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Expanding the Scope of Oligo-pyrrolinone–Pyrrolidines as Protein–Protein Interface Mimics

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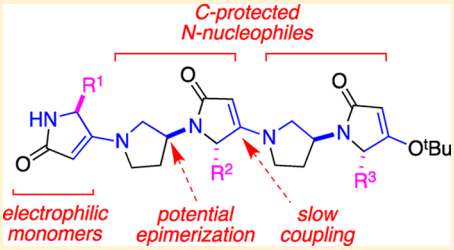
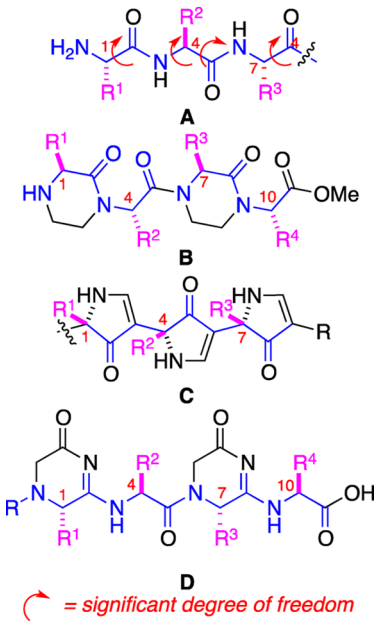
Supporting Information

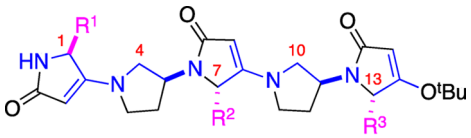
ABSTRACT: Oligo-pyrrolinone–pyrrolidines (generic structure **1**) have the potential to interfere with protein–protein interactions (PPIs), but to reduce this to practice it is necessary to be able to synthesize these structures with a variety of different side chains corresponding to genetically encoded proteins. This paper describes expansion of the synthetic scope of **1**, the difficulties encountered in this process, particularly issues with epimerization and slow coupling rates, and methods to overcome them. Finally, spectroscopic and physicochemical properties as well as proteolytic stabilities of molecules in this series were measured; these data highlight the suitability of oligo-pyrrolinone–pyrrolidines for the development of pharmacological probes or pharmaceutical leads.



INTRODUCTION

Minimalist mimics of secondary structures is a term we used to describe organic scaffolds that present amino acid side chains (usually three) in restricted conformations relative to peptides **A**. Typical examples of these include Hamilton's helical systems beginning with terphenyls and now encompassing a range of variations from his group[1−5] and others[6−12] and Hirschmann–Smith turn and sheet mimics.[13−17] Some specific examples of recent *minimalist mimics* include Arora's oxopiperazines **B**, Smith–Hirschmann pyrrolinones **C**,[17] selected Bartlett's aza-@tide structures **D**,[18,19] and our own oligo-pyrrolinone–pyrrolidines **1**.[20]





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Several attributes shared by the generalized structures **B**–**D** and **1** enhance their value as minimalist mimics. First, they all must occupy more narrowly defined regions of conformational space than the peptides **A** because their backbones have fewer significant degrees of freedom (red arrows). Second, they are not composed of repeating secondary amide units, enhancing the likelihood that their derivatives will be proteolytically stable, cell permeable, and orally bioavailable,[21] though each of these parameters is partly determined by the side chains. Their relative conformational rigidities,[22] and the fact that they will not form polycationic ammonium species under physiological conditions, are also desirable features with respect to retarding proteolysis and promoting absorption. Third, water solubilities of compounds having these structures will tend to be promoted by their heteroatoms and heterocycles, relative to structures featuring mostly benzenoid rings. Fourth, each of these minimalist mimic scaffolds have side chains arranged in *1*, *4*, *7*, *10* ..... relationships (we colloquially refer to this feature of some minimalist mimics as *side-chain periodicities*). The fact that this is the same side-chain periodicity as peptides and proteins is almost certainly advantageous when attempting to mimic consecutive amino acid side chains. Finally, and related to this, all these structures presumably could be made from amino acids, and this may facilitate preparations of systems with more

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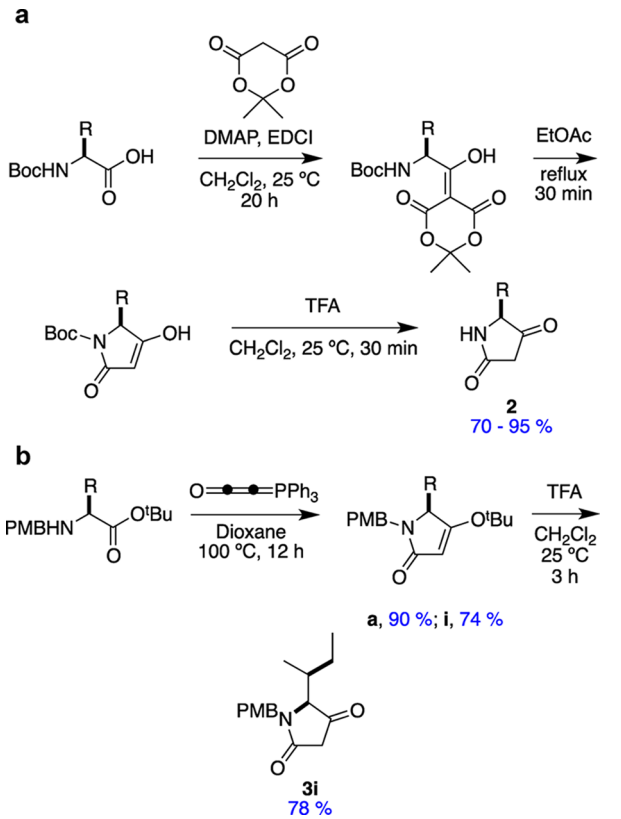
functionalized, less synthetically accessible, side chains like those found in Arg, His, Trp, and Cys.

In practice, the fact that structures like **B**–**D** *could* be made from functionalized amino acids is very different to actually demonstrating the methods and conditions by which they *can* be prepared with these side chains. Just as protocols in peptide synthesis have taken decades to develop and refine, syntheses of any of these systems with different combinations of side chains corresponding to a diverse set of amino acids are difficult. Consequently, one key objective of this article is to describe syntheses of compounds **1** incorporating a variety of amino acid side chains. We have not prepared a set of systems that shows all 20 naturally encoded amino acids can be incorporated, but have made enough to support an immediate need for further validating the concept of exploring key orientations (EKO).[23] This technique features data mining to match preferred conformations of a minimalist mimic with PPI interface regions.

RESULTS AND DISCUSSION

Preparation of Tetramic Acid Derivatives 2. The first of two routes to the requisite tetramic acid synthons involved BOC-protected amino acids as starting materials in condensation/cyclization processes involving Meldrum's acid as shown in Scheme 1a.[24−27] This strategy has the advantage that many

Scheme 1. Two Methods for Preparing Tetramic Acid Derivatives: (a) Meldrum's Acid Approach; (b) Bestmann's Ylide[28−30] Route



BOC-protected amino acids with appropriate side-chain protection are commercially available and inexpensive relative to other protected amino acid derivatives. Moreover, the condensation/cyclization process is experimentally convenient because strictly anhydrous conditions are not required. However, this "Meldrum's acid route" described above also has drawbacks. In our hands, the condensation procedure does not proceed to completion for some amino acids (e.g., Phe) unless excess carboxylate activating agents are used (e.g., 2.0–2.5 equiv of EDCI, even though some procedures have reported using less EDCI).[27] Consequently, a procedure was devised wherein an aqueous work up was used to remove excess EDCI and DMAP salts after first step of the process, and the crude material was used in the next transformation without further purification.

The procedures described above were based on work by Tønder and co-workers[24−27] who made these products but exclusively using hydrophobic amino acids for which the purified products could be obtained via precipitation from ether. The current studies showed this approach was not universally applicable to *functionalized* side chains, depending on the scale, the side-chain, and residual TFA levels in the crude material. Thus, isolation of some tetramic acids (e.g., that from Trp) required chromatograpy, but several others (Met/Phe/Thr and Leu) were crystallized directly after rigorous removal of TFA. Overall, we find this "Meldrum's acid route" to be the most useful entry point into tetramic acids with *NH N*-termini; it tends to be practical because many of the requisite BOC-protected amino acids starting materials are commercially available.

Scheme 1b shows an alternative route to tetramic acid derivatives based on cumulative literature, largely originating from Schobert's laboratory,[29−33] regarding additions of (triphenylphosphoranylidine)ketene (the Bestmann ylide) to *N*-alkyl α-amino esters. These routes are conceptually different from those based on Meldrum's acid insofar as the starting materials are *C*- rather than *N*-protected. Removal of Ph_3PO after the cyclization in method **b** can be problematic, particularly for >5 g scale. When trying to overcome this difficulty, we found the *N*-PMB-protected products tend to be ether soluble, thus facilitating precipitation of Ph_3PO from concentrated ether solutions of the crude material at −20 °C. Samples obtained in this way could be subjected to column chromatography, even on >5 g scales. This approach is more practical, if less elegant, than ones based on supported variants of Bestmann's ylide[33] that involve inconveniently big amounts of solid-phase reagent for large-scale reactions.

Overall, the Bestmann's ylide approach in Scheme 1b is preferred if *N*-capped tetramic acid derivatives are required. *N*-PMB tetramic derivatives are useful in the syntheses described below, and the starting materials can be prepared conveniently from amino acids via reductive amination with 4-methoxybenzaldehyde.

A possible complication for preparation of tetramic acids from aspartic acid derivatives is competing cyclization to give the six-membered ring product in competition with the desired material **2d′**. To explore this possibility, the di-*tert*-butyl ester of aspartic acid was transformed as indicated in reaction 1, but only the five-membered ring compound **2d′** was observed. The corresponding reaction with glutamic acid was not attempted, but in that case the competitive processes would be between formation of a 5-membered ring and a 7-membered ring; the latter is less likely, so side-chain complications are predicted to be even less of a concern.

Table 1 shows all of the tetramic acids isolated in the course of this work and indicates how the one-letter codes for side chains in this paper correspond to those of the parent amino acids.

