, 0.22 mL, 0.22 mmol), and the mixture was stirred at room temperature for 24 h to give a red solution. The mixture was diluted in EtOAc, washed with hydrochloric acid (1 N), saturated sodium bicarbonate, and brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:5) to give **44** (0.585 g, 70%) as a yellow oil: R_f 0.40 (EtOAc/hexanes, 1:3); $[\alpha]_D^{23}$ −81.2 (*c* 1.29, CH_2Cl_2); IR (cm^{-1}) 2971, 2931, 2874, 2232, 1720, 1690, 1456, 1366, 1383, 1307, 1236, 1144, 1090; ^{1}H NMR analysis at room temperature showed a 1.4:1 mixture of rotamers. Major rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.14 (s, 1 H), 4.91 (d, 1 H, J = 9.6 Hz), 4.41 (q, 2 H, J = 7.0 Hz), 2.84 (s, 3 H), 1.98 (bs, 1 H), 1.46 (s, 9 H), 1.39 (t, 3 H, J = 6.9 Hz), 1.10 (d, 3 H, J = 6.6 Hz), 0.90 (d, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 160.8, 155.6, 149.0, 147.4, 128.4, 94.4, 93.8, 80.1, 61.7, 54.4, 31.3, 29.9, 28.3, 19.7, 18.5, 14.3; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.65 (d, 1 H, J = 9.0 Hz), 2.86 (s, 3 H), 1.79 (bs, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 154.8, 80.4, 55.7, 31.6, 29.6, 19.9, 18.8; HRMS (ESI) calcd for $C_{18}H_{26}N_2O_4NaS$ *m*/*z* (M + Na) 389.1511, found 389.1501. Chiral HPLC (Chiralcel OD HPLC column, 250 × 4.6 mm; isocratic elution with 10% 2-propanol in hexanes, 0.8 mL/min; detection at 254 nm) showed two peaks at 8.00 and 8.99 min with a 0.07:100 ratio, and the enantiomeric excess was determined to be 99.9%.

(R)-Ethyl 2-(3-(tert-Butoxycarbonylmethylamino)-4-methylpentyl)thiazole-4-carboxylate (45). A solution of **44** (0.40 g, 1.09 mmol) in MeOH (20 mL) was hydrogenated in an H-Cube (30 bar, 30 °C, 10% Pd/C catalyst, 2 mL/min) for two passages, and TLC analysis showed an incomplete conversion. The hydrogen pressure was raised to 50 bar, and the mixture was recycled on the H-Cube at 1 mL/min for 4 h. Concentration followed by chromatography on SiO_2 (EtOAc/hexanes 1:3) gave **45** (0.34 g, 84%) as a yellow oil: R_f 0.18 (EtOAc/hexanes 1:3); $[\alpha]_D^{23}$ −11.0 (*c* 1.72, CH_2Cl_2); IR (cm^{-1}) 2969, 2932, 2875, 1734, 1716, 1685, 1481, 1366, 1235, 1202, 1150; ^{1}H NMR analysis at room temperature showed a 1.1:1 mixture of rotamers. Major rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.06 (s, 1 H), 4.45−4.40 (m, 2 H), 3.86 (t, 1 H, J = 13.2 Hz), 2.99−2.96 (m, 2 H), 2.66 (s, 3 H), 2.20−2.10 (m, 1 H), 1.88−1.81 (m, 1 H), 1.71−1.67 (m, 1 H), 1.46 (s, 9 H), 1.41 (t, 3 H, J = 7.2 Hz), 0.97 (d, 3 H, J = 6.6 Hz), 0.85 (d, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.8, 161.5, 156.6, 146.9, 126.9, 79.2, 61.41, 61.36, 30.7, 30.5, 30.0, 28.4, 20.1, 19.9, 19.6, 14.4; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.05 (s, 1 H), 3.69 (bs, 1 H), 2.97 (t, 2 H, J = 8.4 Hz), 2.70 (s, 3 H), 1.43 (s, 9 H), 1.40 (t, 3 H, J = 6.9 Hz), 0.96 (d, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.4, 161.4, 156.4, 146.7, 126.8, 30.7, 20.2; HRMS (ESI) calcd for $C_{18}H_{30}N_2O_4NaS$ *m*/*z* (M + Na) 393.1824, found 393.1802.

