Pyrrolidinone-modified di- and tripeptides: highly diastereoselective preparation and investigation of their stability†

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Pyrrolidine-2,4-diones have been identified as a novel starting point for the synthesis of peptide analogues. This paper describes a method for the efficient and diastereoselective incorporation of this moiety into peptide chains to furnish di- and tripeptide analogs. The stability of these pyrrolidinone modified di- and tripeptides was found to be markedly improved when compared to that of a natural peptide. In addition, solid phase peptide synthesis employing a pyrrolidinone containing tripeptide is demonstrated.

Introduction

Peptides are very attractive as drug candidates, due to their selectivity for specific targets and high efficacy as compared to traditional small molecule based drugs, and there is also less concern with the formation of toxic metabolites. However, there are some limitations associated with peptide drugs, the greatest problem being very low bioavailability upon oral administration.[1,2]

The main cause of this limitation is pre-systemic enzymatic degradation and, moreover, the gastrointestinal tract provides an additional barrier. If a peptide drug manages to pass these barriers, enzymatic digestion in the blood stream will occur, resulting in a very low half-life. To overcome these obstacles one must first identify the problems associated with degradation,[3] and in this respect many research groups have developed a range of pseudopeptides to aid drug development.[4]

In our group, we have focused our strategy on site-specific modifications of amino acids in peptides with pyrrolidine-2,4-dione derivatives, a motif which is found in several biologically significant natural products[5] such as dolastatin 15, which contains the *py*Phe-OMe moiety,[6] and mirabimide E, containing a *py*Ala-OMe unit (Fig. 1).[7]

The 5-alkyl-pyrrolidine-2,4-diones (**1**) have an amide group instead of the amine functionality of amino acids, and an acidic OH resembling the carboxylic acid of the natural amino acid, hence the name tetramic acids. The side-chain of the amino acid is retained by this modification, and likewise the chirality is preserved. The rigid 5-membered ring system furthermore gives higher levels of conformational restriction, which can be advantageous in terms of target selectivity. By these site-specific alterations, we believe that we can regulate the enzymatic degradation of peptide drugs, while maintaining the activity which is inherent to the side chain of amino acids. When introducing a pyrrolidinone as replacement for an amino acid, the backbone of the peptide will be altered: the natural amide bond in the peptide chain will be substituted by an imide bond at the nitrogen, and to an amine or alkoxy at the 4-carbonyl of the pyrrolidinone. This will have an effect on the recognition pattern in enzymatic degradation processes as well as the receptor interaction pattern. The optimal scenario is naturally one where the enzymatic degradation is disturbed while the interaction with the receptor target is either unaffected or increased.

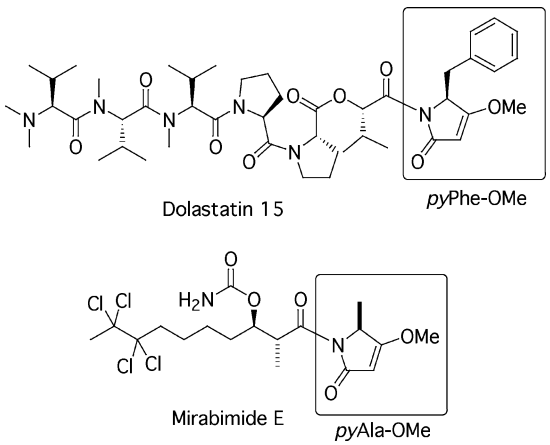

Fig. 1 Natural products containing pyrrolidinones.

To reach these goals we firstly focused on developing the chemistry, which will enable us to introduce diastereo- and chemoselectively a pyrrolidine-2,4-dione motif in a peptide chain. Our strategy is the use of *N*-acylation to funtionalize the amide nitrogen of the pyrrolidine-2,4-dione[8] and a reductive amination process for functionalization at the 4-carbonyl.[9]

This method has enabled us to functionalize pyrrolidinones with amino acids at nitrogen, giving structures of the type H-AA-*py*AA-OH, at an intermediate position of a small peptide, giving structures of the type H-AA-*py*AA-AA-OH, and at the 4-carbonyl of pyrrolidine-2,4-diones, giving structures of the type *py*AA-AA-OH.

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Results and discussion

The synthesis of 5-alkylpyrrolidine-2,4-diones has previously been described in the literature. The primary routes take advantage of amino acids as chiral precursors and ring formation by Dieckmann cyclization, or condensation with Meldrum's acid followed by decarboxylative cyclization.[10]

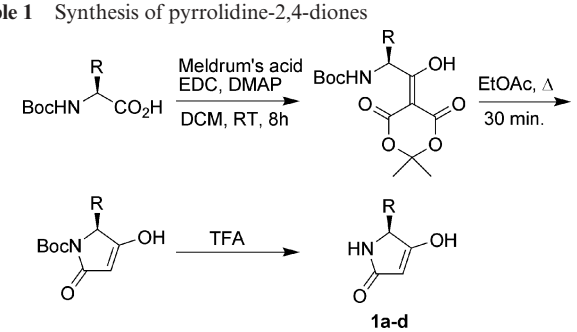
As our strategy requires enantiopure pyrrolidine-2,4-diones, we did not attempt to use the Dieckmann procedure, which previously has been reported to afford epimerization.[11] The procedure using Meldrum's acid was therefore explored, and we found that it furnished enantiopure products, but the final purification was problematic. However, by substituting DCC with EDC as activating agent and using a modified extraction procedure, we could obtain pure gram-scale product in high yield and excellent enantiopurity, without the need for column chromatography (Table 1).

With this convenient procedure at hand, we were ready to incorporate the products into peptides. As these pyrrolidinones possess a weakly acidic α-proton ($pK_a \sim 6.4$),[12] protection for the 5-oxygen was required to allow selective *N*-acylation. We explored different protective groups (TIPS, TPS, TBS, Ts, PMB, SEM, Et, Me) and found that the subsequent acylation was possible in high yield only when simple alkyl groups were used to protect the oxygen (Table 2). Furthermore, we found that the deprotection (10% HBr) of these ethyl enol ethers (*py*AA-OEt) was faster than the naturally occurring methyl enol ethers (*py*AA-OMe), and therefore settled on the ethyl group.

As previous reported routes to *py*AA-OAlk were rather tedious and resulted in low yield,[13] we decided to optimize the reaction with regard to different bases and electrophiles. Conversion of *py*AA-OH to *py*AA-OEt was best performed using KHMDS/18-crown-6 as base and EtOTs as the hard electrophile, ensuring selective *O*-alkylation.

