

HETEROCYCLES, Vol. 97, No. 1, 2018, pp. 333 - 354. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 19th January, 2018, Accepted, 22nd February, 2018, Published online, 6th March, 2018
DOI: 10.3987/COM-18-S(T)22

SYNTHESIS OF TRIPEPTIDES CONTAINING HETEROCYCLIC α -AMINO ACIDS BY USING HETEROSPIROCYCLIC 3-AMINO-2*H*-AZIRINES

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Dedicated to Professor Dr. Kiyoshi Tomioka on the occasion of his 70th birthday

Abstract – By using the ‘azirine/oxazolone method’, di- and tripeptides containing six-membered heterocyclic 4-amino-4-carboxylic acids with a piperidine, tetrahydropyran or tetrahydrothiopyran ring have been synthesized. It has been shown that the corresponding heterospirocyclic 3-(*N*-methyl-*N*-phenylamino)-2*H*-azirines are suitable synthons for these heterocyclic α -amino acids. As expected, the presence of these α,α -disubstituted α -amino acids stabilizes β -turn conformations in the prepared tripeptides of type Z-Phe-Xaa-Val-OR.

INTRODUCTION

The continuing interest in the biological and medicinal chemistry of α,α -disubstituted α -amino acids (C^α -tetrasubstituted α -amino acids) is mainly due to their influence on the conformation of peptides, i.e. their helix-inducing potential.² In recent years, the enantioselective synthesis of chiral representatives was a focus of several research groups.³ Another type which is of interest consists of heterocyclic *n*-amino-*n*-carboxylic acids, for example the 4-amino-4-carboxylate derivatives **1** of piperidine,⁴ tetrahydropyran,⁵ and tetrahydrothiopyran^{5,6} (Figure 1). Although some studies on amino acids of type **1** as structural units in pharmacologically interesting compounds are known,⁷ the major interest concerns their insertion into peptides and the resulting structural properties.⁸

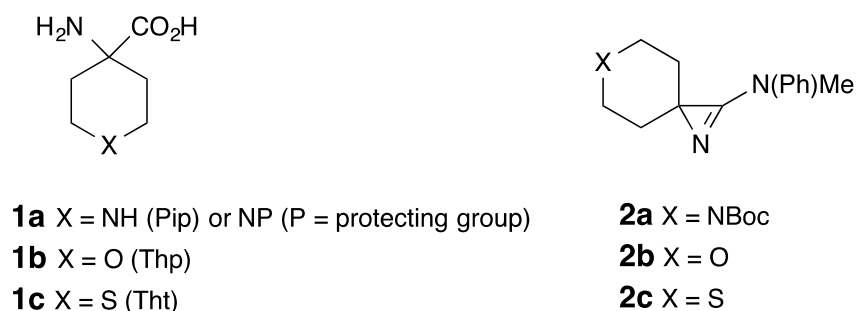
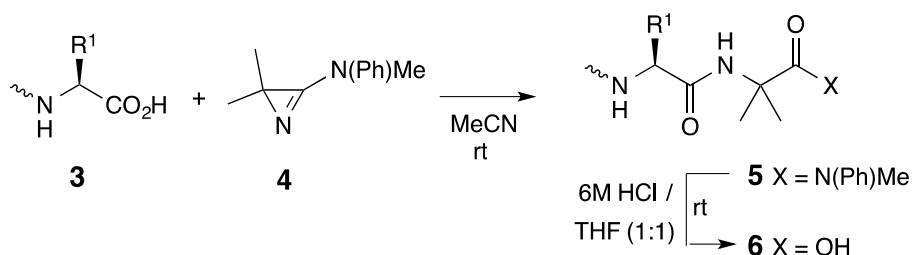


Figure 1. Heterocyclic 4-amino-4-carboxylic acids **1** and heterospirocyclic 3-amino-2*H*-azirines **2** as synthons for **1** in peptide synthesis

In the last few decades, we evolved an alternative method for the introduction of α,α -disubstituted acids into peptides, the so-called ‘azirine/oxazolone method’.⁹ The smooth and efficient coupling of a peptide acid **3** with a 2,2-disubstituted 3-amino-2*H*-azirine of type **4** yields the extended peptide amide **5** (Scheme 1). Subsequent selective hydrolysis via an intermediate 1,3-oxazolone leads to peptide **6**, for the next coupling step. This method was also adapted for solid phase synthesis.¹⁰



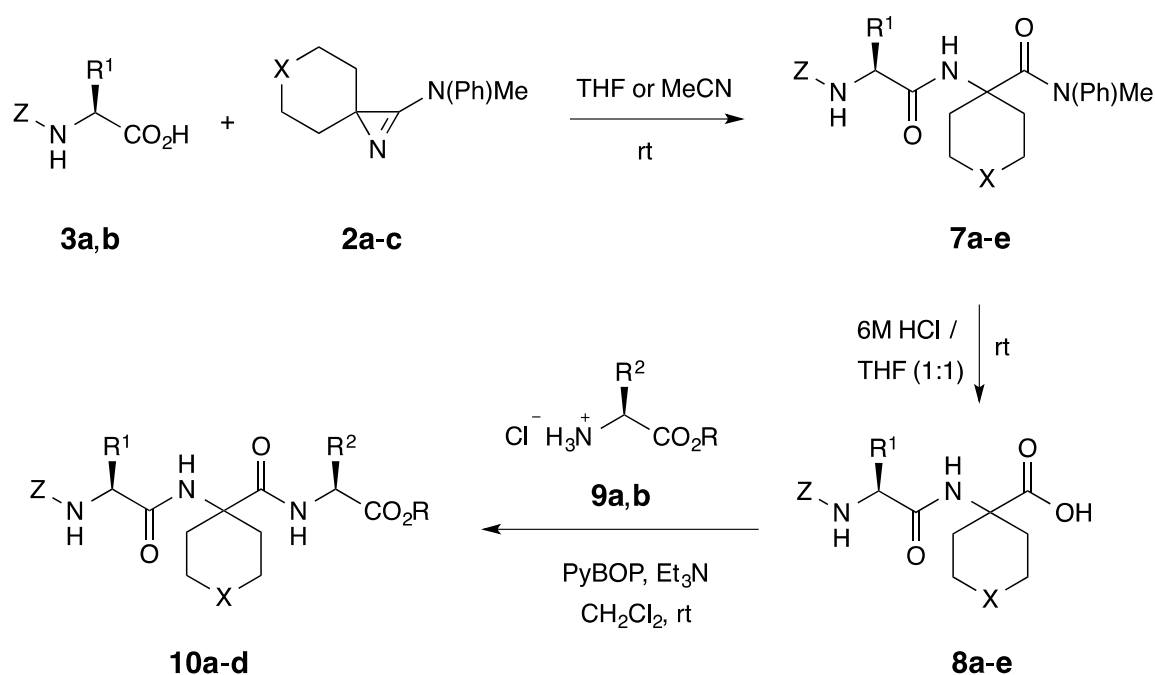
Scheme 1. Coupling of a peptide acid **3** with 3-amino-2*H*-azirine **4** and selective hydrolysis to give the extended peptide **6**

The ‘azirine/oxazolone method’ has been used for the synthesis of α -aminoisobutyric (Aib)-containing peptides (peptaibols),¹¹ cyclodepsipeptides¹² and cyclopeptides.¹³ In addition to the Aib-synthon **4**, other 2,2-disubstituted 3-amino-2*H*-azirines of type **4** with various substituents at C(2) have been prepared. Among them, the synthons for heterocyclic α -amino acids¹⁴ or dipeptides containing a heterocyclic α -amino acid^{14c, 15} are of special interest.

The goal of the present study was the use of the heterospirocyclic 3-amino-2*H*-azirines **2a–2c**^{14b} (Figure as synthons for heterocyclic α -amino acids **1** in the synthesis of tripeptides as useful units for subsequent peptide syntheses.

RESULTS AND DISCUSSION

According to the standard conditions of the ‘azirine coupling’, a solution of equimolar amounts of a heterospirocyclic 3-amino-2*H*-azirine **2** and a *N*-protected proteinogenic α -amino acid **3** (Phe or Val) in acetonitrile was stirred at room temperature for 16 h. After evaporation of the solvent and chromatographic purification (SiO₂, hexane/AcOEt), the dipeptide amide of type **7** was obtained in 80–85% yield (Scheme 2, Table 1). The latter compound was dissolved in THF and the solution was cooled to 0 °C. Then, a solution of 6M HCl/THF (1:1) was added and the mixture was stirred at room temperature for 4 h leading to the selective hydrolysis of the terminal amide group. In the cases of **7b–e**, the dipeptide acids **8b–e** were isolated in 77–94% yield as colorless crystalline materials. On the other hand, the hydrolysis of the *N*-Boc-protected **7a** gave, as expected, **8a** with the deprotected piperidine ring in 62% yield.



Scheme 2. Synthesis of tripeptides **10** (Z-Phe-Xaa-Val-OMe and Z-Val-Xaa-Phe-OEt; Z = benzyloxycarbonyl)

Finally, **8b–e** were coupled with a second proteinogenic amino acid ester **9** (Val or Phe) using PyBOP as a classical peptide coupling reagent. The desired tripeptides **10a–d** with protected amino and carboxyl groups were obtained in 89–96% yield. The structures of all prepared compounds were determined on the basis of their spectroscopic and analytical data. Furthermore, the products were formed without epimerization because the ¹H- as well as ¹³C-NMR analyses showed that in each case only one stereoisomer was present.

Table 1. Synthesis of tripeptides **10** via the ‘azirine/oxazolone method’

3	R ¹	2	X	7	Yield (%) ^{a)}	8	Yield (%) ^{a)}	9	R ²	R	10	Yield (%) ^{a)}
a	Bn	a	NBoc	a	82	a ^{b)}	62	-			-	
a	Bn	b	O	b	80	b	87	a	iPr	Me	a	96
b	iPr	b	O	c	82	c	77	b	Bn	Et	b	92
a	Bn	c	S	d	85	d	94	a	iPr	Me	c	94
b	iPr	c	S	e	82	e	80	b	Bn	Et	d	89

a) Yield of isolated product. b) The dipeptide acid with the deprotected piperidine ring (X = NH) was obtained.

The structures of **10a** and **10c** were established by single crystal X-ray analysis (Figure 2), and for **10c**, the *S,S*-configuration was confirmed by the refinement of the absolute structure parameter. In the crystal, both tripeptides exist in a β -turn conformation with an intramolecular hydrogen bond between N(4)–H of valine and O(11) of the benzyloxycarbonyl (Z) group (graph set motif¹⁷ S(10); Table 2). The other two NH groups, N(7)–H and N(10)–H, form intermolecular hydrogen bonds with the carbonyl O-atoms O(5) and O(2), respectively, in different neighboring molecules (graph set motifs¹⁷ C(5) and C(11), resp.).