Preparation of Pyrrolinone–pyrrolidine Nucleophiles 2. Our preparation of the scaffold systems **1** begin with a Merck

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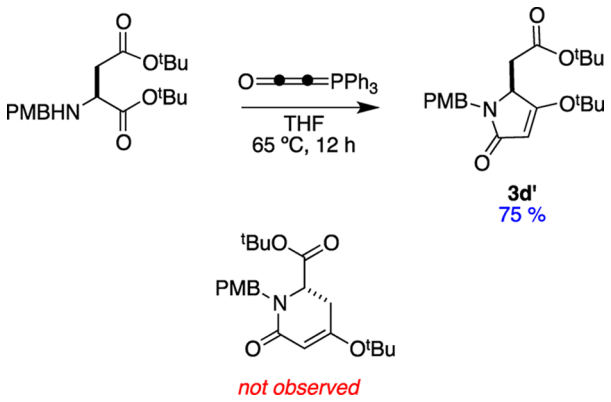
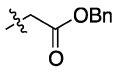
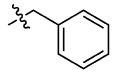
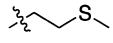
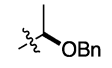
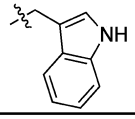
procedure to decarboxylate *trans*-4-hydroxyproline that can afford more than 50 g of crystalline (*R*)-3-hydroxypyrrolidine without



chromatography.[34] *N*-Protection of (*R*)-3-hydroxypyrrolidine followed by nucleophilic displacement of its triflate derivative (under conditions optimized to avoid elimination)[35] gave the amino esters **4** (Scheme 2). Variable amounts of epimerization occurred in these reactions, but this is not a serious problem because all of the esters **4** featured in this paper were obtained as single diastereomers via one crystallization from either ethanol or acetonitrile. The communication that precedes this work reported an X-ray analysis of **4f·HCl** (after crystallization from ethanol) that proved it formed via a single inversion, i.e., without a neighboring group effect.[20] Here, **4i·HCl** (after crystallization from acetonitrile) was similarly shown to be the product of one inversion.

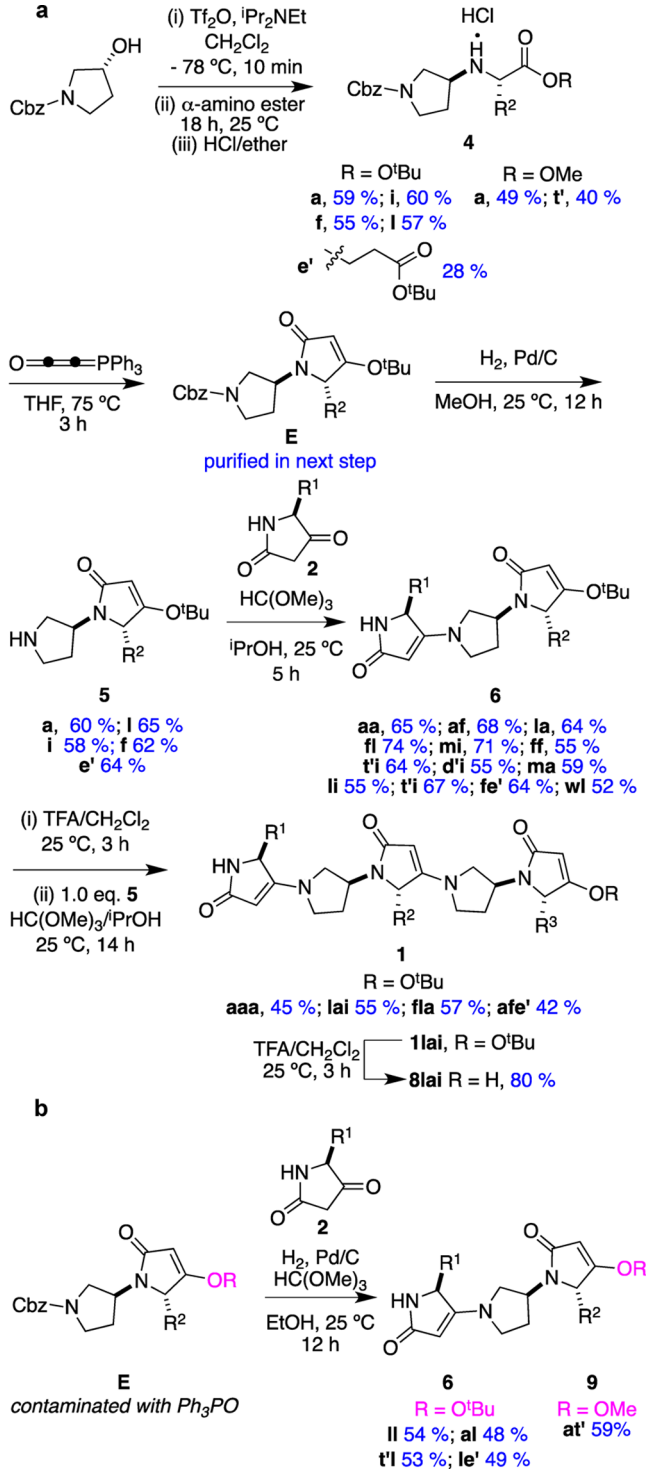
A series of experiments were undertaken to elucidate which of the two chiral centers in structure **4** epimerized in the synthesis of this material. First a mixture that would be formed from "acyclic epimerization", (*S*,*S*)-**4f** + (*R*,*S*)-**4f** (see below),

Table 1. Preparation of Tetramic Acid Derivatives[a]

compound	R^1	yield (%)
2a		92
2d'		71
2f		75
2i		68
3i		78
2l		70
2m		64
2t'		82
2w		58

[a]All tetramic acids were synthesized from the corresponding L-amino acids except **w**, which was made from the D-amino acid.

Scheme 2. (a) Syntheses of the Target Systems 1. (b) One-Pot Modification of the Key Coupling Step



was made by reacting the Cbz-pyrrolidine 3-triflate precursor with (*rac*)-*tert*-butyl phenylalanine. Second, racemic Cbz-pyrrolidine 3-triflate was reacted with optically pure *tert*-butylphenylalanine to form the isomers that would be formed from "ring epimerization", i.e., (*S*,*S*)-**4f** + (*R*,*S*)-**4f** and (*S*,*R*)-**4f** + (*R*,*R*)-**4f**. After removal of the Cbz group, ^{13}C NMR spectroscopy of these mixtures allowed (*S*,*S*)-**4f** and (*R*,*S*)-**4f** to be differentiated [and, of course, the enantiomers (*R*,*R*)-**4f** from (*S*,*R*)-**4f**]. Comparison of that ^{13}C NMR data with analytical

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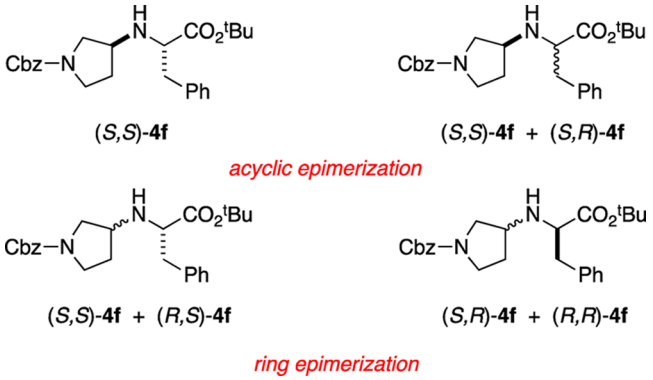

traces from a chiral HPLC column established that (*R*,*S*)-**4f** could be chromatographically distinguished from the other three stereoisomers that eluted at almost the same time under these conditions. Comparison of all this data with ^{13}C NMR and chiral HPLC analyses of the partially epimerized mixture (step 1, Scheme 1) was sufficient to confirm that ring epimerization was the dominant process and acyclic epimerization did not occur to within the limits of detection in the ^{13}C spectra that were accumulated (see the Supporting Information).

Epimerization in the first step of Scheme 1 was found to be dependent upon the side chains involved and the scale of the reaction. Loss of stereochemical integrity in this reaction could occur via minor pathways involving S_N1 mechanisms and/or a neighboring group effect from the carbamate. It was conspicuous in this study because the nucleophile is homochiral. Several papers in the literature feature reactions of *N*-carbamate-protected 3-hydroxypyrrolidine derivatives with *achiral* nucleophiles;[36−43] partial racemization in these reactions would have been less noticeable.

Once crystallized, the hydrochlorides **4** are stable on the bench for at least several weeks. It is convenient that the products are hydrochlorides because this provides the 1 equiv of acid that is required to activate Bestmann's ylide[28−30] in the next step: cyclization to Cbz-protected forms of the pyrrolidines **5**. Intermediates **E** are labeled with a letter and not a number because they were subjected to hydrogenolysis "as is". Epimerization can occur in cyclizations using Bestmann's ylide, but we found it is negated in THF using relatively short reaction times (3 h). Dimerization of the ketene formed from Bestmann's ylide after protonation competes with the desired cyclizations in these processes. To minimize this complication, the solid ylid was added portionwise every 15 min until completion of the reaction (monitored via 1H NMR spectroscopy; see the Supporting Information).

Condensation of the free pyrrolidine-*NH* of **5** with 5-substituted 2,4-pyrrolidinediones (tetramic acids) **2** gave the pyrrolinone−pyrrolidine−pyrrolinone systems **6**. Unfortunately, the free pyrrolidines **5** seem to be basic enough to mediate self-epimerization, at rates dependent on the side chains and the *C*-terminal cap but, in the worst cases, even during storage at −20 °C. For instance, the *tert-butyl* ester **5a** (Me side-chain, O^{t}Bu *C*-terminus) was isolated and characterized as one stereoisomer and then used immediately in the next step without compromising its stereochemical purity, whereas the corresponding *methyl* ester derivatives were far less stable (see below). Similarly, to illustrate dependence of epimerization on side chains, storage of **5a** at −20 °C for 4 d resulted in epimerization, but none was detected for the derivative from Ile (**5i**) under the same conditions. Overall, we conclude the tendencies for compounds **5** to epimerize are suppressed when the side-chain and/or the *O*-substituent are large, probably because this sterically disfavors formation of planar, extended enolates.

Pyrrolidines **5** tend to be protonated by adventitious protons upon standing. One attempt to crystallize the *free base* **5f** using untreated glassware deposited crystals of the corresponding *ammonium chloride* (X-ray analysis). It is important to avoid such protonation events because the salts of **5** do not couple with tetramic acids to give the desired vinylogous ureas **6**. For this reason we routinely purified the free-bases **5** immediately before the next coupling step via column chromatography using an eluent containing 1% Et_3N. Epimerization may occur if the fractions are directly concentrated in that basic medium, but azeotropic removal with toluene prevents epimerization in the rotary evaporation process, because, we hypothesize, the mol fraction of Et_3N is not increased under these conditions.

It is to be anticipated that *C*-terminal tetramate-*OMe* units will be preferred when dealing with those PPI targets where the corresponding *tert*-butyl esters are too bulky and lipophilic to be accommodated. This consideration led us to investigate the methyl tetramate forms of **5**; however, these were found to epimerize faster than the corresponding *tert*-butyl compounds. Consequently, a "one-pot" procedure was developed to transform intermediate **E** directly into the products **9** (methyl forms) and **6** (*tert*-butyl). In the event, the modification that was developed (Scheme 2b) facilitated these reactions without epimerization *and* made it easier to remove triphenylphosphine oxide from the products. Moreover, selective hydrogenolysis of the Cbz group in the presence of *O*-benzyl side-chain protection occurred in this process; that is an advantage in situations where downstream steps require side-chain *O*-benzyl protection.