(S,E)-Ethyl 4-(2-((R)-3-(tert-Butoxycarbonylmethylamino)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoate (47). To a solution of **45** (0.204 g, 0.55 mmol) in EtOH (5 mL) was added 1 N NaOH (1.0 mL, 1.0 mmol), and the solution was stirred at room temperature for 6 h. TLC analysis (EtOAc/hexanes, 1:2) showed a single spot at baseline. The solution was then adjusted to pH 3 with 1 N HCl and extracted with EtOAc (30 mL × 3). The combined organic layers were dried (Na_2SO_4) and concentrated to give crude acid (0.195 g, 100%) as a colorless oil. To a solution of **15** (0.27 g, 0.81 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.30 mL, 4.00 mmol), and the mixture was stirred at room temperature for 4 h. TLC analysis (EtOAc/hexanes, 1:2) showed a single spot at baseline. The mixture was then diluted in ethyl acetate, washed with 0.5 N NaOH (20 mL) and saturated sodium bicarbonate (20 mL), dried (Na_2SO_4), and concentrated to give **46** (0.18 g, 97%) as a colorless oil. To a solution of the acid derived from **45** (90 mg, 0.25 mmol), 1-hydroxybenzotriazole hydrate (50 mg, 0.33 mmol), and DMAP (4.4 mg, 0.035 mmol) in anhydrous dichloromethane (1 mL) was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (63 mg, 0.32 mmol), and the mixture was stirred for 20 min before a solution of **46** (95 mg, 0.41 mmol) in dichloromethane (1 mL) was added. Stirring was continued overnight, and 1 N NaOH (10 mL) was added. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:3) to give **47** (0.135 g, 96% for two steps) as a colorless oil: R_f 0.57 (EtOAc/hexanes, 1:1); $[\alpha]_D^{23}$ +25.6 (*c* 2.14, CH_2Cl_2); IR (cm^{-1}) 3295, 2971, 2931, 1709, 1677, 1537, 1492, 1366, 1253, 1138, 734; ^{1}H NMR analysis at room temperature showed a 1.1:1 mixture of rotamers. Major rotamer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (s, 1 H), 7.48 (d, 1 H, J = 8.0 Hz), 7.29−7.26 (m, 2 H), 7.23−7.19 (m, 3 H), 6.64 (d, 1 H, J = 7.5 Hz), 5.19−5.11 (m, 1 H), 4.20−4.14 (m, 2 H), 3.87 (t, 1 H, J = 11.0 Hz), 3.12−3.07 (m, 1 H), 2.95−2.82 (m, 3 H), 2.67 (s, 3 H), 2.19−2.07 (m, 1 H), 1.83−1.79 (m, 1 H), 1.75 (s, 3 H), 1.71−1.67 (m, 1 H), 1.40 (s, 9 H), 1.27 (t, 3 H, J = 7.0 Hz), 0.98 (d, 3 H, J = 6.5 Hz), 0.86 (d, 3 H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.8, 167.7, 160.3, 156.5, 149.3, 139.1, 136.6, 130.2, 129.4, 128.4, 126.7, 122.6, 60.7, 60.2, 48.7, 40.9, 30.7, 30.5, 30.1, 29.6, 29.2, 28.4, 20.1, 19.9, 14.2, 12.7; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (s, 1 H), 7.50 (d, 1 H, J = 8.5 Hz), 6.65 (d, 1 H, J = 7.5 Hz), 3.67 (bs, 1 H), 2.68 (s, 3 H), 1.74 (s, 3 H), 1.47 (s, 9 H), 0.87 (d, 3 H, J = 6.0 Hz); ^{13}C NMR (CDCl3, 150 MHz) δ 170.6, 167.7, 160.3, 156.4, 149.2, 139.1, 130.3, 129.5, 122.5, 40.9, 30.2, 28.4, 20.3, 19.6; R_f = 0.57 (EtOAc/hexanes, 1:1); HRMS (ESI) calcd for $C_{30}H_{43}N_3O_5NaS$ ([M + Na]$^+$) 580.2821, found 580.2816.