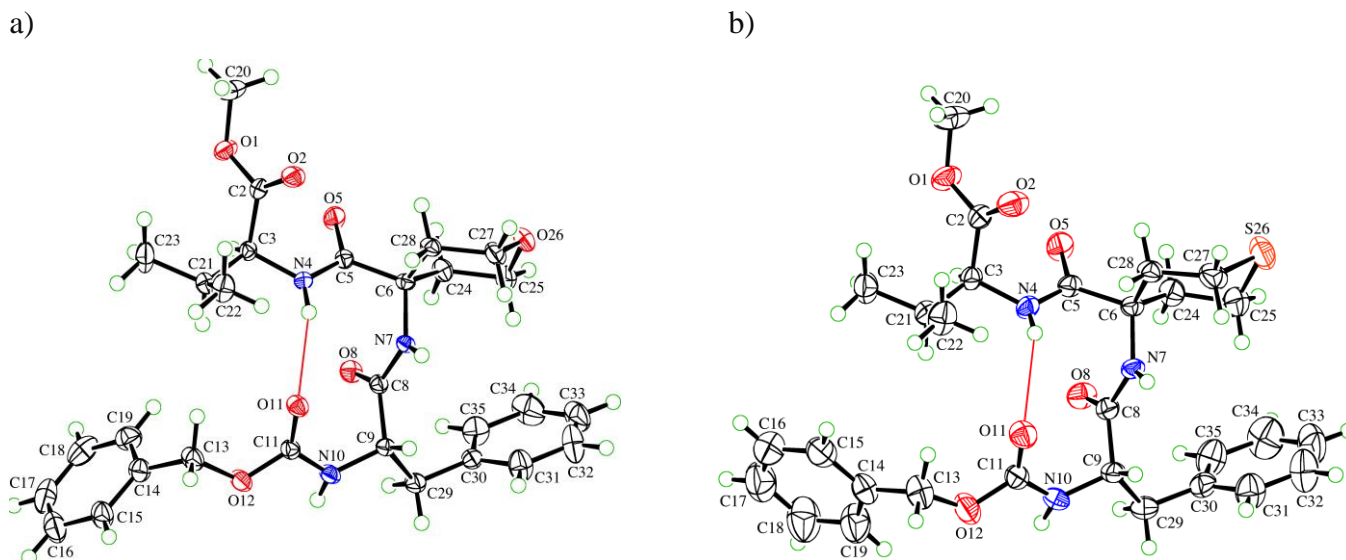
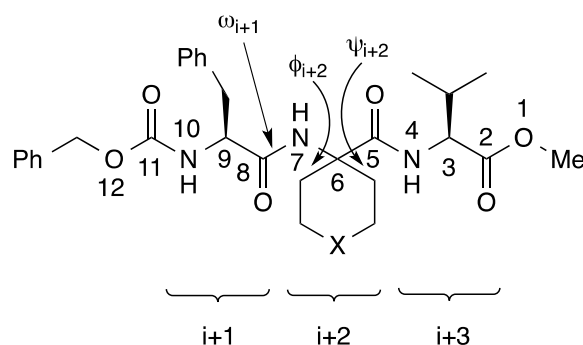


Figure 2. ORTEP plots¹⁶ of the molecular structures of the tripeptides a) Z-Phe-Thp-Val-OMe (**10a**) and b) Z-Phe-Tht-Val-OMe (**10c**). Displacement ellipsoids are drawn at the 50% probability level.

Table 2. Intramolecular hydrogen bonds in the tripeptides **10a**, **10c** and **12a**

Peptide	H·····O [Å]	N·····O [Å]	N–H·····O [°]
10a	2.27(3)	3.096(3)	158(3)
10c	2.44(6)	3.154(5)	148(5)
12a	2.37(3)	3.169(3)	148(2)

The conformations of both tripeptides **10a** and **10c** are very similar, as shown by the similarity of the torsion angles of the peptide backbone (Figure 3, Table 3). But there is a significant difference between these conformations and those of the previously reported tripeptides Z-Aib-Xaa-Aib-N(Ph)Me,^{14b} which form β -turns of type III with the characteristic torsion angles for Aib_{i+1} and Xaa_{i+2}. Whereas the ϕ_{i+2}/ψ_{i+2} values of the heterocyclic amino acids in **10a** and **10c** are also close to those of a β -turn of type III (+60°/+30°), characteristic for α,α -disubstituted α -amino acids, ϕ_{i+1}/ψ_{i+1} for the Phe residue are indicative of a β -turn of type II (−60°/+120°).

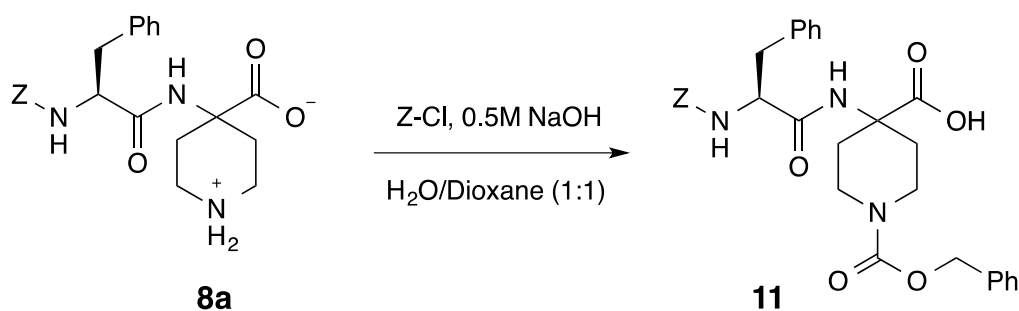
**Figure 3.** Numbering of the atoms (arbitrary) and torsion angles of the peptide backbone of **10a** and **10c****Table 3.** Torsion angles [°] within the backbone of the tripeptides **10a**, **10c** and **12a**

	ω_i	ϕ_{i+1}	ψ_{i+1}	ω_{i+1}	ϕ_{i+2}	ψ_{i+2}	ω_{i+2}
10a	174.3(2)	−52.4(3)	122.8(2)	177.1(2)	54.0(3)	36.9(3)	166.3(2)
10c	177.2(4)	−57.4(5)	122.3(4)	178.5(3)	51.8(4)	40.4(4)	164.1(3)
12a	175.7(2)	−51.9(2)	124.1(2)	175.2(2)	52.0(2)	40.3(2)	162.5(2)

There is a strong indication that the tripeptide derivatives **10a–d** also exist as β -turns in solution, similar to the previously reported tripeptides Z-Aib-Xaa-Aib-N(Ph)Me containing only α,α -disubstituted α -amino acids.^{14b} The three NH signals in the ¹H-NMR spectra in CDCl₃ of all four components could be assigned to N(4)H (7.4–7.1 ppm), N(7)H (6.19–6.57 ppm) and N(10)H (5.34–5.49 ppm) on the basis of their multiplicity and 2D-NMR experiments. It is well known that the chemical shifts of NH groups of peptides involved in intramolecular hydrogen bonds show a small dependence on the solvent polarity,

whereas those exposed to the solvent are influenced significantly.¹⁸ The measurements in CDCl₃ containing 0–12% (D₆)DMSO at ca. 30 °C showed a very small solvent-dependence for N(4)H ($\Delta\delta = 0.1\text{--}0.6$ ppm) but a significant one in the cases of N(7)H and N(10)H ($\Delta\delta = 1.14\text{--}1.50$ ppm). We explain these results with the presence of an intramolecular hydrogen bond N(4)–H \cdots O(11), i.e. a β -turn conformation also in solution.

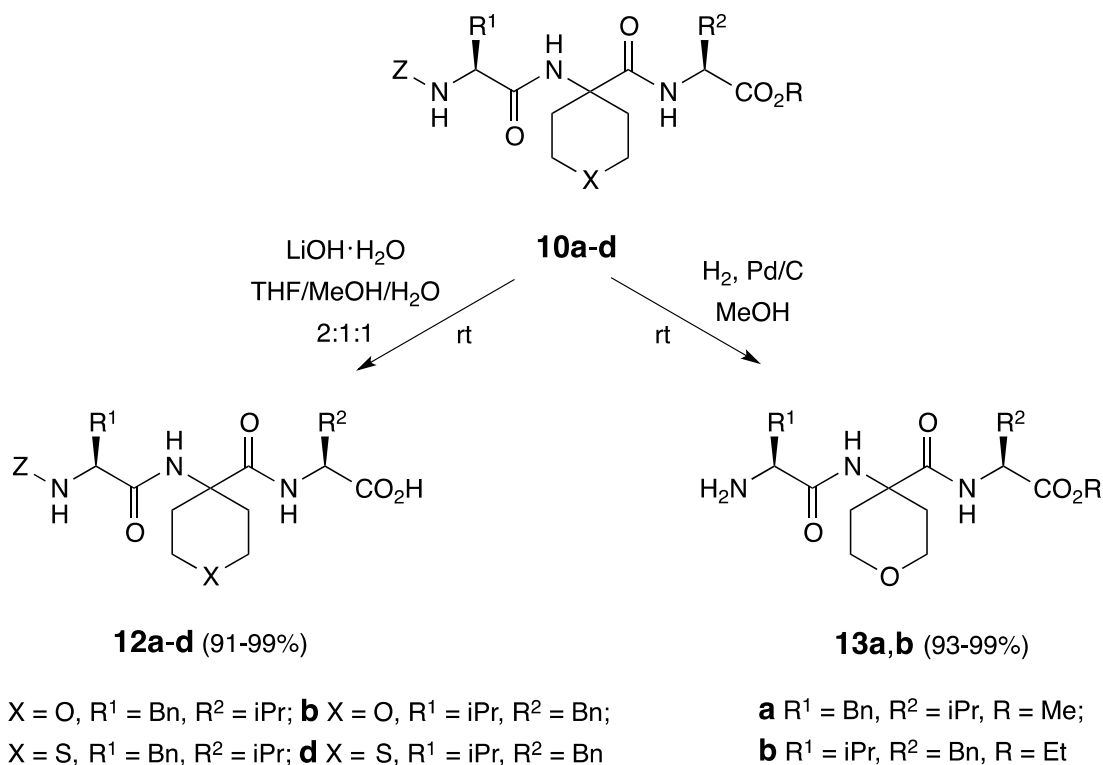
The problem arising in the hydrolysis of the dipeptide amide **7a** bearing the acid-labile Boc-protecting group in the piperidine ring was not unexpected. The attempts to reprotect the resulting dipeptide acid **8a** (X = NH) with (Boc)₂O under standard conditions were unsuccessful. On the other hand, the reaction with Z-chloride led to the N-protected dipeptide acid **11** as a useful building block for peptide synthesis in 39% yield (Scheme 3).



Scheme 3. Reprotection of the piperidine N-atom with benzyloxycarbonyl chloride (Z-Cl)

With the aim of obtaining tripeptide units useful for further peptide synthesis, the selective deprotections of the terminal carboxylic acid and amino group, respectively, were studied. Treatment of **10a–d** with LiOH·H₂O in THF/MeOH/H₂O (2:1:1) at room temperature for 2 h, acidic workup and crystallization led to the tripeptide acids **12a–d** in very good yields (91–99%, Scheme 4). All products were obtained as single diastereoisomers evidenced by a single set of signals in the ¹H- and ¹³C-NMR spectra. Therefore, the saponification proceeded under preservation of the configuration.