Pyrrolinone−Pyrrolidine−Pyrrolinone−Pyrrolidine−Pyrrolinones 1. Optimized conditions for coupling of the pyrrolidine **5** with the tetramic acids **2** as described above worked well for several substrates, but coupling of the same pyrrolidines **5** with the larger electrophiles derived from deprotection of vinylogous carbamates **6** was significantly slower. We therefore investigated reaction of the nucleophiles with mesylates derived from the tetramic acid, with or without catalytic Lewis acids like $Yb(OTf)_3$[44] and ^{i}PrOH/AcOH/molecular sieves,[45] but this did not improve the product yield. Qualitatively, we observed that reaction of the pyrrolidines **5** was faster in alcohol solvents, but MeOH caused methanolysis of the vinylogous urea bonds leading to byproducts, so a more hindered alcohol medium, ^{i}PrOH, was used. Trimethyl orthoformate was added to scavenge water in this reaction; it was more effective than molecular sieves or azeotropic removal. Better product yields were obtained with 1.5 equiv of trimethylorthoformate, though use of this as a cosolvent was not helpful. No epimerization was observed when products **1** or **6** were formed under the conditions specified in Scheme 2a and, most particularly, using a slight excess of the *C*-terminal tetramate electrophile, whereas it *was* problematic in test reactions in which the pyrrolidine nucleophile was used in excess. Fortunately, protecting group requirements for functionalized nucleophiles **5** and electrophiles **2** do not place severe constraints on the reaction; for instance, the unprotected indole side-chain in **2w** tolerated coupling with the nucleophiles **5**. However, when the R^2 substituent is large, e.g., for Thr(OBn) or Ile, the desired reaction is relatively slow, and the product yield is diminished.

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Properties of the Pyrroline–Pyrrolidine Oligomers. Stabilities of the oligomers containing pyrroline-pyrrolidine were challenged under commonly encountered conditions to determine synthetic limitations and constraints for in vitro assays and to reveal how the featured compounds might be changed in vivo. In the first instance, influence of aqueous acid was examined by HPLC for **6t′l** in pH 7.4 phosphate buffer (100 mM) and at pH 4.5 (100 mM acetate buffer) and 25 °C. At pH 7.4 there was no change to **6t′l** over 15 h (after which time the experiment was stopped, see the Supporting Information), but at pH 4.5 this compound hydrolyzed with a half-life of ~40 h^{-1} (Figure 1a). Similarly, **6ll** was completely stable in pH 7.8 10 mM Tris buffer at 55 °C for 60 h (after which time the experiment was stopped).

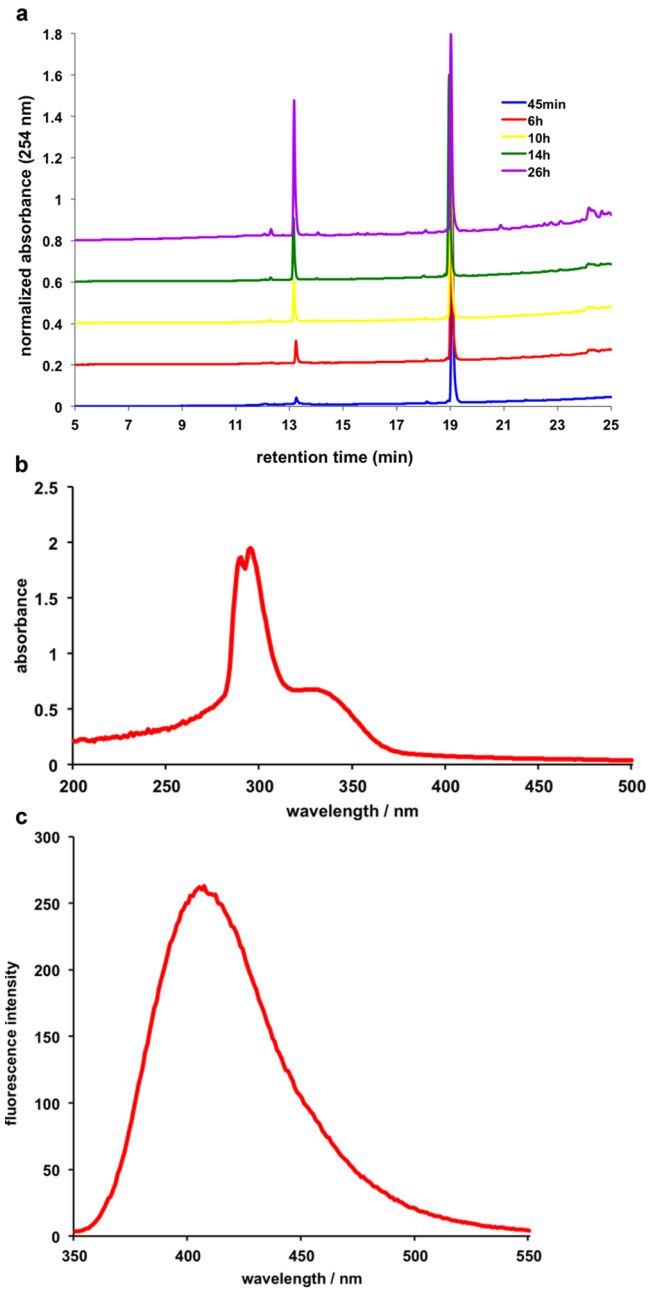


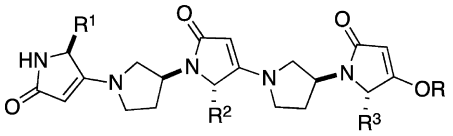
Figure 1. (a) Compound **6t′l** in pH 4.5 100 mM acetate buffer has a half-life of ca. 40 h^{-1} at 25 °C. (b) UV absorbance spectrum of **1fla** in 100 mM acetate buffer pH 4.5. (c) Fluorescence spectrum of the *C*-deprotected form of **6fl**.

Proteolytic stabilities were assessed by treating **1fla** and **6ll** with 63 μM pronase (a mixture of proteases from *Streptomyces griseus* that is routinely used to hydrolyze peptides)[46] at pH 7.8 in 10 mM Tris buffer containing 5 mM $CaCl_2$, at 37 °C. No decomposition of either compound was observed by HPLC over a period of 60 h (after which time the experiment was stopped). Under the same conditions a control tetrapeptide GATV-**DAP** (GATV fluorescently labeled on the *N*-terminus with 4-dimethyolaminophthalimide) was completely hydrolyzed to smaller fragments within 2 h. Collectively, these data indicate the compounds **1** and **6** tend to be stable under neutral aqueous conditions at 37 °C, even in the presence of proteases, but they would be hydrolyzed at decreased pH values, such as in the stomach.

Figure 1 shows these compounds have a maximal UV absorbance at around 295 nm: it has an extinction coefficient of 3.88 × 10^4 M^{-1} cm^{-1}. The products with an alkylated *C*-terminus have no significant fluorescence, but the deprotected forms like the *C*-deprotected form of **6fl** (i.e., **6fl-OH**; Φ = 0.006 in 100 mM acetate buffer, pH 4.5) fluoresce with a low quantum yield, but which might influence assays set to observe around 400 nm.

Two molecular features govern the bioavailability of structures **1**, specifically, parameters of the (i) scaffold unit and (ii) the side chains. Jorgenssen's QikProp program[47,48] was used to simulate rates of permeation into Caco-2 cells (Table 2) in comparison with corresponding peptides

Table 2. Rates of Permeation into Caco-2 Cells Simulated Using QikProp



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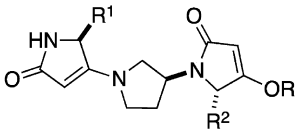

6

	R^1	R^2	R^3	R	PCaco-2 (nm/s)
1	H	H	H	H	4
1	H	H	H	^{t}Bu	15
1	Me	Me	Me	H	6
1	Me	Me	Me	^{t}Bu	10
1	Phe	Leu	Me	H	15
1	Phe	Leu	Me	^{t}Bu	25
1	Leu	Me	Ile	H	12
1	Leu	Me	Ile	^{t}Bu	19
1	H	H	Arg	H	0.3
1	Lys	Me	Me	H	0.7
1	Lys	Me	Asp	H	0.03
1	Lys	Me	Asp	^{t}Bu	0.06
6	H	H		H	34
6	H	H		^{t}Bu	118
6	Me	Me		H	49
6	Me	Me		^{t}Bu	89
6	Leu	Me		H	74
6	Leu	Me		^{t}Bu	218
6	Leu	Lys		H	5
6	Leu	Lys		^{t}Bu	9

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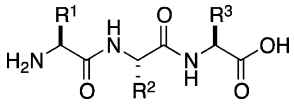
Table 3. Rates of Permeation into Cells Simulated via QikProp



R^1	R^2	R^3	PCaco-2 (nm/s)
H	H	H	1
Me	Me	Me	2
Me	H	Me	2
Leu	Me	Ile	11
Phe	Leu	Me	6

(Table 3) to begin to understand these properties. Rates of >20 nm/s^{-1} are widely considered to be a good indicator of favorable oral bioavailability; standards for permeation into cells would be somewhat less than this.

Table 2 lists simulated rates of permeation into Caco-2 cells for compounds based on the generic structures **1** and **6**, and Table 3 gives the data for peptides with the corresponding side chains. This data covers six compounds **1** that were prepared in this work and six more that have not been prepared yet. Similarly, that same table covers four compounds **6** that were made and four others that were not. None of the peptides are predicted to be bioavailable (PCaco <11 nm/s). However, most of the PCaco data is above the 20 nm/s threshold for the smaller mimics **6** that have about the same molecular masses as the tripeptides. The only exceptions were for the most polar analogues, e.g., ones containing a Lys side-chain. Predicted Caco-2 data for the larger compounds **1** was found to be less, as expected from their larger sizes. Nevertheless, the least polar of these structures had pCaco values in an acceptable region (15 − 25 nm/s). Overall, we conclude that molecules based on **1** and **6** are reasonable candidates for experimental determinations of bioavailabilities, and they are probably much more cell-permeable than closely related peptides.

CONCLUSIONS

Several issues emerged when attempting to expand the scope of the procedures to obtain interface mimics **1**. First, preparation of the requisite tetramic acids with several different side chains is relatively easy. The first real problem encountered in the syntheses was "ring-epimerization" in the *C*-protected *N*-nucleophile syntheses corresponding to fragments **4**. This issue was address by crystallizing those nucleophiles; this proved to be possible for every different side chain R^2 or R^3 examined here. The second issue relates to slow couplings of the nucleophiles **5** with electrophiles like **6**; this was an issue that was improved, and steric constraints regarding the side chains were elucidated. Furthermore, a one-pot procedure was developed for the first key coupling step in the syntheses of **6**.

The target molecules **1** are stable at neutral pH levels around physiological, but have a half-life of about 40 h^{-1} at pH 4.5. They are proteolytically stable, thermally resilient to 55 °C, and potentially cell permeable based on QikProp calculations. The molecular scaffolds **1** and **6** explored in this work have potentially much better cell permeability than the corresponding peptides.

