Synthesis and Applications of $\alpha,\beta$-Dehydroamino Acid-Containing Peptides

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Synthesis and Applications of $\alpha,\beta$-Dehydroamino Acid-Containing Peptides

Diego A. Moya

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

Synthesis and Applications of α,β-Dehydroamino Acid-Containing Peptides

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Doctor of Philosophy

Yaku’amide A (YA) is a linear anticancer peptide that is rich in bulky dehydroamino acids (ΔAAs) and β-hydroxyamino acids (β-OHAAs). In our recent total synthesis of YA, we featured a one-pot anti dehydration–azide reduction–O→N acyl transfer process for the stereospecific construction of Z- and E- ΔIle residues. Despite previous total syntheses and our efforts, the synthesis of YA remains lengthy. Via computational studies, we identified two analogue peptides that closely resemble the conformation of YA. The use of simpler and symmetrical bulky ΔAAs such as dehydrovaline (ΔVal) and dehydroethylnorvaline (ΔEnv) as surrogates of ΔIle, along with azlactone chemistry for their incorporation, significantly decreased the overall number of synthetic steps. Biological studies revealed that our analogues exhibited very similar activity to that of the natural product YA, demonstrating their suitability as mimics and consistency with our computational model.

Despite its utility in the construction of YA analogues, azlactone chemistry is sluggish and moderate to low yielding. For this reason, we have explored strategies to streamline the synthesis of peptides containing Z-dehydroaminobutyric acid (ΔAbu), ΔVal, and Z-dehydrophenylalanine (ΔPhe). The key process is to form the alkene moiety via elimination of a β-sulfonium or β-OHAA embedded within a peptide, avoiding the need to form the alkene moiety via azlactone-dipeptide dehydration and bypassing sluggish amidation/ring opening steps.
β-sheet disruption of Tau-model hexapeptides is a key type of inhibition for modulating Alzheimer’s disease progression. Previous studies replaced key residues with proline, due to its rigidity and lack of amide proton, to inhibit β-sheet formation. Similar to proline, ΔAAs are also known for their rigidity and ability to favor other conformations (e.g. β-hairpin, 310-helix) along with increasing peptide half-life. We have incorporated ΔAbu, ΔVal and dehydrocyclohexylglycine (ΔChg) in a highly aggregative hexapeptide sequence, using previously studied methods, to assess their capabilities as putative β-sheet breakers and to stabilize against proteolysis. Studies are continuing.

Keywords: peptides, synthesis, dehydrations, bulky dehydroamino acids, azlactones, anticancer, computational studies, inhibitory effects, β-sulfonyl, β-hydroxyamino acid, β-sheets, Alzheimer’s disease, proteolysis
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CHAPTER 1: Introduction

1.1 Dehydroamino Acids

α,β-Dehydroamino Acids (ΔAAs) are non-proteinogenic residues found in natural and synthetic peptides. They are characterized by their planarity and lack of chirality, both which profoundly affect the structures of dehydropeptides.\(^1\) ΔAA-containing peptides have been isolated primarily from bacteria but also from plants, fungi, and marine invertebrates, and they exert a wide range of biological activities.\(^2\) ΔAAs can be classified based on size as either small, medium, or bulky. Dehydroalanine (ΔAla) is a small ΔAA, Z-dehydroaminobutyric acid (Z-ΔAbu) is a medium-sized residue, while dehydrovaline (ΔVal) would be considered a bulky residue. Stammer and co-workers found that inserting a range of small and medium-sized ΔAAs into enkephalin mimics protected them from proteolytic degradation.\(^3\) The introduction of A\(_{1,3}\) strain (also known as allylic strain) imparts rigidity to peptide backbones, favoring a folded state over the more rapidly cleaved random coils (Figure 1-1).\(^4\)

![Figure 1-1: Dehydroamino Acids](image)

The coplanarity of the alkene with the neighboring amide group enables π-electron conjugation, acting as the stabilizing force for selected low-energy conformations.\(^1\) Jalan and co-workers have demonstrated that incorporating bulky ΔAAs such as ΔVal and ΔEnv into the turn region of the known Waters’ peptide can significantly enhance its proteolytic stability without altering its β-hairpin structure.\(^5\)
The conventional wisdom regarding $\alpha,\beta$-$\Delta$AAs is that they can undergo many reactions, particularly Michael additions. More recent studies by Joaquin and co-workers found that peptides containing medium and bulky $\alpha,\beta$-$\Delta$AAs are inert, even at elevated temperatures, to conjugate additions by nucleophilic thiols. Altogether, these discoveries suggest that inserting sterically hindered $\Delta$AAs in bioactive peptides at appropriate locations will increase their proteolytic stability without altering their secondary structure or rendering them susceptible to uncatalyzed conjugate additions under physiological conditions.

1.2 Medium $\alpha,\beta$-$\Delta$AA-Containing Natural Products

Many of the peptide natural products that contain medium-sized or trisubstituted $\alpha,\beta$-$\Delta$AAs belong to the diketopiperazines. Albonoursin (Figure 1-2) contains Z-$\Delta$Leu and Z-$\Delta$Phe, isolated in the 1960s from two different actimomycete species, demonstrating activity against bacteria, tumor cells and H1N1 influenza strain.

![Figure 1-2: Albonoursin](image)

Similar to Albonoursin, most diketopiperazines with a $\Delta$Leu, contain the Z isomer of this residue. (3Z, 6E)-1-$N$-Methylalbonoursin is a rare example of a diketopiperazine that contains $E$-$\Delta$Leu. Other diketopiperazines include Lansai C and D and the unknown metabolites 1a and 1b (Figure 1-3).
Figure 1-3: Diketopiperazines

Tentoxin is a cyclic tetrapeptide (Figure 1-4) that contains a Z-ΔPhe residue which was isolated by Templeton and co-workers from the *Alternaria tenuis* fungus.\(^\text{10}\) Work by the Meyer\(^\text{11}\) and Rich\(^\text{12}\) groups contributed to its structure elucidation.

Figure 1-4: Tentoxin

Phomazine A (Figure 1-5) was isolated from the fermentation broth of the endophytic fungus *Phoma* sp. OUCMDZ-1847. In addition to possessing a Z-ΔPhe, it contains an unusual tetrahydroindole component.\(^\text{13}\)

Figure 1-5: Phomazine A
Resormycin (Figure 1-6) was isolated by Igarashi and co-workers from a *Streptomyces platensis* strain in 1997 comprised of a Z-4-chloro-3,5-dihydroxyΔPhe non-coded amino acid.\textsuperscript{14} It also displays herbicidal activity and inhibits the green algae growth.\textsuperscript{15}

![Figure 1-6: Resormycin](image)

In 2004, Son and co-workers isolated Golmaenone (Figure 1-7) from the marine-derived fungus *Aspergillus* sp. It features a benzoyl group attached to an alkene. Golmaenone is known as a potent radical scavenger and UV-A protective agent.\textsuperscript{16}

![Figure 1-7: Golmaenone](image)

Various ΔTrp-containing peptides have also been discovered. Telomycin (Figure 1-8) was isolated by Misiek and co-workers from the culture broth of an unidentified *Streptomyces* species.\textsuperscript{17} Its structural characterization was performed by Sheehan et al. using chemical degradation in combination with various mass spectroscopic methods.\textsuperscript{18}
Kobayashi et al. isolated keramamide F (Figure 1-9), a cyclic antitumor agent, from Okinawan sponge *Theonella genus*. This peptide contains a Z-ΔTrp in addition to various unusual structural features such as (O-methylseryl) thiazole derivative, an isoserine residue, and an α-ketoamide.  

Bulky or tetrasubstituted α,β-ΔAAs are less common residues, but have been found in nature in a number of bioactive peptide natural products. In 1969, Marchand and co-workers isolated lasiodine A (Figure 1-10), an acyclic ΔVal-containing depsipeptide, isolated from the African buckthorn plant *Lasiodiscus marmoratus*. It has demonstrated an inhibitory profile against photophosphorylation in spinach chloroplasts.21
In 1981, Shimada and co-workers isolated antibiotic antrimycin A (2a, Figure 1-11). The ΔIle-containing heptapeptide was obtained from *Streptomyces xanthocidicus*. In 1982, Shirosa and co-workers discovered cirratiomycin A (2b) and B from *Streptomyces cirratus*. Cirratiomycin B is identical to 2a, while cirratiomycin A contains a Leu residue in place of the Ala found in 2a.23

In 1983, Culvenor and co-workers isolated phomopsins A and B (3a and 3b, Figure 1-12). The antimitotic peptides were extracted from the cultivated fungus *Phomopsis leptostromiformis*. Its uncommon structure possesses a 13-membered lactam with an acyclic side chain, six nonproteinogenic amino acids and one E-ΔIle residue. Phomopsin A is known to bind β-tubulin to inhibit the formation of microtubules in livestock via lupinosis activation.25
In the same year, Kemmer and co-workers isolated Myxovalargin A (Figure 1-13) from *Myxococcus fulvus*.\(^{26}\) It was found that the E-ΔIle-containing peptide inhibits the synthesis of bacterial proteins and causes cell membrane damage.\(^ {27}\)

In 1986, Nagaoka and co-workers isolated Azinomycins A and B (Figure 1-14) from *Streptomyces griseofuscus*.\(^ {28}\) These peptides carry a tetrasubstituted ΔAA containing an aziridino[1,2-α]pyrrolidine ring residue. Azinomycin B is known for its ability to form cross-links between DNA strands, demonstrating potency against a variety of tumors.\(^ {29}\)
In 2003, FR225659 (4a) and four related peptides (4b-e, Figure 1-15) were isolated by Fujisawa Pharmaceutical Co. from a strain of fungus obtained from a decayed leaf. Peptide 4a-e inhibit glucagon-stimulated gluconeogenesis.\(^\text{30}\)

**Figure 1-15: FR225659 and related peptides**

In 2006, Fudou, Ojika, and co-workers isolated miuraenamides A and B from the myxobacterial strain SMH-27-4 (Figure 1-16), exhibiting antifungal activity. The exact determination of their structures was published in 2008 along with the isolation of miuraenamides C, F and D.\(^\text{31}\)

**Figure 1-16: Miuraenamides**

In 2010, Yaku’amide A and B (Figure 1-17) were isolated from a deep-sea sponge *Ceratopsion* sp. by Matsunaga et al. Both Yaku’amide A (YA) and Yaku’amide B (YB) possess potent anticancerous activity against P388 murine leukemia cells. Yaku’amide A displays a unique
activity profile against JFCR 39 human cancer line panel, which has suggested a novel mode of action.32

![Yaku’amides A and B](image)

Figure 1-17: Yaku’amides A and B

1.4 Synthesis of Bulky $\alpha,\beta$-Dehydroamino Acids

Through the years, many methods have been employed to deliver $\alpha,\beta$-dehydroamino acids. In 1969, Kishi and co-workers synthesized $\Delta$Val 6 from a penicillamine-containing dipeptide 5. This route33 was inspired by the proposed biosynthetic pathway of the penicillamines and cephalosporins (Scheme 1-1).

![Scheme 1-1: Kishi’s Base-promoted Eliminations](image)

Scheme 1-1: Kishi’s Base-promoted Eliminations

Shin and co-workers established a one-pot synthesis of tripeptide 7b using $\Delta$AA-containing-$N$-carboxyanhydride 7a.34 The established route generated a mixture of alkene isomers which limited its application (Scheme 1-2).
In 1999, Wandless and co-workers developed a two-step route to stereoselectively assemble medium and bulky ΔAAs. Their approach involved the generation of cyclic sulfamidate via \( \text{SOCl}_2 \) from a \( \beta \)-phenylserine derivative \( 8a \). \( E-\Delta \text{Phe} \) \( 8b \) was obtained from a DBU-mediated \( \text{anti} \) elimination (Scheme 1-3).

This method was applied to the synthesis of \( E-\Delta \text{Ile} \) dipeptide \( 9b \) from a \( \beta \)-OH\( \text{Ile} \) dipeptide \( 9a \). Building block \( 9b \) was used for the total synthesis of Phomopsin B in 2007 (Scheme 1-4).
In 2001, Albericio and co-workers found that a copper-carbodiimide elimination method could be used on a resin-bound peptide (Scheme 1.5). Dehydration of diastereomeric peptide 10a only led to the formation of thermodynamically more stable Z isomer of ΔPhe 10b.37

**Scheme 1-5: Albericio’s Z-ΔPhe Synthesis**

In the same year, Coleman and co-workers used a Horner-Wadsworth-Emmons olefination of an aldehyde 11b with a phosphonate 11a to yield the precursor 12 to the desired azinomycin A (Scheme 1-6).38

**Scheme 1-6: Coleman’s Synthesis**

Joullié and co-workers used a copper-carbodiimide approach to synthesize the E-ΔIle residue present in the phomopsin side chain. Alkene 13b was synthesized from compound 13a in the presence of EDC and Cu(OTf)_2 in THF (Scheme 1-7).39
Scheme 1-7: Joullié’s E-ΔIle Synthesis

Inoue and co-workers' approach to ΔIle 14b involved a stereospecific Cu-catalyzed amidation of vinyl iodide 14a. Their cumbersome 6-step process required backbone protection to prevent azlactone formation and alkene isomerization of 15 (Scheme 1-8).41

Scheme 1-8: Inoue’s E-ΔIle Synthesis

In 2014, Castle and co-workers found that Z-ΔIle 17a and E-ΔIle 17b could be acquired via Martin sulfurane-mediated anti dehydrations from β-OH-Ile residues 16a and 16b via a highly asynchronous E2 pathway (Scheme 1-9).42

Scheme 1-9: Castle’s ΔIle Synthesis
In 2020, the Inoue group synthesized YB using a traceless Staudinger ligation method on SPPS. Enamide 19 was made from the ligation of phosphinophenol ester 18 and an alkenyl azide (Scheme 1-10). Developed by the Bertozzi and Raines groups, the ligation to form amide bonds was favored by generating N\textsubscript{2} as the thermodynamic driving force.

Scheme 1-10: Inoue’s Δlle Synthesis

1.5 Summary

A variety of methods have been developed for the synthesis of α,β-dehydroamino acids, from Shin’s carboxyanhydride amidations to Inoue’s Cu-catalysis of vinyl iodides. Despite this progress, a one-size-fits-all approach remains to be discovered. In our lab, we have developed methods for incorporating medium, and bulky α,β-dehydroamino acids into small and medium-sized peptides that possess a wide range of biological activities.

1.6 References


CHAPTER 2: Yaku’amide A and Analogues

2.1 Yaku’amide A

In 2010, Matsunaga and co-workers isolated minute quantities of two cytotoxic compounds, Yaku’amide A (YA or 20, Figure 2-1) and Yaku’amide B (YB or 21). These linear peptides were obtained from the deep-sea sponge Ceratopsion sp. at Yakushinsone, located in the East China Sea.¹ YA and YB present complex structures featuring E- and Z-dehydroisoleucines (ΔIle) and dehydrovaline (ΔVal) as well as several β-OHAAAs (Figure 2-1). YA and YB are known for their potent growth inhibitory effects against P388 murine leukemia cell lines (IC₅₀ = 14 ng/mL for 20 and IC₅₀ = 4 ng/mL for 21).

These results prompted a screening against a JFCR39 cancer cell line panel, which revealed a unique inhibitory profile with an IC₅₀ < 1μM against several cancer cell lines which include breast, colon, gastrointestinal and lung cancers.² Recent studies have suggested that 21 depletes cellular ATP concentrations by binding to the mitochondrial enzyme F₀F₁-ATP synthase and simultaneously inhibiting ATP synthesis and promoting ATP hydrolysis.³ The first total synthesis of 20 was performed by Inoue and co-workers in 2015, involving 86 total steps and a 25-step longest linear sequence.⁴ Prompted by our interest in its synthetically challenging structure, its bioactivity, and its limited supply in nature, we performed a more efficient total synthesis of 20.

Figure 2-1: YA (20) and YB (21)
2.1.1 Retrosynthesis of YA

Our approach featured a one-pot anti dehydration–azide reduction–O→N acyl transfer process for the stereospecific construction of the Z- and E- ΔIle residues. Our path towards the synthesis of natural product 20 is highlighted in Scheme 2-1. We divided 20 into an N-terminal acyl group (NTA) 22, a pentapeptide 23 and a nonapeptide 24. The latter compound could further be dissected into a dipeptide 25, a tripeptide 26, and a tetrapeptide 27.

![Scheme 2-1: Retrosynthesis of 20](image)

2.1.2 Synthesis of Tripeptide 26 and Iodide 32

The synthesis of YA was tackled as a team effort. My contribution to the project involved the synthesis of tripeptide 26 as well as iodide 32. Compounds 28 and 29 were made from a base-free OsO4-catalyzed aminohydroxylation of a chiral mesyloxycarbamate with an enoate.

We began with ester hydrolysis of 28 using Nicolaou’s trimethyltin hydroxide protocol and coupling with racemic β-OHIle 29 to yield the desired dipeptide 30 (Scheme 2-2).
Scheme 2-2: Synthesis of Tripeptide 26

After the coupling, we proceeded to remove our chiral carbamate via a protecting group swap to install a more base-resistant Boc group in compound 31. Na$_2$CO$_3$ was required to prevent TES ether cleavage in the hydrogenolysis reaction. Installing the robust Boc group allowed LiOH to be used for saponifying 31, and subsequent alkylation with enantiopure iodide 32 yielded azide 33. Compound 32 was made from a diazo transfer followed by an Appel reaction to install both azide and iodide from its amino propanol precursor (Scheme 2-3).

Scheme 2-3: Synthesis of Iodide 32

Alcohol 34 was synthesized via a one-pot anti-dehydration of 33 mediated by Martin sulfurane, followed by azide reduction using Lindlar catalyst and O→N acyl transfer facilitated by piperidine. These three transformations occurred in a single pot. Two subsequent oxidations using DMP and Pinnick conditions yielded the desired acid 26.
Acid 26 was then coupled by Concordia Lo to hydrolyzed tetrapeptide 35 to yield desired heptapeptide 36. Hydrolyzed heptapeptide 37 was then coupled to dipeptide 25 to yield nonapeptide 24 in high yields (Scheme 2-4).