The hydrogenolytic deprotection of the N-terminus of the tripeptides **10a,b** by treatment with Pd/C in methanol gave the desired products **13a,b** in high yields (Scheme 4). On the other hand, the analogous reactions with the S-containing tripeptides **10c,d** were unsuccessful, and also under modified conditions, the deprotection could not be achieved. As a consequence, for further peptide synthesis with the Tht-synthon **2c**, another N-protecting group has to be chosen.



Scheme 4. Selective deprotection of the carboxylic acid and amino terminus, respectively, of the tripeptide derivatives **10a–d**

In the case of **12a**, the molecular structure was established by X-ray crystallography (Figure 4). The crystals contain one molecule of water for every peptide molecule. The carboxylic acid group forms an intermolecular hydrogen bond with the O-atom of the water molecule, which in turn forms intermolecular hydrogen bonds with the second amide O-atom and the third amide N-atom of two different peptide molecules. These interactions respectively link the peptide and water molecules in an alternating sequence into extended chains which run parallel to the [001] and [−101] directions. The graph set motifs¹⁷ describing these chains are C₂²(12) and C₂²(13), respectively. N(10)–H forms an intermolecular hydrogen bond with the carboxylate O-atom, O(2), of a neighboring peptide molecule to build a chain which runs parallel to the [001] direction with a graph set motif of C(11). N(7)–H forms an intermolecular hydrogen bond with the amide O-atom, O(5), of a different adjacent peptide molecule thereby building a chain which runs parallel to the [100] direction with a graph set motif of C(5). The combination of all the intermolecular interactions links the molecules into two-dimensional sheets, which lie parallel to the (010) plane. Finally, N(4)–H forms an intramolecular hydrogen bond with the carbonyl O(11)-atom that is seven atoms back along the peptide backbone (graph set S(10)) forming a β-turn (Tables 2 and 3). The conformation of **12a** in the crystal is virtually identical with that of the corresponding peptide ester **10a**.

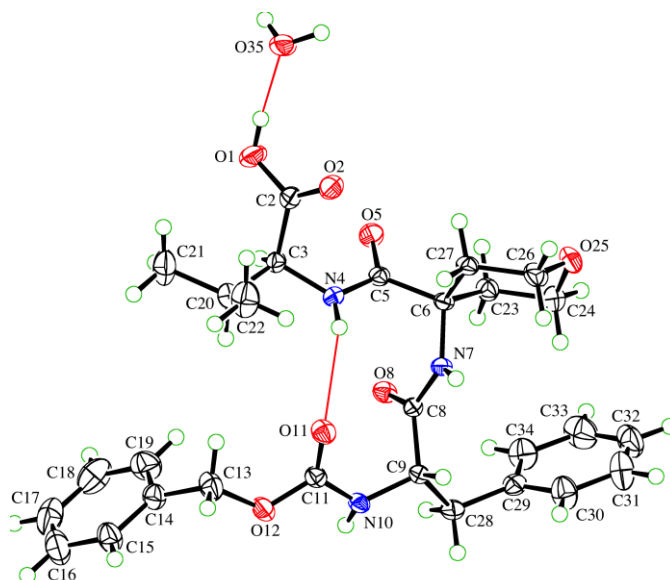


Figure 4. ORTEP plot¹⁶ of the molecular structure of the tripeptide Z-Phe-Thp-Val-OH.H₂O (**12a**). Displacement ellipsoids are drawn at the 50% probability level.

CONCLUSIONS

The present studies show that the heterospicyclic 3-(*N*-methyl-*N*-phenylamino)-2*H*-azirines **2a–c** are suitable building blocks for the synthesis of peptides containing heterocyclic 4-amino-4-carboxylic acids with a piperidine, tetrahydropyran or tetrahydrothiopyran ring. According to the ‘azirine/oxazolone method’, their coupling with Z-Val-OH or Z-Phe-OH occurs smoothly and in high yields. The selective hydrolysis of the terminal amide group of the dipeptides and further coupling with a proteinogenic amino acid ester can be achieved easily in the cases of the O- and S-containing heterocyclic amino acids. On the other hand, the acid catalyzed hydrolysis of the terminal amide group of the piperidine-containing dipeptide leads to the deprotection of the piperidine ring. Whereas the tripeptide with a tetrahydropyran residue can be selectively deprotected at the carboxylic acid and amino terminus, respectively, the hydrogenolytic deprotection of the amino group of the tripeptide with the sulfur-containing heterocycle is not possible. The goal of further studies is the evaluation of different protecting groups in the synthon **2a** for 4-aminopiperidine-4-carboxylate (**1a**) as well as for the used amino acids in coupling reactions with the tetrahydrothiopyran-containing synthon. Finally it has been shown that the presence of the heterocyclic α -amino acids **1b** and **1c** in the prepared tripeptides stabilize β -turn conformations as expected for α,α -disubstituted α -amino acids.

EXPERIMENTAL

General remarks. Melting points were determined using a Mettler FP5 apparatus, and they are

uncorrected. Thin layer chromatography (TLC): Merck silica gel 60 F₂₅₄ plates (0.25 mm); column chromatography (CC): silica gel Merck 60 (0.040–0.063 mm). The IR spectra were recorded on a Perkin-Elmer 297 or Perkin-Elmer 781 spectrophotometer in CDCl₃ or in KBr; absorptions in cm⁻¹. The ¹H- and ¹³C-NMR spectra were measured on a Bruker ARX-300, AM-400 or AMX-600 instrument (300/75.4, 400/100.6, and 600/150.9 MHz, resp.) in CDCl₃ with TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants *J* in Hz. Mass spectra (MS) were recorded on a Finnigan SSQ-700 (CI, NH₃, 150 eV) or Finnigan TSQ-700 (ESI) instrument. Optical rotations [α _D]: Zeiss LEP-A₂ polarimeter, in MeOH at 20–22 °C.

Starting materials. The synthesis of *tert*-butyl 2-[methyl(phenyl)amino]-1,6-diazaspiro[2.5]oct-1-ene-6-carboxylate (**2a**), *N*-methyl-*N*-phenyl-6-oxa-1-azaspiro[2.5]oct-1-en-2-amine (**2b**), and *N*-methyl-*N*-phenyl-6-thia-1-azaspiro[2.5]oct-1-en-2-amine (**2c**) has been described previously.^{14b} The amino acid derivatives *Z*-Phe-OH, *Z*-Val-OH, *H*-Phe-OEt, and *H*-Val-OMe as well as all used reagents were commercially available. Reported yields refer to isolated products.

Abbreviations. AcOEt, ethyl acetate; Boc, *tert*-butyloxycarbonyl; MeCN, acetonitrile; Phe, phenylalanine; Pip, 4-aminopiperidine-4-carboxylic acid; PyBOP, [(benzotriazol-1-yl)oxy]-tripyrrolidinophosphonium hexafluorophosphate; THF, tetrahydrofuran; Thp, 4-aminotetrahydropyran-4-carboxylic acid; Tht, 4-aminotetrahydrothiopyran-4-carboxylic acid; Val, valine; Z, benzyloxycarbonyl.

General procedure for the synthesis of dipeptide amides 7a–e (azirine coupling). To a solution of the corresponding azirine **2** in MeCN or THF at 0 °C, *Z*-Phe-OH or *Z*-Val-OH were added and the mixture stirred at rt for 16 h. Then, the solvent was evaporated and the residue was purified by column chromatography (CC).

Z-Phe-Pip(Boc)-*N*(Ph)Me (**7a**). The reaction of 500 mg (1.58 mmol) azirine **2a** and 473 mg (1.58 mmol) *Z*-Phe-OH in 5 mL MeCN followed by CC (hexane/AcOEt 2:1) gave 890 mg (82%) of **7a**. Colorless solid; mp 126–127 °C. IR (CHCl₃): 3420_w, 3060_w, 3020_w, 3000_m, 2980_w, 2930_w, 2860_w, 1685_s, 1645_m, 1595_m, 1495_s, 1455_m, 1430_m, 1390_w, 1370_m, 1285_m, 1260_m, 1250_m, 1145_m, 1130_w, 1075_w, 1050_w, 1030_w, 995_w, 860_w, 700_m. ¹H-NMR (CDCl₃): 7.37–7.02 (*m*, 15 arom. H, 1 NH); 5.45 (*d*, *J* = 6.7, 1 NH); 5.08, 5.01 (*2d*, *J*_{AB} = 12.1, PhCH₂O); 3.95–3.85 (*m*, HC(2) of Phe); 3.75–3.55 (*m*, 2 H_{eq} of –CH₂NCH₂–); 3.16 (*s*, MeN); 3.02–2.96 (*m*, 2 H_{ax} of –CH₂NCH₂–); 2.65–2.25 (*m*, CH₂ of Phe); 2.09–1.83 (*m*, –CH₂CCH₂–); 1.43 (*s*, Me₃C). ¹³C-NMR (CDCl₃): 171.2, 169.7 (*2s*, 2 C=O); 156.1, 154.5 (*2s*, 2 OCON); 144.6, 136.6, 136.0 (*3s*, 3 arom. C); 129.6, 129.2, 128.9, 128.6, 128.4, 128.1, 127.9, 127.4, 127.2 (*9d*, 15

arom. CH); 79.6 (*s*, Me₃C); 67.2 (*t*, PhCH₂O); 59.0 (*s*, C(4) of Pip); 55.7 (*d*, HC(2) of Phe); 41.3 (*q*, MeN); 39.2, 37.4 (2*t*, –CH₂NCH₂–, CH₂ of Phe); 32.6 (*t*, –CH₂CCH₂–); 28.4 (*q*, Me₃C). ESI-MS: 637 ([*M*+Na]⁺). [α^{21}_D] –3.2 (c 1.189). Anal. Calcd for C₃₅H₄₂N₄O₆ (614.74): C 68.38, H 6.89, N 9.16. Found: C 68.26, H 6.83, N 8.97.

