Probing the Proteolytic Stability of β-Peptides Containing α-Fluoro- and α-Hydroxy-β-Amino Acids

David F. Hook, François Gessier, Christian Noti, Peter Kast, and Dieter Seebach*[a]

One of the benefits of β-peptides as potential candidates for biological applications is their stability against common peptidases. Attempts have been made to rationalize this stability by altering the electron availability of a given amide carbonyl bond through the introduction of polar substituents at the α-position of a single β-amino acid. Such β-amino acids (β-homoglycine, β-homoalanine), containing one or two fluorine atoms or a hydroxy group in the α-position, were prepared in enantiopure form. A versatile method for preparing these α-fluoro-β-amino acids by the homologation of appropriate α-amino acids and C-OH→C-F or C=O→CF_2 substitution with DAST, is described. Consequently, a series of β-peptides possessing an electronically modified residue at the N terminus or embedded within the chain was synthesized, and their proteolytic stability was investigated against a selection of enzymes. All ten β-peptides tested were resilient to proteolysis. Introducing a polar, sterically undemanding group, into the α-position of β-amino acids in a β-peptide chain does not appear to facilitate localized or general enzymatic degradation.

Introduction

The innate ability of β-peptides to adopt secondary structures analogous to their α-peptidic counterparts has encouraged investigations into their potential use as pharmaceutical or agricultural agents. Studies have shown that these homologated α-peptides possess antimicrobial, hemolytic, and antiproliferative activities and are metabolically stable in vivo.[1–8] Moreover, they exhibit remarkable proteolytic stability under conditions under which α-peptides are completely degraded. With the exception of recent proteolytic stability studies performed by Gellman and co-workers on an amphiphilic β-peptide,[4] the most comprehensive studies on β-peptide (and γ-peptide) stability have emerged from our laboratories.[6,9–13] In no case was any degradation observed after 48 h against a range of enzymes, with the substrates apparently exhibiting no inhibitory effect.[14] In addition, the incorporation of a β-amino acid moiety into an α-peptide sequence can beneficially enhance proteolytic stability about the modified residue whilst still retaining significant original biological activity.[15–18] Conversely, the presence of an α-amino acid residue in a β-peptide chain need not impact on proteolytic stability or biological activity.[12]The insertion of a methylene group into an α-amino acid has a clear structural impact; β-peptide chains exhibit additional degrees of conformational freedom as compared to their α-peptidic counterparts. Altered steric arrangements of functional groups and other recognition features of the β-peptidic backbone might, as a result, prevent productive interactions with the relevant sites on a peptidase. Such factors can clearly impede the recognition of β-peptides by peptidases and account for their hydrolytic stability. However, such structural modifications also affect the electronic environment of the amide bonds. In the case of β^3-peptides that lack a substituent in the 2-position of an amino acid, reduced steric hindrance about a given amide carbonyl bond, as compared to α-analogues, is paralleled by a lower reactivity, created by the absence of an adjacent electronegative group (Figure 1). The enhancement of amide carbonyl bond reactivity by a neighboring, sterically undemanding polar group, and the subsequent impact on proteolytic stability are, in part, the focus of this work.

Replacement of hydrogen atoms by fluorine alters the neighboring environment electronically without providing steric constraints. Such modifications can beneficially influence the biological activity and structure of compounds.[19] Thus, it is envisaged that the introduction of a β-amino acid bearing one or two fluorine atoms in the 2-position into a β-peptide chain will enhance the reactivity of the adjacent amide carbonyl bond (Figure 1). Moreover, the introduction of a hydroxyl functionality in the 2-position might provide structural benefits created through hydrogen-bonding effects, again without forcing any severe steric demands. Such α-hydroxy-β-amino acids are present in a variety of biologically active compounds, including protease inhibitors.[20] Ultimately, the presence of an electron-withdrawing group in the 2-position of a β-amino acid within a β-peptide chain could afford bond-specific enzymatic degradation and permit the tuning of β-peptide stability.