(S,E)-Ethyl 4-(2-((R)-3-((2S,3S)-2-(((9H-fluoren-9-yl)methoxy)-carbonylamino)-N,3-dimethylpentanamido)-4-methylpentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoate (48). To a solution of **47** (78 mg, 0.14 mmol) in dichloromethane (1 mL) was added TFA (0.2 mL, 2.66 mmol), and the mixture was stirred at room temperature for 4 h. TLC analysis (EtOAc/hexanes 1:1) showed only one spot at baseline. The mixture was partitioned in a mixture of EtOAc (40 mL) and water (10 mL), and the organic phase was washed with saturated sodium bicarbonate, dried (Na_2SO_4), and concentrated to give the crude amine as a colorless oil. To a solution of the amine in anhydrous dichloromethane (2 mL) was added *N*,*N*-diisopropylethylamine (0.03 mL, 0.18 mmol) followed by Fmoc-Ile-F (64 mg, 0.18 mmol), and the mixture was stirred at room temperature overnight, diluted in ethyl acetate, washed with 1 N NaOH and brine, dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:3) to give **48** (0.10 g, 90% for two steps) as a colorless solid: R_f 0.38 (EtOAc/hexanes, 1:1); mp 69−72 °C; $[\alpha]_D^{23}$ +19.0 (*c* 1.14, CH_2Cl_2); IR (cm^{-1}) 3279, 2965, 2876, 1708, 1634, 1536, 1249, 740; ^{1}H NMR analysis showed a 5:1 mixture of rotamers at room temperature. Major rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.94 (s, 1 H), 7.77 (d, 2 H, J = 7.2 Hz), 7.60 (t, 3 H, J = 6.3 Hz), 7.40 (t, 2 H, J = 7.5 Hz), 7.31−7.27 (m, 4 H), 7.19−7.18 (m, 2 H), 6.72 (d, 1 H, J = 9.0 Hz), 5.60 (d, 1 H, J = 9.6 Hz), 5.20−5.11 (m, 1 H), 4.59 (t, 1 H, J = 8.4 Hz), 4.42−4.35 (m, 3 H), 4.23 (t, 1 H, J = 7.2 Hz), 4.20−4.15 (m, 3 H), 3.14 (dd, 1 H, J = 13.5, 5.7 Hz), 3.00 (s, 3 H),

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2.94 (dd, 1 H, J = 13.5, 7.5 Hz), 2.86−2.82 (m, 3 H), 2.15−2.14 (m, 1 H), 1.88−1.82 (m, 3 H), 1.75 (s, 3 H), 1.64−1.61 (m, 1 H), 1.27 (t, 3 H, J = 6.9 Hz), 1.20−1.15 (m, 1 H), 1.01 (d, 3 H, J = 7.2 Hz), 1.00 (d, 3 H, J = 7.2 Hz), 0.92 (t, 3 H, J = 7.2 Hz), 0.82 (d, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.3, 169.7, 167.8, 160.3, 156.4, 149.5, 143.9, 143.7, 141.24, 141.20, 139.2, 136.7, 130.2, 129.5, 128.4, 127.6, 127.0, 126.7, 125.1, 125.0, 122.6, 119.9, 66.9, 60.7, 55.7, 48.8, 47.1, 40.9, 37.5, 30.2, 30.0, 29.3, 23.9, 20.0, 19.6, 15.9, 14.2, 12.7, 11.2; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88 (s, 1 H), 7.56 (t, 3 H, J = 6.6 Hz), 6.67 (d, 1 H, J = 9.6 Hz), 5.65 (d, 1 H, J = 10.2 Hz), 3.09 (dd, 1 H, J = 13.8, 6.6 Hz), 1.72 (s, 3 H), 1.06 (d, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.6, 169.9, 167.7, 160.2, 156.2, 149.4, 143.6, 136.7, 130.1, 129.4, 128.4, 126.6, 124.9, 123.0, 67.2, 60.6, 55.3, 48.8, 47.1, 37.9, 29.6, 23.4, 20.3, 16.2, 12.6, 11.3; HRMS (ESI) calcd for $C_{46}H_{57}N_4O_6S$ ([M + H]$^+$) 793.3999, found 793.4016.