Z-Phe-Thp-*N*(Ph)Me (**7b**). The reaction of 652 mg (3.015 mmol) azirine **2b** and 905 mg (3.023 mmol) *Z*-Phe-OH in 8 mL MeCN followed by CC (hexane/AcOEt 2:1) gave 1.243 g (80%) of **7b**. Colorless crystals; mp 172–173 °C. IR (CHCl₃): 3420*m*, 3000*m*, 2960*m*, 2860*m*, 1690*s*, 1640*s*, 1595*m*, 1495*s*, 1470*w*, 1450*m*, 1370*m*, 1290*m*, 1110*m*, 1075*m*, 1050*m*, 1030*m*, 1000*m*, 910*w*, 860*w*, 840*w*, 820*w*, 700*m*. ¹H-NMR (CDCl₃): 7.38–7.16, 7.06–7.03 (2*m*, 15 arom. H); 5.69 (*s*, 1 NH); 5.31 (*d*, *J* = 8.0, 1 NH); 5.10, 5.02 (2*d*, *J*_{AB} = 12.2, PhCH₂O); 4.02–3.94 (*m*, HC(2) of Phe); 3.67–3.55, 3.20–3.00 (2*m*, –CH₂OCH₂–); 3.17 (*s*, MeN); 3.00 (*d*, *J* = 7.2, CH₂ of Phe); 2.28–2.15, 1.82–1.77 (2*m*, –CH₂CCH₂–). ¹³C-NMR (CDCl₃): 171.8, 170.3 (2*s*, 2 C=O); 156.7 (*s*, OCON); 145.1, 137.2, 136.6 (3*s*, 3 arom. C); 130.1, 129.9, 129.4, 129.2, 128.9, 128.6, 128.4, 128.1, 127.8 (9*d*, 15 arom. CH); 67.8, 63.5, 63.4 (3*t*, PhCH₂O, –CH₂OCH₂–); 58.8 (*s*, C(4) of Thp); 56.4 (*d*, HC(2) of Phe); 41.8 (*q*, MeN); 37.9, 34.2, 33.9 (3*t*, CH₂ of Phe, –CH₂CCH₂–). ESI-MS: 538 ([*M*+Na]⁺). [α^{21}_D] –4.6 (c 1.060). Anal. Calcd for C₃₀H₃₃N₃O₅ (515.61): C 69.88, H 6.45, N 8.15. Found: C 69.63, H 6.36, N 8.07.

Z-Val-Thp-*N*(Ph)Me (**7c**). The reaction of 641 mg (2.964 mmol) azirine **2b** and 752 mg (2.993 mmol) *Z*-Val-OH in 8 mL MeCN followed by CC (hexane/AcOEt 2:1) gave 1.132 g (82%) of **7c**. Colorless crystals; mp 147–148 °C. IR (CHCl₃): 3420*m*, 3000*m*, 2960*m*, 2930*w*, 2860*w*, 1715*s*, 1685*s*, 1640*s*, 1590*m*, 1495*s*, 1465*m*, 1450*m*, 1430*w*, 1390*w*, 1370*m*, 1350*w*, 1290*m*, 1170*w*, 1150*w*, 1110*m*, 1075*w*, 1035*m*, 1025*m*, 1000*w*, 915*w*, 860*w*, 830*w*, 700*m*. ¹H-NMR (CDCl₃): 7.39–7.27, 7.13–7.11 (2*m*, 10 arom. H); 5.69 (*s*, 1 NH); 5.50 (*d*, *J* = 8.9, 1 NH); 5.18, 5.10 (2*d*, *J*_{AB} = 12.2, PhCH₂O); 3.80–3.67, 3.46–3.37 (2*m*, –CH₂OCH₂–, HC(2) of Val); 3.20 (*s*, MeN); 2.43–2.33, 2.22–1.96, 1.80–1.75 (3*m*, Me₂CH of Val, –CH₂CCH₂–); 0.93, 0.85 (2*d*, *J* = 6.8, Me₂CH of Val). ¹³C-NMR (CDCl₃): 171.1, 170.2 (2*s*, 2 C=O); 156.3 (*s*, OCON); 144.2, 136.2 (2*s*, 2 arom. C); 129.5, 128.5, 128.2, 127.9, 127.8, 127.6 (6*d*, 10 arom. CH); 67.0, 63.3, 62.8 (3*t*, PhCH₂O, –CH₂OCH₂–); 59.6 (*d*, HC(2) of Val); 58.1 (*s*, C(4) of Thp); 41.1 (*q*, MeN); 34.4, 32.7 (2*t*, –CH₂CCH₂–); 31.3 (*d*, Me₂CH of Val); 19.4, 17.1 (2*q*, Me₂CH). ESI-MS: 490 ([*M*+Na]⁺). Anal. Calcd for C₂₆H₃₃N₃O₅ (467.57): C 66.79, H 7.11, N 8.99. Found: C 66.87, H 7.32, N 8.83.

Z-Phe-Tht-*N*(Ph)Me (**7d**). The reaction of 406 mg (1.747 mmol) azirine **2c** and 525 mg (1.754 mmol) *Z*-Phe-OH in 5 mL THF followed by CC (hexane/AcOEt 2:1) gave 793 mg (85%) of **7d**. White crystals; mp 143–144 °C. IR (CHCl₃): 3420*m*, 3060*w*, 3000*m*, 2960*w*, 2920*w*, 1685*s*, 1640*s*, 1590*s*, 1490*s*, 1455*m*, 1445*m*, 1370*m*, 1280*m*, 1255*m*, 1130*m*, 1050*w*, 1030*w*, 975*w*, 910*w*, 820*w*, 700*s*. ¹H-NMR (CDCl₃): 7.36–7.05 (*m*, 15 arom. H); 5.55 (*s*, 1 NH); 5.30 (*d*, *J* = 7.9, 1 NH); 5.10, 5.04 (2*d*, *J*_{AB} = 12.5, PhCH₂O);

3.91–3.84 (*m*, HC(2) of Phe); 3.17 (*s*, MeN); 2.99 (*d*, $J = 6.8$, CH₂ of Phe); 2.45–2.17 (*m*, 4 CH₂). ¹³C-NMR (CDCl₃): 171.6, 169.7 (2*s*, 2 C=O); 156.2 (*s*, OCON); 144.8, 136.7, 136.0 (3*s*, 3 arom. C); 129.6, 129.2, 128.9, 128.6, 128.4, 128.1, 127.8, 127.3, 127.2 (9*d*, 15 arom. CH); 67.3 (*t*, PhCH₂O); 59.9 (*s*, C(4) of Tht); 55.7 (*d*, HC(2) of Phe); 41.5 (*q*, MeN); 37.2 (*t*, CH₂ of Phe); 34.1, 33.9 (2*t*, –CH₂CCH₂–); 23.0, 22.8 (2*t*, –CH₂SCH₂–). CI-MS: 532 (3, [M+1]⁺), 426 (23), 425 (100, [M–N(Ph)Me]⁺). [α²¹_D] –18.7 (c 1.000).

Z-Val-Tht-N(Ph)Me (7e). The reaction of 403 mg (1.734 mmol) azirine **2c** and 438 mg (1.743 mmol) *Z*-Val-OH in 5 mL THF followed by CC (hexane/AcOEt 2:1) gave 692 g (82%) of **7e**. White solid; mp 168–169 °C. IR (CHCl₃): 3410*m*, 3005*m*, 2980*m*, 2930*m*, 1685*s*, 1645*s*, 1595*m*, 1495*s*, 1370*m*, 1280*m*, 1090*w*, 1025*w*, 975*w*, 700*m*. ¹H-NMR (CDCl₃): 7.40–7.10 (*m*, 10 arom. H); 5.50 (*s*, 1 NH); 5.42 (*d*, $J = 8.8$, 1 NH); 5.18, 5.09 (2*d*, $J_{AB} = 12.3$, PhCH₂O); 3.63 (*dd*, $J = 8.8, 4.8$, HC(2) of Val); 3.20 (*s*, MeN); 2.62–2.50, 2.40–2.35, 2.24–2.20, 2.08–2.02 (4*m*, Me₂CH of Val, 4 CH₂); 0.94, 0.90 (2*d*, $J = 6.8$, Me₂CH of Val). ¹³C-NMR (CDCl₃): 171.4, 170.4 (2*s*, 2 C=O); 156.4 (*s*, OCON); 144.5, 136.2 (2*s*, 2 arom. C); 129.6, 128.6, 128.3, 128.1, 127.9, 127.5 (6*d*, 10 arom. CH); 67.2 (*t*, PhCH₂O); 59.8 (*d*, HC(2) of Val); 59.7 (*s*, C(4) of Tht); 41.4 (*q*, MeN); 35.0, 33.8 (2*t*, –CH₂CCH₂–); 31.1 (*d*, Me₂CH of Val); 23.7, 23.3 (2*t*, –CH₂SCH₂–); 19.6, 17.2 (2*q*, Me₂CH). CI-MS: 484 (2, [M+1]⁺), 378 (22), 377 (100, [M–N(Ph)Me]⁺). [α²¹_D] –24.6 (c 0.977).

General procedure for the hydrolysis of dipeptide amides 7a–e. To a solution or suspension of the corresponding dipeptide amide **7** in THF at 0 °C, the same volume of 6M HCl was added and the mixture was stirred at rt for 4 h. Then, the mixture was extracted with CH₂Cl₂, the organic layer was dried with MgSO₄ and the solvent evaporated in vacuo. The residue was purified by CC or crystallization.

Z-Phe-Pip-OH (8a). To a suspension of 1.124 g (1.828 mmol) **7a** in 6 mL THF at 0 °C was added 6M HCl (6 mL). The organic solvent was evaporated and the aqueous solution was cooled to 0 °C. Then, 5M NaOH was added until pH 13, the mixture was washed with Et₂O (2x) and neutralized by addition of 1M HCl. The precipitate was filtered and washed with water to give 630 mg (81%) of **8a**. Colorless powder; mp 240–243 °C. IR (CHCl₃): 3300*m*, 3220*w*, 3140*w*, 3060*w*, 3020*w*, 2960*w*, 2940*w*, 2800*w*, 1690*s*, 1680*s*, 1665*m*, 1585*m*, 1540*m*, 1505*m*, 1495*m*, 1465*m*, 1450*w*, 1435*w*, 1410*m*, 1390*m*, 1360*m*, 1350*m*, 1320*m*, 1290*m*, 1240*w*, 1220*w*, 1185*w*, 1140*m*, 1080*w*, 1055*m*, 1025*w*, 995*w*, 955*w*, 935*w*, 910*w*, 850*w*, 800*w*, 780*w*, 750*m*, 695*m*. ¹H-NMR (CF₃CO₂D): 7.43–7.26 (*m*, 10 arom. H); 5.20 (*s*, PhCH₂O); 4.85–4.70 (*m*, HC(2) of Phe); 3.70–3.05 (*m*, –CH₂NCH₂–, CH₂ of Phe); 2.80–2.25 (*m*, –CH₂CCH₂–). ¹³C-NMR (CF₃CO₂D): 176.1, 174.7 (2*s*, 2 C=O); 158.1 (*s*, OCON); 134.3, 131.1 (2*s*, 2 arom. C); 129.3, 128.8, 128.3, 128.1, 127.6, 127.4 (6*d*, 10 arom. CH); 68.7 (*t*, PhCH₂O); 55.8 (*s*, C(4) of Pip); 55.3 (*d*, HC(2) of Phe); 40.7, 37.3 (2*t*, –CH₂NCH₂–, CH₂ of Phe); 28.1 (*t*, –CH₂CCH₂–). ESI-MS: 448 (93, [M+Na]⁺), 426

(100, $[M+1]^+$).