[a] *Dr. D. F. Hook, Dr. F. Gessier, Dipl.-Chem. C. Noti, Dr. P. Kast, Prof. Dr. D. Seebach*
Laboratorium für Organische Chemie
Eidgenössischen Technischen Hochschule
Wolfgang-Pauli-Strasse 10, 8093 Zürich (Switzerland)
Fax: (+41) 1-632-11-44
E-mail: seebach@org.chem.ethz.ch

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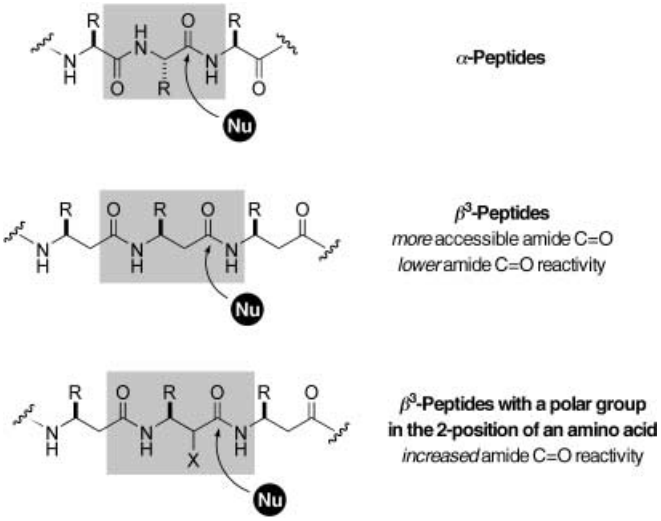


Figure 1. *Structural and electronic consequences of homologating α-peptides. (X = F, 2F, OH; R = any proteinogenic side chain). In the case of α-peptides, an adjacent R group is capable of restricting nucleophile access to the amide C=O bond. In addition, the NHCO of the neighboring residue enhances amide C=O reactivity as compared to an isolated, analogous bond.*

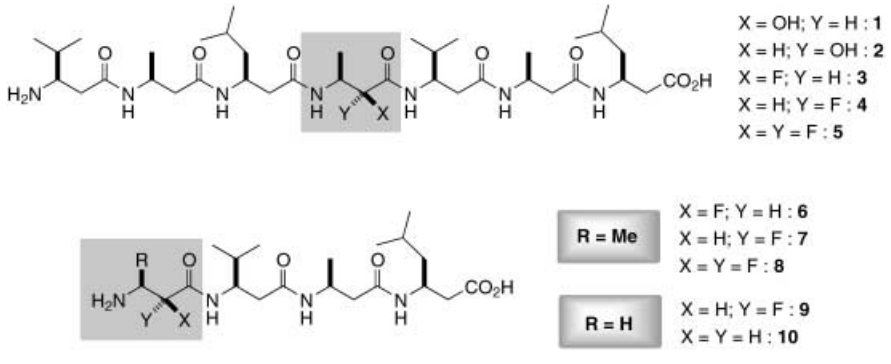
Choice of substrates

β-Peptides **1**–**10**, which possess an internal or an N-terminal β-amino acid moiety substituted with one or two polar groups in the 2-position, were prepared to screen stability toward endo- and exopeptidases, respectively. β-Peptides containing an α-fluoro or an α-hydroxyl β-amino acid of either configuration at the 2-position were required. Such compounds have already supplemented our ongoing studies on the secondary structures of β-peptides.[21]

Choice of enzymes

Clearly, the specificity of a given peptidase for a particular amide carbonyl-bond environment was determined from studies on α-peptides; however, this might have no direct correlation to β-peptides. Consequently, enzymes representing a broad range of residue specificities were utilized and are outlined in the following paragraph.[22]

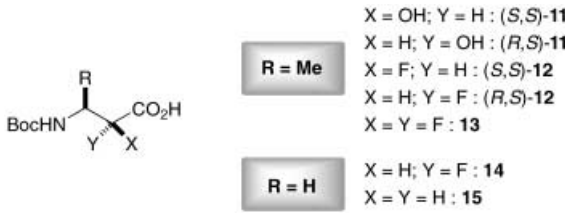
α-*Chymotrypsin* (EC 3.4.21.1) is a serine endopeptidase from bovine pancreas and shows specificity for amide bonds with aromatic or bulky hydrophobic residues in the so-called P1 position.[23,24] *Leucine aminopeptidase*, cytosol, (EC 3.4.11.1) from hog kidney is an N-terminal exopeptidase that cleaves a range of N-terminal residues.[25,26] *Pancreatic elastase* (EC 3.4.21.36) from hog pancreas is a serine endopeptidase that preferentially cleaves bonds adjacent to neutral residues, especially Ala.[27] *Pepsin* (EC 3.4.23.1) is an aspartic endopeptidase from hog stomach. It exhibits a preference for aromatic or bulky hydrophobic residues in the P1 and P1′ positions.[28] *Pronase* (EC 3.4.24.4) is a group of at least ten proteolytic enzymes obtained from the culture supernatant of *Streptomyces griseus* K-1. A mixture of endo- and exopeptidases, it has "broad" (in reality low) specificity and cleaves nearly all peptide bonds, including those adjacent to D-amino acids.[29] *Proteinase K* (EC 3.4.21.64) is a serine endopeptidase produced by the fungus *Tritirachium album* limber. It exhibits "broad" specificity but shows some preference for uncharged aliphatic or aromatic residues in the P1 and P1′ positions, especially Ala.[30] *Subtilisin Carlsberg* (EC 3.4.21.62) from *Bacillus subtilis var. biotecus A* is a serine endopeptidase with "broad" specificity. It nevertheless shows a preference for large, uncharged residues in the P1 position.[31] *Trypsin* (EC 3.4.21.4), from hog pancreas, is a serine endopeptidase that favors Arg or Lys in the P1 position.[32,33]