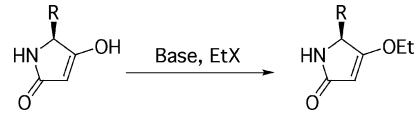
We furthermore found that the use of lithium bases (LiHMDS and *n*-BuLi) with the aid of 12-crown-4 did not provide noticeable amounts of product, which presumably is a result of the insolubility of the anion, while alkyl halides (EtI, EtBr) did not provide any product, which can be ascribed to their soft nature. Among the potassium bases, KHMDS afforded yields in the range of 74–87%, with no racemization of the final product. This was

Table 1 Synthesis of pyrrolidine-2,4-diones



Entry	Compound		R	Isolated yield (%)
1	*py*Ala	(**1a**)	Me	92
2	*py*Val	(**1b**)	*i*-Pr	92
3	*py*Leu	(**1c**)	*i*-Bu	93
4	*py*Phe	(**1d**)	Bn	91

Table 2 Synthesis of 4-ethoxy-3-pyrrolin-2-ones



1a-d 2a-d

Entry	X	R		Base	Solvent	Isolated yield (%)
1	I	Bn	(**2a**)	KO*t*-Bu	DMF	—
2	Br	Bn	(**2a**)	KO*t*-Bu	DMF	—
3	OTs	Bn	(**2a**)	KO*t*-Bu	DMF	26
4	OTs	Bn	(**2a**)	KO*t*-Bu	THF	—
5[a]	OTs	Bn	(**2a**)	KO*t*-Bu	THF	62
6[a]	OTs	Bn	(**2a**)	K_2CO_3	THF	92[b]
7[a]	OTs	Bn	(**2a**)	BuLi	THF	—
8[a]	OTs	Bn	(**2a**)	LHMDS	THF	—
9[a]	OTs	Bn	(**2a**)	KHMDS	THF	84
10[a]	OTs	Me	(**2b**)	KHMDS	THF	87
11[a]	OTs	*i*-Pr	(**2c**)	KHMDS	THF	84
12[a]	OTs	*i*-Bn	(**2d**)	KHMDS	THF	74

[a] Crown ether added. [b] 58% ee.

superior to both KO*t*-Bu in terms of yield and to K_2CO_3 in terms of enantiopurity (Table 2). With this procedure in hand, we now had access to stable protected 4-ethoxy-3-pyrrolin-2-ones (**2a–d**) in high yield without loss of enantiopurity.

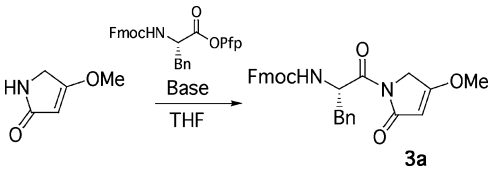
To further functionalize these compounds **2a–d** at nitrogen, we were reluctant to use strong base, as deprotonation at C-5 and formation of an aromatic intermediate leading to racemization of the product was a concern. Traditional peptide coupling reagents, which provide milder reaction conditions, have previously been shown not to facilitate the reaction, as the nucleophilicity of the amide nitrogen is relatively poor,[14c] and even at elevated temperature (60 °C) no product was detected in our studies employing peptide coupling reagents (EDC, HOBt, TEA). We therefore had no choice but to use strong bases as previously reported for similar compounds,[7,14] while paying close attention to the problem of racemization.[15]

To investigate the dependence of racemization on temperature, we conducted a series of experiments on *py*Phe-OEt, measuring the extent of racemization by chiral HPLC. Performing a deprotonation/quench sequence below −45 °C gave no trace of racemization, while this process was quite fast at higher temperatures.[8]

For further coupling, we were interested in developing a route that could be used both in solution and on solid support. Therefore, a procedure that would be compatible with both Boc and Fmoc chemistry was necessary. We were naturally concerned with the use of base in the presence of the base-labile Fmoc protecting group; however, initial experiments and a previous report[5h] had shown that the acylation of the activated amino acids was much faster than the undesired deprotection of the Fmoc group. In initial experiments, we used the commercially available *py*Gly-OMe and Fmoc-Phe-OPfp to optimize the acylation conditions to form the dipeptide analogue (**3a**).

We explored different bases and found that lithium bases were superior to potassium bases in terms of reactivity, BuLi providing the highest yield. However, the highest yield in this series (Table 3, entries 1–8) was not satisfactory. We therefore warmed the reaction mixture to a maximum of −45 °C, which we previously found to

Table 3 Optimization of acylation conditions



Entry	Base[a]	Temp./°C	Isolated yield (%)
1[b]	KO*t*-Bu	−78	8
2[c]	KO*t*-Bu	−78	14
3[b]	KO*t*-Bu	−78	14
4[b]	LiHMDS	−78	62
5[b]	BuLi	−78	69
6[b]	*i*-PrMgCl	−78 → 22	65
7[b]	BuLi	−78	76
8[c]	BuLi	−78	72
9[b]	**BuLi**	**−55 → −45**	**91**

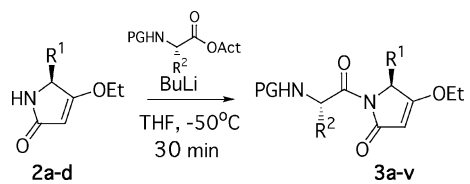
[a] 1.0 eq. base used. [b] 1.0 eq. nucleophile used. [c] 2.0 eq. nucleophile used.

be the maximum temperature before racemization was observed, and found that at these temperatures the yields were now quite satisfactory (Table 3, entry 9).

With this procedure in hand, we synthesized a range of dipeptide analogues (**3a–u**) with *py*AA-OEt at the C-terminus. Both Boc- and Fmoc-protected activated amino acids gave high yields, while no epimerization was observed by ^{1}H NMR (Table 4).

Deprotection of these dipeptide analogues to enable further synthesis was accomplished with either TFA or DBU (depending

Table 4 Synthesis of dipeptide analogues



Compound[a]		Isolated yield (%)[b]
Fmoc-Phe-***py*Gly**-OMe	(**3a**)	91
Fmoc-Val-***py*Ala**–OEt	(**3b**)	92
Fmoc-Leu-***py*Ala**-OEt	(**3c**)	88
Fmoc-Phe-***py*Ala**-OEt	(**3d**)	85
Fmoc-Ala-***py*Val**-OEt	(**3e**)	89
Fmoc-Val-***py*Val**-OEt	(**3f**)	76
Fmoc-Leu-***py*Val**-OEt	(**3g**)	78
Fmoc-Phe-***py*Val**-OEt	(**3h**)	78
Fmoc-Ala-***py*Leu**-OEt	(**3i**)	84
Fmoc-Val-***py*Leu**-OEt	(**3j**)	81
Fmoc-Phe-***py*Leu**-OEt	(**3k**)	80
Fmoc-Ala-***py*Phe**-OEt	(**3l**)	84
Fmoc-Val-***py*Phe**-OEt	(**3m**)	88
Fmoc-Leu-***py*Phe**-OEt	(**3n**)	85
Boc-Ala-***py*Val**-OEt	(**3o**)	85
Boc-Val-***py*Val**-OEt	(**3p**)	86
Boc-Leu-***py*Val**-OEt	(**3q**)	80
Boc-Phe-***py*Val**-OEt	(**3r**)	94
Boc-Ala-***py*Phe**-OEt	(**3s**)	85
Boc-Val-***py*Phe**-OEt	(**3t**)	89
Boc-Leu-***py*Phe**-OEt	(**3u**)	88
Boc-Phe-***py*Phe**-OEt	(**3v**)	92

[a] Fmoc-AA-OPfp and Boc-AA-ONp were used as electrophiles. [b] Only one diastereomer was observed by ^{1}H NMR (de > 95%).

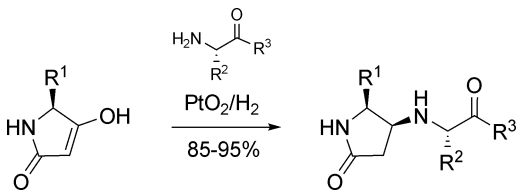
Table 5 Deprotection of dipeptide analogues[a]

Product		Protecting group	Isolated yield (%)
H-Val-*py*Val-OEt	(**4a**)	Fmoc	96
		Boc	97
H-Leu-*py*Val-OEt	(**4b**)	Fmoc	97
		Boc	98
H-Phe-*py*Val-OEt	(**4c**)	Fmoc	95
		Boc	99

[a] DBU and TFA were used for deprotection of Fmoc- and Boc-protected compounds, respectively.

on the protecting group) and provided the free-amine dipeptide analogues (**4a–c**, Table 5) in high yield and with no trace of epimerization. Use of standard Fmoc deprotection conditions (20% piperidine/DMF) caused significant difficulty in purification, while treating the substrate with DBU for prolonged times (>30 min) resulted in polymeric byproducts.