Z-Phe-Thp-OH (**8b**). Treatment of 1.212 g (2.351 mmol) of **7b** in 8 mL THF with 6M HCl (6 mL) at 0 °C followed by CC (CH₂Cl₂/MeOH/AcOH 100:4:1) gave 872 mg (87%) of **8b**. Colorless crystals; mp 109 °C. IR (CHCl₃): 3300_w, 3030_m, 2960_m, 2860_m, 1720_m, 1670_s, 1540_s, 1455_m, 1445_m, 1390_m, 1290_m, 1250_s, 1145_m, 1105_m, 1050_m, 1030_m, 840_m, 740_m. ¹H-NMR (CD₃OD): 7.30–7.18 (*m*, 10 arom. H); 5.01 (*s*, PhCH₂O); 4.49–4.44 (*m*, HC(2) of Phe); 3.72–3.64, 3.54–3.47, 3.39–3.29, 3.12–3.05, 2.90–2.82 (*5m*, –CH₂OCH₂–, CH₂ of Phe); 2.10–1.88 (*m*, –CH₂CCH₂–). ¹³C-NMR (CD₃OD): 176.3, 173.8 (2_s, 2 C=O); 158.1 (*s*, OCON); 138.5, 138.2 (2_s, 2 arom. C); 130.5, 129.5, 129.45, 128.7, 127.8 (5_d, 10 arom. CH); 67.6, 64.4 (2_t, PhCH₂O, –CH₂OCH₂–); 57.7 (*s*, C(4) of Thp); 57.5 (*d*, HC(2) of Phe); 39.1, 33.4, 33.2 (3_t, CH₂ of Phe, –CH₂CCH₂–). ESI-MS: 449 ($[M+Na]^+$). $[\alpha^{21}_D]$ –2.4 (c 1.013). Anal. Calcd for C₂₃H₂₆N₂O₆ (426.47): C 64.78, H 6.14, N 6.57. Found: C 64.54, H 5.99, N 6.79.

Z-Val-Thp-OH (**8c**). Treatment of 1.089 g (2.329 mmol) of **7c** in 6 mL THF with 6M HCl (6 mL) at 0 °C followed by CC (CH₂Cl₂/MeOH/AcOH 100:4:1) gave 682 mg (77%) of **8c**. Colorless crystals; mp 85–86 °C. IR (KBr): 3300_w, 3060_m, 3030_m, 2960_m, 2870_m, 1710_s, 1670_s, 1540_s, 1470_m, 1455_m, 1445_m, 1430_w, 1390_m, 1290_m, 1250_s, 1145_m, 1105_m, 1030_m, 1000_w, 840_m. ¹H-NMR (CD₃OD): 7.35–7.26 (*m*, 5 arom. H); 5.12, 5.07 (2_d, *J*_{AB} = 12.3, PhCH₂O); 3.98 (*d*, *J* = 7.3, H(C2) of Val); 3.75–3.55 (*m*, –CH₂OCH₂–); 2.16–1.93 (*m*, Me₂CH of Val, –CH₂CCH₂–); 0.99, 0.55 (2_d, *J* = 6.8, Me₂CH of Val). ¹³C-NMR (CDCl₃): 174.9, 172.6 (2_s, 2 C=O); 157.0 (*s*, OCON); 136.7 (*s*, 1 arom. C); 128.0, 127.5, 127.3 (3_d, 5 arom. CH); 66.2, 63.0, 62.9 (3_t, PhCH₂O, –CH₂OCH₂–); 60.4 (*d*, HC(2) of Val); 56.0 (*s*, C(4) of Thp); 32.0, 31.6 (2_t, –CH₂CCH₂–); 30.5 (*d*, Me₂CH of Val); 18.2, 17.0 (2_q, Me₂CH). ESI-MS: 779 (100, $[2M+Na]^+$), 401 (58, $M+Na^+$). $[\alpha^{21}_D]$ –15.8 (c 0.943). Anal. Calcd for C₁₉H₂₆N₂O₆ (378.43): C 60.30, H 6.93, N 7.40. Found: C 60.45, H 7.12, N 7.20.

Z-Phe-Tht-OH (**8d**). Treatment of 822 mg (1.546 mmol) of **7d** in 8 mL THF with 6M HCl (8 mL) at 0 °C followed by crystallization from MeOH/Et₂O gave 643 mg (94%) of **8d**. Colorless crystals; mp 136–137 °C. IR (CHCl₃): 3420_m, 3320_m, 3060_m, 3005_m, 2960_m, 2920_m, 1715_s, 1510_s, 1455_m, 1445_m, 1430_m, 1280_m, 1240_m, 1145_w, 1110_w, 1080_w, 1050_w, 910_w, 700_s. ¹H-NMR (CD₃OD): 7.42–7.25 (*m*, 10 arom. H); 5.09 (*s*, PhCH₂O); 4.55 (*dd*, *J* = 9.0, 5.9, HC(2) of Phe); 3.22–3.14, 2.98–2.87, 2.78–2.65, 2.57–2.31, 2.25–2.13 (*5m*, CH₂ of Phe; 4 CH₂). ¹³C-NMR (CD₃OD): 174.1, 171.3 (2_s, 2 C=O); 155.7 (*s*, OCON); 136.0, 135.7 (2_s, 2 arom. C); 128.0, 127.0, 126.5, 126.3, 125.3 (5_d, 10 arom. CH); 65.2 (*t*, PhCH₂O); 56.9 (*s*, C(4) of Tht); 55.1 (*d*, HC(2) of Phe); 36.6 (*t*, CH₂ of Phe); 31.9, 31.8 (2_t, –CH₂CCH₂–); 21.7 (*t*, –CH₂SCH₂–). CI-MS: 460 (13, $[M+NH_4]^+$), 443 (42, $[M+1]^+$), 426 (24), 425 (100, $[M-OH]^+$). $[\alpha^{21}_D]$ –7.7 (c 1.004). Anal. Calcd for C₂₃H₂₆N₂O₅S (442.54): C 62.42, H 5.92, N 6.33, S 7.25. Found: C 62.17, H 6.08, N 6.00, S 6.95.

Z-Val-Tht-OH (**8e**). Treatment of 867 mg (1.793 mmol) of **7e** in 8 mL THF with 6M HCl (8 mL) at 0 °C

followed by crystallization from MeOH/Et₂O gave 565 mg (80%) of **8e**. Colorless crystals; mp 135–136 °C. IR (CHCl₃): 3420_w, 3320_w, 3020_w, 3000_w, 2960_m, 2920_m, 2870_m, 1715_s, 1510_s, 1470_w, 1455_w, 1445_w, 1430_w, 1390_w, 1370_w, 1280_m, 1090_w, 1025_w, 910_w, 700_m. ¹H-NMR (CDCl₃): 7.33–7.28 (*m*, 5 arom. H); 7.07 (*s*, 1 NH); 5.92 (*d*, *J* = 8.3, 1 NH); 5.10 (*s*, PhCH₂O); 4.05–4.00 (*m*, HC(2) of Val); 2.70–2.50, 2.34–2.07 (2*m*, Me₂CH of Val, 4 CH₂); 0.92, 0.88 (2*d*, *J* = 6.9, Me₂CH of Val). ¹³C-NMR (CDCl₃): 175.7, 172.0 (2*s*, 2 C=O); 157.0 (*s*, OCON); 136.0 (*s*, 1 arom. C); 128.4, 128.1, 127.8 (3*d*, 5 arom. CH); 67.2 (*t*, PhCH₂O); 60.4 (*d*, HC(2) of Val); 58.0 (*s*, C(4) of Tht); 33.3, 32.8 (2*t*, –CH₂CCH₂–); 31.5 (*d*, Me₂CH of Val); 23.3, 22.6 (2*t*, –CH₂SCH₂–); 19.2, 17.8 (2*q*, Me₂CH). CI-MS: 412 (40, [M+NH₄]⁺), 396 (21), 395 (100, [M+1]⁺), 377 (44, [M–OH]⁺). [α²¹_D] –9.54 (c 1.007).

General procedure for the coupling of 8b–e with an amino acid ester to give tripeptide esters 10. To a solution of the corresponding dipeptide **8** in CH₂Cl₂, 1.1 mol equivalent of Val-OMe.HCl (**9a**) or Phe-OEt.HCl (**9b**), respectively, 1 equivalent of the coupling reagent PyBOP, and 3 equivalents Et₃N were added at rt. The mixture was stirred at rt for 1–2 h, then, the solvent was evaporated in vacuo, and the residue was purified by CC.

Z-Phe-Thp-Val-OMe (**10a**). The reaction of 708 mg (1.660 mmol) of **8b**, 307 mg (1.831 mmol) **9a**, 868 mg (1.668 mmol) PyBOP, and 700 μL (5.0 mmol) Et₃N in 10 mL CH₂Cl₂ at rt, followed by CC (Et₂O/AcOEt 2:1) gave 864 mg (96%) of **10a**. Colorless crystals; mp 147–148 °C. IR (CHCl₃): 3420_m, 3000_m, 2960_m, 1735_s, 1680_s, 1510_s, 1470_m, 1455_m, 1390_w, 1370_m, 1350_m, 1300_w, 1240_m, 1180_w, 1155_m, 1110_m, 1080_w, 1050_m, 1030_m, 1000_w, 910_w. ¹H-NMR (CDCl₃): 7.41–7.20 (*m*, 10 arom. H); 6.40 (*s*, 1 NH); 5.49 (*d*, *J* = 7.3, 1 NH); 5.07 (*s*, PhCH₂O); 4.47–4.39 (*m*, HC(2) of Phe, HC(2) of Val); 3.73–3.59, 3.37–3.29, 3.21–3.00 (3*m*, –CH₂OCH₂–, CH₂ of Phe); 3.67 (*s*, MeO); 2.23–1.98, 1.73–1.68 (2*m*, –CH₂CCH₂–, Me₂CH of Val); 0.92, 0.88 (2*d*, *J* = 6.8, Me₂CH of Val). ¹³C-NMR (CDCl₃): 172.5, 172.2, 171.5 (3*s*, 3 C=O); 156.2 (*s*, OCON); 136.1, 135.8 (2*s*, 2 arom. C); 129.1, 128.8, 128.5, 128.2, 127.9, 127.2 (6*d*, 10 arom. CH); 67.2 (*t*, PhCH₂O); 63.0, 62.8 (2*t*, –CH₂OCH₂–); 57.9 (*s*, C(4) of Thp); 57.4, 56.8 (2*d*, HC(2) of Phe, HC(2) of Val); 51.9 (*s*, MeO); 37.7, 33.1, 31.5 (3*t*, CH₂ of Phe, –CH₂CCH₂–); 30.8 (*d*, Me₂CH of Val); 19.0, 17.7 (2*q*, Me₂CH of Val). ESI-MS: 562 ([M+Na]⁺). [α²¹_D] –7.9 (c 1.126). Anal. Calcd for C₂₉H₃₇N₃O₇ (539.63): C 64.55, H 6.91, N 7.72. Found: C 64.44, H 6.78, N 7.90.