*(S,E)-4-(2-((R)-3-((2S,3S)-N,3-Dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoic Acid 2,2,2-Trifluoroacetate (**49**).* To a solution of *N*-methyl D-pipecolic acid (23 mg, 0.16 mmol) and pentafluorophenol (48 mg, 0.26 mmol) in anhydrous DMF (0.5 mL) was added EDC hydrochloride (37 mg, 0.19 mmol). The reaction mixture was stirred at room temperature overnight to give a clear yellow solution, which was directly used in the following coupling reaction. To a solution of **48** (98 mg, 0.12 mmol) in dichloromethane (1 mL) was added tris(2-aminoethyl)amine (0.1 mL, 0.64 mmol), and the mixture was stirred at room temperature for 4 h, diluted in EtOAc (50 mL), washed with brine (10 mL) and saturated sodium bicarbonate (10 mL), dried (Na_2SO_4), and concentrated to give the crude amine as a colorless oil that was used without further purification. A solution of activated Mep ester (~0.46 M, 0.45 mL, 0.21 mmol) in DMF prepared above was treated with the crude amine, and the resulting yellow reaction mixture was stirred at room temperature for 18 h, diluted with EtOAc (60 mL), washed with 1 N NaOH (10 mL) and brine (10 mL), dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:3 to 1:1; followed by MeOH/CH_2Cl_2 5:95) to give the coupling product **48a** (79 mg, 92% for two steps) as a colorless foam: R_f 0.32 (MeOH/CH_2Cl_2 5:95); $[\alpha]_D^{23}$ +46.9 (c 1.28, CH_2Cl_2); ^{1}H NMR analysis showed a 10:1 mixture of rotamers at room temperature. Major rotamer: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.98 (s, 1 H), 7.25−7.24 (m, 4 H), 7.21−7.17 (m, 1 H), 6.80 (dd, J = 9.5, 1.5 Hz, 1 H), 5.10 (q, J = 8.0 Hz, 1 H), 4.74 (d, J = 8.5 Hz, 1 H), 4.37 (bs, 1 H), 4.16 (q, J = 7.0 Hz, 2 H), 3.13−3.10 (m, 4 H), 2.99−2.92 (m, 3 H), 2.88−2.72 (m, 2 H), 2.25−2.13 (m, 5 H), 2.01−1.76 (m, 5 H), 1.68 (d, J = 1.5 Hz, 3 H), 1.63−1.55 (m, 3 H), 1.36−1.16 (m, 6 H), 0.99 (d, J = 7.5 Hz, 3 H), 0.97 (d, J = 7.0 Hz, 3 H), 0.90 (t, J = 7.2 Hz, 3 H), 0.79 (d, J = 7.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.2, 173.3, 169.8, 167.8, 160.4, 149.5, 139.2, 136.7, 130.2, 129.5, 128.4, 126.7, 122.6, 69.6, 62.8, 60.7, 58.5, 55.4, 53.1, 48.8, 44.8, 41.0, 37.1, 30.3, 30.2, 30.1, 29.4, 25.0, 24.6, 23.2, 20.1, 19.6, 15.9, 14.2, 12.7, 10.9; characteristic peaks of the minor rotamer ^{1}H NMR (CD_3OD, 500 MHz) δ 7.98 (s, 1 H), 4.79 (d, J = 8.5 Hz, 1 H), 1.10 (d, J = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.9, 167.8, 160.4, 139.2, 136.8, 130.2, 126.7, 122.9, 60.7, 48.9, 31.4, 30.6, 29.6, 20.6, 20.4, 16.6, 11.4. To a solution of the ethyl ester (41 mg, 0.059 mmol) in 95% ethanol (0.5 mL) was added 0.5 N LiOH (0.5 mL, 0.25 mmol), and the mixture was stirred at room temperature for 8 h. HCl (0.1 N, 5 mL, 0.5 mmol) was added, and the mixture was extracted with EtOAc (30 mL × 2). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by chromatography (MeOH/CH_2Cl_2 1:9) to give crude product as a colorless oil. Further purification by semipreparative HPLC (Microsorb C-18 column, 250 × 10 mm; methanol/0.1% TFA in water; methanol gradient from 50% to 100% over 30 min; 2 mL/min; UV detection at 254 nm) afforded the trifluoroacetic acid salt of **49** (36 mg, 91%) as a colorless oil: R_f 0.35 (MeOH/CH_2Cl_2 1:9); $[\alpha]_D^{23}$ +9.0 (c 2.42, MeOH); IR (cm^{-1}); ^{1}H NMR analysis showed a 10.6:1 mixture of rotamers at room temperature. Major rotamer: ^{1}H NMR analysis showed a 10.6:1 mixture of rotamers at room temperature. Major rotamer: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.01 (s, 1 H, Tuv thiazole C*H*), 7.28−7.24 (m, 4 H, Tup *o*-, *o*′-, *m*-, and *m*′-phenyl C*H*s), 7.21−7.17 (m, 1 H, Tup *p*-phenyl C*H*), 6.81 (dd, J = 9.2, 1.2 Hz, 1 H, Tup C*H* = C), 5.11 (q, J = 7.8 Hz, 1 H, Tup C*H*-NH), 4.70 (d, J = 8.0 Hz, 1 H, Ile CH-NH), 4.33 (bs, 1 H, Tuv CH-N), 3.76 (dd, J = 12.0, 2.5 Hz, 1 H, Mep C*H*-N), 3.48 (d, J = 12.5 Hz, 1 H, Mep C*H*2-N a), 3.13−3.05 (m, 5 H, Mep C*H*2-N b, Tuv N-C*H*3, Tup C*H*2-Ph a), 3.00−2.83 (m, 3 H, Tup C*H*2-Ph b, Tuv C*H*2-CH2-CH a, Tuv C*H*2-CH2-CH b), 2.74 (s, 3 H, Mep N-C*H*3), 2.23−2.14 (m, 2 H, Tuv CH2-C*H*2-CH a, Mep β C*H*2 a), 2.04−1.92 (m, 4 H, Mep γ C*H*2 a, Mep δ C*H*2 a, Ile C*H*-CH3, Tuv C*H*-CH3), 1.84−1.73 (m, 3 H, Tuv CH2-C*H*2-CH b, Mep β C*H*2 b, Mep δ C*H*2 b), 1.67 (s, 3 H, Tup C*H*3), 1.64−1.58 (m, 2 H, Mep γ C*H*2 b, Ile C*H*2 a), 1.29−1.20 (m, 1 H, Ile C*H*2 b), 1.04 (d, J = 7.0 Hz, 3 H, Tuv CH-C*H*3 a), 0.99 (d, J = 6.0 Hz, 3 H, Ile CH-C*H*3), 0.94 (t, J = 7.0 Hz, 3 H, Ile CH2-C*H*3), 0.80 (d, J = 6.5 Hz, 3 H, Tuv CH-C*H*3 b); ^{1313}C NMR ($CDCl_3$, 150 MHz) δ 174.6, 171.9, 171.1, 169.1, 162.6, 162.1 (q, J_{C-F} = 36.2 Hz), 150.4, 141.1, 138.6, 131.1, 130.6, 129.4, 127.7, 124.4, 118.0 (q, J_{C-F} = 285.8 Hz), 68.2, 60.3, 56.2, 56.0, 50.9, 49.6, 42.9, 41.7, 37.6, 31.4, 31.0, 30.29, 30.25, 25.6, 24.0, 22.4, 20.5, 20.3, 16.0, 12.9, 11.3; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.99 (s, 1 H), 3.82 (dd, J = 12.8, 3.2 Hz, 1 H), 2.72 (s, 3 H), 1.12 (d, J = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.6, 172.5, 169.2, 141.1, 138.6, 131.2, 130.6, 124.7, 68.2, 64.5, 56.2, 50.8, 43.1, 41.5, 38.2, 32.5, 31.5, 30.5, 28.3, 25.7, 24.3, 20.8, 20.6, 16.4, 11.8; 3288, 2963, 2932, 2876, 1704, 1637, 1542, 1495, 1265, 1237, 732, 700; MS (ESI) m/z 668 ([M + H]$^+$); HRMS (ESI) m/z calcd for $C_{36}H_{54}N_5O_5S$ (M + H) 668.3846, found 668.3832.