As we recently[9] developed a method to access dipeptide analogues by means of reductive amination of *py*AA-OH at C-4 with protected amino acids (Scheme 1), we explored the opportunity to elongate these further to tripeptide analogues.



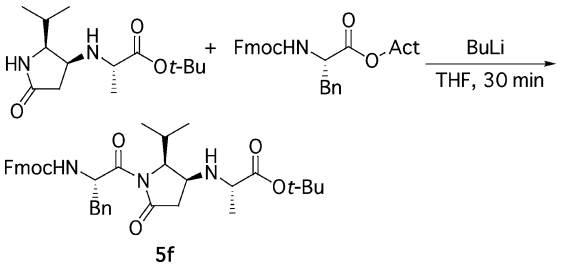
Scheme 1 Reductive amination of dipeptide analogues.

Our starting point was the use of our optimized acylation procedure, which had provided high yields in the synthesis of the dipeptide analogues described above (Table 4). Initial experiments revealed that the reaction was very sensitive to temperature. Interestingly, reactions at lower temperature provided higher yield, whereas for high temperature reactions several byproducts were formed. We therefore increased the amount of nucleophile (which was apparently unstable) to 2 eq., and lowered the temperature to −100 °C, which provided the highest yield in this series (Table 6).

However, we were not satisfied with the use of −100 °C and 2 eq. of the non-commercially available nucleophile, as this can be quite tedious in routine parallel synthesis and even more in scale-up processes. We therefore attempted to optimize the reaction with the use of other bases.

Potassium bases (KHMDS), as previously observed (Table 4), led to a low degree of conversion; however, utilizing a sterically hindered Grignard reagent as base furnished a much more stable nucleophile, which even at room temperature led to very clean product formation with no epimerization. Furthermore, only 1.0 eq. of the nucleophile was now required as a result of the stability of the anion.

Table 6 Optimization of tripeptide synthesis



5f

Entry	Eq. nucleophile	Temp./°C	Isolated yield (%)[a]
1	1.0	−15	28
2	1.0	−25	33
3	1.0	−35	37
4	1.0	−45	36
5	1.0	−55	32
6	1.0	−78	41
7	2.0	−78	63
8	**2.0**	**−100**	**76**

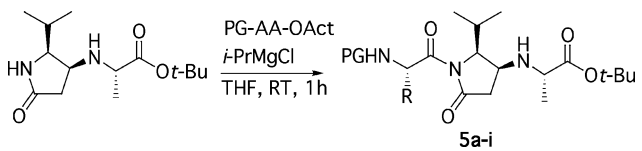
[a] Only one diastereomer was observed by ^{1}H NMR (d.e > 95%).

We then examined the scope of this reaction, and synthesized a range of tripeptide analogues with different amino acids protected as Boc, Fmoc and Cbz derivatives (**5a–i**, Table 7).

Throughout the series products were diastereoisomerically pure as determined by ^{1}H NMR and isolated in good yield. Hence, this method provides a very convenient synthetic route to libraries of tripeptide analogues. When pyrrolidinones with functionalized side chains are to be employed, appropriate protecting groups must be chosen. Thus, for O-protection, *t*-Bu is preferred to the Bn group, since Bn groups can be readily cleaved during PtO_2-mediated reductive amination. For N-protection, a double protection scheme should be employed to avoid NH deprotonation in the acylation step.

To evaluate the proteolytic stability of the new peptide analogues, we conducted a series of peptide stability tests in 50% pooled human plasma (Fig. 2).[16] Evaluating H-Val-*py*Ala-OEt and the corresponding natural peptide H-Val-Ala-OMe showed clear differences in their degradation. After the chosen reaction time of 200 min, only 20% of the natural peptide was intact, while 53% of the pyrrolidinone-modified peptide was intact. From LCMS analysis, we could not determine the degradation products of the natural peptide, while H-Val-*py*Ala-OEt was clearly cleaved at the imide bond.

Table 7 Synthesis of tripeptide analogues



5a-i

Entry[a]	PG	R		Isolated yield (%)[b]
1	Boc	*i*-Pr	(**5a**)	78
2	Boc	*i*-Bu	(**5b**)	90
3	Boc	Bn	(**5c**)	83
4	Fmoc	*i*-Pr	(**5d**)	83
5	Fmoc	*i*-Bu	(**5e**)	85
6	Fmoc	Bn	(**5f**)	88
7	Cbz	*i*-Pr	(**5g**)	82
8	Cbz	*i*-Bu	(**5h**)	83
9	Cbz	Bn	(**5i**)	89

[a] Fmoc-AA-OPfp, Boc-AA-ONp and Cbz-AA-ONp were used as electrophiles. [b] Only one diastereomer was observed by ^{1}H NMR (d.e > 95%).

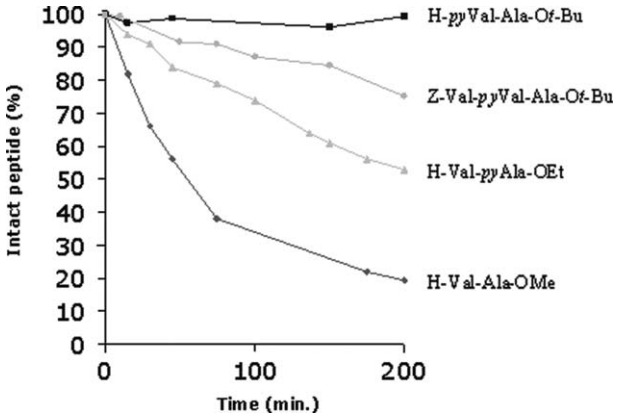


Fig. 2 Degradation curves of selected peptide analogues.‡

We likewise examined *py*Val-Ala-O*t*-Bu, for which no degradation could be observed, indicating the amine bond in this system was very stable. The proteolytic stability of the tripeptide Z-Val-*py*Val-Ala-O*t*-Bu (**5a**) was found to be even more stable than the dipeptide H-Val-*py*Ala-OEt (**4a**), with 76% of **5a** remaining in solution at the end of the 200 min experiment.

To evaluate the compatibility of **5** with solid phase peptide synthesis, **5d** was deprotected and the resulting **6a** was attached to a 2-ClTrt resin preloaded with H-Ser(*t*-Bu)-OH. Deprotection of the amine group and attachment of a phenylalanine residue, followed by cleavage of the peptide from the resin, provided a pentapeptide (**7a**) containing a pyrrolidinone moiety (Scheme 2).