Scheme 2-4: Synthesis of Nonapeptide 24

The total synthesis was completed by Concordia Lo by further coupling the pentapeptide 23 and NTA cap 22 to yield 20 in a 19-steps longest linear sequence with a 3.5% overall yield and 13 chromatographic purifications. Our route compares favorably with Inoue’s solution-phase synthesis of YA, which used a 25-step longest linear sequence (1.9% overall yield). Although Inoue’s linear solid-phase peptide synthesis (SPPS) of YB is more efficient by some metrics (9.1% overall yield),

9 our approach does not require excess amounts of precious intermediates and is convergent. Also, our synthesis is convergent, which is often advantageous compared to a linear approach due to its flexibility and synthesis of fragments that could be useful for analogue synthesis.
2.2 Yaku’amide A Analogues

Despite Inoue’s groundbreaking total synthesis\(^4\) and our efforts,\(^5\) the synthesis of 20 remains lengthy. Controlling the stereochemistry of the unsymmetrical ΔIle residues and preventing E/Z isomerization have posed a synthetic challenge. For this reason, we shifted our attention towards the synthesis of more accessible YA analogues (Figure 2-2). We hypothesized that replacement of the E- and Z-ΔIle residues by bulky ΔAAAs that do not exist as geometrical isomers would not only bypass the isomerization issues but would also streamline the synthesis by enabling the use of simpler azlactone chemistry for dehydration.

![Diagram of Yaku’amide A Analogues](image)

**Figure 2-2: YA Analogues 38 and 39**

We decided to use bulky ΔAAAs such as ΔVal and ΔEnv\(^{10}\) as surrogates of ΔIle due to their proximity in size and their ease of construction due to the lack of geometrical isomers. Each possible analogue of 20 was assigned a three-letter abbreviation, where E represents ΔEnv and V represents ΔVal replacing a ΔIle residue at positions 3, 5 and 10 with the substitutions listed in order from the N- to the C-terminus. With a possible 8 analogues, David Kastner used a computational model to identify which ones closely resemble the 3D structure of 20. The
computational analysis revealed that analogue EVV resembled that of the natural product \(20\) more closely while analogue VEV was selected as second best (Figure 2-2). We reasoned that these analogues could potentially retain the unique inhibitory effects of YA by closely resembling its conformation.

### 2.2.1 Retrosynthesis of Yaku'amide A Analogues

The proposed retrosynthesis (Scheme 2-5) of analogues \(38\) and \(39\) resembles that of the natural product with the disconnections done in similar fashion yielding NTA \(22\), pentapeptides \(40\) (used for EVV analogue) and \(41\) (used for VEV analogue), as well as nonapeptide \(42\). The latter intermediate could further be simplified into a dipeptide \(25\), a tripeptide \(43\), and a tetrapeptide \(27\). Intermediates \(22\), \(25\), and \(27\) (highlighted in blue) were all constructed in the natural product synthesis. This new strategy only required three new subunits: \(40\), \(41\) and \(43\). Thus, our convergent synthetic design significantly streamlined the synthesis of the analogues.

![Scheme 2-5: Retrosynthesis of 38 and 39](image-url)
My contribution to the project involved the synthesis of pentapeptides 40 and 41. While fellow group member Daniel Joaquin was primarily responsible for the tripeptide synthesis, I also synthesized 43 as needed.

2.2.2 Synthesis of Tripeptide 43

The synthesis of tripeptide 43 was achieved via dehydration using azlactone chemistry to yield the bulky ΔAAs (Scheme 2-6).

![Scheme 2-6: Azlactone-Dehydration Chemistry](image)

We began the synthesis (Scheme 2-7) with the ester hydrolysis of 28 using mild conditions and coupled with racemic β-OHVal\(^{11}\) 44 to yield the desired dipeptide 45 as an inconsequential mixture of diastereomers. After the coupling, we proceeded to swap our chiral carbamate for the more base-resistant Boc group in alcohol 46. Saponification of 46 with LiOH yielded the acid which was exposed to the azlactone dehydration-amidation procedure. EDC•HCl activates the carboxylate and dehydrates the tertiary alcohol, prompting an azlactone formation. The azlactone can then be opened via amidation by the addition of D-Ala-OMe and Et.N along with heat to yield a single alkene 47. Finally, saponification of 47 yielded the desired acid 43 used in the synthesis of both EVV and VEV analogues.\(^{12}\)
2.2.3 Synthesis of Pentapeptides 40 and 41

The pentapeptide 40, which is required for analogue EVV, was constructed (Scheme 2-8) using similar chemistry applied to the synthesis of tripeptide 43 (Scheme 2-7). Compounds 44, 48, 49 were previously made via a base-free OsO₄-catalyzed aminohydroxylation of a mesyloxycarbamate with an enoate.⁵ The hydrolysis of β-OHlle derivative 48 was followed by coupling to racemic β-OHEnv-OEt (49a), delivering dipeptide 50a as an inconsequential mixture of diastereomers. Swapping the chiral carbamate for a Boc group furnished 51a. Ester hydrolysis and subsequent azlactone dehydration–amidation with Gly-OMe helped access tripeptide 52a. The modest yield of this reaction is most likely due to the sterically hindered nature of the ∆Env-azlactone intermediate. The remaining amino acids ∆Val and D-Val-OMe were attached to 53a via coupling followed by one-pot dehydration–amidation. Saponification of the resulting pentapeptide 54a yielded acid 40.
Preparation of pentapeptide 41, which was required for the second-choice analogue, resembled the construction of 40 with some highlighted differences. Conversion of dipeptide 51b into ∆Val-containing tripeptide 52b was accomplished using DMAP as an additive to promote ring-opening amidation of the azlactone intermediate. The high yield of this process relative to analogous transformations in the syntheses of 43 (Scheme 2-7) and 58a (Scheme 2-8) is likely due to the use of less-hindered coupling partners (∆Val and Gly-OMe). Conversely, formation of pentapeptide 54b involved the lowest-yielding dehydration–amidation sequence complicated by the highly hindered ∆Env and D-Val-OMe coupling partners. Retro aldol scission of the β-OHEnv residue during saponification of tetrapeptide 44b was mitigated by using methyl ester 40b instead of the ethyl ester 40a employed in synthesizing 40.
The assembly of the YA analogue subunits was conducted by fellow group member Concordia Lo. Tripeptide 43 was then coupled to hydrolyzed tetrapeptide 27 to yield desired heptapeptide 55. Hydrolyzed heptapeptide was then coupled to dipeptide 25 to yield nonapeptide 42 in high yields (Scheme 2-9).

**Scheme 2-9: Synthesis of Nonapeptide 42**

Pentapeptide 40 and 41 were both coupled to nonapeptide 42 to yield tetradecapeptide 56 and 57. Analogues EVV 38 and VEV 39 were yielded by coupling NTA 22 in modest yields (Scheme 2-10).

**Scheme 2-10: Synthesis of Analogues EVV 38 and VEV 39**

### 2.3 Summary

Our YA analogues were submitted for biological testing to our collaborators at Bristol-Myers-Squibb. 72-hour MTS cell proliferation assays revealed that analogues 38 and 39 exhibited
very similar activity to that of the natural product, with the EVV analogue being a better mimic than the VEV analogue. These results demonstrate our analogues’ suitability as mimics and consistency with our computational model and hypothesis. Both analogues also showed encouraging lack of potency against non-cancerous MRC5 cell lines, suggesting they possess a satisfactory therapeutic window for use as anticancer agents. The EVV analogue is suitable as a starting point for designing molecular probes that would help us learn more about YA’s mode of action, binding specificity, and targets.

2.4 References


CHAPTER 3: Synthesis of Peptides Containing $\alpha,\beta$-Dehydroamino Acids

3.1 Introduction

Our syntheses of $\Delta$AA-containing peptides have primarily relied on azlactone (oxazolone) ring-openings for the incorporation of unsaturated residues. The required azlactones can be readily generated from peptides containing a $C$-terminal $\beta$-hydroxyamino acid ($\beta$-OHAA) via tandem dehydration–cyclization. Despite its utility in the construction of yaku’amide A analogues 38 and 39, amidation steps involving azlactones can be sluggish and moderate to low yielding. Furthermore, application of this chemistry to solid-phase peptide synthesis (SPPS) requires a cumbersome solution-phase preparation of azlactone dipeptides. Other methods for incorporating $\Delta$AAs require bulky protecting group chemistry. Thus, the discovery of simple methods to incorporate $\Delta$AAs into peptides using readily accessible amino acid derivatives is warranted.

We have explored strategies to streamline the synthesis of model tripeptides containing medium-sized $Z$-dehydroaminobutyric acid ($\Delta$Abu), $Z$-dehydrophenylalanine ($\Delta$Phe) and bulky dehydrovaline ($\Delta$Val). The key process involves coupling of a suitable $\Delta$AA precursor (e.g., $\beta$-OHAA or $\beta$-SHAA) via standard protocols (Scheme 3-1), followed by dehydration or elimination to generate the embedded alkene moiety within peptides. While these studies were conducted in solution, they lay the groundwork for future studies using SPPS.

Scheme 3-1: Elimination of Embedded $\Delta$AA Precursors
3.2 Elimination Strategies of Model Tripeptides 62, 65, 67 and 70

Although dehydrations of β-OH acids or esters to afford C-terminal ΔAAs are accessible, challenges are met for similar reactions of β-OH amides to afford ΔAAs embedded within peptide chains. However, the feasibility of this concept has been established by our Martin sulfurane-mediated dehydrations of Threonine (Thr) to furnish Z-ΔAbu. The modest yields from these studies demonstrate the need for optimization. We evaluated a host of conditions for dehydration of complex alcohols via E2 anti or E1 mechanisms (Scheme 3-2) on tripeptide models. Our Z-ΔAbu, Z-ΔPhe and ΔVal tripeptide precursors contain the requisite β-OHAA embedded between two glycine (Gly) residues.

Scheme 3-2: Solution-Phase Dehydration of β-OH Precursors

While these conditions (Scheme 3-2) serve to promote alcohol dehydrations, we planned a second approach to ΔVal using sulfide or sulfonium eliminations. The required precursor (Scheme 3-3), contains a Penicillamine (Pen) residue sequestered between two glycines.

Scheme 3-3: Solution-Phase Elimination of a β-SH Precursors
3.2.1 Thr-Containing Tripeptide 62

Model tripeptide 62 (Scheme 3-4) was accessed by coupling commercially available Gly 58 and Z-Thr-OH 59 to obtain dipeptide 60 in high yields. Subsequent hydrogenolysis and coupling with Z-Gly-OH 61 yielded tripeptide 62 in 43% yield over two steps.

![Scheme 3-4: Synthesis of Model Tripeptide 62](image)

3.2.2 β-Phenylserine-Containing Tripeptide 65

For the synthesis of model tripeptide 65 (Scheme 3-5), we made β-Phenylserine (β-OHPhe) 63 following Crich and Banerjee’s procedure.25 Dipeptide 64 was generated from the coupling of 63 and Gly 58 with 64% yield. Hydrogenolysis of dipeptide 64 and subsequent coupling with Z-Gly-OH 61 produced tripeptide 65 in high yields over two steps.

![Scheme 3-5: Synthesis of Model Tripeptide 65](image)

3.2.3 β-OH and Pen-Containing Tripeptides 67 and 70

For the synthesis of tripeptide precursors 67 and 70, two strategies were explored (Scheme 3-6). The top route used an embedded β-OHAA while the bottom route involved a sequestered β-SH amino acid. Synthesis of tripeptide 67 began by coupling racemic β-OHVal-OEt 44 and Z-
Gly 61 to yield dipeptide 66. Ester 66 was then hydrolyzed using trimethyltin hydroxide followed by coupling to Gly-OMe 58 to yield tripeptide 67.

Scheme 3-6: Synthesis of Model Tripeptides 67 and 70

Synthesis of β-SHAA-containing tripeptide 70 began by coupling commercially available Fmoc-Pen(Trt) 68 and Gly-OMe 58 to yield Fmoc-Pen(Trt)-Gly-Ome 69a. Subsequent Fmoc removal was achieved to afford dipeptide 69b in high yields. Z-Gly 61 was then coupled to 69b and subsequent removal of the trityl protecting group yielded tripeptide 70 in 90% yield over two steps.

3.3 Z-dehydroaminobutyric acid (ΔAbu)-Containing Tripeptide 71a

The dehydrations of Thr-containing tripeptide 62 to yield ΔAbu 71a are summarized in Table 3-1. Competition between the desired dehydrated product 71a and a cyclized (oxazoline) byproduct 71b was significant, but each reaction produced single products rather than mixtures. For example, treatment of 62 with MsCl/Et$_3$N followed by DBU in 1,2-dichloroethane (DCE), CH$_3$CN or DMF yields 71b exclusively (Entries 1, 3 and 4). However, using a larger excess of MsCl while switching from DCE to CH$_3$CN in the elimination step furnishes 71a in low yields (Entry 2). Exposing 71a to the conditions of Entry 3 did not produce 71b. This demonstrates that the ΔAA product is not an intermediate to the oxazoline byproduct. Reactions of 62 with Boc$_2$O/DMAP then 1,1,3,3-tetramethylguanidine (TMG) also furnished 71a. Attempts to increase
the temperature of this reaction did not produce either product. Use of EDC•HCl/CuCl conditions in CH$_2$Cl$_2$ produced 71a in high yields (Entries 8-12). Switching from CuCl to Cu(OTf)$_2$ was not fruitful (Entry 13). Use of PPh$_3$ and I$_2$ produced 71b in modest yields.

**Table 3-1: Dehydrations of Threonine-Containing Peptide 62**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Temp</th>
<th>Time (h)</th>
<th>Product*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MsCl/Et$_3$N, then DBU$^b$</td>
<td>rt, then reflux</td>
<td>17</td>
<td>71b (38)</td>
</tr>
<tr>
<td>2</td>
<td>MsCl/Et$_3$N, then DBU$^b$</td>
<td>rt, then reflux</td>
<td>17</td>
<td>71a (31)</td>
</tr>
<tr>
<td>3</td>
<td>MsCl/pyr, then DBU$^d$</td>
<td>rt, then reflux</td>
<td>17</td>
<td>71b (70)</td>
</tr>
<tr>
<td>4</td>
<td>MsCl/Et$_3$N, then DBU$^g$</td>
<td>rt, then reflux</td>
<td>17</td>
<td>71b (20)</td>
</tr>
<tr>
<td>5</td>
<td>Boc$_2$O/DMAP, then TMG$^d$</td>
<td>0 ° C to rt</td>
<td>12</td>
<td>71a (18)</td>
</tr>
<tr>
<td>6</td>
<td>Boc$_2$O/DMAP, then TMG$^d$</td>
<td>0 ° C to rt</td>
<td>24</td>
<td>71a (44)</td>
</tr>
<tr>
<td>7</td>
<td>Boc$_2$O/DMAP, then TMG$^d$</td>
<td>0 ° C to 40 °C</td>
<td>12</td>
<td>nd$^f$</td>
</tr>
<tr>
<td>8</td>
<td>EDC/CuCl, CH$_2$Cl$_2$</td>
<td>rt</td>
<td>16</td>
<td>71b (9)</td>
</tr>
<tr>
<td>9</td>
<td>EDC/CuCl, CH$_2$Cl$_2$–DMF</td>
<td>rt</td>
<td>16</td>
<td>71a (7)</td>
</tr>
<tr>
<td>10</td>
<td>EDC/CuCl, CH$_3$CN</td>
<td>rt</td>
<td>16</td>
<td>71b (7)</td>
</tr>
<tr>
<td>11</td>
<td>EDC/CuCl, CH$_3$Cl</td>
<td>reflux</td>
<td>16</td>
<td>71a (55)</td>
</tr>
<tr>
<td>12</td>
<td>EDC/CuCl, CH$_2$Cl$_2$</td>
<td>rt</td>
<td>40</td>
<td>71a (59)</td>
</tr>
<tr>
<td>13</td>
<td>EDC/Cu(OTf)$_2$, THF</td>
<td>60 °C</td>
<td>1.5</td>
<td>71a (6)</td>
</tr>
<tr>
<td>14</td>
<td>PPh$_3$/I$_2$, CH$_2$Cl$_2$</td>
<td>rt</td>
<td>4</td>
<td>71b (20)</td>
</tr>
</tbody>
</table>

$^a$ Isolated yield in parentheses.

$^b$ CICH$_2$CH$_2$Cl as solvent.

$^c$ CICH$_2$CH$_2$Cl as solvent for mesylation, then CH$_3$CN for elimination; 4 equiv of MsCl used instead of 2 equiv employed in other reactions.

$^d$ CH$_3$CN as solvent.

$^e$ DMF as solvent.

$^f$ Neither product was detected.

The most effective methods in the dehydration of Thr were applied towards the dehydration of β-OH Phe-containing tripeptide 65. Surprisingly, EDC•HCl/CuCl conditions only yielded trace amounts of the desired product. Other conditions such as Boc$_2$O/DMAP then TMG yielded comparable results. Use of DAST/pyridine also proved unsuccessful. The phenyl group on
tripeptide 65 is significantly bulkier than the methyl group of the Thr residue in tripeptide 62, presumably preventing the formation of ΔPhe by this method.

3.4 Dehydrovaline (ΔVal)-Containing Tripeptide 72a

The dehydrations of β-OHVal-containing tripeptide 67 to yield ΔVal-containing tripeptide 72a were also quite challenging due to substantial sterics. The results are summarized in Table 3-2.

Table 3-2: Dehydrations of β-OHVal-Containing Peptide 67

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOCl₂/pyr, CH₂Cl₂</td>
<td>0</td>
<td>1</td>
<td>72a (18)</td>
</tr>
<tr>
<td>2</td>
<td>SOCl₂/pyr, CH₂Cl₂</td>
<td>0</td>
<td>2</td>
<td>72a (14)</td>
</tr>
<tr>
<td>3</td>
<td>SOCl₂/pyr, CH₃CN</td>
<td>0</td>
<td>1</td>
<td>72a (25), 72b (15)</td>
</tr>
<tr>
<td>4</td>
<td>Burgess, CH₃CN</td>
<td>70</td>
<td>12</td>
<td>72a (5)</td>
</tr>
<tr>
<td>5</td>
<td>DAST/pyr, CH₂Cl₂</td>
<td>0</td>
<td>0.5</td>
<td>72a (39)</td>
</tr>
<tr>
<td>6</td>
<td>DAST/pyr, CH₂Cl₂</td>
<td>–30</td>
<td>0.5</td>
<td>72a (27), 72b (50)</td>
</tr>
<tr>
<td>7</td>
<td>DAST/pyr, CH₂Cl₂</td>
<td>0</td>
<td>0.5</td>
<td>72a (13), 72b (58)</td>
</tr>
<tr>
<td>8</td>
<td>DAST/DMAP, CH₂Cl₂</td>
<td>0</td>
<td>0.5</td>
<td>72a (31), 72b (21)</td>
</tr>
<tr>
<td>9</td>
<td>DAST/2,6-lut, CH₂Cl₂</td>
<td>0</td>
<td>0.5</td>
<td>72a (5), 72b (46)</td>
</tr>
<tr>
<td>10</td>
<td>DAST/DBU, CH₂Cl₂</td>
<td>0</td>
<td>0.5</td>
<td>72b (40)</td>
</tr>
<tr>
<td>11</td>
<td>DAST/pyr, PhCH₃</td>
<td>0</td>
<td>0.5</td>
<td>72a (19), 72b (30)</td>
</tr>
<tr>
<td>12</td>
<td>DAST/pyr, THF</td>
<td>0</td>
<td>0.5</td>
<td>72a (8), 72b (25)</td>
</tr>
</tbody>
</table>

*a Isolated yield in parentheses.