Z-Val-Thp-Phe-OEt (**10b**). The reaction of 526 mg (1.390 mmol) of **8c**, 353 mg (1.537 mmol) **9b**, 727 mg (1.397 mmol) PyBOP, and 600 μL (4.3 mmol) Et₃N in 8 mL CH₂Cl₂ at rt, followed by CC (Et₂O/AcOEt 3:1) gave 711 mg (92%) of **10b**. Colorless crystals; mp 125–126 °C. IR (CHCl₃): 3420_m, 3300_m, 3000_m, 2970_m, 2940_m, 2860_m, 1730_s, 1680_s, 1510_s, 1470_m, 1455_m, 1390_m, 1350_m, 1290_m, 1160_m, 1110_m, 1080_w, 1030_m, 980_w, 910_w, 850_w, 825_w, 700_m. ¹H-NMR (CDCl₃): 7.42 (*d*, *J* = 6.6, 1 NH); 7.37–7.12 (*m*,

10 arom. H); 6.57 (*s*, 1 NH); 5.36 (*d*, $J = 7.6$, 1 NH); 5.09 (*s*, PhCH₂O); 4.75 (*q*, $J = 6.8$, HC(2) of Phe); 4.08 (*q*, $J = 7.2$, MeCH₂O); 3.91 (*t*, $J = 7.3$, H(C2) of Val); 3.75–3.64, 3.53–3.46 (*m*, –CH₂OCH₂–); 3.15–2.99 (*m*, CH₂ of Phe); 2.19–1.97, 1.83–1.79 (*m*, Me₂CH of Val, –CH₂CCH₂–); 1.17 (*t*, $J = 7.2$, MeCH₂O); 0.94, 0.90 (*2d*, $J = 6.8$, Me₂CH of Val). ¹³C-NMR (CDCl₃): 172.3, 171.9, 171.3 (3*s*, 3 C=O); 156.6 (*s*, OCON); 136.1, 135.8 (2*s*, 2 arom. C); 129.2, 128.5, 128.3, 128.2, 127.9, 126.8 (6*d*, 10 arom. CH); 67.2 (*t*, PhCH₂O); 63.1, 61.2 (2*t*, –CH₂OCH₂–, MeCH₂O); 61.0 (*d*, HC(2) of Phe); 57.7 (*s*, C(4) of Thp); 53.5 (*d*, HC(2) of Val); 37.9, 32.8, 31.9 (3*t*, CH₂ of Phe, –CH₂CCH₂–); 30.2 (*d*, Me₂CH of Val); 19.4, 17.5 (2*q*, Me₂CH of Val); 13.9 (*q*, MeCH₂O). ESI-MS: 576 (100, [M+Na]⁺), 554 (21, [M+1]⁺). [α²¹_D] –15.6 (c 0.996). Anal. Calcd for C₃₀H₃₉N₃O₇ (553.66): C 65.08, H 7.10, N 7.59. Found: C 65.05, H 7.23, N 7.69.

Z-Phe-Tht-Val-OMe (**10c**). The reaction of 446 mg (1.008 mmol) of **8d**, 187 mg (1.115 mmol) **9a**, 524 mg (1.008 mmol) PyBOP, and 420 μL (3.0 mmol) Et₃N in 2 mL CH₂Cl₂ at rt, followed by CC (hexane/AcOEt 1:1) gave 525 mg (94%) of **10c**. Colorless crystals; mp 160–161 °C. IR (CHCl₃): 3420*m*, 3005*m*, 2960*m*, 2930*m*, 2870*w*, 1740*s*, 1680*s*, 1510*s*, 1455*m*, 1440*m*, 1390*m*, 1370*w*, 1350*w*, 1310*m*, 1280*m*, 1255*m*, 1150*m*, 1080*w*, 1050*w*, 1030*w*, 1000*w*, 945*w*, 910*w*, 700*s*. ¹H-NMR (CDCl₃): 7.37–7.22 (*m*, 10 arom. H, 1 NH); 6.19 (*s*, 1 NH); 5.45 (*d*, $J = 6.9$, 1 NH); 5.11, 5.06 (2*d*, $J_{AB} = 12.2$, PhCH₂O); 4.48–4.35 (*m*, HC(2) of Phe, HC(2) of Val); 3.68 (*s*, MeO); 3.16–3.02 (*m*, CH₂ of Phe); 2.47–2.35, 2.25–2.12 (2*m*, 4 CH₂, Me₂CH of Val); 0.91, 0.88 (2*d*, $J = 6.8$, Me₂CH of Val). ¹³C-NMR (CDCl₃): 172.9, 172.3, 171.1 (3*s*, 3 C=O); 156.3 (*s*, OCON); 136.2, 135.8 (2*s*, 2 arom. C); 129.1, 128.9, 128.5, 128.3, 128.0, 127.3 (6*d*, 10 arom. CH); 67.3 (*t*, PhCH₂O); 59.5 (*s*, C(4) of Tht); 57.3, 56.9 (2*d*, HC(2) of Phe, HC(2) of Val); 51.9 (*q*, MeO); 37.6 (*t*, CH₂ of Phe); 33.7, 32.4 (2*t*, –CH₂CCH₂–); 30.9 (*d*, Me₂CH of Val); 23.0, 22.9 (2*t*, –CH₂SCH₂–); 19.0, 17.7 (2*q*, Me₂CH of Val). ESI-MS: 578 ([M+Na]⁺). [α²¹_D] –17.3 (c 1.013). Anal. Calcd for C₂₉H₃₇N₃O₆S (555.70): C 62.68, H 6.71, N 7.56, S 5.77. Found: C 62.68, H 6.80, N 7.56, S 5.70.

Z-Val-Tht-Phe-OEt (**10d**). The reaction of 497 mg (1.260 mmol) of **8e**, 319 mg (1.389 mmol) **9b**, 655 mg (1.260 mmol) PyBOP, and 520 μL (3.8 mmol) Et₃N in 8 mL CH₂Cl₂ at rt, followed by CC (hexane/AcOEt 1:1) gave 637 mg (89%) of **10d**. Colorless crystals; mp 157–158 °C. IR (CHCl₃): 3660*w*, 3420*m*, 3060*w*, 3000*m*, 2960*m*, 2930*m*, 2870*w*, 1730*s*, 1680*s*, 1510*s*, 1455*w*, 1445*w*, 1390*w*, 1375*w*, 1350*m*, 1280*m*, 1220*s*, 1130*m*, 1110*m*, 1095*m*, 1080*w*, 1030*m*, 950*w*, 860*w*, 700*s*. ¹H-NMR (CDCl₃): 7.37–7.12 (*m*, 10 arom. H, 1 NH); 6.44 (*s*, 1 NH); 5.34 (*d*, $J = 7.5$, 1 NH); 5.10 (*s*, PhCH₂O); 4.74 (*q*, $J = 6.7$, HC(2) of Phe); 4.08 (*q*, $J = 7.1$, MeCH₂O); 3.91 (*t*, $J = 7.3$, HC(2) of Val); 3.14–3.00 (*m*, CH₂ of Phe); 2.69–2.59, 2.50–2.39, 2.25–2.08 (3*m*, Me₂CH of Val, 4 CH₂); 1.17 (*t*, $J = 7.1$, MeCH₂O); 0.96, 0.91 (2*d*, $J = 6.8$, Me₂CH of Val). ¹³C-NMR (CDCl₃): 172.7, 171.6, 171.4 (3*s*, 3 C=O); 156.7 (*s*, OCON); 136.1, 135.8 (2*s*, 2 arom. C); 129.3, 128.5, 128.3, 128.2, 128.0, 126.8 (6*d*, 10 arom. CH); 67.3 (*t*,

PhCH₂O); 61.2 (*t*, MeCH₂O); 61.1 (*d*, HC(2) of Val); 59.3 (*s*, C(4) of Tht); 53.5 (*d*, HC(2) of Phe); 37.8 (*t*, PhCH₂); 33.5, 32.7 (*2t*, -CH₂CCH₂-); 30.0 (*d*, Me₂CH of Val); 23.2 (*t*, -CH₂SCH₂-); 19.5, 17.8 (*2q*, Me₂CH); 14.0 (*q*, MeCH₂O). ESI-MS: 592 ([*M*+Na]⁺). [α^{21}_D] +17.3 (c 1.024). Anal. Calcd for C₃₀H₃₉N₃O₆S (569.72): C 63.24, H 6.90, N 7.38, S 5.63. Found: C 63.06, H 7.13, N 7.35, S 5.79.

Reprotection of the piperidine N-atom of **8a**.

Z-Phe-Pip(*Z*)-OH (**11**). To a solution of 75 mg (0.176 mmol) **8a** in 2 mL 1M NaOH/dioxane at 0 °C were added 60 mg (0.352 mmol) benzyloxycarbonyl chloride (*Z*-Cl). The mixture was stirred at rt for 16 h, then 6M HCl was added until pH \approx 2. The resulting mixture was extracted with AcOEt (2x), the organic phase was dried (MgSO₄) and the solvent evaporated. Purification of the residue by CC (CH₂Cl₂/MeOH 10:1) gave 37 mg (38%) of **11** as colorless resin. ¹H-NMR ((D₆)DMSO): 7.99 (*s*, 1 NH); 7.49 (*d*, *J* = 8.5, 1 NH); 7.39–7.15 (*m*, 15 arom. H); 5.07 (*s*, PhCH₂O); 4.97, 4.92 (*2d*, *J*_{AB} = 12.8, PhCH₂O); 4.40–4.25 (*m*, HC(2) of Phe); 3.70–2.74 (*m*, -CH₂NCH₂-, CH₂ of Phe); 2.15–2.05, 1.80–1.65 (*2m*, -CH₂CCH₂-). ¹³C-NMR (CD₃OD): 171.2 (*s*, 2 C=O); 156.1, 154.8 (*2s*, 2 OCON); 138.5, 137.5, 137.3 (*3s*, 3 arom. C); 129.6, 128.7, 128.6, 128.3, 128.1, 128.0, 127.8, 126.5 (*8d*, 15 arom. CH); 66.3, 65.6 (*2t*, 2 PhCH₂O); 57.8 (*s*, C(4) of Pip); 56.8 (*d*, HC(2) of Phe); 40.4, 38.0 (*2t*, -CH₂NCH₂-, CH₂ of Phe); 33.1, 32.2 (*2t*, -CH₂CCH₂-). CI-MS: 1185 (100, [*2M*-2H+3Na]⁺), 604 (27, [*M*-H+2Na]⁺).

General procedure for the selective deprotection of the carboxylic group of the tripeptide esters **10a–d**.

The corresponding tripeptide **10** was dissolved in THF/MeOH/H₂O (2:1:1) and cooled to 0 °C. Then, LiOH.H₂O was added and the solution was stirred at rt for 2 h. After cooling to 0 °C, 2M HCl was added until pH \approx 1 and the mixture was extracted with CH₂Cl₂ (3x). The combined organic phase was washed with 1M HCl, dried over MgSO₄, and the solvent evaporated. Crystallization of the residue from CHCl₃/hexane or CH₂Cl₂/Et₂O gave the pure tripeptide acids **12**.