Conclusion

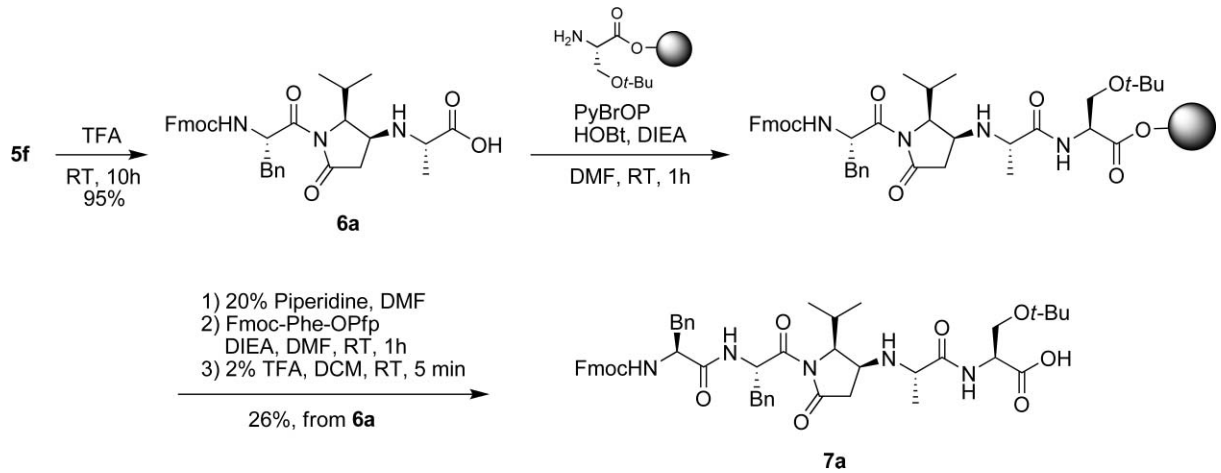
In conclusion, we have developed a methodology for the incorporation of pyrrolidine-diones into peptide chains either at the C- or N-terminus or in an intermediate position in good to excellent yield and with excellent enantio- and diastereoselectivity. The pyrrolidinone-containing tripeptides were shown to be applicable in solid phase peptide synthesis, allowing parallel synthesis and possible identification of biologically active compounds for therapeutic and diagnostic purposes. In support of this, we have examined the proteolytic stability of these, showing markedly increased stability towards human plasma.

Experimental

General methods and materials

NMR spectra were recorded on 400 MHz or 300 MHz Bruker instruments. Chemical shifts are reported in ppm (parts per million) using the solvent residual peak as the internal reference. NMR

‡ A peptide concentration of 0.5 mg mL^{-1} in 50% human plasma was used. Solutions were stirred at 37 °C for 200 min and analyzed by HPLC at appropriate intervals.



Scheme 2 Solid phase peptide synthesis of a pyrrolidinone-containing peptide (polystyrene resin with a 2-ClTrt linker was used as the solid support).

abbreviations are as follows: quin = quintet, sext = sextuplet, sept = septuplet, dsept = double septuplet, oct = octuplet. TLC was performed on aluminium sheets coated with silica gel 60 F_{254}, and visualizing was effected with UV-light and/or 1% ninhydrin in n-BuOH–AcOH. *py*AA-OH (**1a–d**), *py*AA-OEt (**2a–d**) and dipeptides (**3a–n**) were prepared as previously described.[8] Synthesis of *py*Val-Ala-O*t*-Bu using reductive amination was performed as previously described.[9]

Boc-Ala-*py*Val-OEt (3o)

To a solution of *py*Val-OEt (35 mg, 0.21 mmol) in THF (3 mL) was added BuLi (1.6 M, 0.13 mL, 0.21 mmol) and the mixture was stirred for 10 min whereafter Boc-Phe-ONp (71 mg, 0.23 mmol) in THF (2 mL) was added dropwise over 15 min. The mixture was allowed to stir for an additional 10 min and quenched with AcOH (0.1 mL) and evaporated on silica. Column chromatography over silica gel (EtOAc/Hep 1:3) provided the pure product (60 mg, 0.18 mmol, 85%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.21–5.43 (m, 2H), 5.03 (s, 1H), 4.49 (d, $J = 2.6$ Hz, 1H), 3.95–4.10 (m, 2H), 2.46–2.65 (m, 3H), 1.36–1.49 (m, 13H), 1.32 (d, $J = 6.4$ Hz, 3H), 1.10 (d, $J = 7.2$ Hz, 3H), 0.76 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.1, 15.4, 18.2, 18.8, 28.3, 28.5, 50.1, 64.2, 67.8, 79.4, 94.5, 155.0, 170.1, 173.4, 178.7. $[\alpha]_D^{20}$ +5 (*c* 0.63, MeOH). IR (KBr): 3440(s), 2955(m), 1729(s), 1832(s), 1180(m). HRMS (EI$^+$): Calcd. for $C_{17}H_{28}N_2O_5$ [M + H$^+$] 341.2079. Found 341.2079.

Fmoc-Val-*py*Val-OEt (3f)

Following the procedure for **3o** (76%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.70 (d, $J = 7.6$ Hz, 2H), 7.56 (d, $J = 7.6$ Hz, 2H), 7.34 (t, $J = 7.3$ Hz, 2H), 7.26 (t, $J = 7.6$ Hz, 2H), 3.81–5.67 (m, 11H), 1.91–3.29 (m, 4H), 1.30–1.67 (m, 6H), 0.35–1.11 (m, 16H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.63, 14.06, 14.12, 15.11, 16.28, 17.55, 18.90, 19.08, 19.99, 22.67, 23.02, 26.76, 28.51, 29.29, 29.33, 29.54, 29.57, 29.61, 29.63, 30.00, 30.39, 30.56, 31.37, 31.89, 41.00, 47.18, 47.22, 58.73, 64.36, 65.19, 66.93, 67.17, 67.81, 94.59, 119.93, 125.20, 127.04, 127.62, 127.69, 141.27, 143.78, 143.93, 144.03, 156.23, 156.80, 170.19, 171.63, 178.76. $[\alpha]_D^{20}$ +24 (*c* 0.22, MeOH). IR (KBr): 3435(s), 2927(m), 1727(s), 1619(s), 1240(m). HRMS (EI$^+$): Calcd. for $C_{29}H_{34}N_2O_5$ [M + H$^+$] 491.2546. Found 491.2564.

Boc-Val-*py*Val-OEt (3p)

Following the procedure for **3o** (86%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.43 (dd, $J = 9.6$, 4.0 Hz, 1H), 5.26 (d, $J = 9.1$ Hz, 1H), 5.04 (s, 1H), 4.49 (d, $J = 3.0$ Hz, 1H), 3.97–4.10 (m, 2H), 2.84–3.10 (m, 1H), 2.55–2.69 (m, 1H), 1.97–2.15 (m, 1H), 1.36–1.49 (m, 12H), 1.12 (d, $J = 7.1$ Hz, 3H), 1.02 (d, $J = 7.1$ Hz, 3H), 0.84 (d, $J = 7.1$ Hz, 3H), 0.76 (d, $J = 7.1$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 15.2, 16.4, 18.9, 19.9, 28.3, 28.5, 30.4, 58.2, 64.3, 67.8, 79.4, 94.6, 155.8, 170.2, 172.3, 178.7. $[\alpha]_D^{20}$ +46 (*c* 0.71, MeOH). IR (KBr): 3446(s), 2968(m), 1729(s), 1620(s), 1174(m). HRMS (EI$^+$): Calcd. for $C_{19}H_{32}N_2O_5$ [M + H$^+$] 369.2389. Found 369.2402.

Boc-Leu-*py*Val-OEt (3q)

Following the procedure for **3o** (80%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.42 (dt, $J = 10.0$, 3.3 Hz, 1H), 4.99–5.21 (m, 2H), 4.49 (d, $J = 2.5$ Hz, 1H), 4.05 (dt, $J = 2.5$, 7.5 Hz, 1H), 3.99–4.10 (m, 2H), 2.36–2.96 (m, 2H), 1.68–1.86 (m, 1H), 1.41–1.48 (m, 12H), 1.11 (d, $J = 7.6$ Hz, 3H), 1.02 (d, $J = 6.6$ Hz, 3H), 0.92 (d, $J = 6.6$ Hz, 3H), 0.77 (d, $J = 7.1$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 15.3, 18.9, 21.2, 23.6, 24.9, 28.3, 28.5, 41.1, 52.9, 64.2, 67.7, 79.4, 94.5, 155.5, 170.1, 173.6, 178.6. $[\alpha]_D^{20}$ +22 (*c* 0.26, MeOH). IR (KBr): 3449(s), 2978(m), 1725(s), 1629(s), 1180(m). HRMS (EI$^+$): Calcd. for $C_{20}H_{34}N_2O_5$ [M + H$^+$] 383.2546. Found 383.2531.