*b 2 equiv of DAST used instead of 1 equiv employed in other reactions.

Use of SOCl₂/pyr in CH₂Cl₂ afforded dehydrated product 72a in low yields (Entry 1). Switching to CH₃CN solvent produced a separable mixture of 72a and oxazoline 72b in modest yields (Entry 3). Exposure of 67 to the Burgess reagent produced only trace amounts of 72a (Entry 4). Use DAST/pyr in CH₂Cl₂ at 0 °C produced 72a in 39% yield. Unfortunately, efforts to optimize this reaction by decreasing the temperature, using excess reagents and different bases only led to
mixtures, primarily favoring cyclized 72b (Entries 5-12). Exposure of 72a to conditions in Entry 5 did not yield 72b. Once again this indicates that the ΔAA product is not an intermediate to the oxazoline byproduct. The EDC•HCl/CuCl conditions were not examined in this case as previous attempts with them to produce ΔVal in the Yaku’amide A total synthesis were ineffective.

To expand on the results above, we examined the elimination of Pen-containing tripeptide 70 to furnish ΔVal 72a as summarized in Table 3-3. Treatment of 70 with 1,4-diiodobutane/K2CO3 in DMF produced a mixture of 72a and 72b (Entry 1). Efforts to optimize this reaction by involving different bases, solvents, temperatures, and reaction times (Entries 1-8) raised the yield of 72a to 53% (Entry 5). However, substantial amounts of 72b were produced in the optimization process. Ag+−promoted sulfide elimination was also attempted but neither product was produced (Entry 9).

Table 3-3: Eliminations of β-SH-Containing Peptide 70

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Temp</th>
<th>Time (h)</th>
<th>Products*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I(CH2)4I, K2CO3, DMF</td>
<td>rt</td>
<td>4</td>
<td>72a (24), 72b (51)</td>
</tr>
<tr>
<td>2</td>
<td>I(CH2)4I, Na2CO3, DMF</td>
<td>rt</td>
<td>4</td>
<td>72a (31), 72b (32)</td>
</tr>
<tr>
<td>3</td>
<td>I(CH2)4I, Cs2CO3, DMF</td>
<td>rt</td>
<td>4</td>
<td>72a (15), 72b (72)</td>
</tr>
<tr>
<td>4</td>
<td>I(CH2)4I, DBU, DMF</td>
<td>rt</td>
<td>4</td>
<td>72a (40), 72b (52)</td>
</tr>
<tr>
<td>5</td>
<td>I(CH2)4I, DBU, DMF</td>
<td>0 °C</td>
<td>4</td>
<td>72a (53), 72b (46)</td>
</tr>
<tr>
<td>6</td>
<td>I(CH2)4I, DBU, DMF</td>
<td>rt</td>
<td>16</td>
<td>72a (21), 72b (59)</td>
</tr>
<tr>
<td>7</td>
<td>I(CH2)4I, DMAP, DMF</td>
<td>rt</td>
<td>4</td>
<td>72a (28), 72b (5)</td>
</tr>
<tr>
<td>8</td>
<td>I(CH2)4I, DBU, CH3CN</td>
<td>rt</td>
<td>4</td>
<td>72a (34), 72b (29)</td>
</tr>
<tr>
<td>9</td>
<td>MeI/K2CO3, DMF, thenAgNO3/DBN, CH3CN</td>
<td>rt</td>
<td>3</td>
<td>ndb</td>
</tr>
</tbody>
</table>

* Isolated yield in parentheses.

b Neither product was detected

3.5 Summary

We attempted to streamline the synthesis of ΔAA-containing tripeptides by investigating strategies to furnish embedded medium-sized ΔAbu and bulky ΔVal in model tripeptides. We
found that EDC•HCl/CuCl conditions helped furnish ΔAbu from Thr dehydration. Dehydrations of β-OHPhe-containing peptide were fruitless, revealing a gap in current approaches that is worthy of additional study. While DAST-promoted dehydrations and DBU-mediated sulfonium eliminations delivered the desired ΔVal tripeptide in encouraging yields, these reactions still require optimization. Reduction of the formation of oxazoline byproducts is needed. In this respect, it is possible that the conformational flexibility of the Gly-containing precursors is facilitating oxazoline formation. Therefore, more hindered substrates will be the subject of future studies, along with adaptation of these methods to SPPS.

3.6 References


4.1 Introduction

Amyloid-β and tau proteins are two known culprits in Alzheimer’s Disease (AD).\(^1\) AD is characterized by accumulation of these β-sheet-rich proteins into amyloid plaques and neurofibrillary tangles respectively.\(^2\) This cytotoxic assembly causes mental deterioration over time and eventually leads to dementia and death. Mental exercises and drugs such as Donepezil, Rivastigmine, and Memantine have helped minimize AD symptoms, but such treatments have only slowed progression of the disease.\(^3\) Hence, potential therapeutics that can stop and/or reverse AD progression continue to be heavily studied.

β-sheet disruption has the potential to become a key type of inhibition for modulating AD progression.\(^4\) PHF6 80 is a hexapeptide segment of tau protein that readily aggregates by the formation of β-sheets. Proline (Pro) AAs due to their rigidity and lack of amide proton, they inhibit H-bonding which is a key interaction in β-sheets, making these suitable residues as β-sheet breakers. Segal and co-workers determined that replacing five out of six residues in 80 individually with Pro yielded five peptides (P1-P5) that form random coils rather than aggregating (Figure 4-1).\(^5\) The naming of such peptides stem from the residue used (e.g., P for Pro, T for Thr or V for Val) and the substituted position. The Pro-containing analogues were able to disrupt β-sheet formation, reducing its propensity for self-assembly and in other cases inhibiting 80 from amyloidogenic aggregation. Peptides P2 (88) and P3 (89) were also able to disassemble pre-formed aggregates and inhibit induced cytotoxicity of fibrils.
Figure 4-1: ∆AA PHF6 Analougues

α,β-∆AAs are known for their rigidity and ability to favor other certain secondary structures (e.g. β-hairpins, 310-helices) along with increasing peptide half-life with respect to proteolysis. The bulky residues ∆Val and ∆Env have imparted proteolytic stability and helped maintain a β-hairpin structure when inserted in the (i+1) and (i+2) turn regions of the Waters’ peptide. Similarly, replacement of an N-terminal aminoisobutyric (Aib) residue with medium or bulky ∆AAs in Balaram’s tetrapeptides helped maintain a 310-helical structure. However, replacing a different Aib residue in the same peptide lead to a β-sheet-like orientation. Thus, the location of the unsaturated residue in the backbone plays an important role in determining the structure of the peptide. The turn-inducing properties of ∆AAs justified inserting these residues in 80 as putative β-sheet breakers.

4.2 Synthesis of PHF6 and Pro Analougues

We began by synthesizing 80 and proline-containing analogues 88 and 89 via automated solid-phase peptide synthesis (SPPS). The purpose of these peptides is to serve as controls for performing assays/analysis (e.g., thioflavin, CD spectroscopy) that will be used by other group members to study the properties of our dehydropeptides. Using a high loading (0.65 mmol/g) Rink amide resin, commercially available AAs and coupling reagents we were able to synthesize 80 as shown in Scheme 4-1.
Scheme 4-1: Synthesis of PHF6 (80)

Like the synthesis of 80, we used automated SPPS to prepare 88 and 89. For the synthesis of 88, we installed Pro in place of glutamine (Gln) as highlighted in red in Scheme 4-2. For the synthesis of 89, we replaced isoleucine (Ile) with the desired Pro residue as highlighted in blue in a similar scheme below.

Scheme 4-2: Synthesis of P2 (88) and P3 (89)

4.3 Synthesis of ∆AA-Containing PHF6 Peptides Via SPPS

In previous studies, incorporation of ∆AAs to SPPS required a cumbersome solution-phase preparation of azlactone dipeptides. Other known methods for introducing ∆AAs into peptides that bypassed azlactones required unwieldy protecting group chemistry and lengthy construction of dipeptide intermediates for use in SPPS. Inoue and co-workers’ Staudinger ligation allowed installation of ∆AAs into peptides via SPPS but also required use of air-sensitive precursors in high quantities.

In the previous chapter, we investigated strategies to furnish embedded medium-sized ∆Abu and bulky ∆Val in model tripeptides. We found that EDC•HCl/CuCl conditions helped
furnish ∆Abu from threonine (Thr) dehydration, while DBU-mediated sulfonium eliminations of embedded penicillamine (Pen) delivered the desired ∆Val tripeptide via solution-phase. In unpublished studies, Joaquin and co-workers found that ∆Cyclohexylglycine (∆Chg) induces more rigidity to peptides hence increasing stability to proteolysis, compared to other cyclic ∆AAs. The encouraging potential for application of solution-phase methods to more hindered substrates and interesting new results from cyclic ∆AAs justified the adaptation of these methods to SPPS for the introduction of ∆AAs into 80. For on-resin eliminations we considered ∆Abu and ∆Val as suitable initial targets, with a focus on substituting individual positions two and three of the PHF6 sequence.

4.3.1 Synthesis of ∆Abu-Containing Analogues via On-resin Eliminations

We proceeded to synthesize Thr-containing hexapeptides 95 shown in red and 96 shown in blue as ∆Abu analogue precursors using automated SPPS (Scheme 4-3). Like the synthesis of 80, we used a high loading Rink amide resin, commercially available AAs, and the coupling reagents HATU and HOBT in NMP solvent. For Fmoc deprotections we used 20% Piperidine in DMF and subsequently, acetic anhydride was used for capping the N-termini of hexapeptides. Cleavage from resin was achieved using a cocktail of trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/water.

Scheme 4-3: Synthesis of T2 (97) and T3 (98)
We examined the elimination of Thr-containing hexapeptides 95 and 96 to furnish T2 (97) and T3 (98) using previously studied methods from Ch.3. Treatment of 95 and 96 with MsCl/Et3N in 1,2-dichloroethane (DCE) did not yield 97 or 98 respectively at room temperature (rt). Addition of DBU in DCE or DCM with increasing temperature also did not produce either product. Using DCE as solvent for the mesylation step while switching to CH3CN for the elimination did not produce significant results. Trace amounts of 98 were produced when using DCE for the mesylation step while switching to DMF for the elimination stage while no traces were seen for 97.

Use of EDC•HCl/CuCl conditions in a mixture of CH2Cl2-DMF failed to produce either product. Increasing reaction time as well as using a purified batch of CuCl also failed to produce peptides 97 and 98. However, we continued to use the pure batch of CuCl for future eliminations. Addition of DBU with increasing heat and reaction time in DCE and DMF did not yield the desired products. Refluxing in DCE for 3 days yielded trace amounts of 97. On the other hand, EDC/CuCl with DBU procedure worked best for peptide 97. We also subjected peptides 95 and 96 to DBU alone in DCE and DMF respectively which saw no products. These results demonstrating that DBU-mediated elimination reactions alone cannot reach completion without the hydroxyl alkylating first step.

4.3.2 Synthesis of ∆Val-Containing Analogues via On-resin Eliminations

We proceeded to use automated SPPS for the synthesis of Pen-containing hexapeptides 104 shown in red and 105 shown in blue as ∆Val analogue precursors (Scheme 4-4).
We examined the elimination of Pen-containing hexapeptides 104 and 105 to furnish V2 (106) and V3 (107) using previously studied methods from Ch.3. Peptides 104 and 105 were first deprotected using boron trifluoride etherate and dimethylphenylsilane in hexafluoroisopropanol. Peptides were then treated with 1,4-diiodobutane (DIB) in DMF for the alkylation step. DBU in DMF was later added for the elimination step but yielding no positive results. Efforts to optimize this reaction involved increasing reaction time for the elimination step, but also repeating the deprotection and alkylation steps up to five times. This effort proved more effective as trace amounts for both products were seen, it did however fail to deliver the kind of results previously seen on solution-phase.11

4.4 Synthesis of ΔAA-Containing PHF6 Analogues via Azlactone Dehydrations

In 2015, Jiang and co-workers8 developed methods for incorporating bulky α,β-ΔAAs into peptides using SPPS. This method involved an azlactone (containing the unsaturated residue) opening by the amine group of a resin-bound peptide. A similar approach was used by Jalan and co-workers6 in 2017 for the introduction of ΔAAs into the Waters peptide. We justified the use of these methods towards the synthesis of ΔA-containing PHF6 analogues for their encouraging yields and application to hindered substrates.
4.4.1 Synthesis of ΔAbu-Containing Analogues via Azlactone Dehydrations

For the synthesis of 97 and 98, the resin-bound amines were made via automated SPPS. The required azlactones were made in solution-phase as shown on Scheme 4-4. For the synthesis of T2, Azlactone 110 was made in-situ after coupling of commercially available threonine 109a and valine 108 by addition of excess EDC•HCl. The oxazolone was then opened by the resin-bound amine 111 using triethylamine in NMP at high temperatures. Cleavage from resin using TFA/TIPS/water yielded hexapeptide 97. For the synthesis of T3, dipeptide 114 was made from the coupling of Alloc-protected glutamine 113 and thr 109b. Alloc-protected peptide 116 was obtained after hydrolysis, azlactone dehyderation and amidation with resin bound 115.

Scheme 4-5: Synthesis of T2 and T3 via Azlactone Opening

Alloc removal was achieved on resin using Pd(PPh₃)₄ and phenylsilane, and coupling of Fmoc-Val residue to yielded 117. Finally, fmoc deprotection and acetylation yields 125. Cleavage from resin delivered hexapeptide 107.

4.4.2 Synthesis of ΔVal-Containing Analogues via Azlactone Dehydrations

For the synthesis of 106 and 107, the resin bound amines were made via automated SPPS. The required azlactones were made in solution-phase as shown on Scheme 4-5. For the synthesis of V2, commercially available valine 108 and was coupled to β-OHVal 119 to yield dipeptide 120.
Ac-121 was obtained after hydrolysis, azlactone dehydration and amidation with resin bound 111 using triethylamine in NMP at high temperatures. Cleavage from resin yielded hexapeptide 106.

For the synthesis of V3, dipeptide 122 was made from the coupling of Alloc-protected glutamine 113 and β-OHVal 119. Alloc-123 was formed after hydrolysis, azlactone dehydration and amidation with resin-bound 115. Alloc removal and coupling of Fmoc-Val AA yielded peptide 124. Finally, Fmoc deprotection, acetylation and cleavage from resin furnished hexapeptide 107.

Scheme 4-6: Synthesis of V2 and V3 via Azlactone Opening

4.4.3 Synthesis of ∆Chg-Containing Analogues via Azlactone Dehydrations

We used the same methods above for the introduction of ∆Chg at position two (Chg2 or 129) and position three (Chg3 or 134) as shown on Scheme 4-6. For the synthesis of Chg2, commercially available valine 108 and was coupled to β-OHVal-Chg 126 to yield dipeptide 127. Ac-128 was yielded after hydrolysis, azlactone dehydration and amidation with resin bound 111 at high temperatures. Cleavage from resin yielded hexapeptide 129. For the synthesis of Chg3, dipeptide 130 was made from the coupling of Alloc-protected glutamine 113 and β-OHChg 126. Alloc-131 was yielded after hydrolysis, azlactone dehydration and amidation with resin bound 115. Alloc removal and coupling of Fmoc-Val AA afforded peptide 124. Finally, Fmoc deprotection, acetylation and cleavage from resin yielded hexapeptide 134.
4.5 Summary

We attempted to streamline the synthesis of ∆AA-containing PHF6 analogues by investigating elimination strategies on-resin. We found that previously studied solution-phase conditions were futile, revealing a gap in current approaches that is worthy of additional study. Possible issues could be attributed to the steric hindrance of hexapeptide substrates compared to the less hindered Gly-containing tripeptide precursors used in solution-phase studies. Sluggishness of these methods prompted our shift towards azlactone chemistry in SPPS where a considerable change was seen. HPLC purification of peptides are currently underway. Purified peptides will be assessed using ThS assays and CD spectroscopy for their ability to inhibit β-sheet formation, reduce the propensity for self-assembly, and inhibiting 80 from amyloidogenic aggregation.

4.6 References


5.1 General Experimental Details

Dichloromethane, N,N-dimethylformamide, methanol, and tetrahydrofuran were dried by passage through a solvent drying system containing cylinders of activated alumina. Chloroform was dried by storage over a mixture of activated 4Å MS and anhydrous K₂CO₃. Other solvents and reagents were purchased from commercial vendors and used without purification. Flash chromatography was carried out using 60–230 mesh silica gel. ¹H NMR spectra were acquired on Varian or Bruker 500 MHz spectrometer with chloroform (7.27 ppm) or dimethyl sulfoxide (2.50 ppm) as internal references. Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), br s (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). ¹³C NMR spectra were acquired on a spectrometer operating at 125 MHz with chloroform (77.23 ppm) or dimethyl sulfoxide (39.51 ppm) as internal references. Infrared spectra were obtained on an FT-IR spectrometer. Mass spectral data were obtained using ESI techniques.

5.2 Experimental Procedures and Spectral Data

Ethyl (2R*,3S*)-3-hydroxy-3-methyl-2-((S)-3-methyl-2-(((R)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)butanamido)pentanoate (30). A suspension of 28² (94.0 mg, 0.178 mmol) and Me₃SnOH (81.0 mg, 0.448 mmol, 2.5 equiv) in hexanes (8 mL, pretreated with Na₂SO₄ for 6 h) under Ar was stirred at 60 °C for 48 h. The solvent was concentrated in vacuo, and the residue was treated with Et₂O (10 mL). The mixture was filtered through Celite, (washed with 60 mL of Et₂O), and the filtrate was concentrated in
**vacuo** to afford the crude acid as a colorless oil that was used directly in the next step without further purification.