EXPERIMENTAL SECTION

General Experimental Methods. All reactions were carried out under an inert atmosphere (nitrogen or argon where stated) with dry solvents under anhydrous conditions. Glassware for anhydrous reactions were dried in an oven at 140 °C for minimum 6 h prior to use. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at a high commercial quality (typically 97% or higher) and used without further purification, unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV, ceric ammonium molybdate, and/or potassium permanganate stains. Flash column chromatography was performed using silica gel 60 (Silicycle, 230−400 mesh) as per the Still protocol. ^{1}H and ^{13}C spectra were recorded on a 300 MHz spectrometer and were calibrated using residual nondeuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16, DMSO-d_6: ^{13}C NMR = 39.52, CD_3OD: ^{1}H NMR = 3.31, ^{13}C NMR = 49.00). The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad, dd = doublet of doublet, app = apparent. IR spectra were recorded using NaCl plates. Melting points were recorded on an automated melting point apparatus and are uncorrected. Optical rotations were obtained on a polarimeter at the D line of sodium.

All of the HPLC analyses were carried out with UV detection monitored at 254 nm. Analytical reversed-phase HPLC analyses were performed with a 150 × 4.6 mm C-18 column using gradient conditions (10−90% acetonitrile in water, flow rate = 0.75 mL/min). Chiralpak AD (250 × 4.6 mm ID) column was utilized for the chiral HPLC analysis (hexanes: isopropyl alcohol 85:15, flow rate = 1 mL/min).

QikProp 3.5 from Schrödinger (2012) was used to evaluate pharmaceutically relevant properties for compounds **1** and **6** and tripeptides with various side chains.

General Procedure for the Synthesis of *N*-PMB α-Amino Ester Hydrochlorides. To a solution of the α-amino ester hydrochloride (40.0 mmol) in methanol (50 mL) at 0 °C was added triethylamine (5.6 mL, 40.0 mmol, 1.0 equiv) dropwise. *p*-Methoxybenzaldehyde (5.82 mL, 48.0 mmol, 1.2 equiv) was added, and the reaction was allowed to proceed at 25 °C for 2 h. Sodium borohydride (3.02 g, 80.0 mmol, 2.0 equiv) was carefully added portionwise at 0 °C over 60 min. The reaction was stirred for 30 min at 25 °C and concentrated. The residue was partitioned between Et_2O (250 mL) and saturated $NaHCO_3$ (150 mL). The aqueous layer was extracted twice with Et_2O (75 mL), and the combined organic layers were washed with brine (75 mL), dried over $MgSO_4$, and filtered. The filtrate was cooled to 0 °C and treated with a 4 M solution of HCl in dioxane (10 mL, dropwise addition) to precipitate the product, which was collected by filtration.

(*S*)-*tert*-Butyl 2-((4-methoxybenzyl)amino)propanoate (*N*-PMB Ala *tert*-butyl ester·HCl): white solid, 10.4 g, 86%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 2H), 4.05 (m, 2H), 3.69 (s, 3H), 3.45 (m, 1H), 1.59 (d, *J* = 7.2 Hz, 3H), 1.50 (s, 9H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 160.2, 132.1, 122.4, 114.3, 83.9, 55.2, 53.8, 48.6, 27.9, 15.7; IR (film, cm^{-1}) 1735, 1613, 1562, 1421, 1390, 1257, 1090; MS (ESI-TOF) *m*/*z* calcd for $C_{15}H_{23}NO_3$ $(M + H)^+$ 266.18, found 266.19

(2*S*,3*S*)-*tert*-Butyl 2-((4-methoxybenzyl)amino)-3-methylpentanoate (*N*-PMB Ile *tert*-butyl ester·HCl): white solid, 8.1 g, 79%; $[\alpha]^{20}$ +15.5 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.52 (br s, 1H), 9.60 (br s, 1H), 7.58 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.32 (d, *J* = 13.2 Hz, 1H), 4.15 (d, *J* = 13.5 Hz, 1H), 3.77 (s, 3H), 3.38 (br s, 1H), 2.45−2.32 (m, 1H), 1.50 (s, 10H), 1.45−1.27 (m, 1H), 1.14 (d, *J* = 6.9 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 160.4, 132.5, 121.7, 114.4, 84.3, 61.7, 55.2, 49.7, 36.1, 28.1, 26.8, 15.0, 11.6; IR (film, cm^{-1}) 1732, 1614, 1557, 1421, 1393, 1257, 1092, 1031; MS (ESI-TOF) *m*/*z* calcd for $C_{18}H_{30}NO_3$ $(M + H)^+$ 308.22, found 308.29

(*S*)-Di-*tert*-butyl 2-((4-methoxybenzyl)amino)succinate (*N*-PMB Asp(O^{t}Bu) *tert*-butyl ester·HCl): light yellow solid, 8.1 g, 74%; mp = 112.0−113.3 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (dd, J_1 = 2.1 Hz, J_2 = 6.6 Hz, 2H), 6.87 (dd, J_1 = 2.1 Hz, J_2 = 6.6 Hz, 2H),

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3.87−3.82 (m, 4H), 3.67 (d, J = 12.6 Hz, 1H), 3.51 (dd, J_1 = 5.7 Hz, J_2 = 6.9 Hz, 1H), 2.63 (dd, J_1 = 5.7 Hz, J_2 = 15.6 Hz, 1H), 2.54 (dd, J_1 = 6.9 Hz, J_2 = 15.6 Hz, 1H), 1.92 (b, 1H), 1.50 (s, 9H), 1.47 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 170.3, 158.7, 132.0, 129.5, 113.7, 81.3, 80.8, 57.8, 55.3, 51.4, 39.5, 28.1; IR (film, cm^{-1}) 3420(br), 2981, 1735, 1614, 1517, 1371, 1255, 1154; HRMS (ESI-TOF) m/z calcd for $C_{20}H_{31}NO_5$ (M + H)$^+$ 366.2275, found 366.2274 (0.3 ppm)

General Procedure for the Cyclization of *N*-PMB α-Amino Ester Hydrochlorides. To a stirred suspension of the PMB-protected α-amino ester hydrochloride (5.0 mmol) in anhydrous dioxane (0.25 M) under Argon was added Bestmann's ylide (11.0 mmol, 2.2 equiv) in one portion at 25 °C. The reaction was heated at 100 °C under Argon for 12 h. Upon cooling, the reaction mixture was concentrated and resuspended in 50 mL of ether. The suspension was stirred vigorously for 30 min, and the solids were removed by filtration. The filtrate was concentrated to ∼10 mL and placed at −20 °C for 14 h following which the crystallized Ph_3PO byproduct was removed by filtration. The filtrate was concentrated to obtain an oil which was purified by flash chromatography.

(S)-4-tert-Butoxy-1-(4-methoxybenzyl)-5-methyl-1H-pyrrol-2(5H)-one: colorless oil, 1.30 g, 90%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 9.0 Hz, 2H), 5.03−4.96 (m, 2H), 3.96 (d, J = 15.0 Hz, 1H), 3.76 (s, 3H), 3.66 (q, J = 6.7 Hz, 1H), 1.39 (s, 9H), 1.18 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 172.0, 158.8, 130.0, 129.2, 113.9, 94.9, 81.4, 56.6, 55.2, 42.3, 27.4, 16.0; MS (ESI-TOF) m/z calcd for $C_{17}H_{24}NO_3$ (M + H)$^+$ 290.17, found 290.10.

(S)-4-tert-Butoxy-5-((S)-sec-butyl)-1-(4-methoxybenzyl)-1H-pyrrol-2(5H)-one: colorless oil, 1.23 g, 74%; $[\alpha]^{20}$ +11.8 (c 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 5.18−5.10 (m, 2H), 3.88 (d, J = 15.3 Hz, 1H), 3.82 (s, 3H), 3.72 (d, J = 3.0 Hz, 1H), 1.96−1.82 (m, 1H), 1.45 (s, 9H), 1.55−1.33 (m, 2H), 0.89 (t, J = 7.4 Hz, 3H), 0.77 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 170.8, 158.8, 129.9, 129.3, 113.9, 96.5, 81.6, 63.4, 55.2, 42.3, 34.9, 27.5, 25.7, 12.9, 12.6; IR (film, cm^{-1}) 1682, 1514, 1372, 1329, 1246, 1171; MS (ESI-TOF) m/z calcd for $C_{20}H_{30}NO_3$ (M + H)$^+$ 332.22, found 332.23

*(S)-tert-Butyl 2-(3-tert-butoxy-1-(4-methoxybenzyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)acetate (**2d′**):* colorless oil, 1.54 g, 75%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (dd, J_1 = 2.1 Hz, J_2 = 6.6 Hz, 2H), 6.86 (dd, J_1 = 2.1 Hz, J_2 = 6.6 Hz, 2H), 5.10 (s, 1H), 5.05 (d, J = 15.3 Hz), 4.07 (t, J = 5.1 Hz), 4.03 (d, J = 15.3 Hz), 3.81 (s, 3H), 2.62 (dd, J_1 = 5.1 Hz, J_2 = 15.3 Hz, 1H), 2.51 (dd, J_1 = 5.1 Hz, J_2 = 15.3 Hz, 1H), 1.45 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 170.2, 169.0, 158.9, 129.7, 129.3, 114.0, 95.7, 82.1, 81.3, 57.8, 55.2, 42.8, 36.6; IR (film, cm^{-1}) 2979, 2361, 1731, 1615, 1513, 1368, 1248, 1147; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{31}NO_5$ (M + Na)$^+$ 412.2094, found 412.2101 (1.7 ppm).

Procedure for the Syntheses of 3i. (*S*)-4-*tert*-Butoxy-5-((*S*)-*sec*-butyl)-1-(4-methoxybenzyl)-1*H*-pyrrol-2(5*H*)-one (1 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0 °C. TFA (5 mL) was added, and the reaction was allowed to warm to room temperature. After being stirred for 2 h, toluene (25 mL) was added and the solution was concentrated. Residual TFA was azeotroped two times with toluene (25 mL × 2), and the residue was purified by flash chromatography (50% ethyl acetate in dichloromethane) to afford (*S*)-5-((*S*)-*sec*-butyl)-1-(4-methoxybenzyl)pyrrolidine-2,4-dione (3i) in 78% yield: colorless oil, 215.4 mg, 78%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 9.0 Hz, 2H), 5.22 (d, J = 14.7 Hz, 1H), 3.82 (d, J = 14.7 Hz, 1H), 3.74 (s, 3H), 3.61 (d, J = 3.3 Hz, 1H), 2.92 (s, 2H), 1.90−1.78 (m, 1H), 1.49−1.33 (m, 2H), 0.86−0.73 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.5, 170.4, 159.5, 129.8, 126.6, 114.3, 68.6, 55.2, 43.1, 42.4, 35.1, 25.1, 13.4, 12.0; IR (film, cm^{-1}) 1770, 1514, 1418, 1248, 1034; MS (ESI-TOF) m/z calcd for $C_{16}H_{22}NO_3$ (M + H)$^+$ 276.15, found 276.19

General Procedure for the Syntheses of Tetramic Acids 2. A modified literature procedure was used. To a stirred solution of meldrum's acid (476 mg, 3.3 mmol, 1.1 equiv) and DMAP (550 mg, 4.5 mmol, 1.5 equiv) at 0 °C in dichloromethane (30 mL) was added *N*-Boc-amino acid (3.0 mmol, 1.0 equiv) in one portion. EDCI (1.2 g, 7.2 mmol, 2.4 equiv) was added in one portion, and the reaction mixture was stirred at 25 °C for 14 h. The yellow reaction mixture was transferred to a separatory funnel, diluted with EtOAc (80 mL), and washed with cold 5% $KHSO_4$ (3 × 100 mL) and brine (75 mL). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was refluxed for 30 min under N_2. Upon concentration, the residue was dissolved in dichloromethane (5 mL) and cooled to 0 °C. TFA (5 mL) was added, and the reaction was stirred for 30 min. Toluene (25 mL) was added, and the solution was concentrated. Residual TFA was azeotroped two times with toluene (25 mL each), and then 5 mL ether was added. After the mixture was stirred for 10 min, the solid product was collected by filtration.