Boc-Phe-*py*Val-OEt (3r)

Following the procedure for **3o** (94%): Following the procedure for **3o** (94%)^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.13–7.40 (m, 5H), 5.14–5.75 (m, 2H), 5.05 (s, 1H), 4.51 (d, $J = 2.5$ Hz, 1H), 3.98–4.13 (m, 2H), 3.03–3.26 (m, 1H), 2.50–2.86 (m, 2H), 1.44 (t, $J = 7.1$ Hz, 3H), 1.22–1.39 (m, 9H), 1.12 (d, $J = 7.1$ Hz, 3H), 0.77 (d, $J = 6.6$ Hz, 3H) ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 15.8, 19.2, 28.6, 28.9, 38.3, 55.8, 64.7, 68.2, 79.8, 94.9, 127.0, 128.7, 130.0, 137.3, 155.5, 170.7, 172.3, 179.2. $[\alpha]_D^{20}$ +50 (*c* 0.24, MeOH). IR (KBr): 3372(m), 2979(m), 1726(s), 1619(s), 1315(s). HRMS (EI$^+$): Calcd. for $C_{23}H_{32}N_2O_5$ [M + H$^+$] 417.2389. Found 417.2404.

Boc-Ala-*py*Phe-OEt (3s)

Following the procedure for **3o** (85%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.22–7.18 (m, 3H), 7.02 (d, J = 6.6 Hz, 2H), 5.40–5.28 (m, 2H), 4.80–4.77 (m, 2H), 4.06–3.89 (m, 2H), 3.55 (dd, J = 5.1, 13.6 Hz, 1H), 3.12 (d, J = 13.6 Hz, 1H), 1.48 (s, 9H), 1.45 (t, J = 7.1 Hz), 1.34 (d, J = 6.6 Hz) ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 18.3, 28.4, 34.7, 50.1, 60.0, 67.7, 79.5, 94.7, 127.1, 128.2, 129.8, 134.1, 155.2, 169.3, 173.9, 177.2. $[\alpha]_D^{20}$ +120 (*c* 0.17, MeOH). IR (KBr): 3436(s), 1727(s), 1674(s), 1621(s), 1304(m). HRMS (EI$^+$): Calcd. for $C_{21}H_{28}N_2O_5$ [M + H$^+$] 389.2076. Found 389.2068.

Boc-Val-*py*Phe-OEt (3t)

Following the procedure for **3o** (89%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.16–7.26 (m, 3H), 6.99 (d, J = 6.6 Hz, 2H), 5.44 (dd, J = 9.9, 3.8 Hz, 1H), 5.24 (d, J = 10.1 Hz, 1H), 4.73–4.82 (m, 2H), 3.87–4.09 (m, 2H), 3.57 (dd, J = 13.6, 5.1 Hz, 1H), 3.12 (dd, J = 13.9, 2.3 Hz, 1H), 2.04–2.17 (m, 1H), 1.49 (s, 9H), 1.45 (t, J = 7.3 Hz, 3H), 1.03 (d, J = 7.1 Hz, 3H), 0.86 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 16.1, 19.9, 28.3, 30.1, 34.7, 58.1, 60.0, 67.6, 79.4, 94.8, 127.0, 128.2, 129.7, 134.0, 155.8, 169.4, 172.8, 177.0. $[\alpha]_D^{20}$ +130 (*c* 0.21, MeOH). IR (KBr): 3452(s), 2966(m), 1735(s), 1622(s), 1378(s), 1165(m). HRMS (EI$^+$): Calcd. for $C_{23}H_{32}N_2O_5$ [M + H$^+$] 417.2389. Found 417.2381.

Boc-Leu-*py*Phe-OEt (3u)

Following the procedure for **3o** (88%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.18–7.26 (m, 3H), 6.90–7.08 (m, 2H), 5.46 (t, J = 11.6 Hz, 1H), 5.13 (d, J = 8.6 Hz, 1H), 4.68–4.85 (m, 2H), 3.89–4.09 (m, 2H), 3.56 (dd, 1H), 3.12 (dd, J = 13.6, 2.0 Hz, 1H), 1.74–1.86 (m, 1H), 1.32–1.51 (m, 14H), 1.01 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 21.0, 23.6, 24.9, 28.4, 34.6, 41.0, 53.0, 60.0, 67.6, 79.4, 94.7, 127.1, 128.2, 129.8, 134.1, 155.7, 169.3, 174.3, 177.1. $[\alpha]_D^{20}$ +73 (*c* 0.76, MeOH). IR (KBr): 3336(m), 1732(s), 1688(s), 1386(m), 737(m). HRMS (EI$^+$): Calcd. for $C_{24}H_{34}NO_5$ [M + H$^+$] 431.2546. Found 431.2554.

Removal of the Boc group

H-Val-*py*Val-OEt (4a). To a solution of Boc-Val-*py*Val-OEt (**6**) (41 mg, 0.11 mmol) in DCM (3 mL) was added TFA (3 mL) and the residual mixture was stirred for 15 min at RT. The mixture was evaporated and residual TFA was removed by solid phase extraction (PS–H_2CO_3) to provide the pure product (29 mg, 0.11 mmol, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.18 (br. s, 4H), 5.15 (d, J = 3.0 Hz, 1H), 5.10 (s, 1H), 4.50 (d, J = 2.5 Hz, 1H), 3.99–4.16 (m, 2H), 2.56–2.69 (m, 1H), 2.23–2.35 (m, 1H), 1.45 (t, J = 7.1 Hz, 3H), 1.16 (d, J = 7.1 Hz, 3H), 1.11 (d, J = 7.1 Hz, 3H), 0.96 (d, J = 7.1 Hz, 3H), 0.70 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 14.6, 15.1, 18.9, 19.2, 28.4, 29.1, 58.2, 64.7, 68.2, 94.3, 167.8, 170.5, 179.8. $[\alpha]_D^{20}$ +88 (*c* 0.37, $CHCl_3$). IR (KBr): 3437(m), 2976(m), 1698(s), 1614(s), 1318(m), 1203(s). HRMS (EI$^+$): Calcd. for $C_{14}H_{24}N_2O_5$ [M + H$^+$] 269.1865. Found 269.1874.

H-Leu-*py*Val-OEt (4b). Following the procedure for **4a** (98%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.28 (s, 1H), 5.10 (dd, J = 9.6, 3.5 Hz, 1H), 4.55 (s, 1H), 4.06–4.22 (m, 2H), 2.62–2.76 (m, 1H), 1.60–1.84 (m, 3H), 1.43 (t, J = 7.1 Hz, 3H), 1.17 (d, J = 7.6 Hz, 3H), 1.04 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.1 Hz, 3H), 0.79 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 15.5, 19.3, 21.1, 24.0, 25.5, 29.8, 40.6, 53.7, 66.0, 69.9, 95.3, 170.2, 172.4, 181.7. $[\alpha]_D^{20}$ +30 (*c* 0.16, MeOH). IR (KBr): 3436(s), 1691(s), 1619(s), 1339(m), 1224(m), 757(m). HRMS (EI$^+$): Calcd. for $C_{15}H_{26}N_2O_3$ [M + H$^+$] 283.2022. Found 283.2029.