A solution of the crude acid in anhydrous CH$_2$Cl$_2$ (2.5 mL) at 0 °C under Ar was treated with amine 29$^3$ (35.1 mg, 0.200 mmol, 1.1 equiv), HOBt (ca. 20% H$_2$O content, 45.2 mg, 0.268 mmol, 1.5 equiv), and EDC•HCl (51.0 mg, 0.266 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C under Ar for 2 h. The reaction was quenched by the addition of sat aq NaHCO$_3$ (2 mL) and H$_2$O (2 mL), and the layers were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (6 × 4 mL), and the combined organic layers were dried (Na$_2$SO$_4$) and concentrated *in vacuo*. Flash chromatography (5 mL of SiO$_2$, 0–1.5% MeOH in CH$_2$Cl$_2$ gradient elution) afforded 30 (102.8 mg, 0.157 mmol, 88%) as a colorless oil that was a 1:1 mixture of diastereomers: $^1$H NMR (CDCl$_3$, 500 MHz, minor rotamers present, data for major rotamer of each diastereomer) δ 7.61 (d, $J$ = 7.5 Hz, 2H), 7.44–7.34 (m, 3H), 7.32 and 7.17 (2d, $J$ = 8.1 and 8.4 Hz, 1H), 6.28 and 6.26 (2s, 1H), 6.07 and 6.01 (2d, $J$ = 7.8 and 6.8 Hz, 1H), 4.61 and 4.47 (2d, $J$ = 8.6 and 8.3 Hz, 1H), 4.28–4.19 (m, 2H), 4.17 and 4.09 (2d, $J$ = 7.9 and 7.1 Hz, 1H), 2.62 and 2.58 (2s, 1H), 1.61–1.58 (m, 2H), 1.35–1.10 (m, 12H), 1.05–0.88 (m, 12H), 0.75–0.59 (m, 6H); $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 171.6 and 171.2, 170.0 and 169.6, 154.6 and 154.5, 133.3 and 133.2, 129.63, 129.56 (2C), 129.56 (2C), 99.5, 83.5, 76.1 and 75.6, 74.2 and 73.6, 64.0 and 63.2, 61.6 and 61.5, 58.6 and 58.4, 31.6 and 31.4, 27.4 and 27.1, 26.6 and 25.4, 23.6 and 23.4, 14.1, 7.9 and 7.8, 7.0 (3C), 6.4 (3C); IR (film) $\nu_{\text{max}}$ 3356, 2957, 2877, 1734, 1666, 1506, 1373, 1202, 1059, 1021 cm$^{-1}$; HRMS (ESI) $m/z$ 655.2139 (MH$^+$, C$_{28}$H$_{45}$Cl$_3$N$_2$O$_7$SiH$^+$ requires 655.2140).
Ethyl \((2R^*,3S^*)\)-2-((S)-2-(((\textit{tert}-\textit{butoxycarbonyl})amino)-3-methyl-3-((\textit{triethylsilyl})oxy)butanamido)-3-hydroxy-3-methylpentanoate \((31)\). A suspension of carbamate \(30\) (83.7 mg, 0.128 mmol) in a mixture of THF (1.5 mL) and sat aq NaHCO\(_3\) (0.8 mL) was treated with 10\% Pd/C (10 mg, 0.12 wt equiv) and Boc\(_2\)O (29.0 mg, 0.133 mmol, 1.04 equiv) sequentially at rt under Ar. The resulting mixture was stirred at rt under H\(_2\) (100 psi) for 15 h, diluted with H\(_2\)O (3 mL), and extracted with EtOAc (5 \times 5 mL). The combined organic layers were dried (Na\(_2\)SO\(_4\)) and concentrated \textit{in vacuo}. Flash chromatography (10 mL of SiO\(_2\), 0–1.5\% MeOH in CH\(_2\)Cl\(_2\) gradient elution) afforded \(31\) (60.1 mg, 0.119 mmol, 93\%) as a colorless oil that was a 1:1 mixture of diastereomers: \(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 7.34 and 7.14 (2 br s, 1H), 5.45 (br s, 1H), 4.62 and 4.49 (2d, \(J = 8.4\) and 8.4 Hz, 1H), 4.29–4.16 (m, 2H), 4.14 and 4.04 (2 br s, 1H), 2.75 and 2.65 (2 br s, 1H), 1.60–1.53 (m, 2H), 1.45 and 1.44 (2s, 9H), 1.37 and 1.35 (2s, 3H), 1.31 (t, \(J = 7.2\) Hz, 3H), 1.28 (s, 3H), 1.20 and 1.19 (2s, 3H), 0.98 (t, \(J = 8.0\) Hz, 9H), 0.95–0.91 (m, 3H), 0.72–0.64 (m, 6H); \(^{13}\)C NMR (CDCl\(_3\), 125 MHz) \(\delta\) 171.7, 171.3 and 170.9, 156.1, 80.0 and 79.7, 76.4 and 75.5, 74.2 and 73.7, 63.7 and 62.6, 61.5 and 61.4, 58.5 and 58.2, 31.5 and 31.4, 28.3 (3C), 27.6, 27.2 and 26.7, 23.6 and 23.4, 14.2 and 14.1, 7.9 and 7.8, 7.0 (3C), 6.5 (3C); IR (film) \(v_{\text{max}}\) 3373, 2976, 2877, 2361, 1725, 1664, 1501, 1367, 1164, 1051 cm\(^{-1}\); HRMS (ESI) \(m/z\) 505.3311 (MH\(^+\), C\(_{24}\)H\(_{48}\)N\(_2\)O\(_7\)SiH\(^+\) requires 505.3309).

\((R)\)-2-azido-1-iodopropane \((32)\). A solution of \((R)-(--)\)-2-amino-1-propanol (222 \(\mu\)L, 214 mg, 2.85 mmol), K\(_2\)CO\(_3\) (386.7 mg, 2.80 mmol), and CuSO\(_4\)•5H\(_2\)O (7.7 mg, 0.031 mmol) in H\(_2\)O (9 mL) and MeOH (18 mL) at rt under Ar was treated with a solution of TfN\(_3\) in CH\(_2\)Cl\(_2\) (prepared according to the procedure of Lundquist and Pelletier\(^4\)) ca. 0.42 M, 13.3 mL, ca. 5.6
mmol). The resulting mixture was stirred at rt for 48 h. The layers were separated, and the aqueous layer was extracted with CH2Cl2 (4 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na2SO4), and concentrated in vacuo. The crude azido alcohol was used in the next step without further purification.

A solution of PPh3 (931 mg, 3.55 mmol) in anhydrous CH2Cl2 (30 mL) at rt under Ar was treated with imidazole (520 mg, 7.64 mmol) followed by I2 (1.44 g, 5.67 mmol), stirred for 5 min, then treated dropwise with the crude azido alcohol. The resulting mixture was refluxed for 48 h, cooled to rt, and treated with sat aq. Na2SO3 (20 mL). It was stirred until the color changed from black to yellow, at which time the layers were separated. The aqueous layer was extracted with CH2Cl2 (4 × 20 mL), and the combined organic layers were washed with brine (20 mL), dried (Na2SO4), and concentrated in vacuo. Flash chromatography (100 mL of SiO2, 0−0.5% EtOAc in hexanes gradient elution) afforded 32 (449 mg, 2.13 mmol, 75% over 2 steps) as a light yellow oil: [α]25D −29 (c 0.90, CHCl3); 1H NMR (CDCl3, 500 MHz) δ 3.65–3.56 (m, 1H), 3.28–3.18 (m, 2H), 1.38 (d, J = 6.5 Hz, 3H); 13C NMR (CDCl3, 125 MHz) δ 57.8, 19.9, 9.7; IR (film) νmax 2922, 2851, 2105, 1261 cm−1.

(R)-2-azidopropyl (2S*,3R*)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methyl-3-((triethylsilyl)oxy)butanamido)-3-hydroxy-3-methylpentanoate (33). A solution of ester 31 (21.3 mg, 0.0422 mmol) in t-BuOH (400 µL) and H2O (150 µL) at 0 °C was treated with LiOH•H2O (8.8 mg, 0.210 mmol, 5.0 equiv), then stirred at rt for 3 h. The resulting mixture was acidified to pH 4~5 by the addition of 2 N HCl, diluted with H2O (2 mL), and extracted with EtOAc (2 × 6 mL). The combined organic layers were dried (Na2SO4) and concentrated in vacuo.
The crude carboxylic acid (17.5 mg, 0.0367 mmol, 87%) was used directly without further purification.

A solution of the crude carboxylic acid (17.5 mg, 0.0367 mmol) and iodide 32 (15.5 mg, 0.0735 mmol, 2.0 equiv) in anhydrous DMF (600 µL) at rt under Ar was treated with Cs₂CO₃ (9.6 mg, 0.0295 mmol, 0.80 equiv). The resulting mixture was stirred at 80 °C under Ar for 16 h, diluted with H₂O (2 mL), and extracted with EtOAc (6 × 2 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (5 mL of SiO₂, 0–1% MeOH in CH₂Cl₂ gradient elution) afforded 33 (15.5 mg, 0.0277 mmol, 75%; 66% from 31) as a colorless oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.37 and 7.16 (2 br s, 1H), 5.48 and 5.43 (2 br s, 1H), 4.63 and 4.51 (2d, J = 7.8 Hz and 8.1 Hz, 1H), 4.19–4.01 (m, 3H), 3.85–3.74 (m, 1H), 2.56 and 2.45 (2 br s, 1H), 1.67–1.54 (m, 2H), 1.45 (s, 9H), 1.37 and 1.35 (2s, 3H), 1.34–1.31 (m, 3H), 1.29 and 1.28 (2s, 3H), 1.24 and 1.23 (2s, 3H), 0.98 (t, J = 7.8 Hz, 9H), 0.96–0.91 (m, 3H), 0.72–0.64 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.3 and 171.0, 170.5, 156.1, 79.9 and 79.8, 76.3 and 75.6, 74.0 and 73.6, 67.8 and 67.4, 63.7 and 62.6, 58.7 and 58.4, 55.7 and 55.5, 31.6 and 31.5, 28.3 (3C), 27.5 and 27.2, 26.7 and 25.4, 23.7 and 23.4, 16.1 and 15.9, 7.9 and 7.8, 7.0 (3C), 6.5 (3C); IR (film) νₘₐₓ 3410, 2976, 2360, 2120, 1670, 1507, 1367, 1163 cm⁻¹; HRMS (ESI) m/z 560.3468 (MH⁺, C₂₅H₄₉N₅O₇SiH⁺ requires 560.3480).

BocHN

H

OTES

O

N

H

34

tert-Butyl ((6R,12R,Z)-9-(butan-2-ylidene)-3,3-diethyl-13-hydroxy-5,5,12-trimethyl-7,10-dioxo-4-oxa-8,11-diaza-3-silatridecan-6-yl)carbamate (34). A solution of alcohol 33 (22.5 mg, 0.0402 mmol) in anhydrous CHCl₃ (300 µL) at 0 °C under Ar was treated dropwise with a solution of Martin sulfurane (54.1 mg, 0.0804 mmol, 2.0 equiv) in anhydrous
CHCl₃ (600 μL). The resulting mixture was stirred at 0 °C under Ar for 1 h, warmed to rt, and concentrated in vacuo. The residue was dissolved in THF (1.0 mL) and H₂O (100 μL), then treated with Lindlar catalyst (217.1 mg). The resulting suspension was stirred at rt under H₂ (1 atm) for 20 h, at which point reduction of the azide was complete as evidenced by MS. The H₂ was replaced by Ar (1 atm), piperidine (110 μL, 94.8 mg, 1.11 mmol, 28 equiv) was added to the mixture, and it was stirred at rt for 24 h. The mixture was then treated with sat aq NaHCO₃ (5 mL) and extracted with EtOAc (5 × 6 mL). The combined organic extracts were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (8 mL of SiO₂, 0–4% MeOH in CH₂Cl₂ gradient elution) afforded 34 (16.8 mg, 0.0326 mmol, 81%, 12:1 dr) as a white film: [α]²⁵D +16.6 (c 0.5, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 7.61 (s, 1H), 6.32 (br s, 1H), 5.45 (br s, 1H), 4.16–4.09 (m, 1H), 4.05 (d, J = 5.5 Hz, 1H), 3.81–3.73 (m, 1H), 3.49–3.41 (m, 2H), 2.18–2.08 (m, 2H), 1.99 (s, 3H), 1.45 (s, 9H), 1.37 (s, 3H), 1.31 (s, 3H), 1.20 (d, J = 6.9 Hz, 3H), 1.04 (t, J = 7.6 Hz, 3H), 0.99 (t, J = 8.0 Hz, 9H), 0.67 (q, J = 7.8 Hz, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.2, 166.6, 156.5, 141.0, 124.1, 80.5, 75.7, 66.5, 63.7, 48.0, 28.3 (3C), 27.3, 26.7, 26.3, 17.7, 16.7, 11.7, 7.0 (3C), 6.5 (3C); IR (film) νmax 3265, 2924, 2854, 1649, 1517, 1367, 1170, 1051 cm⁻¹; HRMS (ESI) m/z 516.3441 (MH⁺, C₂₅H₄₉N₃O₆SiH⁺ requires 516.3469).

Ethyl 3-Hydroxy-3-methyl-2-(((S)-3-methyl-2-(((R)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethyilsilyl)oxy)butanamido)butanoate (45). A suspension of 28² (175.0 mg, 0.332 mmol) and Me₃SnOH (288.0 mg, 1.592 mmol, 4.8 equiv) in hexanes (10 mL, pretreated with Na₂SO₄ for 12 h) under Ar was stirred at 60 °C for 48 h. The solvent was concentrated in vacuo, and the residue was treated with Et₂O (12 mL). The mixture
was filtered through Celite, (washed with 50 mL of Et₂O), and the filtrate was concentrated in vacuo to afford the crude carboxylic acid as a pale yellow oil that was used directly in the next step without further purification.

A solution of the crude carboxylic acid in anhydrous CH₂Cl₂ (3.0 mL) at 0 °C under Ar was treated with amine 44 (109 mg, 0.676 mmol, 2.0 equiv), HOBt (ca. 20% H₂O content, 114 mg, 0.675 mmol, 2.0 equiv), and EDC•HCl (130 mg, 0.678 mmol, 2.0 equiv). The resulting mixture was stirred at 0 °C under Ar for 4 h. The reaction was quenched by the addition of sat aq NaHCO₃ (3 mL) and H₂O (2 mL), and the layers were separated. The aqueous layer was extracted with CH₂Cl₂ (4 × 5 mL), and the combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (10 mL of SiO₂, 0–1.5% MeOH in CH₂Cl₂ gradient elution) afforded 45 (181 mg, 0.282 mmol, 85%) as a colorless oil that was a 1:1 mixture of diastereomers: \(^1\)H NMR (CDCl₃, 500 MHz, minor rotamers present, data for major rotamer of each diastereomer) δ 7.61 (d, \(J = 7.6\) Hz, 2H), 7.44–7.34 (m, 3H), 7.14 (d, \(J = 8.3\) Hz, 1H), 6.32 (s, 1H), 6.10 and 6.04 (2d, \(J = 7.6\) and 6.7 Hz, 1H), 4.49 and 4.40 (2d, \(J = 8.7\) and 8.1 Hz, 1H), 4.28–4.17 (m, 2H), 4.16 (d, \(J = 7.7\) Hz, 1H), 2.64 and 2.62 (2s, 1H), 1.42 and 1.41 (2s, 3H), 1.32–1.24 (m, 6H), 1.21 and 1.20 (2s, 3H), 1.19 (s, 3H), 1.03–0.94 (m, 9H), 0.74–0.64 (m, 6H); \(^{13}\)C NMR (CDCl₃, 125 MHz) δ 171.4 and 170.9, 170.0 and 169.6, 154.7 and 154.5, 133.0, 129.7 (2C), 129.5, 127.9 (2C), 99.7 and 99.6, 83.6 and 83.5, 72.0 and 71.4, 63.7, 62.9, 61.6 and 61.5, 60.3, 27.5 and 27.3, 27.1 and 26.7, 26.4 and 26.1, 25.0, 14.2 and 14.1, 7.0 (3C), 6.4 (3C); IR (film) \(\nu_{\max}\) 3349, 2956, 2876, 1738, 1667, 1505, 1375, 1202, 1059 cm\(^{-1}\); HRMS (ESI) \(m/z\) 641.1984 (MH\(^+\), \(\text{C}_{27}\text{H}_{43}\text{Cl}_3\text{N}_2\text{O}_7\text{SiH}^+\) requires 641.1978).
Ethyl 2-((S)-2-((tert-Butoxycarbonyl)amino)-3-methyl-3-((triethylsilyl)oxy) butanamido)-3-hydroxy-3-methylbutanoate (46). A suspension of 45 (80.0 mg, 0.125 mmol) in a mixture of THF (2.0 mL) and sat aq NaHCO₃ (1.0 mL) was treated with 10% Pd/C (30.2 mg, 0.38 wt equiv) and Boc₂O (28.1 mg, 0.129 mmol, 1.03 equiv) at rt under Ar. The resulting mixture was stirred at rt under H₂ (200 psi) for 24 h, then diluted with H₂O (4 mL), and extracted with EtOAc (4 × 6 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (11 mL of SiO₂, 0–1.5% MeOH in CH₂Cl₂ gradient elution) afforded 46 (56.1 mg, 0.114 mmol, 92%) as a colorless oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.33 and 7.14 (2 br s, 1H), 5.48 and 5.45 (2 br s, 1H), 4.57 and 4.48 (2d, J = 8.3 and 8.4 Hz, 1H), 4.28–4.19 (m, 2H), 4.15 and 4.04 (2 br s, 1H), 2.86 and 2.78 (2 br s, 1H), 1.45 and 1.44 (2s, 9H), 1.38 and 1.35 (2s, 3H), 1.32–1.25 (m, 12H), 0.98 (t, J = 7.9 Hz, 9H), 0.71–0.65 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.0, 170.9, 156.1, 79.7, 76.4, 75.4, 72.1 and 71.7, 61.4, 60.2 and 60.1, 28.2 (3C), 27.5, 27.2, 26.8, 26.6 and 26.5, 14.1, 7.0 (3C), 6.4 (3C); IR (film) νmax 3257, 2954, 2876, 1747, 1646, 1513, 1366, 1165, 1052 cm⁻¹; HRMS (ESI) m/z 491.3152 (MH⁺, C₂₅H₄₆N₂O₇SiH⁺ requires 491.3147).