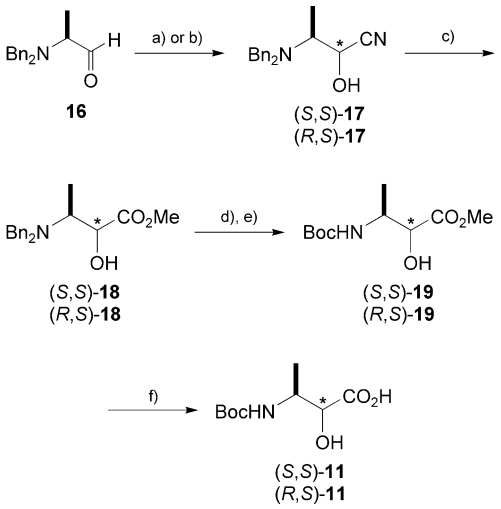
Results

Preparation of *N*-protected *N*-Boc-β-amino acids 11–14

Except for commercially available *N*-Boc-β-homoglycine (**15**), which is commonly referred to as *N*-Boc-β-alanine, all other β-amino acids (Scheme 1) had to be prepared. As outlined in a previous communication,[21] preparation of β-amino acids (*S*,*S*)-**11** and (*R*,*S*)-**11** started with aldehyde **16**, obtained in three steps from L-alanine (Scheme 2).[34,35] Treatment with trimethylsilylcyanide in the presence of an appropriate Lewis acid provided access to diastereoisomeric cyanohydrins (*S*,*S*)-**17** and (*R*,*S*)-**17** in high selectivity.[36] Acid hydrolysis in the presence of methanol afforded the key 2-hydroxy esters (*S*,*S*)-**18** and (*R*,*S*)-**18**, with subsequent protecting-group manipulations realizing



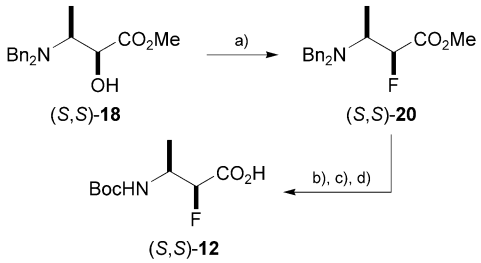
Scheme 1. *Formulae of α-heterosubstituted* N*-Boc-β-amino acids **11**–**15** used for the preparation of β-peptides **1**–**10**.*



Scheme 2. *Preparation of the* N*-Boc-protected carboxylic acids (*S,S*)-**11** and (*R,S*)-**11**. a) Me_3SiCN, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −10 °C, 38% for (S,S)-**11**; b) Me_3SiCN, $TiCl_4$, CH_2Cl_2, −78 °C, 23% for (R,S)-**11**; c) HCl/MeOH, RT, 12 h, 76% for (S,S)-**18** and 88% for (R,S)-**18**; d) H_2, Pd/C, TFA, MeOH, RT, 24 h; e) Boc_2O, Et_3N, MeOH, RT, 12 h, 72% for (S,S)-**19** and 92% for (R,S)-**19** over 2 steps; f) $LiOH{\cdot}H_2O$, EtOH/H_2O, RT, 1 h, 65% for (S,S)-**11** and 94% for (R,S)-**11**.*

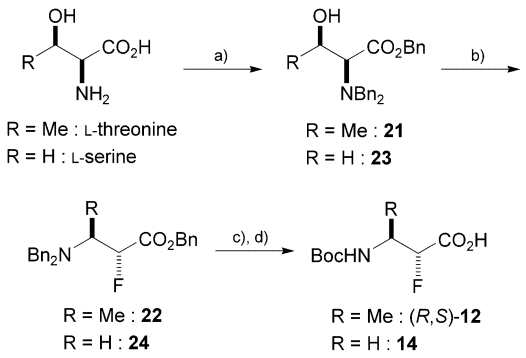
α-hydroxy esters (*S,S*)-**19** and (*R,S*)-**19**. Saponification furnished the desired *N*-Boc-α-hydroxy-β-amino acids (*S,S*)-**11** and (*R,S*)-**11**.