*(S)-5-((1H-Indol-3-yl)methyl)pyrrolidine-2,4-dione (**2w**):* white solid, 407.3 mg, 58%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (br s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.38−7.32 (m, 1H), 7.26−7.18 (m, 1H), 7.17−7.10 (m, 1H), 7.25 (br s, 1H), 6.93 (d, J = 2.4 Hz, 1H), 4.23 (dd, J = 7.5, 3.3 Hz, 1H), 3.01 (dd, J = 14.9, 7.9 Hz, 1H), 2.89 (d, J = 22.2 Hz, 1H), 2.68 (dd, J = 22.2, 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.3, 171.4, 136.2, 126.9, 123.5, 122.5, 119.9, 118.6, 111.5, 109.2, 64.7, 40.9, 28.1; IR (film, cm^{-1}) 3400 (br), 2918, 1767, 1684, 1361, 1236, 1093, 745; HRMS (ESI-TOF) m/z calcd for $C_{13}H_{12}N_2O_2$ (M + Li)$^+$ 235.1020, found 235.1028 (3.4 ppm).

*(S)-5-((S)-sec-Butyl)pyrrolidine-2,4-dione (**2i**):* white solid, 316.4 mg, 68%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (br s, 1H), 3.90 (d, J = 3.6 Hz, 1H), 2.95 (m, 2H), 1.91−1.84 (m, 1H), 1.38−1.27 (m, 2H), 0.99 (d, J = 6.9 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.7, 172.3, 69.1, 41.7, 37.7, 24.3, 15.3, 11.6; IR (film, cm^{-1}) 3200 (br), 2957, 1766, 1697, 1471, 1363, 1291; HRMS (ESI-TOF) m/z calcd for $C_8H_{13}NO_2$ (M + H)$^+$ 156.1019; found 156.1018 (0.6 ppm).

General Procedure for the Syntheses of 4. To a solution of Cbz-protected pyrrolidin-3-ol (2.57 g, 11.6 mmol) in dry dichloromethane (15 mL) at −78 °C was added diisopropylethylamine (2.2 mL, 12.8 mmol, 1.1 equiv) dropwise. Freshly distilled (P_2O_5) triflic anyhydride (2.0 mL, 12.1 mmol, 1.05 equiv) was added using a syringe pump at rate of 4 mL/h ensuring that the bath temperature did not exceed −70 °C. The reaction mixture turned pink. On complete addition of triflic anyhydride, the reaction was stirred for 10 min. A solution of amino acid *tert*-butyl ester (17.4 mmol, 1.5 equiv) in dichloromethane (15 mL) was then added at a rate of 30 mL/h. The reaction was stirred for 10 min at −78 °C and allowed to warm to 25 °C. After 18 h, the reaction mixture was transferred to a separatory funnel and diluted with dichloromethane (125 mL). The organic layer was extracted with saturated sodium bicarbonate (2 × 150 mL) and brine (1 × 100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 1:9 ethyl acetate/dichloromethane; ceric ammonium molybdate stain and UV for visualization) to afford the product as a mixture of diastereomers. To a solution of the mixture in dry ether (70 mL) at 0 °C was added HCl in dioxane (4 M, 1.1 equiv) dropwise. Removal of the ether by filtration afforded a hydroscopic white solid. The solid was dissolved in dichloromethane (10 mL) and transferred to a 50 mL erlenmeyer flask. Hexanes (35 mL) was slowly added to form a second layer. The flask was placed at −20 °C for 12 h to afford a white, nonhydroscopic solid. The resulting solid was collected by filtration and to afford the required pure single diastereomer.

*(S)-Di-tert-butyl 2-(((S)-1-((benzyloxy)carbonyl)pyrrolidin-3-yl)-amino)pentanedioate (**4e′**):* white crystals, crystallized from hot MeCN (∼12 mL/g), 1.62 g after crystallization, 28%; mp = 171−172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.26 (m, 5H), 5.16−5.02 (m, 2H), 3.98−3.86 (m, 1H), 3.85−3.62 (m, 4H), 3.44−3.24 (m, 1H), 2.72−2.42 (m, 4H), 2.39−2.22 (m, 2H), 1.50 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 166.4, 154.4, 136.4, 128.5, 128.2, 128.09, 128.03, 127.9, 85.2, 81.1, 67.2 and 67.1, 59.2, 55.7 and 54.9, 47.5 and 47.1, 44.3 and 44.0, 30.7, 28.9, 28.1, 28.0, 25.4, 25.2. Note: Carbon spectra show more than the expected number of peaks due to restricted rotation about the NH−C═O bond. This was confirmed by hydrogenolysis of the product (see the Supporting Information); IR (film, cm^{-1}) 2978, 2628, 1714, 1697, 1417, 1153;

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HRMS (ESI-TOF) m/z calcd for $C_{25}H_{39}N_2O_6$ $(M + H)^+$ 463.2808, found 463.2817 (1.9 ppm)

*(S)-Benzyl 3-(((2S,3R)-3-(benzyloxy)-1-methoxy-1-oxobutan-2-yl)amino)pyrrolidine-1-carboxylate (**4t′**):* white crystals, crystallized from hot MeCN (~ 14 mL/g), 1.85 g after crystallization, 40%; mp = 164−166 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.21 (m, 10 H), 5.16−4.98 (m, 2H), 4.72−4.56 (m, 2H), 4.50−4.39 (m, 1H), 4.01−3.90 (m, 1H), 3.88−3.78 (m, 2H), 3.78 (m, 3H), 3.70−3.59 (m, 1H), 3.34−3.19 (m, 1H), 2.63−2.40 (m, 1H), 2.40−2.27 (m, 1H), 1.38 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 154.4, 137.3, 136.5, 128.51, 128.48, 128.28, 128.12, 128.05, 127.9, 73.2, 71.5, 67.0, 63.3, 57.3 and 56.7, 53.4, 48.1 and 47.6, 44.2 and 43.9, 28.5 and 27.7, 16.7; IR (film, cm^{-1}) 2953, 2886, 2636, 1747, 1213, 1123, 741; HRMS (ESI-TOF) m/z calcd for $C_{24}H_{31}N_2O_5$ $(M + H)^+$ 427.2233, found 427.2219 (3.3 ppm)

General Procedure for the Syntheses of 5. The recrystallized hydrochloride salt **4** (0.97 mmol) was suspended in dry THF (10 mL), and Bestmann's ylide (recrystallized from PhMe, 352 mg, 1.2 equiv) was added in one portion. The reaction mixture was heated to 75 °C under an argon atmosphere. After 30 min, a second portion of Bestmann's ylide (59 mg, 0.2 equiv) was added, and this process was repeated four additional times at 15 min intervals to complete the addition of 2.2 equiv of ylide. The reaction was monitored by NMR spectroscopy. After completion of reaction (~ 3 h), the solvent was evaporated. Upon cooling, the THF was removed in vacuo, and the residue was loaded onto a short SiO_2 column. Elution with 5% EtOAc/CH_2Cl_2 (to remove traces of unreacted starting material) followed by 50% % EtOAc/CH_2Cl_2 afforded a mixture of the cyclized product and triphenylphosphine oxide. The mixture was directly utilized in the next step.

General Procedure for the Hydrogenolysis of the *N*-Cbz Group. To a solution of the substrate in methanol (0.1 M) under nitrogen was added 10 wt % of Pd/C (0.1 equiv Pd). The reaction was placed under an atmosphere of hydrogen (1 atm, balloon) for 12 h and purged with N_2. The reaction mixture was filtered over a Celite pad and concentrated to afford the crude product. The crude product was purified by flash chromatography (SiO_2, 3% MeOH/CH_2Cl_2 → 3% MeOH/CH_2Cl_2 containing 1% Et_3N) to afford the product.

*tert-Butyl 3-((S)-3-tert-butoxy-5-oxo-1-((S)-pyrrolidin-3-yl)-2,5-dihydro-1H-pyrrol-2-yl)propanoate (**5e′**):* colorless oil, 225.4 mg, 64% over two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.23 (s, 1H), 4.22−4.10 (m, 1H), 3.95−3.89 (m, 1H), 3.63−3.51 (m, 3H), 3.50−3.36 (m, 1H), 2.50−2.29 (m, 1H), 2.12−1.94 (m, 5H), 1.44 (s, 9H), 1.41 (s, 9H) (Note: Complete removal of residual solvent (e.g., Et_3N, CH_2Cl_2) used in chromatography was not done to avoid epimerization. After characterization, the sample was immediately carried to the next step.); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 171.9, 171.1, 96.7, 83.1, 81.0, 61.8, 51.9, 49.5, 45.8, 45.2, 30.1, 28.1, 27.4, 23.4; IR (film, cm^{-1}) 2980, 2935, 2870, 1728, 1682, 1614, 1369, 1339, 1258, 1167, 844, 808; HRMS (ESI-TOF) m/z calcd for $C_{19}H_{33}N_2O_4$ $(M + H)^+$ 353.2440, found 353.2431 (2.6 ppm)

General Procedure for the Syntheses of 6. These compounds were prepared via the general procedure described previously.[49]

*(S)-4-tert-Butoxy-5-methyl-1-((S)-1-((S)-2-(2-(methylthio)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-1H-pyrrol-2(5H)-one (**6ma**):* pale yellow oil, 231.9 mg, 59%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.37 (br s, 1H), 5.00 (app s, 1H), 4.56 (d, J = 1.2 Hz, 1H), 4.37−4.25 (m, 2H), 3.94−3.82 (m, 1H), 3.64−3.52 (m, 1H), 3.53−3.38 (m, 2H), 3.38−3.30 (m, 1H), 2.57 (t, J = 7.3 Hz, 2H), 2.53−2.40 (m, 1H), 2.28−2.13 (m, 2H), 2.11 (s, 3H), 1.90−1.75 (m, 1H), 1.45 (s, 9H), 1.35 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 172.7, 172.1, 165.4, 95.5, 89.2, 81.9, 77.3, 57.9, 56.3, 51.7, 50.1, 47.6, 31.9, 29.9, 27.4, 18.0, 15.7; IR (film, cm^{-1}) 3214 (br), 2958, 2873, 1653, 1599, 1480, 1456, 1399, 1373, 1339, 1302, 1259, 1214, 1168, 1096, 880, 840, 781, 757; HRMS (ESI-TOF) m/z calcd for $(M + H)^+$ $C_{20}H_{32}N_3O_3S$ 394.2164, found 394.2175 (2.7 ppm).