Methyl (2-((S)-2-((tert-Butoxycarbonyl)amino)-3-methyl-3-((triethylsilyl)oxy)butanamido)-3-methylbut-2-enoyl)-D-alaninate (47). A solution of 46 (40.1 mg, 0.0817 mmol) in t-BuOH (800 μL) was treated with LiOH•H₂O (17.4 mg, 0.415 mmol, 5.1 equiv) and H₂O (300 μL), then stirred at rt for 6 h. The resulting mixture was acidified to pH
4~5 by the addition of 1 N HCl, diluted with H2O (2 mL), and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na2SO4) and concentrated \textit{in vacuo}. The crude carboxylic acid was used directly without further purification.

A solution of the crude carboxylic acid in anhydrous DMF (2 mL) was treated with EDC•HCl (156.5 mg, 0.816 mmol, 10.0 equiv) and stirred at rt under Ar for 24 h. The resulting mixture was treated with D-Ala-OMe•HCl (113.5 mg, 0.813 mmol, 10.0 equiv), Et3N (180 μL, 131 mg, 1.29 mmol, 15.9 equiv), and additional DMF (1 mL), then stirred at 70 °C under Ar for 48 h. The mixture was diluted with H2O (8 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (Na2SO4) and concentrated \textit{in vacuo}. Flash chromatography (12 mL of SiO2, 0–1.5% MeOH in CH2Cl2 gradient elution) afforded 47 (24.6 mg, 0.0464 mmol, 57% from 46) as a yellow film: [α]25D +15.1 (c 0.6, CHCl3); \(^1\)H NMR (CDCl3, 500 MHz, mixture of rotamers) δ 7.64 and 7.59 (2 br s, 1H), 6.91 and 6.83 (br s and d, J = 6.2 Hz, 1H), 5.47 (br s, 1H), 4.60 (q, J = 7.1 Hz, 1H), 4.10 and 4.04 (2d, J = 5.9 and 5.9 Hz, 1H), 3.74 and 3.73 (2s, 3H), 2.07 and 2.04 (2s, 3H), 1.78 (s, 3H), 1.45 and 1.44 (2s, 9H), 1.38 (d, J = 6.9 Hz, 3H), 1.34 (s, 3H), 1.29 and 1.26 (2s, 3H), 0.98 (t, J = 7.9 Hz, 9H), 0.67 (q, J = 7.8 Hz, 6H); \(^13\)C NMR (CDCl3, 125 MHz, mixture of rotamers) δ 173.33 and 173.27, 169.7 and 169.5, 165.6 and 165.3, 139.5 and 138.6, 129.2 and 127.6, 124.0 and 123.6, 80.3, 75.5, 63.9, 52.3 and 52.2, 48.22 and 48.17, 34.4, 28.2 (3C), 27.3 and 27.2, 26.7, 20.6 and 20.5, 17.9 and 17.8, 6.9 (3C), 6.5 (3C); IR (film) ν\textsubscript{max} 3257, 2954, 2876, 1697, 1513, 1366, 1165, 1052 cm\textsuperscript{-1}; HRMS (ESI) \textit{m/z} 530.3249 (MH\textsuperscript{+}, C25H47N3O7SiH\textsuperscript{+} requires 530.3256).
Ethyl 3-Ethyl-3-hydroxy-2-((2S,3R)-3-methyl-2-(((R)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)pentanamido)pentanoate (50a). A suspension of 48\textsuperscript{3} (127.5 mg, 0.2357 mmol) and Me\textsubscript{3}SnOH\textsuperscript{9} (171.1 mg, 0.9462 mmol, 4.0 equiv) in hexanes (8 mL, pretreated with Na\textsubscript{2}SO\textsubscript{4} for 6 h) was stirred at 60 °C under Ar for 72 h. The mixture was concentrated \textit{in vacuo}, and the residue was treated with Et\textsubscript{2}O (3 mL). The mixture was filtered through Celite, (washed with 10 mL of Et\textsubscript{2}O), and the filtrate was concentrated \textit{in vacuo} to afford the crude acid as a colorless oil that was used directly in the next step without further purification.

The crude acid prepared above was dissolved in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (2 mL), cooled to 0 °C under Ar, then treated with amine 49a\textsuperscript{6} (67.2 mg, 0.355 mmol, 1.5 equiv), HOBt (ca. 20% H\textsubscript{2}O content, 60.0 mg, 0.355 mmol, 1.5 equiv), and EDC•HCl (67.7 mg, 0.353 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 18 h. The reaction was quenched by the addition of sat aq NaHCO\textsubscript{3} (1 mL), the layers were separated, and the aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 × 4 mL). The combined organic layers were dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated \textit{in vacuo}. Flash chromatography (44 mL of SiO\textsubscript{2}, 0–1.5% MeOH in CH\textsubscript{2}Cl\textsubscript{2} gradient elution) afforded 50a (142.4 mg, 0.2081 mmol, 88%) as a white film that was a 1:1 mixture of diastereomers: \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 500 MHz, minor rotamers present, data for major rotamer of each diastereomer) δ 7.61 (d, \textit{J} = 6.8 Hz, 2H), 7.44–7.35 (m, 3H), 7.25 and 7.09 (2d, \textit{J} = 8.6 and 8.7 Hz, 1H), 6.28 and 6.26 (2s, 1H), 6.01 and 5.92 (2d, \textit{J} = 8.0 and 7.5 Hz, 1H), 4.63 and 4.56 (2d, \textit{J} = 8.8 and 8.7 Hz 1H), 4.28–4.15 (m, 3H), 2.45 and 2.40 (2 br s, 1H), 1.63–1.45 (m, 6H), 1.31 (t, \textit{J} = 7.2 Hz, 3H), 1.25 and 1.23 (2s, 3H), 1.02–0.92 (m, 9H), 0.90–0.79 (m, 9H), 0.75–0.63 (m, 6H); \textsuperscript{13}C NMR
(CDCl₃, 125 MHz) δ 172.04 and 171.98, 170.1 and 169.8, 154.7 and 154.5, 133.52 and 133.45, 130.2, 129.8 (2C), 128.0 (2C), 99.7, 83.71 and 83.67, 83.7, 78.7, 78.2, 76.4 and 76.1, 62.1 and 61.7, 57.4 and 57.1, 32.8 and 31.8, 28.74 and 28.69, 26.8 and 26.6, 24.11 and 24.08, 14.3, 8.9 and 8.7, 7.84 and 7.79, 7.7, 7.3 (3C), 6.8 (3C); IR (film) ν max 3353, 3286, 2964, 2878, 2359, 1732, 1661, 1505, 1377, 1200, 1067 cm⁻¹; HRMS (ESI) m/z 683.2445 (MH⁺, C₃₀H₄₉Cl₃N₂O₇SiH⁺ requires 683.2447).

Ethyl 2-((2S,3R)-2-(((tert-Butoxycarbonyl)amino)-3-methyl-3((triethylsilyl)oxy)pentanamido)-3-ethyl-3-hydroxypentanoate (51a). A suspension of carbamate 50a (142.4 mg, 0.2081 mmol) in THF–sat aq NaHCO₃ (2:1, 2.3 mL) was treated sequentially with 10% Pd/C (22.1 mg, 0.16 wt equiv) and Boc₂O (49.9 mg, 0.229 mmol, 1.1 equiv). The resulting mixture was stirred at rt under H₂ (200 psi) for 24 h, diluted with H₂O (3 mL), and extracted with EtOAc (3 × 7 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (44 mL of SiO₂, 0–1.5% MeOH in CH₂Cl₂ gradient elution) afforded 51a (104.0 mg, 0.1952 mmol, 94%) as a white film that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.39 and 7.34 (2 br s, 1H), 7.02 (br s, 1H), 5.39 and 5.31 (d and br s, J = 7.2 Hz, 1H), 4.60 and 4.53 (2d, J = 8.7 Hz and 8.8 Hz, 1H), 4.21–4.11 (m, 3H), 2.50 and 2.48 (2 br s, 1H), 1.55–1.41 (m, 6H), 1.40 and 1.39 (2s, 9H), 1.29–1.22 (m, 5H), 0.96–0.80 (m, 18H), 0.69–0.59 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.1, 171.0 and 170.7, 156.2 and 155.9, 80.0 and 79.8, 78.9 and 78.2, 76.5 and 76.1, 74.3, 61.5, 57.2 and 56.8, 33.0 and 31.9, 28.6, 28.4 (3C), 26.7, 24.2, 14.3, 8.9 and 8.7, 7.8 and 7.7, 7.74, 7.3 (3C), 6.9 and 6.8 (3C);
IR (film) \( \nu_{\text{max}} \) 3359, 2968, 2878, 2360, 2342, 1722, 1662, 1505, 1367, 1166, 1023 cm\(^{-1} \); HRMS (ESI) \( m/z \) 533.3614 (MH\(^+\), C\(_{26}\)H\(_{52}\)N\(_2\)O\(_7\)SiH\(^+\) requires 533.3617).

Methyl \((2S,3R)-2-((\text{tert-Butoxycarbonyl})amino)-3\text{-methyl-3-}
((\text{triethylsilyl})\text{oxy})\text{pentanamido})-3\text{-ethylypent-2-enoyl}\text{glycinate (52a).} \) A solution of ester 51a (45.0 mg, 0.0845 mmol) in \( t\)-BuOH–H\(_2\)O (3:1, 3 mL) was treated with LiOH•H\(_2\)O (17.7 mg, 0.422 mmol, 5.0 equiv) at 0 °C, then stirred at rt for 5 h. The resulting mixture was acidified to pH 4~5 by the addition of 1 N HCl, diluted with H\(_2\)O (2 mL), and extracted with CH\(_2\)Cl\(_2\) (\( 3 \times 5 \) mL). The combined organic layers were dried (Na\(_2\)SO\(_4\)) and concentrated \textit{in vacuo}. The crude carboxylic acid was used directly without further purification.

A solution of the crude carboxylic acid in anhydrous DMF (3 mL) was treated with EDC•HCl (162.0 mg, 0.8451 mmol, 10 equiv) under Ar and stirred at rt for 24 h. The resulting mixture was treated with Gly-OMe•HCl (106.1 mg, 0.8451 mmol, 10 equiv), Et\(_3\)N (190 \( \mu \)L, 138 mg, 1.36 mmol, 16 equiv), and additional anhydrous DMF (1 mL), then stirred under Ar at 80 °C for 48 h. The solution was cooled to rt, diluted with EtOAc (10 mL), and washed with brine (\( 10 \times 10 \) mL) to remove the DMF. The combined organic layers were dried (Na\(_2\)SO\(_4\)) and concentrated \textit{in vacuo}. Flash chromatography (20 mL of SiO\(_2\), 0–1.5% MeOH in CH\(_2\)Cl\(_2\) gradient elution) afforded 52a (23.0 mg, 0.0412 mmol, 49%) as a pale yellow oil: [\( \alpha \)]\(_{D}^{25}\) 3.3 (c 1.7, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\), 500 MHz) \( \delta \) 7.41 (br s, 1H), 7.01 (br s, 1H), 5.31 (br s, 1H), 4.05–3.98 (m, 2H), 3.91 (dd, \( J = 12.5, 5.5 \) Hz, 1H), 3.66 (s, 3H), 2.41–2.32 (m, 2H), 2.06 (q, \( J = 7.6 \) Hz, 2H), 1.64–1.58 (m, 2H), 1.37 (s, 9H), 1.29 (s, 3H), 1.03 (t, \( J = 7.4 \) Hz, 3H), 0.97–0.87 (m, 12H), 0.85–0.78...
(m, 3H), 0.60 (q, J = 7.9 Hz, 6H); \(^{13}\text{C}\) NMR (CDCl\(_3\), 125 MHz) \(\delta\) 170.5, 170.2, 166.3, 156.4, 150.2, 123.0, 80.3, 78.1, 61.6, 52.2, 41.3, 32.7, 28.3 (3C), 28.0, 24.23, 24.18, 13.2, 8.8, 7.1 (3C), 6.7 (3C); IR (film) \(\nu_{\text{max}}\) 3335, 2962, 2877, 2359, 1682, 1505, 1367, 1169, 1066, 1008 cm\(^{-1}\); HRMS (ESI) \(m/z\) 538.3570 (MH\(^+\), C\(_{27}\)H\(_{51}\)N\(_3\)O\(_7\)SiH\(^+\) requires 538.3569).

![Chemical structure of compound 53a](image)

Ethyl (5R,6S)-6-((\textit{tert}-Butoxycarbonyl)amino)-3,3,5-triethyl-15-(2-hydroxypropan-2-yl)-5-methyl-7,10,13-trioxo-9-(pentan-3-ylidene)-4-oxa-8,11,14-triaza3-silahexadecan-16-oate (53a). A solution of tripeptide 52a (10.6 mg, 0.0190 mmol) in \(t\)-BuOH (600 \(\mu\)L) was treated with LiOH•H\(_2\)O (4.3 mg, 0.10 mmol, 5.4 equiv) and H\(_2\)O (200 \(\mu\)L), then stirred at rt for 3 h. The resulting mixture was acidified to pH 4~5 by the addition of 1 N HCl, diluted with H\(_2\)O (2 mL), and extracted with EtOAc (2 \(\times\) 3 mL). The combined organic layers were dried (Na\(_2\)SO\(_4\)) and concentrated \textit{in vacuo}. The crude carboxylic acid was used directly without further purification.

The crude acid was dissolved in anhydrous CH\(_2\)Cl\(_2\) (2.0 mL), cooled to 0 °C under Ar, then treated with amine 44\(^5\) (4.9 mg, 0.030 mmol, 1.6 equiv), HOBt (ca. 20% H\(_2\)O content, 4.8 mg, 0.028 mmol, 1.5 equiv) and EDC•HCl (5.8 mg, 0.030 mmol, 1.6 equiv). The resulting mixture was stirred at rt under Ar for 15 h. The reaction was quenched by the addition of sat aq NaHCO\(_3\) (1 mL), the layers were separated, and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (4 \(\times\) 4 mL). The combined organic layers were dried (Na\(_2\)SO\(_4\)) and concentrated \textit{in vacuo}. Flash chromatography
23 mL of SiO₂, 0–4% MeOH in CH₂Cl₂ gradient elution) afforded 53a (10.5 mg, 0.0153 mmol, 80%) as a colorless oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.64–7.50 (m, 2H), 7.34–7.26 (m, 1H), 7.01 and 6.94 (2 br s, 1H), 5.48 (br s, 1H), 4.54 and 4.51 (2d, J = 9.4 and 9.2 Hz, 1H), 4.20–4.05 (m, 3H), 4.02–3.98 and 3.94–3.90 (2m, 1H), 3.73–3.64 (m, 1H), 2.44–2.28 (m, 2H), 2.12–2.05 (m, 2H), 1.62–1.54 (m, 2H), 1.38 and 1.37 (2s, 9H), 1.30–1.26 (m, 3H), 1.23–1.17 (m, 9H), 1.04–1.00 (m, 3H), 0.98 (t, J = 7.6 Hz, 3H), 0.92 (t, J = 7.9 Hz, 9H), 0.86–0.78 (m, 3H), 0.63–0.57 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.3, 170.5, 170.4, 159.8, 156.7, 150.9, 130.0, 81.0, 77.8, 72.0, 62.2 and 62.0, 61.2 and 61.0, 60.5, 43.2, 32.8, 28.3 (3C), 27.3, 26.8, 24.4, 24.3, 24.2, 14.2 and 14.1, 13.2, 12.1, 8.8, 7.1 (3C), 6.7 (3C); IR (film) ᶦmax 3371, 2911, 2868, 2214, 1750, 1631, 1590, 1389, 1311, 1298, 1170 cm⁻¹; HRMS (ESI) m/z 687.4361 (MH⁺, C₃₃H₆₂N₄O₉SiH⁺ requires 687.4359).

Methyl (2-(2-(2-((2S,3R)-2-((tert-Butoxycarbonyl)amino)-3-methyl-3-((triethylsilyl)oxy)pentanamido)-3-ethylpent-2-enamido)acetamido)-3-methylbut2-enoyl)-D-valinate (54a). A solution of ester 53a (13.8 mg, 0.0201 mmol) in t-BuOH-H₂O (3:1, 1 mL) was treated with LiOH•H₂O (4.3 mg, 0.10 mmol, 5.1 equiv), then stirred at rt for 5 h. The resulting mixture was acidified to pH 4–5 by the addition of 1 N HCl, diluted with H₂O (2 mL), and extracted with CH₂Cl₂ (3 × 4 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The crude carboxylic acid was used directly without further purification.
A solution of the crude carboxylic acid in anhydrous DMF (1 mL) was treated with EDC•HCl (39.0 mg, 0.203 mmol, 10.1 equiv) and stirred at rt under Ar for 24 h, at which point the azlactone intermediate was formed according to MS. The resulting mixture was treated with D-ValOMe•HCl (34.1 mg, 0.203 mmol, 10.1 equiv), Et₃N (46 µL, 33 mg, 0.33 mmol, 16 equiv), and additional anhydrous DMF (1 mL), then stirred at 80 °C under Ar for 48 h. The solution was cooled to rt, diluted with EtOAc (5 mL), and washed with brine (10 × 8 mL) to remove the DMF. The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (10 mL of SiO₂, 0–10 % MeOH in CH₂Cl₂ gradient elution) afforded 54a (7.1 mg, 0.0094 mmol, 47%) as a yellow oil: [α]²⁵D –10.9 (c 1.1, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 8.48 (br s, 1H), 7.68 (br s, 1H), 6.94 (d, J = 8.0 Hz, 1H), 5.44 (br s, 1H), 5.35 (br s, 1H), 4.47 (dd, J = 8.0, 5.8 Hz, 1H), 4.19 (dd, J = 16.9, 6.4 Hz, 1H), 4.00 (d, J = 5.1 Hz, 1H), 3.81–3.73 (m, 1H), 3.71 (s, 3H), 2.53–2.35 (m, 2H), 2.25–2.12 (m, 2H), 2.12 (s, 3H), 2.09–2.00 (m, 1H), 1.81 (s, 3H), 1.70–1.62 (m, 2H), 1.45 (s, 9H), 1.33 (s, 3H), 1.10 (t, J = 7.5 Hz, 3H), 1.04 (t, J = 7.6 Hz, 3H), 1.01–0.96 (m, 12H), 0.94–0.82 (m, 6H), 0.68 (q, J = 7.9 Hz, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 173.2, 171.1, 169.0, 166.2, 165.9, 154.5, 148.0, 141.8, 130.2, 130.0, 81.2, 77.2 (obscured by solvent), 62.1, 58.0, 52.1, 44.1, 32.1, 29.5, 28.5 (3C), 27.4, 25.7, 24.6, 22.9, 21.0, 19.3, 18.5, 13.4, 12.4, 8.9, 7.3 (3C), 6.9 (3C); IR (film) νmax 3312, 2965, 2983, 2855, 1698, 1623, 1510, 1359, 1168 cm⁻¹; HRMS (ESI) m/z 754.4783 (MH⁺, C₃₇H₆₇N₅O₉SiH⁺ requires 754.4781).