One of the most useful and effective methods for the preparation of organofluorine compounds involves the treatment of alcohols with (diethylamino)sulfur trifluoride (DAST).[37,38] Exposure of the hydroxy ester (*S,S*)-**18** to (diethylamino)sulfur trifluoride (DAST) afforded fluoro derivative (*S,S*)-**20** with overall retention of configuration at C-2 (Scheme 3); fluoride ring-



Scheme 3. *Preparation of the* N*-Boc-protected (2S,3S)-3-amino-2-fluorobutanoic acid [(S,S)-**12**]. a) DAST, 0 °C, CH_2Cl_2, 3 h, 85%; b) $LiOH{\cdot}H_2O$, EtOH/H_2O, RT, 1 h; c) H_2, Pd/C, MeOH, RT, 24 h; d) Boc_2O, Et_3N, MeOH, RT, 12 h, 87% over 3 steps.*

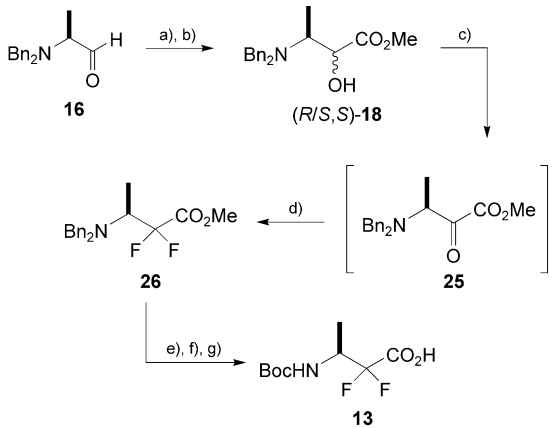
opening of the intermediate aziridinium ion occurring with good regio- and stereoselectivity (*de* 10:1).[39,40] Established transformations furnished the required α-fluoro-β-amino acid (*S,S*)-**12**. Attempts to apply an analogous protocol to the preparation of (*R,S*)-**12** from (*R,S*)-**18** were not successful; an equimolar mixture of regioisomers was obtained, resulting from nonselective aziridinium ring opening by fluoride. Consequently, a revised approach toward (*R,S*)-**12** was adopted, starting from L-threonine (Scheme 4).[39] Treatment with benzyl bromide and base afforded alcohol **21**, from which, on addition of



Scheme 4. *Preparation of the* N*-Boc-protected carboxylic acids (*R,S*)-**12** and **14**. a) K_2CO_3, NaOH, BnBr, H_2O, reflux, 1 h, 41% for **21** and 43% for **23**; b) DAST, RT, THF, 30 min, 60% for **22** and 90% for **24**; c) H_2, Pd/C, MeOH, RT, 24 h; d) Boc_2O, Et_3N, MeOH, RT, 12 h, 65% for (R,S)-**12** and 93% for **14** over 2 steps.*

DAST, the expected 2-fluorinated ester **22** was produced. "Global" benzyl deprotection and Boc protection of the free amino group provided (*R,S*)-**12**. *N*-Boc-α-fluoro-β-homoglycine (**14**) was prepared in an analogous manner from L-serine. Benzyl protection afforded hydroxy ester **23**, permitting a DAST-mediated OH→F substitution to yield **24**, which was subsequently converted to the desired product **14**.[41,42]

Several asymmetric syntheses aimed at preparing 2,2-difluoro-3-amino acids based on the Reformatsky reaction have been published.[43–45] However, our approach to compounds of this type has utilized the homologation–DAST methodology outlined for the monofluorinated β-amino acids. As such, nonstereospecific addition of cyanide to aldehyde **16** and subsequent hydrolysis with methanolic HCl afforded **18** as a mixture of epimers (Scheme 5). Swern oxidation to the α-ketoester **25** proceeded without racemization if chromatographic purification and long periods of storage were avoided. Treatment of the crude product **25** with DAST furnished the 2,2-difluoroester **26** in 94% enantiopurity. Subsequent protecting-group manipulations provided the desired *N*-Boc-β-amino acid **13**.