*(S)-4-tert-Butoxy-5-((S)-sec-butyl)-1-((S)-1-((S)-2-isobutyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-1H-pyrrol-2(5H)-one (**6li**):* pale yellow solid, 115.1 mg, 55%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70 (br s, 1H), 4.99 (s, 1H), 4.52 (d, J = 1.5 Hz, 1H), 4.14 (d, J = 9.6 Hz, 1H), 4.07−3.88 (m, 1H), 3.86 (d, J = 2.7 Hz, 1H), 3.83−3.69 (m, 1H), 3.46−3.15 (m, 3H), 2.74−2.52 (m, 1H), 1.87−1.47 (m, 5H), 1.42 (s, 9H), 1.40−1.35 (m, 1H), 1.0−0.89 (m, 6H), 0.75 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.3, 170.4, 166.7, 97.4, 88.3, 82.0, 65.8, 55.7, 52.6, 49.6, 47.4, 42.2, 36.4, 27.4, 26.0, 25.9, 23.7, 21.4, 12.6, 12.4; HRMS (ESI-TOF) m/z calcd for $C_{24}H_{40}N_3O_3$ $(M + H)^+$ 418.3070, found 418.3083 (3.1 ppm).

*(S)-1-((S)-1-((R)-2-((R)-1-(Benzyloxy)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-4-tert-butoxy-5-methyl-1H-pyrrol-2(5H)-one (**6t′a**):* white solid, 212.6 mg, 67%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.19 (m, 5H), 6.29 (br s, 1H), 4.94 (s, 1H), 4.62−4.54 (m, 2H), 4.43 (d, J = 11.7 Hz, 1H), 4.39−4.26 (m, 1H), 4.24 (d, J = 1.5 Hz, 1H), 3.87−3.76 (m, 1H), 3.69−3.58 (m, 1H), 3.38−3.24 (m, 3H), 3.23−3.09 (m, 1H), 2.30−2.04 (m, 2H), 1.42 (s, 9H), 1.23 (d, J = 6.6 Hz, 3H), 1.17 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.7, 172.7, 172.0, 163.8, 138.1, 128.3, 127.8, 127.7, 95.2, 90.6, 81.8, 74.2, 71.1, 61.2, 57.3, 51.0, 50.9, 48.1, 29.0, 27.4, 18.2, 15.3; IR (film, cm^{-1}) 3250 (br), 2978, 2872, 1597, 1398, 1375, 1257, 1213, 1167, 1096, 735; MS (ESI-TOF) m/z calcd for $C_{26}H_{36}N_3O_4$ $(M + H)^+$ 454.27, found 454.26

*tert-Butyl 3-((S)-1-((S)-1-((S)-2-benzyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-3-tert-butoxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)propanoate (**6fe′**):* colorless oil that turned into a solid under vacuum, 134.0 mg, 64%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.13 (m, 5H), 5.23 (br s, 1H), 5.03 (s, 1H), 4.52 (s, 1H), 4.32 (dd, J = 9.9, 3.0 Hz, 1H), 0.3.04−4.14 (m, 1H), 4.04−3.95 (m, 1H), 3.82−3.66 (m, 1H), 3.65−3.53 (m, 1H), 3.52−3.30 (m, 2H), 3.25 (dd, J = 13.5, 3.0 Hz, 1H), 2.66−2.41 (m, 2H), 2.30−2.16 (m, 1H), 2.15−1.95 (m, 4H), 1.44 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 173.3, 172.3, 170.1, 165.5, 136.8, 129.1, 128.8, 128.6, 127.1, 97.0, 88.8, 82.3, 80.9, 60.7, 58.4, 52.1, 49.9, 47.7, 39.4, 28.1, 27.4, 24.8; IR (film, cm^{-1}) 3250 (br), 2978, 2931, 2872, 1724, 1667, 1601, 1395, 1371, 1339, 1371, 1339, 1258, 1165, 1151, 702; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{42}N_3O_5$ $(M + H)^+$ 524.3124, found 524.3115 (1.8 ppm).

*(S)-1-((S)-1-((S)-2-((1H-Indol-3-yl)methyl)-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-4-tert-butoxy-5-isobutyl-1H-pyrrol-2(5H)-one (**6wl**):* pale yellow solid, 127.4 mg, 52%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.79 (s, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 7.06 (t, J = 7.2 Hz, 1H), 6.95 (d, J = 1.5 Hz, 1H), 5.35 (br s, 1H), 5.00 (s, 1H), 4.56 (s, 1H), 4.38−4.28 (m, 1H), 4.26−4.12 (m, 1H), 3.94−3.76 (m, 2H), 3.76−3.55 (m, 2H), 3.49−3.31 (m, 2H), 2.69 (dd, J = 14.7, 9.6 Hz, 1H), 2.59−2.41 (m, 1H), 2.28−2.13 (m, 1H), 1.91−1.74 (m, 1H), 1.69−1.56 (m, 2H), 1.44 (s, 9H), 0.94 (d, J = 0.9 Hz, 3H), 0.92 (d, J = 0.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4, 173.4, 171.9, 166.2, 136.6, 126.9, 123.4, 121.9, 119.3, 118.0, 111.9, 110.4, 96.2, 88.9, 82.1, 61.4, 57.5, 52.4, 50.9, 47.8, 39.6, 30.2, 28.8, 27.4, 24.1, 24.0, 23.1; IR (film, cm^{-1}) 3414, 3246 (br), 2976, 2928, 2868, 1647, 1597, 1422, 1341, 1167, 908, 735; HRMS (ESI-TOF) m/z calcd for $C_{29}H_{39}N_4O_3$ $(M + H)^+$ 491.3022, found 491.3035 (2.6 ppm)

General Procedure for the One-Pot Syntheses of 6. To a solution of the tetramic acid substrate (1.3 mmol) and *N*-Cbz protected dimer (1.0 mmol) in ethanol (0.1 M) under nitrogen was added trimethylorthoformate (1.5 equiv) and 10 wt % of Pd/C (0.2 equiv Pd). The reaction was stirred under an atmosphere of H_2 (1 atm, balloon) for 12 h. The reaction was purged with N_2 for a few minutes and filtered over a pad of Celite. The filtrate was concentrated to obtain the crude product, which was purified by flash chromatography (4−5% MeOH/CH_2Cl_2) to afford the trimers as a white solid.

*(S)-4-tert-Butoxy-5-isobutyl-1-((S)-1-((S)-2-isobutyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-1H-pyrrol-2(5H)-one (**6ll**):* white solid, 157.7 mg, 54%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78 (br s, 1H), 4.97 (s, 1H), 4.51 (d, J = 1.5 Hz, 1H), 4.21−3.97 (m, 2H), 3.87 (dd, J = 6.3, 3.8 Hz, 1H), 3.79−3.64 (m, 1H), 3.46−3.33 (m, 2H), 3.32−3.14 (m, 1H), 2.65−2.43 (m, 1H), 2.23−2.06 (m, 2H), 1.87−1.67 (m, 2H), 1.67−1.53 (m, 3H), 1.42 (s, 9H), 0.99−0.83 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 173.3, 171.8, 166.8, 96.1, 88.3, 82.0, 61.3, 55.8, 52.5, 49.8, 47.4, 42.1, 39.7, 27.4, 25.8, 24.1, 23.9, 23.7, 23.0, 21.3; IR (film, cm^{-1}) 3211 (br), 2955, 1661, 1651, 1601,

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1472, 1371, 1341, 1268, 1167, 731; HRMS (ESI-TOF) m/z calcd for $C_{24}H_{40}N_3O_3$ $(M + H)^+$ 418.3070, found 418.3067 (0.6 ppm).

*(S)-4-tert-Butoxy-5-isobutyl-1-((S)-1-((S)-2-methyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-1H-pyrrol-2(5H)-one (**6al**):* white solid, 180.1 mg, 48%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.72 (br s, 1H), 4.97 (s, 1H), 4.48 (d, J = 1.2 Hz, 1H), 4.20 (q, J = 6.7 Hz, 1H), 4.14−3.99 (m, 1H), 3.87 (dd, J = 6.4, 3.8 Hz, 1H), 3.77−3.62 (m, 1H), 3.51−3.33 (m, 2H), 3.33−3.16 (m, 1H), 2.62−2.43 (m, 1H), 2.19−2.06 (m, 1H), 1.87−1.71 (m, 1H), 1.65−1.53 (m, 2H), 1.42 (s, 9H), 1.35 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.3, 171.9, 167.3, 96.1, 87.7, 82.0, 61.2, 52.9, 52.5, 49.8, 47.5, 39.7, 27.7, 27.4, 24.1, 23.9, 23.0, 19.0; IR (film, cm^{-1}) 3304 (br), 2976, 2871, 1653, 1601, 1397, 1339, 1258, 1167, 731; HRMS (ESI-TOF) m/z calcd for $C_{21}H_{34}N_3O_3$ $(M + H)^+$ 376.2600, found 376.2610 (2.6 ppm).

*(5S)-1-((3S)-1-(2-((R)-1-(Benzyloxy)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-4-tert-butoxy-5-isobutyl-1H-pyrrol-2(5H)-one (**6t′l**):* white solid, 131.2 mg, 53%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.21 (m, 5H), 5.78 (br s, 1H), 4.98 (s, 1H), 4.64−4.55 (m, 2H), 4.44 (d, J = 11.7 Hz, 1H), 4.21 (d, J = 1.5 Hz, 1H), 4.19−4.07 (m, 1H), 3.83−3.74 (m, 1H), 3.52−3.27 (m, 3H), 3.24−3.11 (m, 1H), 2.41−2.25 (m, 1H), 2.14−2.05 (m, 1H), 1.99−1.86 (m, 1H), 1.82−1.69 (m, 1H), 1.63−1.51 (m, 2H), 1.44 (s, 9H), 1.19 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.6, 173.3, 171.8, 163.8, 138.2, 128.3, 127.8, 127.6, 96.0, 90.3, 81.9, 73.8, 71.0, 61.3, 60.8, 51.9, 50.8, 48.1, 39.7, 28.1, 27.4, 24.0, 23.1, 15.7; IR (film, cm^{-1}) 3227 (br), 2976, 2868, 1599, 1339, 1256, 1167, 1098, 737; HRMS (ESI-TOF) m/z calcd for $C_{29}H_{42}N_3O_4$ $(M + H)^+$ 496.3175, found 496.3171 (0.9 ppm).