Ethyl 3-Hydroxy-3-methyl-2-((2S,3R)-3-methyl-2-(((R)-2,2,2-trichloro1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)pentanamido)butanoate (50b). A suspension of 48² (200 mg, 0.370 mmol) and Me₃SnOH⁹ (401 mg, 2.22 mmol, 6.0 equiv)
in hexane (25 mL, pretreated with Na₂SO₄ for 6 h) was stirred at 70 °C under Ar for 72 h. The mixture was concentrated in vacuo, and the residue was treated with Et₂O (10 mL). The mixture was filtered through Celite, (washed with 60 mL of Et₂O), and the filtrate was concentrated in vacuo to afford the crude acid as a colorless oil that was used directly in the next step without further purification.

The crude acid prepared above (ca. 0.370 mmol) was dissolved in anhydrous CH₂Cl₂ (2 mL), cooled to 0 °C under Ar, then treated with amine 44₅ (77.6 mg, 0.481 mmol, 1.3 equiv), HOBt (ca. 20% H₂O content, 100 mg, 0.592 mmol, 1.6 equiv), and EDC·HCl (107 mg, 0.558 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C under Ar for 3 h. The reaction was quenched by the addition of sat aq NaHCO₃ (1 mL), the layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (6 × 4 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (88 mL of SiO₂, 0–1.5% MeOH in CH₂Cl₂ gradient elution) afforded 50b (203 mg, 0.309 mmol, 84%) as a colorless oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz, minor rotamers present, data for major rotamer of each diastereomer) δ 7.61 (d, J = 6.4 Hz, 2H), 7.45–7.35 (m, 3H), 7.28 and 7.13 (2d (one is partially obscured by solvent), J = 8.1 and 7.3 Hz, 1H), 6.28 and 6.26 (2s, 1H), 6.01 and 5.93 (2d, J = 8.1 and 7.3 Hz, 1H), 4.55 and 4.48 (2d, J = 8.6 and 8.6 Hz, 1H), 4.29–4.17 (m, 3H), 2.69 (br s, 1H), 1.66–1.43 (m, 2H), 1.31 (t, J = 7.2 Hz, 3H), 1.30–1.22 (m, 9H), 0.99 (t, J = 7.9 Hz, 9H), 0.90–0.80 (m, 3H), 0.68 (q, J = 7.5 Hz, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.5 and 171.3, 170.4 and 170.1, 154.7 and 154.6, 133.5 and 133.4, 130.2, 129.8 (2C), 128.1 (2C), 99.6, 83.7, 78.7 and 78.2, 72.4 and 71.8, 62.2 and 62.1, 61.8, 60.4 and 60.3, 32.8 and 31.9, 27.2 and 26.94, 26.87 and 26.7, 24.1, 14.4, 8.9 and 8.7, 7.3 (3C), 6.8 (3C); IR (film) νmax 3352, 2923, 2361, 1734, 1668, 1506, 1377, 1065 cm⁻¹; HRMS (ESI) m/z 655.2139 (MH⁺, C₂₈H₄₅Cl₃N₂O₇SiH⁺ requires 655.2135.
Ethyl 2-((2S,3R)-2-((tert-Butoxycarbonyl)amino)-3-methyl-3((triethoxysilyl)oxy)pentanamido)-3-hydroxy-3-methylbutanoate (51b). A suspension of carbamate 50b (87.0 mg, 0.133 mmol) in THF–sat aq NaHCO₃ (2:1, 10 mL) was treated sequentially with 10% Pd/C (43.5 mg, 0.50 wt equiv) and Boc₂O (116 mg, 0.532 mmol, 4.0 equiv). The resulting mixture was stirred at rt under H₂ (600 psi) for 15 h, diluted with H₂O (1 mL) and sat aq NaHCO₃ (1 mL), and extracted with EtOAc (5 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (98 mL of SiO₂, 0–1.5% MeOH in CH₂Cl₂ gradient elution) afforded 51b (64.0 mg, 0.127 mmol, 96%) as a colorless oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.39 and 7.08 (2 br s, 1H), 5.42 and 5.36 (d and br s, J = 7.2 Hz, 1H), 4.56 and 4.49 (2d, J = 8.8 Hz and 8.6 Hz, 1H), 4.28–4.17 (m, 2.5H), 4.15–4.09 (m, 0.5H), 2.84 (br s, 1H), 1.69–1.60 and 1.58–1.46 (2m, 2H), 1.45 (s, 9H), 1.35–1.27 (m, 6H), 1.26 (s, 3H), 1.25 (s, 3H), 1.01–0.94 (m, 9H), 0.92–0.84 (m, 3H), 0.74–0.62 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.7, 171.0 and 170.9, 156.4 and 156.2, 80.6 and 80.5, 74.0 and 73.9, 72.4 and 72.2, 61.9 and 61.7, 60.2 and 60.0, 59.1 and 58.6, 30.72 and 30.66, 28.5 (3C), 27.0, 26.8 and 26.7, 24.2 and 23.8, 14.3, 8.0, 7.3 and 7.0 (3C), 6.8 and 6.6 (3C); IR (film) νmax 3371, 2923, 2359, 1718, 1652, 1506, 1457, 1368, 1164 cm⁻¹; HRMS (ESI) m/z 505.3299 (MH⁺, C₂₄H₄₈N₂O₇SiH⁺ requires 505.3309).

Methyl (2-((2S,3R)-2-((tert-Butoxycarbonyl)amino)-3-methyl-3-((triethoxysilyl)oxy)pentanamido)-3-methylbut-2-enoyl)glycinate (52b). A solution of ester 51b
(116.0 mg, 0.2298 mmol) in t-BuOH (800 μL) was treated with LiOH•H₂O (48.0 mg, 1.14 mmol, 5.0 equiv) and H₂O (200 μL) at 0 °C, then stirred at 0 °C to rt for 4 h. The resulting mixture was acidified to pH 4~5 by the addition of 1 N HCl, diluted with H₂O (2 mL), and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The crude carboxylic acid was used directly in the next step without further purification.

A solution of the crude carboxylic acid in anhydrous CH₂Cl₂ (2 mL) was treated with EDC•HCl (441.0 mg, 2.300 mmol, 10.0 equiv) and stirred at rt under Ar for 24 h. The resulting mixture was treated with brine (4 mL) and extracted with CH₂Cl₂ (3 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The crude azlactone was used directly in the next step without further purification.

A solution of the crude azlactone in anhydrous DMF (2 mL) was treated with DMAP (60.0 mg, 0.491 mmol, 2.1 equiv, Gly-OMe•HCl (290.0 mg, 2.310 mmol, 10.1 equiv), and Et₃N (480 μL, 348 mg, 3.44 mmol, 15.0 equiv). The resulting solution was stirred at 80 °C under Ar for 72 h. The solution was diluted with H₂O (8 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 5 mL), dried (Na₂SO₄), and concentrated in vacuo. Flash chromatography (50 mL of SiO₂, 0–3% MeOH in CH₂Cl₂ gradient elution) afforded 52b (87.0 mg, 0.164 mmol, 71%) as a white solid: [α]²⁵D –3.6 (c 0.84, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 7.49 (s, 1H), 7.03 (br s, 1H), 5.39 (br s, 1H), 4.13–4.05 (m, 2H), 4.02 (dd, J = 18.0, 5.5 Hz, 1H), 3.74 (s, 3H), 2.10 (s, 3H), 1.79 (s, 3H), 1.67–1.56 (m, 2H), 1.44 (s, 9H), 1.37 (s, 3H), 0.99 (t, J = 7.9 Hz, 9H), 0.92 (t, J = 7.6 Hz, 3H), 0.68 (q, J = 7.9 Hz, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.4, 170.2, 166.3, 156.7, 142.7, 123.5, 80.6, 78.3, 62.0, 52.4, 41.5, 32.9, 28.5 (3C), 24.5, 21.5, 20.9, 8.9, 7.3 (3C), 6.9 (3C); IR (film) vₘₐₓ 3328, 2955, 2877, 1667, 1526, 1369, 1212, 1008 cm⁻¹; HRMS (ESI) m/z 530.3239 (MH+, C₂₅H₄₇N₃O₇SiH⁺ requires 530.3250).
Methyl (5R,6S)-6-((tert-Butoxycarbonyl)amino)-3,3,5-triethyl15-(3-hydroxypentan-3-yl)-5-methyl-7,10,13-trioxo-9-(propan-2-ylidene)-4-oxa-8,11,14-triaza-3-silahexadecan-16-oate (53b). A solution of tripeptide 52b (20.0 mg, 0.0378 mmol) in t-BuOH (800 μL) was treated with LiOH•H₂O (7.9 mg, 0.19 mmol, 5.0 equiv) and H₂O (200 μL), then stirred at rt for 4 h. The resulting mixture was neutralized to pH ~7 by the addition of 1 N HCl, diluted with H₂O (2 mL), and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The crude carboxylic acid was used directly without further purification.

The crude acid was dissolved in anhydrous CH₂Cl₂ (4.0 mL), cooled to 0 °C under Ar, then treated with amine 49b (9.9 mg, 0.056 mmol, 1.5 equiv), HOBt (ca. 20% H₂O content, 10.2 mg, 0.060 mmol, 1.6 equiv), and EDC•HCl (10.9 mg, 0.0569 mmol, 1.5 equiv). The resulting mixture was stirred at rt under Ar for 5 h. The reaction was quenched by the addition of sat aq NaHCO₃ (1 mL), the layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (6 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (30 mL of SiO₂, 0–4% MeOH in CH₂Cl₂ gradient elution) afforded 53b (18.0 mg, 0.0267 mmol, 71%) as a white film that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz, minor rotamers present, data for major rotamer of each diastereomer) δ 7.81 and 7.63 (2 br s, 1H), 7.63 and 7.47 (br s and d, J = 6.9 Hz, 1H), 7.01 and 6.96 (2 br s, 1H), 5.56 and 5.50 (2 br s, 1H), 4.75 and 4.73 (2s, 1H), 4.29–4.12 (m, 1H), 4.08 (d, J = 4.6 Hz, 1H), 3.95–3.81 (m, 1H), 3.73 and 3.71 (2s, 3H), 2.10 and 2.05 (2s, 3H), 1.83 and 1.81 (2s, 3H), 1.74–1.50 (m, 7H), 1.45 (s, 9H), 1.36 (s, 3H), 0.99 (t, J = 7.9 Hz, 9H), 0.93 and 0.92 (2t, J = 7.4 and 7.4 Hz, 6H), 0.84 and 0.83 (2t, J = 7.4
and 7.4 Hz, 3H), 0.72–0.65 (m, 6H); $^1$H NMR (CDCl$_3$, 125 MHz) $\delta$ 172.1, 171.6 and 171.5, 171.3, 165.3 and 165.0, 157.1 and 157.0, 139.8 and 139.6, 123.8 and 123.5, 81.2 and 81.0, 78.3 and 77.9, 76.5, 62.4 and 62.2, 57.6, 52.3 and 52.1, 43.3, 33.0 and 32.7, 28.5 (3C), 27.6, 27.3 and 27.2, 24.6, 21.0, 20.8, 9.0 and 8.9, 8.1, 8.0, 7.3 (3C), 6.9 (3C); IR (film) $v_{\text{max}}$ 3358, 2927, 2877, 2358, 1738, 1651, 1511, 1463, 1368, 1300, 1242, 1170 cm$^{-1}$; HRMS (ESI) $m/z$ 673.4229 (MH$^+$, C$_{32}$H$_{60}$N$_4$O$_9$SiH$^+$ requires 673.4208).

![BocHN HN OTES O N H OMe 54b](image)

Methyl (2-(2-((2S,3R)-2-((tert-Butoxycarbonyl)amino)-3-methyl-3-((triethylsilyl)oxy)pentanamido)-3-methylbut-2-enamido)acetamido)-3-ethylen-2-eno)-D-valinate (54b). A solution of ester 53b (14.0 mg, 0.0208 mmol) in $t$-BuOH (800 μL) was treated with LiOH•H$_2$O (8.7 mg, 0.21 mmol, 10 equiv) and H$_2$O (200 μL), then stirred at rt for 3 h. The resulting mixture was acidified to pH 7 by the addition of 1 N HCl, diluted with H$_2$O (2 mL), and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated in vacuo. The crude carboxylic acid was used directly without further purification.

A solution of the crude carboxylic acid in anhydrous CH$_2$Cl$_2$ (2 mL) was treated with EDC•HCl (40.0 mg, 0.209 mmol, 10.0 equiv) and stirred at rt under Ar for 24 h. The resulting mixture was treated with brine (4 mL) and extracted with CH$_2$Cl$_2$ (3 × 5 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated in vacuo. The crude azlactone was used directly in the next step without further purification.

A solution of the crude azlactone in anhydrous DMF (2 mL) was treated with DMAP (5.1 mg, 0.042 mmol, 2.0 equiv), D-Val-OMe•HCl (35.0 mg, 0.209 mmol, 10.0 equiv), and Et$_3$N (43.5
μL, 31.6 mg, 0.312 mmol, 15.0 equiv). The resulting mixture was stirred at 65 °C under Ar for 72 h, then diluted with H2O (4 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine (2 × 5 mL), dried (Na2SO4) and concentrated in vacuo. Flash chromatography (30 mL of SiO2, 0–4% MeOH in CH2Cl2 gradient elution) afforded 54b (5.0 mg, 0.0066 mmol, 32%) as a white film: [α]25D –4.6 (c 0.57, CHCl3); 1H NMR (CDCl3, 500 MHz, data for major rotamer) δ 8.39 (br s, 1H), 7.63 (br s, 1H), 7.03 (d, J = 7.4 Hz, 1H), 5.46 (br s, 1H), 5.40 (br s, 1H), 4.50 (dd, J = 8.2, 5.4 Hz, 1H), 4.24–4.17 (m, 1H), 4.11–4.04 (m, 1H), 4.03 (dd, J = 15.3, 5.2 Hz, 1H), 3.71 (s, 3H), 2.25–2.12 (m, 4H), 2.09 (s, 3H), 1.82 (s, 3H), 1.76–1.53 (m, 3H), 1.45 (s, 9H), 1.26 (s, 3H), 1.10 (t, J = 7.4 Hz, 3H), 1.04 (t, J = 7.6 Hz, 3H), 1.02–0.95 (m, 12H), 0.93–0.80 (m, 6H), 0.68 (q, J = 7.9 Hz, 6H); 13C NMR (CDCl3, 125 MHz, data for major rotamer) δ 172.9, 170.8, 169.5, 166.1, 165.8, 157.2, 138.0, 136.7, 123.3, 119.6, 81.2, 77.2 (overlap with solvent), 62.1, 57.9, 52.0, 44.0, 32.1, 31.0, 28.5 (3C), 24.6, 22.9, 21.0, 19.3, 18.6, 18.3, 14.4, 13.6, 12.3, 8.9, 7.3 (3C), 6.9 (3C); IR (film) νmax 3303, 2957, 2924, 2853, 1665, 1515, 1367, 1166, 1068, 1007 cm–1; HRMS (ESI) m/z 754.4792 (MH+, C37H67N5O9SiH+ requires 754.4786).

Methyl ((Benzyloxy)carbonyl)-L-threonylglycinate (60). A solution of Z-Thr-OH (59, 1.014 g, 4.00 mmol) was dissolved in anhydrous THF–DMF (5:1, 30 mL), cooled to 0 °C under Ar, and then treated with Gly-OMe•HCl (58, 536 mg, 4.27 mmol, 1.1 equiv), HOBt (ca. 20% H2O content, 806.8 mg, 4.78 mmol, 1.2 equiv), EDC•HCl (1.07 g, 5.58 mmol, 1.4 equiv), and NaHCO3 (801.1 mg, 9.54 mmol, 2.4 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 48 h. The reaction was quenched by the addition of sat aq NaHCO3 (10 mL) and H2O (20 mL), and the aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were dried (Na2SO4) and concentrated in vacuo. Flash chromatography (30 mL of SiO2, 0–
4% MeOH in CH₂Cl₂ gradient elution) afforded 60 (1.25 g, 3.85 mmol, 96%) as a white solid: 

\[ \alpha \]$_{25}^D$ –28.7 (c 5.3, CHCl₃); $^1$H NMR (CDCl₃, 500 MHz) δ 7.39–7.31 (m, 5H), 6.96 (br s, 1H), 5.75 (d, $J$ = 7.6 Hz, 1H), 5.15 (s, 2H), 4.45–4.40 (m, 1H), 4.19 (d, $J$ = 6.6 Hz, 1H), 4.10–4.00 (m, 2H), 3.76 (s, 3H), 3.09 (br s, 1H), 1.22 (d, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (CDCl₃, 125 MHz) δ 171.7, 170.5, 157.1, 136.1, 128.8 (2C), 128.5, 128.2 (2C), 67.5, 67.2, 59.0, 52.7, 41.3, 18.4; IR (film) $\nu_{\text{max}}$ 3330, 3032, 2953, 2359, 1717, 1663, 1539, 1437, 1217, 1090 cm⁻¹; HRMS (ESI) m/z 325.1399 (MH+, C$_{15}$H$_{20}$N$_2$O$_6$H+ requires 325.1394).