H-Phe-*py*Val-OEt (4c). Following the procedure for **4a** (99%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.39 (br. s, 3H), 7.15–7.36 (m, 5H), 5.34 (dd, J = 8.6, 3.5 Hz, 1H), 5.04 (s, 1H), 4.39 (d, J = 2.0 Hz, 1H), 3.97–4.15 (m, 2H), 3.33 (dd, J = 14.1, 3.5 Hz, 1H), 2.40–2.62 (m, 1H), 1.45 (t, J = 7.1 Hz, 3H), 1.06 (d, J = 7.1 Hz, 3H), 0.68 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 15.1, 19.1, 28.9, 36.7, 55.8, 65.0, 68.7, 94.5, 128.2, 129.5, 130.0, 134.2, 167.7, 170.8, 180.3. $[\alpha]_D^{20}$ +96 (*c* 0.62, MeOH). IR (KBr): 3436(s), 1691(s), 1619(s), 1373(m), 1224(m), 701(m). HRMS (EI$^+$): Calcd. for $C_{18}H_{24}N_2O_3$ [M + H$^+$] 317.1865. Found 317.1861.

Removal of the Fmoc group

H-Val-*py*Val-OEt (4a). To a solution of Fmoc-Val-*py*Val-OEt (**3f**) (38 mg, 0.08 mmol) in DCM (3 mL) at room temperature was added DBU (0.02 mL, 0.14 mmol) and the mixture was stirred for 5 min, at which point 3 mL silica was added and the mixture evaporated at reduced pressure. Column chromatography (0→5% MeOH in EtOAc) afforded the pure product as a low-melting solid. Yield (20 mg, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.18 (br. s, 4H), 5.15 (d, J = 3.0 Hz, 1H), 5.10 (s, 1H), 4.50 (d, J = 2.5 Hz, 1H), 3.99–4.16 (m, 2H), 2.56–2.69 (m, 1H), 2.23–2.35 (m, 1H), 1.45 (t, J = 7.1 Hz, 3H), 1.16 (d, J = 7.1 Hz, 3H), 1.11 (d, J = 7.1 Hz, 3H), 0.96 (d, J = 7.1 Hz, 3H), 0.70 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 14.6, 15.1, 18.9, 19.2, 28.4, 29.1, 58.2, 64.7, 68.2, 94.3, 167.8, 170.5, 179.8. $[\alpha]_D^{20}$ +88 (*c* 0.37, $CHCl_3$). IR (KBr): 3437(m), 2976(m), 1698(s), 1614(s), 1318(m), 1203(s). HRMS (EI$^+$): Calcd. for $C_{14}H_{24}N_2O_5$ [M + H$^+$] 269.1865. Found 269.1874.

H-Leu-*py*Val-OEt (4b). Following the procedure for **4a** (97%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.28 (s, 1H), 5.10 (dd, J = 9.6, 3.5 Hz, 1H), 4.55 (s, 1H), 4.06–4.22 (m, 2H), 2.62–2.76 (m, 1H), 1.60–1.84 (m, 3H), 1.43 (t, J = 7.1 Hz, 3H), 1.17 (d, J = 7.6 Hz, 3H), 1.04 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.1 Hz, 3H), 0.79 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 15.5, 19.3, 21.1, 24.0, 25.5, 29.8, 40.6, 53.7, 66.0, 69.9, 95.3, 170.2, 172.4, 181.7. $[\alpha]_D^{20}$ +30 (*c* 0.16, MeOH). IR (KBr): 3436(s), 1691(s), 1619(s), 1339(m), 1224(m), 757(m). HRMS (EI$^+$): Calcd. for $C_{15}H_{26}N_2O_3$ [M + H$^+$] 283.2022. Found 283.2029.

H-Phe-*py*Val-OEt (4c). Following the procedure for **4a** (95%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.39 (br. s, 3H), 7.15–7.36 (m, 5H), 5.34 (dd, J = 8.6, 3.5 Hz, 1H), 5.04 (s, 1H), 4.39 (d, J = 2.0 Hz, 1H), 3.97–4.15 (m, 2H), 3.33 (dd, J = 14.1, 3.5 Hz, 1H), 2.40–2.62 (m, 1H), 1.45 (t, J = 7.1 Hz, 3H), 1.06 (d, J = 7.1 Hz, 3H), 0.68 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 15.1, 19.1, 28.9, 36.7, 55.8, 65.0, 68.7, 94.5, 128.2, 129.5, 130.0, 134.2, 167.7, 170.8, 180.3. $[\alpha]_D^{20}$ +96 (*c* 0.62, MeOH). IR (KBr): 3436(s), 1691(s), 1619(s), 1373(m), 1224(m), 701(m). HRMS (EI$^+$): Calcd. for $C_{18}H_{24}N_2O_3$ [M + H$^+$] 317.1865. Found 317.1861.

Boc-Val-*py*Val-Ala-OtBu (5a)

To a solution fo *py*Val-Ala-O*t*-Bu (50 mg, 0.18 mmol) in THF 3 mL was added *i*-PrMgCl (2.0 M in THF, 0.090 mL, 0.18 mmol). The mixture was allowed to stir for 10 min, at which point a solution of Boc-Val-ONp (69 mg, 0.20 mmol) in THF (2 mL) was added. The mixture was stirred for 1 h, and then AcOH (0.1 mL) was added, and the mixture was diluted with DCM (20 mL), washed with 10% $NaHCO_3$ (20 mL) and evaporated under reduced pressure. Column chromatography over silica gel EtOAc–heptane (1 : 4) provided the pure compound (68 mg, 0.14 mmol, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.36 (dd, J = 9.6, 4.0 Hz, 1H), 5.12 (d, J = 9.6 Hz, 1H), 4.50–4.65 (m, 1H), 3.75–3.91 (m, 1H), 3.63 (q, J = 6.9 Hz, 1H), 2.90 (dd, J = 16.9, 12.4 Hz, 1H), 2.68 (dd, J = 17.2, 8.1 Hz, 1H), 2.22–2.38 (m, 1H), 1.89–2.05 (m, 1H), 1.47–1.54 (m, 12H), 1.42 (s, 9H), 1.08 (d, J = 6.6 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 7.1 Hz, 3H), 0.83 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.3, 17.2, 18.1, 19.9, 22.1, 27.9, 28.1, 28.3, 30.0, 36.9, 54.0, 56.6, 58.7, 61.7, 80.1, 84.7, 115.7, 126.2, 141.4, 141.4, 156.1, 161.4, 161.7, 161.8, 170.9, 171.5, 174.1. $[\alpha]_D^{20}$ +6 (*c* 0.40, MeOH). IR (KBr): 3436(s), 2924(m), 1720(m), 1246(m). HRMS (EI$^+$): Calcd. for $C_{24}H_{43}N_3O_6$ [M + H$^+$] 470.3230. Found 470.3226.

Boc-Leu-*py*Val-Ala-O*t*-Bu (5b)

Following the procedure for **5a** (90%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.38 (dt, J = 10.0, 3.3 Hz, 2H), 4.78–5.09 (m, 1H), 4.32 (dd, J = 7.1, 4.0 Hz, 1H), 3.33 (td, J = 10.2, 7.3 Hz, 1H), 3.15 (q, J = 7.1 Hz, 1H), 2.59 (s, 1H), 2.57 (s, 1H), 2.17–2.30 (m, 1H), 1.63–1.84 (m, 2H), 1.46 (s, 9H), 1.40 (s, 9H), 1.32 (dd, J = 11.1, 4.0 Hz, 1H), 1.24 (d, J = 7.1 Hz, 3H), 1.04 (d, J = 7.1 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 7.1 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.7, 19.6, 21.0, 21.7, 23.5, 24.8, 27.7, 28.0, 28.2, 39.6, 40.9, 53.3, 54.2, 56.2, 63.0, 79.4, 81.5, 155.5, 173.1, 175.3, 175.8. $[\alpha]_D^{20}$ −13 (*c* 6.40, MeOH). IR (KBr): 3436(s), 2975(m), 1721(s), 1368(m), 1168(m). HRMS (EI$^+$): Calcd. for $C_{25}H_{45}N_3O_6$ [M + H$^+$] 484.3387. Found 484.3383.