*tert-Butyl 3-((S)-3-tert-butoxy-1-((S)-1-((S)-2-isobutyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)propanoate (**6le′**):* white solid, 119.9 mg, 49%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.71 (br s, 1H), 5.02 (s, 1H), 4.51 (s, 1H), 4.29−4.10 (m, 2H), 4.01−3.94 (m, 1H), 3.67−3.54 (m, 1H), 3.48−3.32 (m, 2H), 3.32−3.19 (m, 1H), 2.57−2.37 (m, 2H), 2.26−1.96 (m, 5H), 1.80−1.67 (m, 1H), 1.66−1.53 (m, 1H), 1.43 (s, 9H), 1.41 (s, 9H), 0.95 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.2, 172.2, 170.0, 166.7, 97.0, 88.4, 82.3, 80.8, 60.7, 55.7, 52.0, 49.7, 47.4, 42.1, 28.1, 27.4, 25.8, 24.7, 23.7, 21.4; IR (film, cm^{-1}) 3123 (br), 2976, 2871, 1724, 1670, 1600, 1395, 1369, 1341, 1258, 1167, 922, 887, 847, 731; HRMS (ESI-TOF) m/z calcd for $C_{27}H_{44}N_3O_5$ $(M + H)^+$ 490.3281; found 490.3270 (2.2 ppm).

*(S)-5-((R)-1-(Benzyloxy)ethyl)-4-methoxy-1-((S)-1-((S)-2-methyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-1H-pyrrol-2(5H)-one (**9at′**):* white solid, 104.1 mg, 51%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.24 (m, 5H), 5.68 (br s, 1H), 5.04 (s, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.50−4.41 (m, 2H), 4.11−3.96 (m, 3H), 3.78 (s, 3H), 3.78−3.70 (m, 1H), 3.67−3.55 (m, 1H), 3.33−3.21 (m, 2H), 3.21−3.08 (m, 1H), 2.42−2.25 (m, 1H), 2.01−1.88 (m, 1H), 1.30 (d, J = 6.6 Hz, 3H), 1.18 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 175.0, 172.7, 167.2, 137.7, 128.6, 128.0, 127.7, 95.8, 87.7, 74.0, 71.1, 64.5, 58.3, 53.6, 52.8, 49.7, 47.3, 27.2, 19.0, 14.8; IR (film, cm^{-1}) 3260 (br), 2928, 2868, 1670, 1595, 1396, 1361, 1238, 1099, 995, 733, 696; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{30}N_3O_4$ $(M + H)^+$ 412.2236, found 412.2277 (3.7 ppm)

General Procedure for the Syntheses of 1. These compounds were prepared via the general procedure described previously.[49]

*(S)-1-((S)-1-((S)-2-Benzyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)-pyrrolidin-3-yl)-4-((S)-3-((S)-3-tert-butoxy-2-methyl-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)pyrrolidin-1-yl)-5-isobutyl-1H-pyrrol-2(5H)-one (**1fla**):* white solid, 99.7 mg, 54%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.14 (m, 5H), 5.11 (br s, 1H), 4.96 (s, 1H), 4.58 (s, 1H), 4.50 (s, 1H), 4.33−4.23 (m, 2H), 4.24−4.08 (m, 2H), 3.86 (q, J = 6.6 Hz, 1H), 3.63−3.20 (m, 8H), 2.66−2.38 (m, 4H), 2.26−2.08 (m, 2H), 1.77−1.59 (m, 3H), 1.42 (s, 9H), 1.32 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 174.2, 172.7, 172.1, 165.5, 163.5, 137.0, 129.1, 128.8, 127.1, 95.5, 90.6, 88.6, 82.0, 60.2, 58.5, 57.8, 52.9, 51.7, 50.3, 49.9, 47.6, 47.5, 39.4, 38.2, 27.4, 24.1, 23.9, 23.1, 18.0; HRMS (MALDI-TOF) m/z calcd for $C_{36}H_{50}N_5O_4$ $(M + H)^+$ 616.3857; found 616.3865 (1.3 ppm).

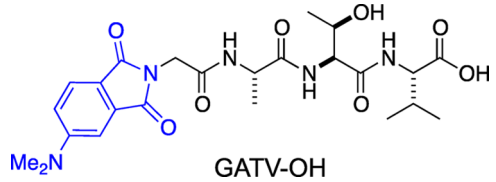
*tert-Butyl 3-((S)-1-((S)-1-((S)-2-benzyl-1-((S)-1-((S)-2-methyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-3-tert-butoxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)propanoate (**1afe′**):* pale yellow solid, 86.6 mg, 42%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25−7.01 (m, 5H), 5.72 (br s, 1H), 5.00 (s, 1H), 4.41 (s, 1H), 4.38 (s, 1H), 4.32 (t, J = 4.5 Hz, 1H), 4.25−4.16 (s, 1H), 4.15−4.05 (s, 1H), 4.01−3.82 (m, 2H), 3.71−3.42 (m, 3H), 3.42−3.17 (m, 4H), 3.17−3.05 (m, 2H), 2.91 (dd, J = 14.3, 5.3 Hz, 1H), 2.55−2.29 (m, 2H), 2.25−2.13 (m, 1H), 2.12−1.95 (m, 5H), 1.42 (s, 9H), 1.40 (s, 9H), 1.31 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 174.3, 173.3, 172.3, 170.1, 167.3, 163.5, 135.1, 129.3, 128.3, 127.1, 96.9, 91.1, 87.4, 82.4, 80.8, 61.2, 60.6, 53.4, 52.9, 52.0, 50.1, 50.0, 47.6, 47.4, 37.6, 28.1, 27.4, 24.7, 19.0; IR (film, cm^{-1}) 3123 (br), 2976, 2871, 1724, 1670, 1600, 1395, 1369, 1341, 1258, 1167, 922, 887, 847, 731; HRMS (MALDI-TOF) m/z calcd for $C_{39}H_{54}N_5O_6$ $(M + H)^+$ 688.4067; found 688.4045 (3.2 ppm).

General Procedure for the Deprotection of *tert*-Butyl Group. *(3′S,5S)-5-((S)-sec-Butyl)-1′-((S)-1-((S)-1-((S)-2-Isobutyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)pyrrolidin-3-yl)-2-methyl-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)-[1,3′-bipyrrolidine]-2,4-dione (**8lai**):* off-white solid, 84.2 mg, 80%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.01 (br s, 1H), 4.65−4.48 (m, 2H), 4.27−4.02 (m, 4H), 3.93 (d, J = 3.0 Hz, 1H), 3.77 (t, J = 9.5 Hz, 1H), 3.68−3.51 (m, 3H), 3.50−3.37 (m, 3H), 3.37−3.22 (m, 1H), 2.98 (s, 2H), 2.69−2.42 (m, 2H), 2.36−2.11 (m, 2H), 1.93−1.78 (m, 1H), 1.77−1.48 (m, 2H), 1.47−1.32 (m, 3H), 1.03−0.92 (m, 12 H), 0.89 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.1, 176.2, 173.2, 169.7, 167.1, 165.0, 89.7, 87.8, 71.4, 56.2, 55.9, 53.2, 52.2, 51.8, 50.1, 49.7, 47.4, 47.3, 43.3, 41.9, 37.5, 27.8, 25.8, 25.3, 23.7, 21.4, 18.6, 13.3, 12.1; HRMS (MALDI-TOF) m/z calcd for $C_{29}H_{44}N_5O_4$ $(M + H)^+$ 526.3393, found 526.3381 (2.3 ppm)

General Procedure for the pH Stability Assay. Procedure: Stock solutions of trimer **6t′l** (200 μM) in 100 mM acetate buffer pH 4.5 (containing less than 5% DMSO) and 100 mM phosphate buffer pH 7.4 (containing less than 5% DMSO) were prepared and stored at 25 °C. Samples (25 μL) were injected into a RP-HPLC system (see general methods) at regular intervals.

General Procedure for the Heat Stability Assay. Procedure: A stock solution of **1fla** in the protease assay buffer (pH 7.8) was prepared as described above and incubated at 55 °C. Samples (200 μL) were aliquoted, periodically diluted with 200 μL of MeCN and 600 μL of protease assay buffer II, and analyzed by RP-HPLC (see the General Experimental Methods).

Protease Assay. Procedure: Two buffers were used in the protease stability assay. Buffer I consisted of 50 mM Tris, 10 mM $CaCl_2$, pH 7.8 in 50% (v/v) glycerol. Buffer II consisted of 50 mM Tris, 10 mM $CaCl_2$, pH 7.8. Pronase (3.2 mg) from *Streptomyces griseus* (commercial supplier) was dissolved in buffer I (1 mL) to obtain a stock solution of 160 μM (assuming an average molecular weight of 20 kDa). Pentamer **1fla** or trimer **6ll** (2 mg) was dissolved in 50 μL of DMSO and diluted with 500 μL of buffer I and then buffer II to make up 2 mL of solution to obtain the sample stock solutions (1.6 mM **1fla**, 2.4 mM **6ll**). The stock solutions were filtered through a 0.2 μL membrane filter to remove particulate matter for HPLC analysis. To the respective sample stock solutions, 70 μL of Pronase stock solution was added and gently mixed to obtain the reaction mixture, which was incubated at 37 °C.



GATV-OH

The positive control was prepared was follows. Pronase stock solution (70 μM) was added to a solution of the *N*-labeled tetrapeptide GATV−OH (3.8 mM, 2 mL), which was prepared in a manner similar to that for the sample stock solution above. The reaction mixture was incubated at 37 °C.

Samples (200 μL) were aliquoted periodically, quenched with 200 μL of MeCN to halt the reaction, diluted with 600 μL of buffer II,

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and analyzed by RP-HPLC (see the General Experimental Methods). Under the described conditions, the control peptide completely decomposed in 2 h.

General Procedure for X-ray Structure Determination. A microscope was used to identify a suitable colorless multifaceted crystal with very well-defined faces with dimensions (max, intermediate, and min) 0.05 mm × 0.03 mm × 0.01 mm from a representative sample of crystals of the same habit. The crystal mounted on a nylon loop was then placed in a cold nitrogen stream maintained at 110 K.

A X-ray diffractometer was employed for crystal screening, unit cell determination, and data collection. The goniometer was controlled using the FRAMBO software suite.[50] The sample was optically centered with the aid of a video camera such that no translations were observed as the crystal was rotated through all positions. The detector was set at 6.0 cm from the crystal sample (MWPC Hi-Star Detector, 512 × 512 pixel). The X-ray radiation employed was generated from a Cu sealed X-ray tube (K_α = 1.54184 Å with a potential of 40 kV and a current of 40 mA) fitted with a graphite monochromator in the parallel mode (175 mm collimator with 0.5 mm monocapillary optics).