Methyl ((Benzyloxy)carbonyl)glycyl-L-threonylglycinate (62). A suspension of dipeptide 60 (198.3 mg, 0.6114 mmol) in MeOH (7 mL) under Ar was treated with 10% Pd/C (125.8 mg, 0.63 wt equiv). The resulting mixture was stirred vigorously at rt under H₂ (500 psi) for 72 h. The mixture was filtered through Celite, (washed with excess MeOH), and the filtrate was concentrated in vacuo to afford the crude amine (104.5 mg, 0.549 mmol, 90%) as a white solid that was used directly in the next step without further purification.

A solution of Z-Gly-OH (61, 231.1 mg, 1.105 mmol) was dissolved in anhydrous THF–DMF (5:1, 30 mL), cooled to 0 °C under Ar, then treated with crude amine (216.5 mg, ca. 1.138 mmol, 1.03 equiv), HOBt (ca. 20% H₂O content, 219.6 mg, 1.300 mmol, 1.2 equiv), EDC•HCl (305.1 g, 1.592 mmol, 1.4 equiv), and NaHCO₃ (222.8 mg, 2.652 mmol, 2.4 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 48 h. The reaction was quenched by the addition of sat aq NaHCO₃ (10 mL) and H₂O (20 mL), and the aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (30 mL of SiO₂, 0–4% MeOH in CH₂Cl₂ gradient elution) afforded 62 (198.2 mg, 0.520 mmol, 47%) as a white solid: [α]$_{25}^D$ –18.9 (c 1.7, CHCl₃); $^1$H NMR (CDCl₃, 500 MHz) δ
7.48 (br s, 1H), 7.39–7.28 (m, 6H), 5.89 (br s, 1H), 5.10 (s, 2H), 4.49 (d, \( J = 7.5 \) Hz, 1H), 4.36 (br s, 1H), 4.06–3.84 (m, 5H), 3.71 (s, 3H), 1.16 (d, \( J = 6.0 \) Hz, 3H); \(^{13}\)C NMR (CDCl\(_3\), 125 MHz) \( \delta \) 171.4, 170.7, 170.5, 157.1, 136.2, 128.8 (2C), 128.5, 128.3 (2C), 67.5, 67.1, 57.8, 52.7, 44.7, 41.3, 18.6; IR (film) \( \nu_{\text{max}} \) 3324, 2924, 2853, 1654, 1539, 1456, 1375, 1215, 1115, 1051 cm\(^{-1}\); HRMS (ESI) \( m/z \) 382.1598 (MH\(^+\), \( C_{17}H_{23}N_3O_7H^+ \) requires 382.1614).

**Methyl ((2S,3R)-2-((tert-Butoxycarbonyl)amino)-3-hydroxy-3-phenylpropanoyl)glycinate (64).** A solution of Boc-\( \beta \)-OH\textsubscript{Phe-OH} \(^8\) (63, 784.0 mg, 2.787 mmol, \( 1.06 \) equiv) in anhydrous THF–DMF (5:1, 24 mL) was cooled to 0 °C under Ar, then treated with 58 (331.0 mg, 2.636 mmol), HOBt (ca. 20% H\(_2\)O content, 419.9 mg, 2.486 mmol, 0.94 equiv), EDC•HCl (696.7 mg, 3.634 mmol, 1.4 equiv) and NaHCO\(_3\) (537.3 mg, 6.396 mmol, 2.4 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 24 h. The reaction was quenched by the addition of sat aq NaHCO\(_3\) (10 mL), and the aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were washed with brine, dried (Na\(_2\)SO\(_4\)) and concentrated \textit{in vacuo}. Flash chromatography (40 mL of SiO\(_2\), 0–5% MeOH in CH\(_2\)Cl\(_2\) gradient elution) afforded 64 (581.9 mg, 1.651 mmol, 62%) as a white solid: \([\alpha]_{25}^{\text{D}} \) –8.8 (c 2.6, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\), 500 MHz) \( \delta \) 7.39 (d, \( J = 7.5 \) Hz, 2H), 7.34 (t, \( J = 7.4 \) Hz, 2H), 7.29–7.25 (m, 1H), 7.15 (br s, 1H), 5.45–5.41 (m, 2H), 4.48 (d, \( J = 7.9 \) Hz, 1H), 4.17 (dd, \( J = 18.1, 5.8 \) Hz, 1H), 3.96 (dd, \( J = 18.2, 5.0 \) Hz, 1H), 3.77 (s, 3H), 3.73 (br s, 1H), 1.32 (s, 9H); \(^{13}\)C NMR (CDCl\(_3\), 125 MHz) \( \delta \) 171.7, 170.4, 156.3, 139.6, 128.6 (2C), 127.9, 126.0 (2C), 80.7, 72.5, 59.7, 52.7, 41.5, 28.3 (3C); IR (film) \( \nu_{\text{max}} \) 3853, 3342, 2924, 2852, 2359, 1668, 1506, 1367, 1090 cm\(^{-1}\); HRMS (ESI) \( m/z \) 353.1712 (MH\(^+\), \( C_{17}H_{24}N_2O_6H^+ \) requires 353.1707).
Methyl (2S,3R)-2-((Benzyloxy)carbonyl)aminoacetamido)-3-hydroxy-3-phenylpropanoyl)glycinate (65). A solution of dipeptide 64 (445.9 mg, 1.265 mmol) in anhydrous CH₂Cl₂ (20 mL) at 0 °C under Ar was treated with HCl (4.0 M in dioxane, 5 mL, 20 mmol, 16 equiv). The resulting mixture was stirred at rt for 3 h, then concentrated in vacuo. The crude amine was used directly in the next reaction without further purification.

A solution of Z-Gly-OH (61, 109.3 mg, 0.522 mmol, 1.0 equiv) in anhydrous THF–DMF (5:1, 6 mL) at 0 °C under Ar was treated with crude amine (127.9 mg, ca. 0.507 mmol, 1 equiv), HOBt (ca. 20% H₂O content, 95.3 mg, 0.564 mmol, 1.1 equiv), EDC•HCl (140.1 mg, 0.731 mmol, 1.4 equiv), and NaHCO₃ (102.9 mg, 1.22 mmol, 2.4 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 24 h. The reaction was quenched by the addition of sat aq NaHCO₃ (10 mL), and the aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were washed with brine (15 mL), dried (Na₂SO₄), and concentrated in vacuo. Flash chromatography (40 mL of SiO₂, 0–5% MeOH in CH₂Cl₂ gradient elution) afforded 65 (148.8 mg, 0.336 mmol, 66%) as a white solid: [α]²⁵D -39.1 (c 1.1, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 7.42–7.27 (m, 10H), 7.05 (br s, 1H), 5.45 (s, 2H), 5.36 (d, J = 6.9 Hz, 1H), 5.18–5.10 (m, 2H), 4.47 (d, J = 7.5 Hz, 1H), 4.20 (dd, J = 18.1, 5.9 Hz, 1H), 3.97 (dd, J = 18.2, 4.8 Hz, 1H), 3.78 (s, 3H), 3.78–3.72 (m, 2H), 3.41 (br s, 1H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.8, 171.2, 171.1, 157.2, 139.5, 136.0, 128.8 (2C), 128.7 (2C), 128.6 (2C), 128.3, 128.0, 126.0 (2C), 72.6, 67.7, 59.6, 52.8, 45.0, 41.5; IR (film) ν max 3862, 3321, 2920, 2850, 2363, 1710, 1670, 1511, 1369, 1090 cm⁻¹; HRMS (ESI) m/z 444.1768 (MH⁺, C₂₂H₂₅N₃O₇H⁺ requires 444.1771).
Ethyl 2-((Benzyloxy)carbonyl)amino)acetamido)-3-hydroxy-3-methylbutanoate (66). A solution of Z-Gly-OH (61, 567.8 mg, 2.714 mmol) in anhydrous THF–DMF (5:1, 30 mL) at 0 °C under Ar was treated with β-OHVal-OEt (44, 451.2 mg, 2.799 mmol, 1.0 equiv), HOBT (ca. 20% H2O content, 551.9 mg, 3.268 mmol, 1.2 equiv), EDC•HCl (707.1 g, 3.689 mmol, 1.4 equiv), and NaHCO3 (542.8 mg, 6.461 mmol, 2.4 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 48 h. The reaction was quenched by the addition of sat aq NaHCO3 (10 mL) and H2O (20 mL), and the aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were dried (Na2SO4) and concentrated in vacuo. Flash chromatography (30 mL of SiO2, 0–4% MeOH in CH2Cl2 gradient elution) afforded 66 (956.1 mg, 2.713 mmol, 99%) as a yellow oil: 1H NMR (CDCl3, 500 MHz) δ 7.39–7.29 (m, 5H), 6.98 (br s, 1H), 5.60 (br s, 1H), 5.13 (s, 2H), 4.52 (d, J = 8.8 Hz, 1H), 4.29–4.15 (m, 2H), 3.97–3.91 (m, 2H), 3.76 (br s, 1H), 1.32–1.20 (m, 9H); 13C NMR (CDCl3, 125 MHz) δ 171.2, 169.3, 152.5, 136.3, 128.6 (2C), 128.2, 128.1 (2C), 72.0, 67.2, 61.7, 59.9, 44.4, 26.7 (2C), 14.1; IR (film) νmax 3343, 3065, 3034, 2980, 1731, 1530, 1455, 1375, 1214, 1049 cm⁻¹; HRMS (ESI) m/z 353.1689 (MH+, C17H24N2O6H+ requires 353.1712).

Methyl 2-((Benzyloxy)carbonyl)amino)acetamido)-3-hydroxy-3-methylbutanoyl)glycinate (67). A suspension of 66 (453.1 mg, 1.286 mmol) and Me3SnOH (716.2 mg, 3.961 mmol, 3.1 equiv) in THF (pretreated with Na2SO4 for 12 h, 8 mL) under Ar was stirred at 40 °C for 4 days. The mixture was concentrated in vacuo, and the residue was treated with Et2O (12 mL). The resulting mixture was filtered through Celite (washed with 50 mL of
Et₂O), and the filtrate was concentrated in vacuo to afford the crude carboxylic acid as a pale yellow oil that was used directly in the next step without further purification.

A solution of the crude carboxylic acid (ca. 1.286 mmol) in anhydrous THF–DMF (5:1, 24 mL) at 0 °C under Ar was treated with Gly-OMe•HCl (58, 180.8 mg, 1.440 mmol, 1.1 equiv), HOBt (ca. 20% H₂O content, 293.2 mg, 1.736 mmol, 1.4 equiv), EDC•HCl (354.1 g, 1.847 mmol, 1.4 equiv), and NaHCO₃ (258.7 mg, 3.080 mmol, 2.4 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 24 h. The reaction was quenched by the addition of sat aq NaHCO₃ (15 mL) and H₂O (30 mL), and the aqueous layer was extracted with EtOAc (5 × 40 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (30 mL of SiO₂, 1–4% MeOH in CH₂Cl₂ gradient elution) afforded 67 (269.8 mg, 0.6823 mmol, 53%) as a white solid: ¹H NMR (CDCl₃, 500 MHz) δ 7.68 (br s, 1 H), 7.47 (d, J = 8.3 Hz, 1H), 7.38–7.28 (m, 5H), 6.04 (br s, 1H), 5.12–5.06 (m, 2H), 4.51 (d, J = 8.6 Hz, 1H), 4.26–4.12 (br s, 1H), 4.03 (dd, J = 18.0, 6.0 Hz, 1H), 3.98–3.82 (m, 3H), 3.69 (s, 3H), 1.30 (s, 3H), 1.16 (s, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.6, 170.4, 170.2, 156.9, 136.4, 128.7 (2C), 128.3 (2C), 128.2, 72.1, 67.2, 59.3, 52.5, 44.5, 41.2, 27.3, 25.6; IR (film) ν max 3066, 2979, 2316, 2091, 1731, 1439, 1215, 1083 cm⁻¹; HRMS (ESI) m/z 396.1770 (MH⁺, C₁₈H₂₅N₃O₇H⁺ requires 396.1771).

**Methyl (R)-(2-Amino-3-methyl-3-(tritylthio)butanoyl)glycinate (69b).** A solution of Fmoc-Pen(Trt)-OH (68, 187.8 mg, 0.306 mmol) in anhydrous DMF (4 mL) at 0 °C under Ar was treated with HBTU (118.7 mg, 0.313 mmol, 1.0 equiv), iPr₂NEt (157 μL, 116 mg, 0.901 mmol, 2.9 equiv), and Gly-OMe•HCl (58, 41.1 mg, 0.327 mmol, 1.1 equiv). The resulting mixture was stirred to rt for 2 h. The mixture was diluted and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with NH₄Cl (3 × 10 mL), NaHCO₃ (2 × 10 mL), and
brine (10 mL), then passed through a pad of SiO₂ rinsing with EtOac (15 mL) and concentrated in vacuo. Fmoc-Pen(Trt)-Gly-OMe 69a was afforded in quantitative yield (theoretical yield = 209.6 mg) as a white film: [α]^{25}_D +45.4 (c 3.10, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 7.77 (t, J = 7.9 Hz, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.63–7.54 (m, 2H), 7.45–7.37 (m, 2H), 7.35–7.18 (m, 15H), 6.18 (br s, 1H), 5.62 (d, J = 5.2 Hz, 1H), 4.42–4.24 (m, 3H), 4.24–4.19 (m, 1H), 4.05–3.99 (m, 1H), 3.94 (dd, J = 18.3, 4.9 Hz, 1H), 3.71 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.0, 169.6, 156.2, 144.9 (3C), 141.44 (2C), 141.40 (2C), 130.1 (3C), 129.8 (2C), 128.1 (3C), 128.0 (3C), 127.9 (3C), 127.2 (2C), 127.0 (3C), 125.3 (2C), 120.1 (2C), 68.5, 67.3, 61.7, 52.5, 47.2, 41.4, 41.1, 26.4 (2C); IR (film) νₓ max 3315, 2926, 2359, 2342, 1750, 1700, 1521, 1213, 1033 cm⁻¹; HRMS (ESI) m/z 685.2729 (MH⁺, C₄₂H₄₀N₂O₅SH⁺ requires 685.2731).

A solution of 69a (209.6 mg, 0.306 mmol) in iPr₂NEt–CH₃CN (1:4, 2.5 mL) at rt was stirred until starting material was consumed as determined by TLC monitoring, then concentrated in vacuo. Flash chromatography (30 mL of SiO₂, 0–3% MeOH in CH₂Cl₂ gradient elution) afforded 69b (136.6 mg, 0.295 mmol, 97% over two steps) as a yellow oil.

Methyl (R)-(2-(2-(((Benzyloxy)carbonyl)amino)acetamido)-3-mercapto-3-methylbutanoyl)glycinate (70). A solution of Z-Gly-OH (61, 92.1 mg, 0.440 mmol, 1.0 equiv) in anhydrous DMF (5 mL) at 0 °C was treated with HBTU (164.1 mg, 0.433 mmol, 1.0 equiv), iPr₂NEt (212 µL, 157 mg, 1.22 mmol, 2.8 equiv), and 69b (198.5 mg, 0.429 mmol). The resulting mixture was stirred at 0 °C to rt for 2 h, then diluted with H₂O (15 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with NH₄Cl (3 × 10 mL), NaHCO₃ (2 × 10 mL), and brine (10 mL), then concentrated in vacuo. Z-Gly-Pen(Trt)-Gly-OMe
was afforded as a white film (260.2 mg, ca. 0.398 mmol) that was used directly in the next reaction without further purification.

A solution of BF$_3$•Et$_2$O (10 μL, 11.5 mg, 0.0810 mmol, 0.2 equiv) and dimethylphenylsilane (238 μL, 212 mg, 1.55 mmol, 3.9 equiv) in hexafluoroisopropanol (1.5 mL) at rt was treated with Z-Gly-Pen(Trt)-Gly-OMe (260.2 mg, ca. 0.398 mmol) was stirred until starting material was consumed as determined by TLC monitoring, then quenched with sat aq NaHCO$_3$ (2 mL) and extracted with EtOAc (2 x 15 mL), then dried over Na$_2$SO$_4$ and concentrated $\textit{in vacuo}$. Flash chromatography (30 mL of SiO$_2$, 0–3% MeOH in CH$_2$Cl$_2$ gradient elution) afforded 70 (149.2 mg, 0.363 mmol, 85% over two steps) as a yellow oil: [α]$^{25}$D –10.4 (c 3.30, CHCl$_3$); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.39–7.30 (m, 5H), 7.13 (d, $J = 8.5$ Hz, 1H), 7.03 (br s, 1H), 5.55 (br s, 1H), 5.14 (s, 2H), 4.54 (d, $J = 9.1$ Hz, 1H), 4.06 (dd, $J = 18.1$, 5.8 Hz, 1H), 4.00–3.90 (m, 3H), 3.74 (s, 3H), 2.60 (s, 1H), 1.53 (s, 3H), 1.30 (s, 3H); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 170.2, 169.8, 169.5, 156.9, 136.2, 128.8 (2C), 128.5, 128.4 (2C), 67.6, 60.7, 52.6, 46.1, 44.8, 41.3, 31.0, 28.7; IR (film) $\nu_{\text{max}}$ 3734, 3306, 2359, 2342, 1653, 1540, 1456, 1279, 1212 cm$^{-1}$; HRMS (ESI) m/z 412.1533 (MH$^+$, C$_{18}$H$_{25}$N$_3$O$_6$S$^+$ requires 412.1542).

Methyl (Z)-(2-(2-(((Benzyloxy)carbonyl)amino)acetamido)but-2-enoxy)glycinate (71a). A solution of 62 (30.0 mg, 0.0787 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) at rt under Ar was treated with EDC•HCl (16.4 mg, 0.0856 mmol, 1.1 equiv) and CuCl (2.4 mg, 0.024 mmol, 0.3 equiv). The resulting mixture was stirred at rt for 40 h, then washed with H$_2$O (2 x 3 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated $\textit{in vacuo}$. Flash chromatography (30 mL of SiO$_2$, 1–3% MeOH in CH$_2$Cl$_2$ gradient elution) afforded 71a (24.0 mg, 0.0660 mmol, 84%) as a light yellow oil: $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.75 (br s, 1H), 7.40–7.31 (m, 5H), 7.00–...
(br s, 1H), 6.65 (d, J = 6.5 Hz, 1H), 5.71 (br s, 1H), 5.13 (s, 2H), 4.03 (d, J = 5.1 Hz, 2H), 3.94 (d, J = 5.6 Hz, 2H), 3.73 (s, 3H), 1.71 (d, J = 6.6 Hz, 3H); 13C NMR (CDCl3, 125 MHz) δ 170.8, 168.8, 165.2, 157.3, 136.1, 132.0, 128.77 (2C), 128.74, 128.5, 128.3 (2C), 67.6, 52.6, 45.2, 41.6, 13.9; IR (film) νmax 3316, 2926, 2853, 1715, 1532, 1455, 1217, 1049 cm⁻¹; HRMS (ESI) m/z 364.1495 (MH⁺, C17H21N3O6H⁺ requires 364.1508).