Boc-Phe-*py*Val-Ala-O*t*-Bu (5c)

Following the procedure for **5a** (83%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.13–7.34 (m, 5H), 5.61–5.74 (m, 1H), 5.17 (d, J = 8.6 Hz, 1H), 4.19–4.31 (m, 1H), 3.15 (q, J = 7.1 Hz, 2H), 2.74–3.08 (m, 2H), 2.44–2.65 (m, 2H), 2.20–2.28 (m, 1H), 1.48 (s, 9H), 1.25 (d, J = 7.1 Hz, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.7, 19.6, 21.6, 27.8, 28.1, 28.2, 38.5, 39.5, 54.1, 55.2, 56.2, 63.1, 76.7, 77.0, 77.2, 77.3, 79.6, 81.5, 126.8, 128.3, 129.5, 136.3, 155.1, 173.4, 173.9, 175.3. $[\alpha]_D^{20}$ −45 (*c* 1.20, MeOH). IR (KBr): 3435(s), 1723 (s), 1368(s), 1169(s), 700(w). HRMS (EI$^+$): Calcd. for $C_{28}H_{43}N_3O_6$ [M + H$^+$] 518.3230. Found 518.3244.

Fmoc-Val-*py*Val-Ala-O*t*-Bu (5d)

Following the procedure for **5a** (83%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 (d, J = 7.6 Hz, 2H), 7.61 (d, J = 7.6 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 7.3 Hz, 2H), 5.52 (dt, J = 10.0, 2.8 Hz, 1H), 5.15–5.46 (m, 1H), 4.08–4.46 (m, 5H), 3.76 (s, 1H), 3.31–3.42 (m, 1H), 3.19 (q, J = 6.9 Hz, 1H), 2.57–2.67 (m, 1H), 2.16–2.33 (m, 1H), 1.68–1.83 (m, 1H), 1.48 (s, 9H), 1.27 (d, J = 7.1 Hz, 3H), 0.87–1.09 (m, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.7, 19.5, 20.9, 21.6, 21.7, 22.8, 23.5, 24.7, 24.8, 27.7, 28.0, 39.4, 41.2, 41.7, 47.1, 52.3, 53.8, 54.3, 56.2, 63.1, 66.9, 81.7, 119.9, 125.1, 125.1, 127.0, 127.6, 127.7, 141.3, 143.7, 143.9, 156.1, 173.3, 173.7, 175.0, 175.3. $[\alpha]_D^{20}$ +16 (*c*2.90, MeOH). IR (KBr): 3351(m), 2960(s), 1726(s), 1518(m), 1151(m). HRMS (EI$^+$): Calcd. for $C_{34}H_{45}N_3O_6$ [M + H$^+$] 592.3387. Found 592.3383.

Fmoc-Leu-*py*Val-Ala-O*t*-Bu (5e)

Following the procedure for **5a** (85%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 (d, J = 7.6 Hz, 2H), 7.61 (d, J = 7.1 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.32 (d, J = 7.1 Hz, 2H), 5.52 (s, 2H), 4.20–4.48 (m, 4H), 3.32–3.42 (m, 1H), 3.20 (q, J = 7.1 Hz, 1H), 2.56–2.66 (m, 2H), 2.23–2.34 (m, 1H), 2.00–2.11 (m, 1H), 1.48 (s, 9H), 0.76–1.12 (m, 14H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 16.1, 18.8, 19.5, 19.9, 21.6, 22.7, 27.6, 28.0, 29.0, 30.5, 31.8, 39.5, 47.1, 54.4, 56.3, 59.0, 63.3, 67.0, 81.7, 119.9, 125.1, 127.0, 127.6, 141.3, 143.8, 143.9, 156.4, 173.54, 173.46, 175.3. $[\alpha]_D^{20}$ +16 (*c* 2.90, MeOH). IR (KBr): 3435(s), 2967(w), 1723(s), 1697(s), 1452(w), 740(w). HRMS (EI$^+$): Calcd. for $C_{35}H_{47}N_3O_6$ [M + H$^+$] 606.3543. Found 606.3514.

Fmoc-Phe-*py*Val-Ala-O*t*-Bu (5f)

Following the procedure for **5a** (88%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, J = 7.6 Hz, 2H), 7.55 (t, J = 7.3 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.17–7.34 (m, 7H), 5.71–5.83 (m, 1H), 5.22–5.51 (m, 1H), 3.93–4.37 (m, 4H), 3.06–3.25 (m, 3H), 2.75–2.91 (m, 1H), 2.43–2.58 (m, 2H), 2.09–2.30 (m, 1H), 1.49–1.53 (m, 9H), 1.26 (d, J = 7.1 Hz, 3H), 1.05 (d, J = 7.1 Hz, 3H), 0.92 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 18.7, 19.6, 21.5, 22.7, 27.8, 28.1, 29.0, 31.8, 38.6, 39.4, 47.1, 50.3, 54.2, 55.6, 56.2, 63.2, 65.1, 66.9, 81.5, 119.9, 120.0, 124.7, 125.1, 127.0, 127.6, 128.4, 129.5, 136.0, 141.2, 141.5, 143.8, 143.8, 144.3, 155.5, 173.2, 173.5, 175.3. $[\alpha]_D^{20}$ +20 (*c* 1.20, MeOH). IR (KBr): 3436(s), 2968(m), 1727(s), 1368(m), 1222(m). HRMS (EI$^+$): Calcd. for $C_{38}H_{45}N_3O_6$ [M + H$^+$] 640.3387. Found 640.3399.

Cbz-Val-*py*Val-Ala-O*t*-Bu (5g)

Following the procedure for **5a** (82%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28–7.41 (m, 5H), 5.51 (dd, J = 9.6, 4.0 Hz, 1H), 5.43 (d, J = 9.1 Hz, 1H), 5.10 (s, 2H), 4.23–4.32 (m, 1H), 3.26–3.37 (m, 1H), 3.17 (q, J = 6.7 Hz, 1H), 2.51–2.65 (m, 2H), 2.18–2.33 (m, 1H), 1.95–2.09 (m, 1H), 1.47 (s, 9H), 1.45 (s, 9H), 1.25 (d, J = 7.1 Hz, 3H), 1.06 (d, J = 7.1 Hz, 3H), 1.02 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 7.1 Hz, 3H), 0.80 (d, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.0, 18.8, 19.6, 20.0, 21.6, 27.6, 28.0, 30.4, 39.6, 54.3, 56.2, 59.0, 63.3, 66.9, 81.5, 128.1, 128.5, 136.4, 156.2, 173.3, 173.5, 175.3. $[\alpha]_D^{20}$ +17 (*c* 2.50, MeOH). IR (KBr): 3435(s), 2968(m), 1726(s), 1369(m), 1151(m). HRMS (EI$^+$): Calcd. for $C_{27}H_{41}N_3O_6$ [M + H$^+$] 504.3074. Found 504.3086.