Z-Phe-Thp-Val-OH (**12a**). The saponification of 150 mg (0.278 mmol) **10a** in 4 mL THF/MeOH/H₂O and crystallization from CH₂Cl₂/Et₂O gave 133 mg (91%) of **12a**. Colorless crystals; mp 132–133 °C. IR (CHCl₃): 3420*m*, 3300*m*, 3000*m*, 2970*m*, 2930*m*, 2860*m*, 1715*s*, 1680*s*, 1515*s*, 1470*m*, 1455*m*, 1445*m*, 1390*m*, 1350*m*, 1300*m*, 1240*m*, 1150*m*, 1110*m*, 1080*w*, 1040*w*, 1030*w*, 890*m*, 700*m*. ¹H-NMR (CDCl₃): 7.38–7.18 (*m*, 10 arom. H); 6.62 (*s*, 1 NH); 5.80 (*brs*, 1 NH); 5.01 (*s*, PhCH₂O); 4.55–4.48 (*m*, HC(2) of Phe); 4.41 (*dd*, *J* = 8.2, 4.9, HC(2) of Val); 3.69–3.57, 3.34–3.26, 3.14–3.06 (*3m*, -CH₂OCH₂-, CH₂ of Phe); 2.23–2.04, 1.65–1.60 (*2m*, -CH₂CCH₂-, Me₂CH of Val); 0.95, 0.88 (*2d*, *J* = 6.8, Me₂CH of Val). ¹³C-NMR (CDCl₃): 174.4, 173.4, 172.3 (*3s*, 3 C=O); 156.5 (*s*, OCON); 136.2, 135.8 (*2s*, 2 arom. C); 129.2, 128.9, 128.6, 128.4, 127.9, 127.3 (*6d*, 10 arom. CH); 67.3 (*t*, PhCH₂O); 63.1, 63.0 (*2t*, -CH₂OCH₂-); 58.0 (*s*, C(4) of Thp); 57.8, 56.6 (*2d*, HC(2) of Phe, HC(2) of Val); 38.3, 33.2, 31.3 (*3t*,

CH₂ of Phe, –CH₂CCH₂–); 30.5 (*d*, Me₂CH of Val); 19.2, 17.7 (*2q*, Me₂CH of Val). ESI-MS: 548 ([*M*+1]⁺). [$\alpha^{21}\text{D}$] –5.8 (c 1.010). Anal. Calcd for C₂₈H₃₅N₃O₇ (525.60): C 63.99, H 6.71, N 7.99. Found: C 63.68, H 6.70, N 7.99.

Z-Val-Thp-Phe-OH (**12b**). The saponification of 105 mg (0.190 mmol) **10b** in 4 mL THF/MeOH/H₂O and crystallization from CHCl₃/hexane gave 91 mg (91%) of **12b**. Colorless crystals; mp 155–157 °C. IR (CHCl₃): 3300*m*, 3030*m*, 2960*m*, 2880*w*, 1730*s*, 1670*s*, 1530*m*, 1500*w*, 1420*m*, 1390*w*, 1350*m*, 1280*m*, 1230*m*, 1170*m*, 1105*m*, 1030*m*, 830*w*, 780*w*, 740*m*, 700*m*. ¹H-NMR (CD₃OD): 7.29–7.13 (*m*, 10 arom. H); 5.07, 5.03 (*2d*, *J*_{AB} = 12.4, PhCH₂O); 4.58 (*t*, *J* = 6.6, HC(2) of Phe); 3.92 (*d*, *J* = 7.4, H(C2) of Val); 3.74–3.52, 3.52–3.34 (*2m*, –CH₂OCH₂–); 3.14–2.99 (*m*, CH₂ of Phe); 2.15–1.97, 1.88–1.84 (*2m*, Me₂CH of Val, –CH₂CCH₂–); 0.98, 0.96 (*2d*, *J* = 6.6, Me₂CH of Val). ¹³C-NMR (CDCl₃): 174.9, 174.4, 174.3 (*3s*, 3 C=O); 158.6 (*s*, OCON); 138.1, 137.8 (*2s*, 2 arom. C); 130.3, 129.3, 129.2, 128.9, 128.7, 127.6 (*6d*, 10 arom. CH); 67.7 (*t*, PhCH₂O); 64.3, 64.1 (*2t*, –CH₂OCH₂–); 62.3 (*d*, HC(2) of Phe); 58.8 (*s*, C(4) of Thp); 55.2 (*d*, HC(2) of Val); 38.3, 34.0, 31.9 (*3t*, CH₂ of Phe, –CH₂CCH₂–); 31.4 (*d*, Me₂CH of Val); 20.0, 18.9 (*2q*, Me₂CH of Val). ESI-MS: 1117 (46, [2*M*–2H+3Na]⁺), 570 (32, [*M*–H+2Na]⁺), 548 (100, [*M*+Na]⁺). [$\alpha^{21}\text{D}$] –9.3 (c 1.009). Anal. Calcd for C₂₈H₃₅N₃O₇ (525.60): C 63.99, H 6.71, N 7.99. Found: C 63.91, H 6.91, N 8.03.

Z-Phe-Tht-Val-OH (**12c**). The saponification of 150 mg (0.270 mmol) **10c** in 4 mL THF/MeOH/H₂O and crystallization from CHCl₃/hexane gave 145 mg (99%) of **12c**. Colorless crystals; mp 102–103 °C. IR (KBr): 3390*m*, 3340*m*, 3140*m*, 3060*m*, 3015*m*, 2960*m*, 2930*m*, 1710*s*, 1695*s*, 1660*s*, 1650*s*, 1550*s*, 1540*s*, 1530*s*, 1505*m*, 1495*m*, 1450*m*, 1370*w*, 1360*m*, 1340*w*, 1310*m*, 1260*m*, 1240*m*, 1210*m*, 1155*m*, 1095*w*, 1070*w*, 1040*m*, 1030*w*, 1015*w*, 940*w*, 920*w*, 910*w*, 740*m*, 695*m*. ¹H-NMR (CDCl₃): 7.95 (*s*, 1 NH); 7.44 (*d*, *J* = 8.1, 1 NH); 7.35–7.20 (*m*, 10 arom. H, 1 NH); 5.08, 5.03 (*2d*, *J*_{AB} = 12.5, PhCH₂O); 4.52–4.46, 4.26–4.20 (*2m*, HC(2) of Phe, HC(2) of Val); 3.04–2.95 (*m*, CH₂ of Phe); 2.63–2.44, 2.33–1.98 (*2m*, 4 CH₂, Me₂CH of Val); 0.92, 0.86 (*2d*, *J* = 6.8, Me₂CH of Val). ¹³C-NMR (CD₃OD): 175.9, 174.7, 174.1 (*3s*, 3 C=O); 158.5 (*s*, OCON); 138.1, 138.0 (*2s*, 2 arom. C); 130.4, 129.7, 129.5, 129.0, 128.8, 128.1 (*6d*, 10 arom. CH); 67.7 (*t*, PhCH₂O); 61.1 (*s*, C(4) of Tht); 59.5, 58.0 (*2d*, HC(2) of Phe, HC(2) of Val); 51.9 (*q*, MeO); 38.6 (*t*, CH₂ of Phe); 35.8, 32.1 (*2t*, –CH₂CCH₂–); 31.7 (*d*, Me₂CH of Val); 23.9, 23.7 (*2t*, –CH₂SCH₂–); 19.6, 18.9 (*2q*, Me₂CH of Val). ESI-MS: 1106 (23, [2*M*+Na]⁺), 564 (100, [*M*+Na]⁺). [$\alpha^{21}\text{D}$] –13.1 (c 1.096). Anal. Calcd for C₂₈H₃₅N₃O₆S·H₂O (559.24): C 60.09, H 6.66, N 7.561. Found: C 59.96, H 6.53, N 7.47.

Z-Val-Tht-Phe-OH (**12d**). The saponification of 171 mg (0.300 mmol) **10d** in 4 mL THF/MeOH/H₂O and crystallization from CHCl₃/hexane gave 152 mg (94%) of **12d**. Colorless crystals; mp 192–194 °C. IR (KBr): 3420*s*, 3020*m*, 2960*m*, 2940*m*, 2920*m*, 2870*m*, 1730*s*, 1710*s*, 1695*s*, 1670*s*, 1650*s*, 1555*s*, 1530*s*, 1515*s*, 1505*s*, 1475*m*, 1450*m*, 1430*m*, 1390*m*, 1370*w*, 1350*m*, 1330*m*, 1280*s*, 1210*s*, 1160*m*, 1140*m*,

1120m, 1100m, 1080w, 1040m, 980w, 930w, 855m, 740m, 695m, 650m. ¹H-NMR (CD₃OD): 7.30–7.13 (m, 10 arom. H); 5.07 (s, PhCH₂O); 4.61–4.56, 3.96–3.92 (2m, HC(2) of Phe, HC(2) of Val); 3.15–2.98 (m, CH₂ of Phe); 2.84–2.71, 2.51–2.40, 2.26–1.97 (3m, Me₂CH of Val, 4 CH₂); 1.01, 0.96 (2d, *J* = 6.8, Me₂CH of Val). ¹³C-NMR (CDCl₃): 175.6, 174.5, 174.4 (3s, 3 C=O); 158.9 (s, OCON); 138.4, 138.0 (2s, 2 arom. C); 130.4, 129.5, 129.4, 129.0, 128.9, 127.7 (6d, 10 arom. CH); 67.8 (t, PhCH₂O); 62.7 (d, HC(2) of Phe); 60.9 (s, C(4) of Tht); 55.3 (d, HC(2) of Val); 38.4 (t, PhCH₂); 35.3, 33.2 (2t, –CH₂CCH₂–); 31.3 (d, Me₂CH of Val); 24.2, 23.9 (2t, –CH₂SCH₂–); 20.1, 19.0 (2q, Me₂CH). ESI-MS: 1106 (100, [2M+Na]⁺), 564 (88, [M+Na]⁺). [α²¹_D] –16.2 (c 1.077).

General procedure for the selective deprotection of the amino group of the tripeptide esters 10a,b.

To a solution of the corresponding tripeptide **10** in MeOH was added 0.1 mass-equiv. of Pd/C (10%). This mixture was stirred under an H₂-atmosphere at rt until disappearance of **10** (TLC). After filtration via Celite, the solvent of the filtrate was evaporated and the residue dried in HV.