*(S,E)-Ethyl 4-(2-((R)-3-((S)-2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-N,3-dimethylbutanamido)-4-methylpentyl)-thiazole-4-carboxamido)-2-methyl-5 phenylpent-2-enoate (**47a**).* To a solution of **47** (57 mg, 0.10 mmol) in dichloromethane (1 mL) was added TFA (0.2 mL, 2.66 mmol), and the mixture was stirred at room temperature for 4 h. TLC analysis (EtOAc/hexanes, 1:1) showed only one spot at baseline. The mixture was diluted with a mixture of EtOAc (40 mL) and water (10 mL), washed with saturated $NaHCO_3$ (10 mL × 2), dried (Na_2SO_4), and concentrated to give the crude amine as a colorless oil. A solution of the amine in anhydrous dichloromethane (2 mL) and diisopropylethylamine (0.02 mL, 0.12 mmol) was treated with Fmoc-Val-F (79 mg, 0.11 mmol), stirred at room temperature overnight, diluted with EtOAc (40 mL), washed with 1 N NaOH (10 mL) and brine (10 mL), dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:3) to give the tripeptide ester **47a** (70 mg, 88% for two steps) as a colorless oil: R_f = 0.39 (EtOAc/hexanes, 1:1); $[\alpha]_D^{23}$ +20.8 (c 0.78, CH_2Cl_2); IR (cm^{-1}) 3292, 2964, 2874, 1708, 1637, 1536, 1494, 1450, 1236, 1028, 740; ^{1}H NMR analysis showed a 5:1 mixture of rotamers at room temperature. Major rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.94 (s, 1 H), 7.77 (d, J = 7.8 Hz, 2 H), 7.60−7.56 (m, 3 H), 7.40 (t, J = 7.2 Hz, 2 H), 7.32−7.29 (m, 3 H), 7.24−7.23 (m, 3 H), 6.72 (d, J = 9.6 Hz, 1 H), 5.67 (d, J = 9.6 Hz, 1 H), 5.20−5.14 (m, 1 H), 4.56 (t, J = 8.1 Hz, 1 H), 4.44−4.33 (m, 3 H), 4.24 (t, J = 6.6 Hz, 1 H), 4.19−4.12 (m, 3 H), 3.13 (dd, J = 13.8, 5.4 Hz, 1 H), 2.99 (s, 3 H), 2.95 (dd, J = 13.5, 7.8 Hz, 1 H), 2.89−2.79 (m, 3 H), 2.15−2.11 (m, 1 H), 2.07−2.03 (m, 1 H), 1.92−1.84 (m, 1 H), 1.76 (s, 3 H), 1.27 (t, J = 7.2 Hz, 3 H), 1.04 (d, J = 6.6 Hz, 3 H), 1.00 (d, J = 7.2 Hz, 3 H), 0.98 (d, J = 7.2 Hz, 3 H), 0.82 (d, J = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.2, 169.8, 167.8, 160.3, 156.5, 149.5, 143.9, 143.7, 141.23, 141.21, 139.2, 136.6, 130.2, 129.5, 128.4, 127.6, 127.0, 126.7, 125.09, 125.05, 122.7, 119.9, 67.0, 60.7, 56.4, 48.8, 47.1, 40.9, 31.1, 30.2, 30.0, 29.3, 20.2, 20.0, 19.8, 19.6, 17.4, 14.2, 12.7; characteristic peaks of the minor rotamer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.90 (s, 1 H), 6.68 (d, J = 11.4 Hz, 1 H), 5.73 (d, J = 13.2 Hz, 1 H), 1.71 (s, 3 H), 0.94 (d, J = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.5, 169.9, 167.7, 156.3, 143.7, 136.8, 130.1, 129.4, 128.3, 126.6, 125.0, 123.1, 67.2, 60.6, 55.6, 48.9, 47.1, 31.2, 29.6, 20.3, 20.1, 16.8, 12.6; HRMS (ESI) m/z calcd for $C_{45}H_{54}N_4O_6NaS$ (M + Na) 801.3662, found 801.3667.