Cbz-Leu-*py*Val-Ala-O*t*-Bu (5h)

Following the procedure for **5a** (83%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28–7.39 (m, 5H), 5.48 (dt, J = 10.0, 2.8 Hz, 1H),

5.33 (d, $J = 9.1$ Hz, 1H), 5.10 (s, 2H), 4.32 (dd, $J = 7.1$, 4.0 Hz, 1H), 3.29–3.39 (m, 1H), 3.16 (q, $J = 6.7$ Hz, 1H), 2.60 (d, $J = 10.1$ Hz, 2H), 2.19–2.31 (m, 1H), 1.72–1.83 (m, 1H), 1.47 (s, 9H), 1.25 (d, $J = 7.1$ Hz, 3H), 1.03 (dd, $J = 11.9$, 6.8 Hz, 6H), 0.86–0.95 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.7, 19.6, 21.0, 21.6, 23.5, 24.8, 27.8, 28.0, 39.5, 41.3, 53.8, 54.3, 56.2, 63.1, 66.8, 81.5, 128.0, 128.4, 136.4, 155.9, 173.2, 175.0, 175.3. $[a]_D^{20}$ −8 (*c* 1.60, MeOH). IR (KBr): 3436(s), 1487(s), 1375(s), 1069(w), 846(w). HRMS (EI$^+$): Calcd. for $C_{28}H_{43}N_3O_6$ [M + H$^+$] 518.3230. Found 518.3246.

Cbz-Phe-*py*Val-Ala-O*t*-Bu (5i)

Following the procedure for **5a** (89%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.08–7.37 (m, 10H), 5.70–5.85 (m, 1H), 5.41 (d, $J = 8.6$ Hz, 1H), 5.00 (s, 2H), 4.14–4.31 (m, 1H), 3.12–3.22 (m, 2H), 3.00–3.10 (m, 1H), 2.83 (dd, $J = 13.1$, 8.1 Hz, 1H), 2.41–2.57 (m, 2H), 2.11–2.28 (m, 1H), 1.48 (s, 9H), 1.25 (d, $J = 6.6$ Hz, 3H), 0.95–1.08 (m, 3H), 0.67–0.94 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.7, 19.6, 21.5, 27.8, 28.1, 38.6, 39.4, 54.1, 55.6, 56.2, 63.2, 66.7, 81.5, 126.9, 128.0, 128.4, 128.4, 129.4, 136.0, 136.3, 155.5, 173.2, 173.5, 175.2. $[a]_D^{20}$ +41 (*c* 2.20, MeOH). IR (KBr): 3378(s), 2973(m), 1725(s), 1669(s), 1358(m). HRMS (EI$^+$): Calcd. for $C_{31}H_{41}N_3O_6$ [M + H$^+$] 552.3074. Found 552.3068.

Peptide stability

Peptide or peptide analogue (5 mg) was added to a temperature-equilibriated solution of 50% human plasma (37 °C, 10 mL, the lyophilized material from SigmaAldrich was dissolved in Millipore water) was added and the mixture stirred vigorously at 37 °C. At appropriate times samples were taken and analyzed by HPLC.

Fmoc-Phe-*py*Val-Ala-OH (6a)

A solution of Fmoc-Phe-*py*Val-Ala-O*t*-Bu (**5f**) (23 mg, 0.036 mmol) in TFA (2 mL) was stirred at RT for 10 h. Evaporation of the mixture provided the pure product as a clear oil (20 mg, 0.034 mmol, 95%). ^{1}H NMR (MeOD, 300 MHz): δ 0.92 (d, $J = 6.78$ Hz, 3H), 1.11 (d, $J = 6.78$ Hz, 3H), 1.60 (d, $J = 7.16$ Hz, 3H), 2.13 (s, 1H), 2.80 (m, 3H), 3.09 (dd, $J = 13.56$, 4.90 Hz, 1H), 4.12 (m, 5H), 4.60 (dd, $J = 6.78$, 4.14 Hz, 1H), 5.65 (dd, $J = 9.80$, 4.52 Hz, 1H), 7.28 (m, 9H), 7.54 (d, $J = 7.16$ Hz, 2H), 7.73 (d, $J = 7.54$ Hz, 2H). ^{13}C NMR (MeOD, 75 MHz): δ 14.9, 18.8, 23.2, 28.6, 35.7, 38.1, 52.6, 56.4, 57.6, 62.4, 67.9, 120.8, 126.2, 127.8, 128.0, 128.1, 128.7, 129.3, 130.5, 138.3, 142.4, 145.0, 158.3, 171.9, 172.2, 175.1 HRMS (EI$^+$): Calcd. for $C_{34}H_{38}N_3O_6$ [M + H$^+$] 584.2761. Found 584.2745.

Fmoc-Phe-Phe-*py*Val-Ala-Ser(*t*-Bu)-OH (7a)

To a solution of Fmoc-Phe-*py*Val-Ala-OH (**6a**) (58 mg, 0.10 mmol) in DMF (2 mL) was added bromotris(pyrrolidino)-phosphonium hexafluorophosphate (46 mg, 0.10 mmol), *N*-hydroxybenzotriazole (14 mg, 0.10 mmol) and *N*,*N*-diisopropylethylamine (0.03 mL, 0.18 mmol). The mixture was stirred for 5 min at room temperature, added to a suspension of H-Ser(*t*-Bu)-2-ClTrt resin (1.1 mmol g^{-1}, 120 mg) in DMF (2 mL) and shaken for 1 h at room temperature. The resin was washed with DMF (3 × 5 mL), treated with 20% piperidine/DMF (3 × 5 min) and washed with DMF (3 × 5 mL). To the resin in DMF (4 mL) was added Fmoc-Phe-OPfp (150 mg, 0.27 mmol) and *N*,*N*-diisopropylethylamine (0.01 mL, 0.06 mmol), and the mixture was shaken for 1 h at room temperature. The resin was washed with DMF (3 × 5 mL) and treated with 2% TFA in DCM. The resin was washed with DCM (3 × 5 mL). The combined DCM fractions were evaporated and purified by preparative HPLC (MeCN–H_2O) to afford the pure product as a white solid (23 mg, 26 mmol, 26%). ^{1}H NMR (MeOD, 300 MHz): δ 0.91 (d, $J = 7.16$ Hz, 3H), 1.08 (d, $J = 6.78$ Hz, 3H), 1.18 (s, 9H), 1.43 (d, $J = 7.16$ Hz, 3H), 2.16 (m, 1H), 2.57 (dd, $J = 17.33$, 8.29 Hz, 1H), 2.77 (m, 3H), 3.05 (m, 2H), 3.62 (m, 3H), 3.87 (dd, $J = 9.42$, 4.14 Hz, 1H), 4.26 (m, 5H), 4.60 (t, $J = 3.58$ Hz, 1H), 5.88 (m, 1H), 7.26 (m, 14H), 7.55 (dd, $J = 7.16$, 2.26 Hz, 2H), 7.77 (d, $J = 7.54$ Hz, 2H), 8.35 (d, $J = 7.54$ Hz, 1H). ^{13}C NMR (MeOD, 75 MHz): δ 18.02, 18.83, 22.74, 27.39, 28.31, 37.83, 38.17, 38.70, 47.95, 54.03, 54.25, 55.48, 57.16, 57.23, 62.48, 63.10, 67.79, 74.30, 120.59, 125.94, 126.03, 127.37, 127.64, 127.87, 128.47, 129.09, 130.09, 130.23, 137.63, 138.15, 142.21, 144.84, 144.93, 157.72, 172.66, 173.51, 173.65, 173.94. Mp 149–152 °C. HRMS (EI$^+$): Calcd. for $C_{50}H_{60}N_5O_9$ [M + H$^+$] 874.4386. Found 874.4380.

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