The rotation exposure indicated acceptable crystal quality and the unit cell determination was undertaken. 2100 data frames were taken at widths of 0.5° with an exposure time of 10 s. Over 6000 reflections were centered and their positions were determined. These reflections were used in the autoindexing procedure to determine the unit cell. A suitable cell was found and refined by nonlinear least-squares and Bravais lattice procedures and reported in Table 1. No supercell or erroneous reflections were observed.

After careful examination of the unit cell, a standard data collection procedure was initiated. This procedure consists of collection of one hemisphere of data collected using ω scans involving the collection 0.5° frames at fixed angles for ϕ, 2θ, and χ (2θ = −28°, χ = 54.73°, 2θ = −90°, χ = 54.73°), while varying ω. Addition data frames were collected to complete the data set. Each frame was exposed for 10 s. The total data collection was performed for duration of approximately 24 h at 110 K. No significant intensity fluctuations of equivalent reflections were observed.

Data Reduction, Structure Solution, and Refinement. Integrated intensity information for each reflection was obtained by reduction of the data frames with the program SAINT.[51] The integration method employed a three-dimensional profiling algorithm and all data were corrected for Lorentz and polarization factors, as well as for crystal decay effects. Finally the data was merged and scaled to produce a suitable data set. The absorption correction program SADABS[52] was employed to correct the data for absorption effects.

Systematic reflection conditions and statistical tests for the data suggested the space group $P2_1$. A solution was obtained readily using SHELXTL (SHELXS).[53] All non-hydrogen atoms were refined with anisotropic thermal parameters. The Hydrogen atoms bound to carbon were placed in idealized positions [C−H = 0.96 Å, U_{iso}(H) = 1.2U_{iso}(C)]. The structure was refined (weighted least-squares refinement on F^2) to convergence. X-seed was employed for the final data presentation and structure plots.[54]

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for all new compounds. X-ray crystallographic data (CIF) for compound **4i·HCl**. HPLC chromatograms for stability assays. Photophysical properties and quantum yields of tetramic acids. HPLC and NMR study of epimerization in the syntheses of **4**. All Qikprop predicted properties of compounds **1** and **6** and tripeptides.This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Yin, H.; Lee, G.; Sedey, K. A.; Kutzki, O.; Park, H. S.; Orner, B. P.; Ernst, J. T.; Wang, H.-G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 10191.
(2) Davis, J. M.; Truong, A.; Hamilton, A. D. *Org. Lett.* **2005**, *7*, 5405.
(3) Ernst, J. T.; Becerril, J.; Park, H.; Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 535.
(4) Davis, J. M.; Tsou, L. K.; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326.
(5) Rodriguez, J. M.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 8614.
(6) Ahn, J.-M.; Han, S.-Y. *Tetrahedron Lett.* **2007**, *48*, 3543.
(7) Shaginian, A.; Whitby, L. R.; Hong, S.; Hwang, I.; Farooqi, B.; Searcey, M.; Chen, J.; Vogt, P. K.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 5564.
(8) Moisan, L.; Odermatt, S.; Gombosuren, N.; Carella, A.; Rebek, J., Jr. *Eur. J. Org. Chem.* **2008**, 1673.
(9) Maity, P.; Koenig, B. *Org. Lett.* **2008**, *10*, 1473.
(10) Marimganti, S.; Cheemala, M. N.; Ahn, J.-M. *Org. Lett.* **2009**, *11*, 4418.
(11) Tosovska, P.; Arora, P. S. *Org. Lett.* **2010**, *12*, 1588.
(12) Lee, Ji, H.; Zhang, Q.; Jo, S.; Chai Sergio, C.; Oh, M.; Im, W.; Lu, H.; Lim, H.-S. *J. Am. Chem. Soc.* **2011**, *133*, 676.
(13) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B., III. *J. Am. Chem. Soc.* **1992**, *114*, 9217.
(14) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengeler, P. A.; Hamley, P.; Smith, A. B., III; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550.
(15) Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Amos B. Smith, I. *J. Am. Chem. Soc.* **1992**, *114*, 9699.
(16) Mowery, B. P.; Prasad, V.; Kenesky, C. S.; Angeles, A. R.; Taylor, L. L.; Feng, J.-J.; Chen, W.-L.; Lin, A.; Cheng, F.-C.; Smith, A. B., III; Hirschmann, R. *Org. Lett.* **2006**, *8*, 4397.
(17) Smith, A. B., III; Charnley, A. K.; Hirschmann, R. *Acc. Chem. Res.* **2011**, *44*, 180.
(18) Hammond, M. C.; Bartlett, P. A. *J. Org. Chem.* **2007**, *72*, 3104.
(19) Hammond, M. C.; Harris, B. Z.; Lim, W. A.; Bartlett, P. A. *Chem. Biol.* **2006**, *13*, 1247.
(20) Raghuraman, A.; Ko, E.; Burgess, K. *J. Am. Chem. Soc.* **2011**, *133*, 12350.
(21) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
(22) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
(23) Ko, E.; Perez, L. M.; Ioerger, T. R.; Burgess, K. *Chem. Commun.* **2013**, submitted.
(24) Jouin, P.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177.
(25) Courcambeck, J.; Bihel, F.; De Michelis, C.; Quelever, G.; Kraus, J. L. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1421.
(26) Ma, D.; Ma, J.; Ding, W.; Dai, L. *Tetrahedron Asymmetry* **1996**, *7*, 2365.
(27) Hosseini, M.; Kringelum, H.; Murray, A.; Tonder, J. E. *Org. Lett.* **2006**, *8*, 2103.

(28) Bestmann, H. J. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 349.
(29) Schobert, R.; Dietrich, M.; Mullen, G.; Urbina-Gonzalez, J.-M. *Synthesis* **2006**, 3902.
(30) Schobert, R.; Boeckman, R. K., Jr.; Pero, J. E. *Org. Synth.* **2005**, *82*, 140.
(31) Schobert, R.; Jagusch, C. *Tetrahedron* **2005**, *61*, 2301.
(32) Schobert, R.; Schlenk, A. *Bioorg. Med. Chem.* **2008**, *16*, 4203.
(33) Schobert, R.; Jagusch, C.; Melanophy, C.; Mullen, G. *Org. Biomol. Chem.* **2004**, *2*, 3524.
(34) Houghton, P. G.; Humphrey, G. R.; Kennedy, D. J.; Roberts, D. C.; Wright, S. H. B. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1421.
(35) Corruble, A.; Davoust, D.; Desjardins, S.; Fressigne, C.; Giessner-Prettre, C.; Harrison-Marchand, A.; Houte, H.; Lasne, M.-C.; Maddaluno, J.; Oulyadi, H.; Valnot, J.-Y. *J. Am. Chem. Soc.* **2002**, *124*, 15267.
(36) Sanchez, J. P.; Domagala, J. M.; Heifetz, C. L.; Priebe, S. R.; Sesnie, J. A.; Trehan, A. K. *J. Med. Chem.* **1992**, *35*, 1764.
(37) Zhang, H.; Mitsumori, S.; Utsumi, N.; Imai, M.; Garcia-Delgado, N.; Mifsud, M.; Albertshofer, K.; Cheong, P. H.-Y.; Houk, K. N.; Tanaka, F.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2008**, *130*, 875.
(38) Bell, I. M.; Gallicchio, S. N.; Abrams, M.; Beshore, D. C.; Buser, C. A.; Culberson, J. C.; Davide, J.; Ellis-Hutchings, M.; Fernandes, C.; Gibbs, J. B.; Graham, S. L.; Hartman, G. D.; Heimbrook, D. C.; Homnick, C. F.; Huff, J. R.; Kassahun, K.; Koblan, K. S.; Kohl, N. E.; Lobell, R. B.; Lynch, J. J.; Miller, P. A.; Omer, C. A.; Rodrigues, A. D.; Walsh, E. S.; Williams, T. M. *J. Med. Chem.* **2001**, *44*, 2933.
(39) Hoehne, M.; Robins, K.; Bornscheuer, U. T. *Adv. Synth. Catal.* **2008**, *350*, 807.
(40) Rejman, D.; Kovackova, S.; Pohl, R.; Dracinsky, M.; Fiedler, P.; Rosenberg, I. *Tetrahedron* **2009**, *65*, 8513.
(41) Pouliquen, M.; Blanchet, J.; De Paolis, M.; Rema Devi, B.; Rouden, J.; Lasne, M.-C.; Maddaluno, J. *Tetrahedron: Asymmetry* **2010**, *21*, 1511.
(42) Tice, C. M.; Zhao, W.; Krosky, P. M.; Kruk, B. A.; Berbaum, J.; Johnson, J. A.; Bukhtiyarov, Y.; Panemangalore, R.; Scott, B. B.; Zhao, Y.; Bruno, J. G.; Howard, L.; Togias, J.; Ye, Y.-J.; Singh, S. B.; McKeever, B. M.; Lindblom, P. R.; Guo, J.; Guo, R.; Nar, H.; Schuler-Metz, A.; Gregg, R. E.; Leftheris, K.; Harrison, R. K.; McGeehan, G. M.; Zhuang, L.; Claremon, D. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6725.
(43) Macdonald, S. J. F.; Clarke, G. D. E.; Dowle, M. D.; Harrison, L. A.; Hodgson, S. T.; Inglis, G. G. A.; Johnson, M. R.; Shah, P.; Upton, R. J.; Walls, S. B. *J. Org. Chem.* **1999**, *64*, 5166.
(44) Phillips, S. T.; Rezac, M.; Abel, U.; Kossenjans, M.; Bartlett, P. A. *J. Am. Chem. Soc.* **2002**, *124*, 58.
(45) Hosseini, M.; Grau Jakob, S.; Sorensen Kasper, K.; Sotofte, I.; Tanner, D.; Murray, A.; Tonder Janne, E. *Org. Biomol. Chem.* **2007**, *5*, 2207.
(46) Jurasek, L.; Johnson, P.; Olafson, R. W.; Smillie, L. B. *Can. J. Biochem.* **1971**, *49*, 1195.
(47) Jorgensen, W. L.; Duffy, E. M. *Bioorg. Med. Chem. Let* **2000**, *10*, 1155.
(48) Duffy, E. M.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2000**, *122*, 2878.
(49) Raghuraman, A.; Ko, E.; Perez, L. M.; Ioerger, T. R.; Burgess, K. *J. Am. Chem. Soc.* **2011**, *133*, 12350.
(50) *APEX2 "Program for Data Collection on Area Detectors"*, BRUKER AXS Inc., 5465 East Cheryl Parkway, Madison, WI.
(51) *SAINT, "Program for Data Reduction from Area Detectors"*, BRUKER AXS Inc., 5465 East Cheryl Parkway, Madison, WI.
(52) SADABS: Sheldrick, G. M. *"Program for Absorption Correction of Area Detector Frames"*, BRUKER AXS Inc., 5465 East Cheryl Parkway, Madison, WI.
(53) SHELXTL: Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112.
(54) X-Seed - A software tool for supramolecular crystallography. Barbour, L. J. *J. Supramol. Chem.* **2001**, *1*, 189.