(S,E)-4-(2-((R)-3-((S)-N,3-Dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)butanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpent-2-enoic Acid 2,2,2-Trifluoroacetate

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(**50**). To a solution of **47a**(68 mg, 0.087 mmol) in dichloromethane (1 mL) was added tris(2-aminoethyl)amine (0.1 mL, 0.64 mmol), and the mixture was stirred at room temperature for 4 h, diluted in EtOAc (50 mL), washed with brine (10 mL) and saturated sodium bicarbonate (10 mL), dried (Na_2SO_4), and concentrated to give the crude amine as a colorless oil that was used without further purification. A DMF solution of Mep-OC_6F_5 (0.46 M, 0.35 mL, 0.16 mmol) was added to the amine, and the resulting yellow solution was stirred at room temperature for 18 h. The mixture was dissolved in EtOAc (60 mL), washed with 1 N NaOH (10 mL) and brine (10 mL), dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (EtOAc/hexanes, 1:3 to 1:1; followed by MeOH/CH_2Cl_2 5:95) to give the tetrapeptide ester **47b** (54 mg, 91% for two steps) as a colorless oil: R_f 0.35 (MeOH/CH_2Cl_2 5:95); $[\alpha]_D^{23}$ +42.0 (*c* 2.46, CH_2Cl_2); IR (cm^{-1}) 3385, 3288, 2963, 2940, 2874, 1708, 1665, 1637, 1536, 1496, 1265, 983, 732, 700; ^1H NMR analysis showed a 10:1 mixture of rotamers at room temperature. Major rotamer: ^1H NMR (CD_3OD, 500 MHz) δ 7.98 (s, 1 H), 7.25−7.24 (m, 4H), 7.20−7.16 (m, 1 H), 6.78 (dd, *J* = 9.2, 1.2 Hz, 1 H), 5.13−5.08 (m, 1 H), 4.68 (d, *J* = 8.0 Hz, 1 H), 4.34 (bs, 1 H), 4.16 (q, *J* = 7.0 Hz, 2 H), 3.13−3.02 (m, 5 H), 2.96−2.92 (m, 2 H), 2.89−2.83 (m, 2 H), 2.31 (bs, 3 H), 2.21−2.08 (m, 2 H), 2.03−1.94 (m, 1 H), 1.72−1.56 (m, 3 H), 1.88−1.78 (m, 3 H), 1.68 (d, J = 1.5 Hz, 3 H), 1.63−1.57 (m, 2 H), 1.40−1.35 (m, 1 H), 1.27 (t, *J* = 7.0 Hz, 3 H), 1.02−0.97 (m, 9 H), 0.79 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.2, 173.0, 169.9, 167.9, 160.4, 149.5, 139.2, 136.8, 130.2, 129.6, 128.5, 126.7, 122.7, 69.7, 60.8, 58.5, 55.4, 54.3, 48.9, 44.8, 41.0, 30.9, 30.3, 30.1, 29.5, 25.0, 23.2, 20.1, 19.9, 19.6, 18.4, 14.2, 12.7; characteristic peaks of the minor rotamer ^1H NMR (CD_3OD, 500 MHz) δ 7.98 (s, 1 H), 4.72 (d, *J* = 8.0 Hz, 1 H), 1.10 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.0, 167.8, 160.4, 139.2, 136.8, 130.3, 126.7, 123.0, 60.8, 48.9, 41.0, 31.4, 30.6, 29.6, 20.6, 20.5, 20.4; MS (ESI) *m*/*z* 704 ($[M + Na]^+$), 682 ($[M + H]^+$); HRMS (ESI) *m*/*z* calcd for $C_{37}H_{56}N_5O_5S$ ($[M + H]^+$) 682.4002, found 682.4050.