H-Phe-Thp-Val-OMe (**13a**). Hydrogenolysis of 154 mg (0.285 mmol) of **10a** in 5 mL MeOH at rt gave 115 mg (99%) of **13a**. White crystals; mp 136–137 °C. IR (CHCl₃): 3420m, 3300s, 2960m, 1740s, 1675m, 1655s, 1540m, 1510m, 1470m, 1455m, 1440m, 1390m, 1370m, 1300m, 1255m, 1190m, 1150m, 1110m, 1075w, 1050w, 1030m, 1010w, 990w, 970w, 930w, 700m. ¹H-NMR (CDCl₃): 7.91 (d, *J* = 8.3, 1 NH); 7.79 (s, 1 NH); 7.37–7.23 (m, 5 arom. H); 4.47 (dd, *J* = 8.3, 5.1, HC(2) of Val); 3.83–3.75 (m, 2H of –CH₂OCH₂–); 3.72 (s, MeO); 3.64 (dd, *J* = 9.8, 3.8, HC(2) of Phe); 3.59–3.51 (m, 2 H of –CH₂OCH₂–); 3.32 (dd, *J* = 13.8, 3.8, 1 H of CH₂ of Phe); 2.72 (dd, *J* = 13.8, 9.8, 1 H of CH₂ of Phe); 2.34–2.16, 2.03–1.98 (2m, –CH₂CCH₂–, Me₂CH of Val); 0.96, 0.93 (2d, *J* = 6.9, Me₂CH of Val). ¹³C-NMR (CDCl₃): 175.5, 173.1, 172.3 (3s, 3 C=O); 137.5 (s, 1 arom. C); 129.3, 128.9, 127.1 (3d, 5 arom. CH); 66.44, 66.41 (2t, –CH₂OCH₂–); 57.6 (s, C(4) of Thp); 57.5, 56.8 (2d, HC(2) of Phe, HC(2) of Val); 52.0 (s, MeO); 40.7, 32.7, 32.5 (3t, CH₂ of Phe, –CH₂CCH₂–); 30.9 (d, Me₂CH of Val); 19.2, 17.8 (2q, Me₂CH of Val). CI-MS: 407 (20), 406 (100, [M+1]⁺). [α²¹_D] +17.3 (c 1.000). Anal. Calcd for C₂₁H₃₁N₃O₅ (405.49): C 62.20, H 7.71, N 10.36. Found: C 62.21, H 7.74, N 10.43.

H-Val-Thp-Phe-OEt (**13b**). Hydrogenolysis of 102 mg (0.184 mmol) of **10b** in 2 mL MeOH at rt gave 72 mg (93%) of **13b**. White foam. IR (CHCl₃): 3420w, 3300m, 3000m, 2970m, 2860m, 1740s, 1675s, 1510s, 1470m, 1455m, 1445m, 1390m, 1375m, 1350m, 1300m, 1240m, 1160m, 1110m, 1080m, 1030m, 860w, 820w, 700m. ¹H-NMR (CDCl₃): 7.87 (d, *J* = 7.4, 1 NH); 7.76 (s, 1 NH); 7.29–7.15 (m, 5 arom. H); 4.47 (q, *J* = 7.5, HC(2) of Phe); 4.14 (q, *J* = 7.1, MeCH₂O); 3.82–3.67, 3.62–3.48, 3.22–3.14, 3.05–2.97 (4m, –CH₂OCH₂–, HC(2) of Val, CH₂ of Phe); 2.36–2.21, 2.16–1.91 (2m, Me₂CH of Val, –CH₂CCH₂–); 1.22 (t, *J* = 7.1, MeCH₂O); 0.99, 0.81 (2d, *J* = 6.9, Me₂CH of Val). ¹³C-NMR (CDCl₃): 175.4, 172.8, 171.4 (3s, 3 C=O); 136.3 (s, 1 arom. C); 129.3, 128.2, 126.7 (3d, 5 arom. CH); 63.4, 63.3 (2t, –CH₂OCH₂–); 61.2 (t,

MeCH₂O); 61.1 (*d*, HC(2) of Phe); 57.2 (*s*, C(4) of Thp); 53.5 (*d*, HC(2) of Val); 37.8, 32.6, 32.2 (3*t*, CH₂ of Phe, -CH₂CCH₂-); 30.3 (*d*, Me₂CH of Val); 19.7, 15.8 (2*q*, Me₂CH of Val); 14.0 (*q*, MeCH₂O). CI-MS: 421 (23), 406 (100, [M+1]⁺). [α^{21}_D] -2.1 (c 1.026). Anal. Calcd for C₂₂H₃₃N₃O₅ (419.52): C 62.99, H 7.93, N 10.02. Found: C 62.41, H 7.64, N 9.74.

X-Ray Crystal Structure Determination of 10a, 10c and 12a.¹⁹ All measurements were made on a Rigaku AFC5R diffractometer using graphite-monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$) and a 12 kW rotating anode generator.²⁰ The intensities of three standard reflections were measured after every 150 reflections and remained stable throughout each data collection. The intensities were corrected for Lorentz and polarization effects.²¹ Azimuthal scans of several reflections indicated no need for an absorption correction. Equivalent reflections, other than Friedel pairs, were merged. The data collection and refinement parameters are given below and views of the molecules are shown in Figures 2 and 4. Each structure was solved by direct methods using SHELXS-86,²² which revealed the positions of all non-hydrogen atoms. The asymmetric unit of **12a** contains one water molecule in addition to the peptide molecule. The non-hydrogen atoms were refined anisotropically. All of the amide, hydroxy and water H-atoms were placed in the positions indicated by difference electron density maps and their positions were allowed to refine together with individual isotropic displacement parameters. All remaining H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 U_{eq} of its parent C-atom (1.5 U_{eq} for the methyl groups). The refinement of each structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function $\sum w(F_o^2 - F_c^2)^2$. Corrections for secondary extinction were not applied. For **10c**, refinement of the absolute structure parameter²³ yielded a value of -0.06(6), which confidently confirms that the refined model corresponds with the true enantiomorph. For the other structures, the absence of significant anomalous scattering elements meant that the precision of the absolute structure parameter was too low to be indicative; the enantiomer used in the refinement was based on the configuration expected from the chemical synthesis. Neutral atom scattering factors for non-H-atoms were taken from ref.²⁴, and the scattering factors for H-atoms were taken from ref.²⁵ Anomalous dispersion effects were included in F_c ; the values for f' and f'' were those of ref.²⁷ The values of the mass attenuation coefficients are those of ref.²⁸ All calculations were performed using the SHELXL-2017²⁹ program.

Crystal data for **10a**: C₂₉H₃₇N₃O₇, $M = 539.61$, crystallized from acetonitrile, colorless, prism, crystal dimensions 0.15 \times 0.18 \times 0.43 mm, monoclinic, space group $P2_1$, $Z = 2$, reflections for cell determination 25, 2θ range for cell determination 23–33°, $a = 6.137(3) \text{ \AA}$, $b = 25.640(2) \text{ \AA}$, $c = 9.561(2) \text{ \AA}$, $\beta = 107.92(3)^\circ$, $V = 1431.4(9) \text{ \AA}^3$, $T = 173(1) \text{ K}$, $D_x = 1.252 \text{ g}\cdot\text{cm}^{-3}$, $\mu(\text{MoK}\alpha) = 0.090 \text{ mm}^{-1}$, scan type ω ,

$2\theta_{(\max)} = 60^\circ$, total reflections measured 4633, symmetry independent reflections 4269, reflections with $I > 2\sigma(I)$ 3235, reflections used in refinement 4269, parameters refined 367, restraints 1, $R(F)$ [$I > 2\sigma(I)$ reflections] = 0.0453, $wR(F^2)$ [all data] = 0.1283 ($w = [\sigma^2(F_o^2) + (0.064P)^2 + 0.0064P]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$), goodness of fit 1.039, final Δ_{\max}/σ 0.000, $\Delta\rho$ (max; min) = 0.25; $-0.25 \text{ e } \text{\AA}^{-3}$.

Crystal data for **10c**: $\text{C}_{29}\text{H}_{37}\text{N}_3\text{O}_6\text{S}$, $M = 555.67$, crystallized from acetonitrile, colorless, prism, crystal dimensions $0.30 \times 0.30 \times 0.45 \text{ mm}$, triclinic, space group $P1$, $Z = 1$, reflections for cell determination 25, 2θ range for cell determination $39\text{--}40^\circ$, $a = 6.1404(10) \text{ \AA}$, $b = 9.529(2) \text{ \AA}$, $c = 13.951(3) \text{ \AA}$, $\alpha = 71.994(15)^\circ$, $\beta = 89.050(18)^\circ$, $\gamma = 73.43(2)^\circ$, $V = 741.8(3) \text{ \AA}^3$, $T = 273(1) \text{ K}$ (lower temperatures damaged the crystals), $D_X = 1.244 \text{ g}\cdot\text{cm}^{-3}$, $\mu(\text{MoK}\alpha) = 0.154 \text{ mm}^{-1}$, scan type $\omega/2\theta$, $2\theta_{(\max)} = 55^\circ$, total reflections measured 7195, symmetry independent reflections 6784, reflections with $I > 2\sigma(I)$ 5398, reflections used in refinement 6784, parameters refined 367, restraints 3, $R(F)$ [$I > 2\sigma(I)$ reflections] = 0.0553, $wR(F^2)$ [all data] = 0.1634 ($w = [\sigma^2(F_o^2) + (0.1092P)^2]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$), goodness of fit 1.039, final Δ_{\max}/σ 0.002, $\Delta\rho$ (max; min) = 0.39; $-0.37 \text{ e } \text{\AA}^{-3}$.

Crystal data for **12a**: $\text{C}_{28}\text{H}_{35}\text{N}_3\text{O}_7\cdot\text{H}_2\text{O}$, $M = 543.60$, crystallized from chloroform/hexane, colorless, plate, crystal dimensions $0.15 \times 0.42 \times 0.50 \text{ mm}$, monoclinic, space group $P2_1$, $Z = 2$, reflections for cell determination 25, 2θ range for cell determination $37\text{--}40^\circ$, $a = 6.159(4) \text{ \AA}$, $b = 25.829(3) \text{ \AA}$, $c = 9.502(3) \text{ \AA}$, $\beta = 108.26(3)^\circ$, $V = 1435.6(10) \text{ \AA}^3$, $T = 173(1) \text{ K}$, $D_X = 1.258 \text{ g}\cdot\text{cm}^{-3}$, $\mu(\text{MoK}\alpha) = 0.092 \text{ mm}^{-1}$, scan type ω , $2\theta_{(\max)} = 55^\circ$, total reflections measured 3566, symmetry independent reflections 3363, reflections with $I > 2\sigma(I)$ 3070, reflections used in refinement 3363, parameters refined 378, restraints 1, $R(F)$ [$I > 2\sigma(I)$ reflections] = 0.0336, $wR(F^2)$ [all data] = 0.0845 ($w = [\sigma^2(F_o^2) + (0.0392P)^2 + 0.2482P]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$), goodness of fit 1.017, final Δ_{\max}/σ 0.000, $\Delta\rho$ (max; min) = 0.18; $-0.18 \text{ e } \text{\AA}^{-3}$.

ACKNOWLEDGEMENTS

We thank the *Swiss National Science Foundation*, the *Stiftung für wissenschaftliche Forschung an der Universität Zürich*, and *F. Hoffmann-La Roche AG, Basel*, for financial support

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