Scheme 5. *Preparation of the* N*-Boc-protected (3S)-3-amino-2,2-difluorobutanoic acid (**13**). a) acetone cyanohydrin, KCN cat., Bu_4NI cat., hexane/H_2O, RT, 2 h; b) HCl/MeOH, RT, 12 h, 80% over 2 steps; c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 90 min; d) DAST, CH_2Cl_2, RT, 4 h, 81% over 2 steps; e) $LiOH{\cdot}H_2O$, EtOH/H_2O, RT, 1 h; f) H_2, Pd/C, MeOH, RT, 24 h; g) Boc_2O, Et_3N, MeOH, RT, 12 h, 80% over 3 steps.*

Synthesis of β-peptides 1–10

Boc deprotection of the known tripeptide **27**[46] preceded a HATU-mediated coupling involving the acids **11**–**15**, which gave the terminally protected tetrapeptides **28**–**34** (Scheme 6). Removal of the *N*- and C-terminal protecting groups and purification by reversed-phase HPLC produced β-peptides **6**–**10** as their TFA salts in useful amounts and in excellent purity. An analogous peptide coupling involving tripeptide acid **35**[9] with Boc-deprotected tetrapeptides **28**–**32** furnished fully protected heptapeptides **36**–**40**. Final Boc and benzyl deprotection afforded, after purification, the required β-peptides **1**–**5** for screening against endopeptidic stability.

Proteolytic degradation studies

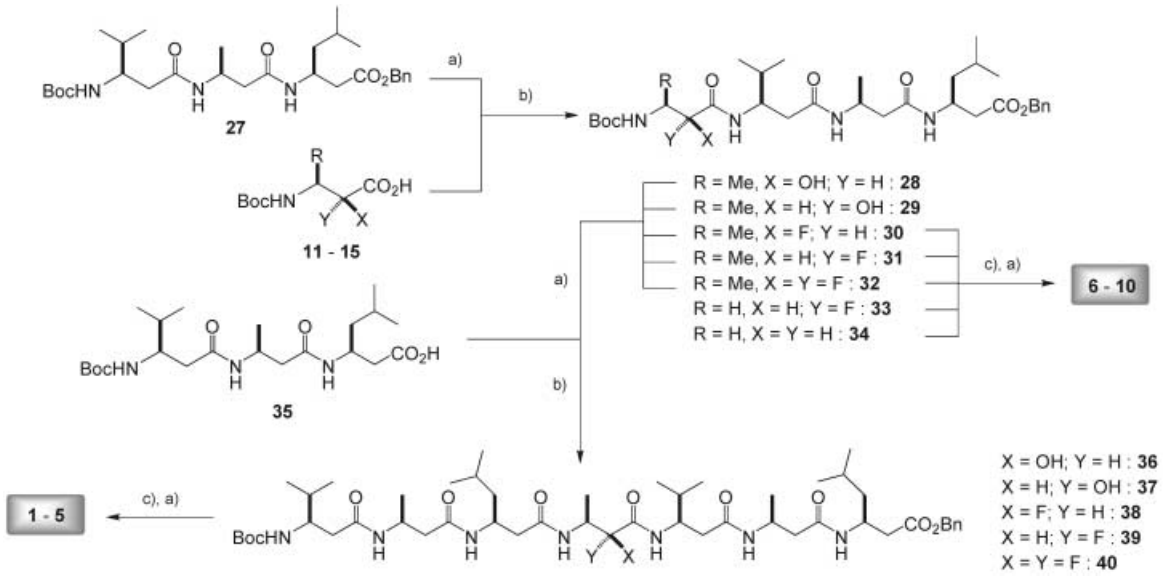
The proteolytic activity of the chosen enzymes was verified prior to each degradation experiment. The α-peptide H-(Val-Ala-Leu)$_2$-OH (**41**) was selected as a test substrate;[9] its sequence generally mimicking the β-peptides to be tested. With the exception of trypsin, complete degradation of α-peptide **41** was observed within 1 hour at an enzyme concentration lower than that to which the β-peptide substrates were subsequently exposed (Table 1, Figure 2). Pronase (EC 3.4.24.4) is known to contain five serine-type proteases, two Zn^{2+} endopeptidases, two Zn^{2+} leucine aminopeptidases, and one Zn^{2+} carboxypeptidase.[29] As such there is no one pH optimum; in this study hydrolysis assays involving pronase were performed at pH 7.8 and pH 9.0.

Internally substituted β-peptides **1**–**5** were incubated in the presence of peptidases at 37 °C in an appropriately composed buffer solution for five days. These proteolytic stability assays were terminated by the addition of acetic acid, with the exception of pepsin (addition of pH 11 buffer solution) and analyzed by reversed-phase HPLC. Prior to quenching, an aliquot was removed from each reaction mixture, and α-peptide **41** added. With the known exception of trypsin, residual enzyme activity was exhibited in all cases, characterized by the disappearance of **41**. The results are expressed in Table 1 and by the representative HPLC chromatograms (Figure 2). It is clear that, whereas the α-peptide is degraded (with the exception of the reaction with trypsin), the β-peptides were intact after the allotted time, with no evidence to suggest degradation. Minor peaks (due to inherent enzyme impurities or the products of enzyme cannibalism?) in the post-enzyme chromatograms were also present in a control experiment, in which the enzyme in the absence of substrate was incubated under analogous conditions to those employed in the study.β-Peptides **6**–**8** bearing an N-terminal α-fluoro-β^3-homoalanine residue were screened against the exopeptidic activities of pronase and leucine aminopeptidase. The results mirror those of the internal C-2-substituted β-peptides: no degradation was observed after eight