To a solution of **47b** (43 mg, 0.063 mmol) in 95% ethanol (0.5 mL) was added 0.5 N LiOH (0.5 mL, 0.25 mmol), and the mixture was stirred at room temperature for 8 h. HCl (1 N, 5 mL, 5.0 mmol) was added, and the mixture was extracted with EtOAc (30 mL × 2). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by chromatography on SiO_2 (MeOH/CH_2Cl_2 5:95) to give **50** (37 mg, 90%) as a colorless oil: R_f 0.35 (MeOH/CH_2Cl_2 5:95); τ_R 21.3 min (Microsorb C-18 column, 250 × 10 mm; methanol/0.1% TFA in water; methanol gradient from 50% to 100% over 30 min; 2 mL/min; UV detection at 254 nm); $[\alpha]_D^{23}$ +7.3 (*c* 2.83, MeOH); IR (cm^{-1}) 3284, 2960, 2937, 2873, 1702, 1638, 1542, 1494, 1230; ^1H NMR analysis showed a 11:1 mixture of rotamers at room temperature. Major rotamer: ^1H NMR (CD_3OD, 500 MHz) δ 8.01 (s, 1 H, Tuv thiazole C*H*), 7.29−7.24 (m, 4 H, Tup *o*-, *o*′-, *m*-, and *m*′-phenyl C*H*s), 7.21−7.17 (m, 1 H, Tup *p*-phenyl C*H*), 6.80 (dd, *J* = 9.5, 1.5 Hz, 1 H, Tup C*H* = C), 5.14−5.09 (m, 1 H, Tup C*H*-NH), 4.65 (d, *J* = 7.5 Hz, 1 H, Val C*H*-NH), 4.31 (bs, 1 H, Tuv C*H*-N), 3.78 (dd, *J* = 12.2, 3.2 Hz, 1 H, Mep C*H*-N), 3.47 (d, *J* = 12.5 Hz, 1 H, Mep C*H*2-NH a), 3.14−3.06 (m, 5 H, Tuv N-C*H*3, Mep C*H*2-N b, Tup C*H*2-Ph a), 3.00−2.83 (m, 3 H, Tup C*H*2-Ph b, Tuv C*H*2-CH2-CH a, Tuv C*H*2-CH2-CH b), 2.74 (s, 3 H, Mep N-C*H*3), 2.23−2.09 (m, 3 H, Tuv CH2-C*H*2-CH a, Mep β C*H*2 a, Val C*H*-CH3), 2.02−1.99 (m, 1 H, Tuv C*H*-CH3), 1.94−1.92 (m, 2 H, Mep γ C*H*2 a, Mep δ C*H*2 a), 1.81−1.76 (m, 3 H, Tuv CH2-C*H*2-CH b, Mep β C*H*2 b, Mep δ C*H*2 b), 1.68 (d, *J* = 2.0 Hz, 3 H, Tup C*H*3), 1.62−1.55 (m, 1 H, Mep γ C*H*2 b), 1.05 (d, *J* = 7.0 Hz, 3 H, Tuv CH-C*H*3 a), 1.02 (d, *J* = 7.5 Hz, 3 H, Val CH-C*H*3 a), 0.99 (d, *J* = 7.0 Hz, 3 H, Val CH-C*H*3 b), 0.80 (d, *J* = 6.5 Hz, 3 H, Tuv CH-C*H*3 b); ^{13}C NMR (CD_3OD, 150 MHz) δ 174.5, 172.5, 171.1, 169.2, 162.6, 150.3, 141.2, 138.6, 131.2, 130.6, 129.4, 127.7, 124.4, 68.2, 60.4, 57.2, 56.2, 50.8, 42.9, 41.6, 31.4, 31.3, 31.0, 30.3, 24.0, 22.4, 20.5, 20.3, 19.9, 18.4, 12.9; characteristic peaks of the minor rotamer: ^1H NMR (CD_3OD, 600 MHz) δ 7.99 (s, 1 H), 4.70 (d, *J* = 9.0 Hz, 1 H), 3.83 (dd, *J* = 12.0, 4.0 Hz, 1 H), 2.72 (s, 3 H), 1.12 (d, *J* = 6.5 Hz, 3 H), 0.76 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR (CD_3OD, 150 MHz) δ 172.5, 131.2, 124.8, 68.3, 57.0, 55.9, 43.1, 41.5, 32.5, 31.5, 30.5, 28.3, 20.7, 20.6, 20.2, 18.7, 16.9; HRMS (ESI) *m*/*z* calcd for $C_{35}H_{52}N_5O_5S$ (M + H) 654.3689, found 654.3631.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01314.

^1H and ^{13}C NMR spectra and HPLC chromatograms for % ee determination (PDF)

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Notes

The authors declare no competing financial interest.

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