Methyl (2-((((Benzyloxy)carbonyl)amino)methyl)-5-methyl-4,5-dihydrooxazole-4-carbonyl)glycinate (71b). This compound was obtained as a yellow oil that was an unwanted byproduct of some of the reactions listed in Table 1: 1H NMR (CDCl3, 500 MHz) δ 7.40–7.32 (m, 5H), 7.00 (br s, 1H), 5.33 (br s, 1H), 5.16 (s, 2H), 4.80 (quin, J = 6.6 Hz, 1H), 4.18 (d, J = 7.6 Hz, 1H), 4.07–4.01 (m, 4H), 3.76 (s, 3H), 1.50 (d, J = 6.2 Hz, 3H); HRMS (ESI) m/z 364.1497 (MH⁺, C17H21N3O6H⁺ requires 364.1508).

Methyl (2-((((Benzyloxy)carbonyl)amino)acetamido)-3-methylbut-2-enoyl)glycinate (72a). From 67: A solution of 67 (17.4 mg, 0.046 mmol) in anhydrous CH₂Cl₂ (5 mL) at 0 °C under Ar was added dropwise to a mixture of DAST (6.6 μL, 8.1 mg, 0.050 mmol, 1.1 equiv) and pyridine (4.1 μL, 4.0 mg, 0.051 mmol, 1.1 equiv). The resulting mixture was stirred at rt for 30 min, then washed with NaHCO₃ (4 × 10 mL). The organic layer was dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (30 mL of SiO₂, 1–4% MeOH in CH₂Cl₂ gradient elution) afforded 72a (6.5 mg, 0.017 mmol, 38%) as a white film.

From 70: To a solution of 70 (14.1 mg, 0.0343 mmol) and DBU (33 μL, 34 mg, 0.22 mmol, 6.4 equiv) in anhydrous DMF (2 mL) was added 1,4-diiodobutane (7.2 μL, 17 mg, 0.055
mmol, 1.6 equiv). The resulting mixture was stirred at 0 °C under Ar for 4 hr, then diluted with EtOAc (10 mL) and washed with brine (15 mL). The organic layer was dried (Na2SO4) and concentrated in vacuo. Flash chromatography (30 mL of SiO2, 1–4% MeOH in CH2Cl2 gradient elution) afforded 72a (6.8 mg, 0.018 mmol, 53%) as a white film and 72b (5.9 mg, 0.016 mmol, 46%) as a light-yellow oil.

Data for 72a: 1H NMR (CDCl3, 500 MHz) δ 7.61 (br s, 1H), 7.40–7.30 (m, 5H), 6.98 (br s, 1H), 5.63 (br s, 1H), 5.13 (s, 2H), 4.04 (d, J = 4.6 Hz, 2H), 3.93 (d, J = 5.6 Hz, 2H), 3.74 (s, 3H), 2.08 (s, 3H), 1.74 (s, 3H); 13C NMR (CDCl3, 125 MHz) δ 171.7, 170.0, 166.6, 156.9, 149.6, 136.3, 128.8 (2C), 128.5, 128.4 (2C), 120.8, 67.5, 52.7, 44.7, 41.2, 25.6, 22.9; IR (film) νmax 3313, 2926, 2854, 2360, 1717, 1666, 1569, 1496, 1363, 1214, 1048 cm−1; HRMS (ESI) m/z 378.1662 (MH+, C18H23N3O6H+ requires 378.1665).

Methyl (2-(((Benzyloxy)carbonyl)amino)methyl)-5,5-dimethyl- 4,5-dihydrooxazole-4-carbonyl)glycinate (72b). This compound was obtained as a light yellow oil that was an unwanted byproduct of many of the reactions listed in Tables 2 and 3: 1H NMR (CDCl3, 500 MHz) δ 7.38–7.30 (m, 5H), 6.61 (br s, 1H), 5.41 (br s, 1H), 5.15 (d, J = 12.0 Hz, 1H), 5.11 (d, J = 12.1 Hz, 1H), 4.24 (dd, J = 18.2, 6.6 Hz, 1H), 4.13 (dd, J = 18.3, 5.1 Hz, 1H), 4.00 (dd, J = 18.3, 5.7 Hz, 1H), 3.89 (dd, J = 18.2, 4.6 Hz, 1H), 3.75 (s, 3H), 3.15 (s, 1H), 1.44 (s, 3H), 1.43 (s, 3H); HRMS (ESI) m/z 378.1660 (MH+, C18H23N3O6H+ requires 378.1665).

General Procedures for Solid-Phase Peptide Synthesis
Fully Automated Synthetic Strategy of Linear Precursors. Rink amide MBHA resin 100–200 mesh (loading 0.65 mmol/g, 161.5 mg, 0.105 mmol). Sequence elongation was performed on a microwave-assisted solid-phase peptide synthesizer Purepep Chorus (Gyros Protein Technologies, Tucson, AZ, U.S.A.) following the Fmoc-protected AA strategy. Reaction temperatures were monitored by an internal fiber-optic sensor. Both deprotection and coupling reactions were performed in a Teflon vessel applying microwave energy under nitrogen bubbling. After the first Fmoc-deprotection, the following amino acids orthogonally protected were added automatically from C- to N-terminal: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(t-Bu)-OH, Fmoc-Val-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Thr-OH, Fmoc-Pen(Trt)-OH, Fmoc-Gln(Trt)-OH, in the presence of the coupling reagent HATU and base DIEA. The Fmoc protected AA MW-SPPS cycle consisted in (1) swelling in DCM (5.25 mL) for 10 min; (2) Fmoc-deprotection by 20% (v/v) piperidine/DMF (5.25 mL); (3) washings with DMF (5 × 5.25 mL); (4) coupling with the Fmoc-protected amino acids (1.0 equiv, 300 mM in NMP), HATU (1.0 equiv, 300 mM in NMP), DIEA (2.0 equiv, 600 mM in NMP); (5), washings with DMF (5 × 5.25 mL). Peptide elongation was performed by repeating the MW-SPPS cycle for each amino acid. Both deprotection and coupling reactions were performed reaching 80 °C.

Fmoc Deprotection. The resin (100 µmol) was treated with piperidine (20% solution in DMF, 5.0 mL) and allowed to stand for 5 min. The solution was drained from the resin using a vacuum manifold, and additional piperidine (20% solution in DMF, 5.0 mL) was added. The resulting mixture was stirred twice at 80 °C in a microwave oven for 4 min. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF (5 × 10 mL).
**Peptide coupling.** Fmoc-Val-OH (500 µmol, 5 equiv) and HBTU (190 mg, 500 µmol, 5 equiv) were dissolved by vortexing in a 0.1 M HOBt solution in NMP (5.0 mL, 500 µmol, 5 equiv). iPr₂NEt (176 µL, 1000 µmol, 10 equiv) was added to this solution, and it was allowed to stand for ca. 1 min. The solution was added to the resin (100 µmol), and the resulting mixture was stirred at 70 °C in a microwave oven for 10 min. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF (5 × 10 mL). The N-terminal amino acid of each peptide (i.e., Arg) was coupled by gently stirring the solution at rt under Ar for ca. 10–12 h after the microwave heating was completed.

**Coupling of azlactones with resin-bound peptides.** A solution of azlactone (500 µmol, 5 equiv, typically employed crude without purification by flash chromatography) and Et₃N (150 µL, 1.08 mmol, 10 equiv) in NMP (10 mL) was added to the resin-bound peptide (100 µmol). The resulting mixture was gently stirred at 80 °C for 24–72 h. The solution was drained from the resin using a fritted polypropylene syringe into a 20 mL glass vial (note: the unreacted azlactone present in the solution can be reused), and the resin was rinsed with DMF (5 × 10 mL). Any unreacted amines were then capped by addition of a solution of Ac₂O (1.0 mL, 1.08 g, 10.6 mmol, 106 equiv) and Et₃N (200 µL, 145.2 mg, 1.44 mmol, 14.4 equiv.) in CH₂Cl₂ (5 mL), followed by stirring at rt under Ar for 90 min and washing with CH₂Cl₂ (5 × 10 mL).

**Alloc deprotection.** The resin-bound peptide (100 µmol) was placed under an Ar atmosphere and treated with a solution of PhSiH₃ (700 µL, 613.9 mg, 5.67 mmol, 56.7 equiv) in CH₂Cl₂ (3 mL) with stirring, followed by addition of a solution of Pd(PPh₃)₄ (56.8 mg, 49.2 µmol, 0.49 equiv) in CH₂Cl₂ (3 mL). The resulting mixture was stirred at rt under Ar for 20 min. The resin was rinsed with CH₂Cl₂ (5 × 10 mL), and the deprotection protocol was repeated once.
Acetylation of the N-terminus of resin-bound peptides. The N-terminus of each peptide was capped by addition of a solution of Ac₂O (1.0 mL, 1.08 g, 10.6 mmol, 106 equiv) and Et₃N (200 µL, 145.2 mg, 1.44 mmol, 14.4 equiv.) in CH₂Cl₂ (5 mL), followed by stirring at rt under Ar for 2 h. The resin was then rinsed with CH₂Cl₂ (5 × 10 mL).

Cleavage of peptide from resin and purification. The peptide-resin was added to a fritted polypropylene syringe, washed with DMF (3 × 3 mL) and DCM (3 × 3 mL) and dried under a vacuum manifold. Peptide cleavage from the resin and concomitant deprotection of the acid sensitive amino acid side-chains were carried out with a TFA/TIS/H₂O (10 mL, 99:0.5:0.5) cocktail. The mixture was maintained for ca. 4 h at rt under magnetic stirring (150 rpm). The resin was washed with fresh TFA (1 mL) and filtered. The peptide was precipitated by filtering the mixture and pouring the filtrate into cold Et₂O (40 mL) and the precipitate was collected by centrifugation. The crude peptide was freeze-dried in 1:1 CH₃CN/H₂O (4 mL), lyophilized and purified by HPLC.

\[
\text{O} \quad \text{NH}_2
\]
\[
\text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{H} \quad \text{N} \quad \text{O} \\
\text{H} \quad \text{N} \quad \text{O} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{H} \quad \text{N} \quad \text{O} \\
\text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{O} \\
\text{H} \quad \text{N} \quad \text{O} \quad \text{H} \quad \text{N} \quad \text{O} \\
\text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{O} \quad \text{O}
\]

\((S)-2-(((S)-2-acetamido-3-methylbutanamido)-N^1-((2S,3S)-1-(((S)-1-(((S)-1,6-diamino-1-oxohexan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)pentanediamide (80).\) The residue was purified via reverse-phase HPLC (Phenomenex, 250 × 30 mm, 1% TFA in H₂O following a 22-27% MeCN [1% TFA] gradient over 23 min, 15 mL/min flow rate, UV detection at 220 nm, \(t_R = 8.5\) min) afforded 80 as a white powder: \(^1\)H NMR (DMSO-\(d_6\), 500 MHz) \(\delta\) 9.12 (br s, 1H), 8.13 (d, \(J = 7.5\) Hz, 1H), 7.98 (d, \(J = 7.3\) Hz, 1H), 7.92 (d, \(J = 7.9\) Hz, 2H), 7.81 (d, \(J = 8.2\) Hz, 1H), 7.76 (d, \(J = 8.9\) Hz, 1H), 7.26 (br s, 1H), 7.16 (br s, 1H), 7.05
(br s, 1H), 7.00 (d, \( J = 8.1 \) Hz, 2H), 6.77 (br s, 1H), 6.61 (d, \( J = 8.2 \) Hz, 2H), 4.45 (m, 1H), 4.23 (m, 1H), 4.20–4.06 (m, 4H), 2.93–2.83 (m, 1H), 2.77–2.60 (m, 3H), 2.15–1.99 (m, 2H), 1.97–1.78 (m, 5H), 1.76–1.59 (m, 3H), 1.55–1.35 (m, 4H), 1.33–1.17 (s, 6H), 0.89–0.68 (2m, 18H); \(^{13}\)C NMR (DMSO-\(d_6\), 125 MHz) \( \delta \) 173.2, 173.1, 171.2, 171.1, 170.84, 170.82, 169.5, 157.9, 155.8, 129.9, 127.5, 114.8, 57.74, 57.7, 56.9, 54.0, 52.3, 52.2, 38.7, 36.3, 31.5, 30.5, 30.4, 27.6, 26.8, 24.3, 22.5, 22.2, 19.2, 19.1, 18.21, 18.15, 15.2, 10.9; HRMS (ESI) \( m/z \) 790.4812 (MH\(^+\), \( C_{38}H_{63}N_9O_9H^+ \) requires 790.4836).

![Chemical structure](image)

\((S)-1-(acetyl-L-valyl)-N-((2S,3S)-1-(((S)-1-(((S)-1-((S)-1,6-diamino-1-oxohexan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)pyrrolidine-2-carboxamide (88). The residue was purified via reverse-phase HPLC (Phenomenex, 250 × 30 mm, 1% TFA in H\(2\)O following a 26-31% MeCN [1% TFA] gradient over 23 min, 15 mL/min flow rate, UV detection at 220 nm, \( t_R = 6.8 \) min) afforded 88 as a white powder: \(^1\)H NMR (DMSO-\(d_6\), 500 MHz) \( \delta \) 9.14 (br s, 1H), 8.06 (d, \( J = 8.3 \) Hz, 1H), 7.93 (m, 3H), 7.66 (d, \( J = 9.0 \) Hz, 1H), 7.15 (br s, 1H), 7.05 (br s, 1H), 7.00 (d, \( J = 8.4 \) Hz, 2H), 6.61 (d, \( J = 8.4 \) Hz, 2H), 4.46 (m, 1H), 4.37 (m, 1H), 4.28 (t, \( J = 8.4 \) Hz, 1H), 4.12 (t, \( J = 8.4 \) Hz, 3H), 3.77–3.69 (m, 1H), 3.60–3.51 (m, 1H), 2.92–2.84 (m, 1H), 2.78–2.62 (m, 4H), 2.03–1.85 (m, 4H), 1.83 (s, 3H), 1.79–1.59 (m, 4H), 1.55–1.41 (m, 4H), 1.23 (s, 4H), 0.93–0.70 (2m, 18H); \(^{13}\)C NMR (DMSO-\(d_6\), 125 MHz) \( \delta \) 173.2, 171.4, 171.0, 170.8, 170.7, 170.1, 169.2, 155.8, 129.9, 127.6, 118.6, 116.2, 114.8, 59.1, 57.5, 57.1, 55.7, 54.0, 52.2, 47.2, 38.7, 36.4, 36.1, 31.4, 30.7, 30.1, 29.0, 26.7, 24.5, 24.3, 22.3, 22.2, 19.1, 19.0, 18.6, 18.0, 15.3, 10.9; HRMS (ESI) \( m/z \) 759.4791 (MH\(^+\), \( C_{38}H_{62}N_8O_8H^+ \) requires 759.4778).
(S)-1-(acetyl-L-valyl-L-glutaminyl)-N-(((S)-1-(((S)-1-
((S)-1,6-diamino-1-oxohexan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-
3-methyl-1-oxobutan-2-yl)pyrrolidine-2-carboxamide (89). The residue was purified via
reverse-phase HPLC (Phenomenex, 250 × 30 mm, 1% TFA in H2O following a 15-20% MeCN
[1% TFA] gradient over 23 min, 15 mL/min flow rate, UV detection at 220 nm, tR = 9.2 min)
afforded 89 as a white powder: 1H NMR (DMSO-d6, 500 MHz) δ 9.18 (br s, 1H), 8.10 (d, J = 7.1
Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.66 (m, 2H), 7.27 (br s, 1H), 7.17 (br s, 1H), 7.07 (br s, 1H),
7.00 (d, J = 8.2 Hz, 2H), 6.82 (br s, 1H), 6.61 (d, J = 8.2 Hz, 2H), 4.47–4.35 (m, 3H), 4.18–4.10
(m, 2H), 4.03 (t, J = 7.8 Hz, 1H), 3.72–3.56 (m, 4H), 2.94–2.86 (m, 1H), 2.78–2.62 (m, 3H), 2.17–
2.05 (m, 2H), 1.99–1.78 (m, 9H), 1.77–1.61 (m, 3H), 1.55–1.44 (m, 3H), 1.34–1.18 (m, 3H), 0.85–
0.71 (m, 12H); 13C NMR (DMSO-d6, 125 MHz) δ 174.0, 173.3, 171.7, 171.2, 171.0, 170.9, 170.2,
169.4, 155.8, 130.1, 127.7, 116.2, 114.9, 59.1, 58.1, 57.5, 54.2, 52.2, 49.9, 46.9, 38.8, 36.4, 31.5,
30.9, 30.53, 30.49, 29.0, 27.0, 26.7, 24.6, 22.6, 22.2, 19.3, 19.2, 18.3, 18.2, 8.3; HRMS (ESI) m/z
774.4511 (MH+, C37H59N9O9H+ requires 774.4523).

5.3 References

15, 1518.


16, 4044.


5.4 Spectra
90 MHz, CDCl₃
$^{1}H$ NMR spectrum (125 MHz, CDCl$_3$)

- 171.2940
- 171.0070
- 170.5377
- 156.1347

$^{13}C$ NMR spectrum (125 MHz, CDCl$_3$)

- 80.0636
- 79.9443
- 79.7590
- 76.3108
- 65.5487
- 74.0332
- 73.8075
- 73.6136
- 67.7694
- 67.4436
- 63.6901
- 62.6970
- 58.7029
- 58.4408
- 55.6981
- 55.4926

- 31.5769
- 31.4828
- 28.2680
- 27.5363
- 27.5170
- 26.7445
- 25.4020
- 23.6865
- 23.4041

- 16.1069
- 15.9331

- 7.9061
- 7.9260
- 7.0034
- 6.4514
122

HO

Cbz

H

N

O

OMe

125 MHz, CDCl3

60

171.6848
170.4609

157.0557

136.1492

129.7816
128.4976
128.2474

136

67.9467
67.1864

58.9702

52.7294

41.3392

18.3788
$^{71a}$ 125 MHz, CDCl$_3$