Table 1. *Degradation experiments on β-peptides with an internal α-heterosubstituted β-amino acid.*[a]

Enzyme	**41**[b]	**1**	**2**	**3**	**4**	**5**
α-chymotrypsin	+	–	–	–	–	–
pancreatic elastase	+	–	–	–	–	–
pepsin	+	–	–	–	–	–
pronase (pH 7.8)	+	–	–	–	–	–
pronase (pH 9.0)	+	–	–	–	–	–
proteinase K	+	–	–	–	–	–
subtilisin carlsberg	+	–	–	–	–	–
trypsin[c]	–	–	–	–	–	–

[a] Reaction mixtures were incubated at 37 °C for 5 d. [b] Reaction mixtures were incubated at 37 °C for 1 h. [c] Trypsin favors positively charged residues in the P1 position (see *Choice of enzymes* section). This probably accounts for the stability of the hydrophobic α-peptide **41** under the conditions used. A + sign denotes that degradation was observed, – that no degradation was detectable.



Scheme 6. *Preparation of β-tetrapeptides **6**–**10** and β-heptapeptides **1**–**5**. a) TFA, CH_2Cl_2, 0 °C, 1 h; b) HATU, NMM, DMF, RT, 12 h; c) H_2, Pd/C, MeOH, RT, 12 h.*

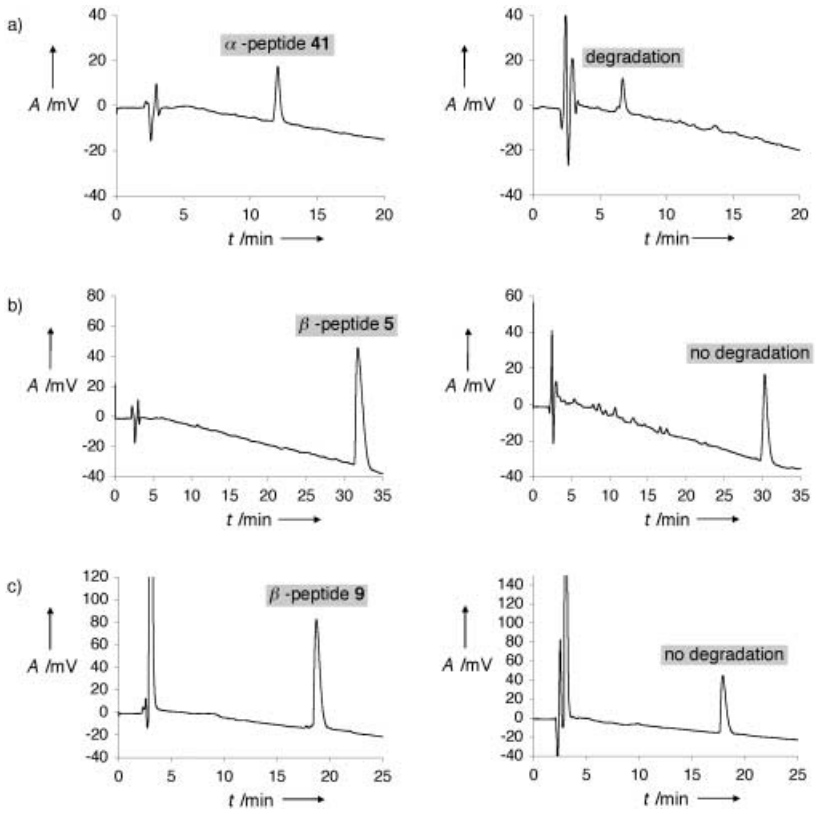


Figure 2. *Reversed-phase HPLC analysis before (left) and after (right) treatment with leucine aminopeptidase. Post-enzyme treated chromatograms were recorded after 1 h, 5 d, and 8 d, respectively.*

days at 37 °C (Table 2, Figure 2). Additionally, compounds bearing an N-terminal α-fluoro-β-homoglycine or an unsubstituted β-homoglycine residue (β-peptides **9** and **10**, respectively) also resisted hydrolysis.

Table 2. *Degradation experiments on β-peptides with an N-terminal α-heterosubstituted β-amino acid.*[a]

Enzyme	**41**[b]	**6**	**7**	**8**	**9**	**10**
pronase (pH 7.8)	+	–	–	–	–	–
pronase (pH 9.0)	+	–	–	–	–	–
leucine aminopeptidase	+	–	–	–	–	–

[a] Reaction mixtures were incubated at 37 °C for 8 d. [b] Reaction mixtures were incubated at 37 °C for 1 h. A + sign denotes that degradation was observed, – that no degradation was detectable.

Discussion

In addition to necessary interactions involving the backbone of a peptide, amide bond cleavage by the action of a peptidase requires recognition of the side chains at appropriate subsites of the enzyme. Although the most important side chains for this purpose are generally those in the P1 and P1′ positions, neighboring residues can have a profound impact on recognition by a peptidase and hence the rate of hydrolysis. Such influences can be envisaged to be more significant for the action of endopeptidases rather than exopeptidases. However, as is evident from the results presented, the resistance to proteolysis of an amide carbonyl group with a polar group in the 2-position, either at the N terminus or embedded in the central position of a β-heptapeptide chain, is not compromised. In the case of the mono-2-substituted β-peptides **1**–**4**, the relative configurations about the modified amide bond do not impact on proteolytic stability. By testing both C-2 epimers, any beneficial or indeed unfavorable interactions that may be created by the hetero group of a particular configuration with the active site of the enzyme are considered. By analogy, the 2,2-difluoro-substituted β-peptide **5**, in which the greatest electronic modification to the neighboring amide carbonyl group is in effect, the stability of the β-peptide as a whole is maintained. It is of course feasible that the presence of a polar group in the α-position impedes binding with the active site of the enzyme through structural and/or electronic interactions even if such a group results in a better substrate for proteolytic degradation.

Despite its name, leucine aminopeptidase (EC 3.4.22.1) hydrolyzes a range of N-terminal α-amino acids, including glycine. Most α-peptides possessing a free amino group at the N terminus are cleaved at measurable rates, although there is a marked reduction in rate when a D-amino acid is present in the penultimate position; N-terminal D-amino acids are not cleaved at all.[25] β-Homoglycine (β-alanine) is one of the few β-amino acids that occur naturally in mammals and is generally presented in the form of the dipeptide carnosine.[47] In fact, there are some enzymes capable of cleaving such an N-terminal residue from an α-peptide chain.[47–50] It was discovered in this work that β-peptide **10**, which possesses an N-terminal β-homoglycine moiety, was stable to leucine aminopeptidase; the neighboring β-amino acid residues seemingly impeding any potential hydrolysis. β-Peptide **9**, which bears an N-terminal α-fluoro-β-homoglycine residue was also resilient; consequently the enhanced electrophilicity of the terminal amide bond did not affect stability. Both β-peptides were also hydrolytically stable to the exopeptidic activity of pronase, under both pH conditions. Based on these observations, it was not wholly surprising that β-peptides **6**–**8**, with an N-terminal α-fluoro-β^3-homoalanine amino acid residue, also remained

intact after exposure to leucine aminopeptidase or pronase. A non-2-substituted N-terminal β^3-homoalanine residue is known to be stable to both enzymes.[11]

Hemolysis assays

Internal C-2 substituted β-peptides **1**–**5** were screened for hemolytic activity. Only **1** evoked some lysis of human and rat erythrocytes at 300 μM; no substantial activities were observed at concentrations up to 100 μM. These results are in keeping with previous observations on the low hemolytic behavior of β-peptides.[51]

Conclusion

The stability of β-peptides used in this study supplements previously obtained results. Modifications to the β-peptide structure to alter the electronic environment of a particular amide carbonyl group seemingly have no influence on general proteolytic stability. It is perhaps the case that inherent stability cannot be influenced by localized peptide-backbone manipulations and that a more contiguous modification is required. Our attempts to present an amide bond within a β-peptide chain that will be specifically targeted by a peptidase have not, on the basis of electronic modifications to the backbone and the scope of this study, proved successful.

Experimental Section

Proteolytic degradation experiments

Reagents and enzymes: The following reagents and enzymes were obtained commercially: Trizma Base (tris[hydroxymethyl]aminomethane, Sigma), NaCl (J. T. Baker), $CaCl_2 \cdot 2H_2O$ (Merck), $MgCl_2 \cdot 6H_2O$ (Fluka), glycerol (Fluka), AcOH (Fluka), and fuming HCl 37% (J. T. Baker). Water used for preparing buffers and other solutions was of Nanopure quality. Individual buffer component solutions were autoclaved prior to use.

α-Chymotrypsin (EC 3.4.21.1) from bovine pancreas (Fluka, product No. 27270), lyophilized powder, used as a 160 μM stock solution in Tris-HCl (50 mM), pH 7.8, 50% *v/v* glycerol, NaCl (100 mM), $CaCl_2$ (10 mM). Leucine aminopeptidase, cytosol, (EC 3.4.11.1, 45 μM) from hog kidney (Fluka, product No. 61860), 45 μM crystalline suspension in NH_4SO_4 (3.8 M), pH 8.0, used as received as a stock solution. Pancreatic elastase (EC 3.4.21.36) from hog pancreas (Fluka, product No. 45125), lyophilized powder, used as a 160 μM stock solution in Tris-HCl (50 mM), pH 7.3, 60% *v/v* glycerol, NaCl (100 mM), $CaCl_2$ (10 mM). Pepsin (EC 3.4.23.1) from hog stomach (Fluka, product No. 77152), lyophilized powder used as a freshly prepared solution (160 μM in AcOH/HCl (10 mM), pH 2.0). Pronase (EC 3.4.24.4) from *Streptomyces griseus* (Fluka, product No. 81748), lyophilized power used as a 160 μM stock solution in Tris-HCl (50 mM), pH 7.8, 50% *v/v* glycerol, $CaCl_2$ (10 mM). Proteinase K (EC 3.4.21.64) from *Tritirachium album* (Fluka, product No. 82456), suspension in Tris-HCl (10 mM), pH 7.5, 40% *v/v* glycerol, $Ca(OAc)_2$ (1 mM) used as a 160 μM stock solution in Tris-HCl (50 mM), pH 7.8, 50% *v/v* glycerol, $CaCl_2$ (5 mM). Subtilisin Carlsberg (EC 3.4.21.14) from *Bacillus subtilis var. biotecus A* (Fluka, product No. 82490, 160 μM), lyophilized powder used as a stock solution in Tris-HCl (50 mM), pH 7.8, 50% *v/v* glycerol, NaCl (100 mM), $CaCl_2$ (10 mM). Trypsin (EC 3.4.21.4) from hog pancreas (Fluka, product No. 93615, 160 μM), powder, used as a stock solution in Tris-HCl (50 mM), pH 7.8, 50% *v/v* glycerol, NaCl (100 mM), $CaCl_2$ (20 mM).

All enzyme stock solutions were stored at −20 °C, with the exception of leucine aminopeptidase (0 °C), and remained proteolytically active for at least 6 months.

1 M Tris-HCl Stock Solution: Trizma base (121.1 g) was dissolved in water. The desired pH was obtained by addition of fuming HCl. Glycine–NaOH Buffer Solution (0.2 M, pH 11): Glycine (1.50 g) and NaOH (0.80 g) were dissolved in water (100 mL).

Devices

pH measurements: All buffer solutions were pH tested at 25 °C with a digital Metrohm 632 pH meter that had been calibrated by using a calibration buffer set (Fluka).

HPLC analysis: Reversed-phase HPLC (RP-HPLC) analyses were performed on a Knauer HPLC system K 1000 (pump type 64, EuroChrom 2000 integration package, degaser, UV detector K 2000 (variable-wavelength monitor)) on a Macherey–Nagel C_8 column (Nucleosil 100–5 C_8 (250×4 mm)).

Assays: The following reaction buffers were used to assay the peptides used in this study: Tris-HCl (50 mM), pH 8.0, NaCl (100 mM), $CaCl_2$ (10 mM) for α-chymotrypsin, pancreatic elastase and subtilisin Carlsberg; Tris-HCl (58 mM), pH 9.0, $CaCl_2$ (5.8 mM) for leucine aminopeptidase; AcOH–HCl (10 mM), pH 2.0 for pepsin; Tris-HCl (50 mM), pH 7.8 and pH 9.0, $CaCl_2$ (10 mM) for pronase; Tris-HCl (50 mM), pH 8.0, $CaCl_2$ (5 mM) for proteinase K; Tris-HCl (50 mM), pH 8.0, NaCl (100 mM), $CaCl_2$ (20 mM) for trypsin.

All peptides employed in the degradation studies were used as trifluoroacetic acid (TFA) salts as obtained after lyophilization.

General procedures: A stock solution of α-peptide **41** (1.5 mM) was freshly prepared prior to use in Tris-HCl (50 mM), pH 7.8. A portion (4 μL) of this substrate stock solution was subsequently diluted by addition of the appropriate reaction buffer (33.5 μL) to establish the desired conditions (160 μM substrate, 37.5 μL). Stock solutions of β-peptides **1**–**10** (1.4 mM) were also freshly prepared prior to use in Tris-HCl (50 mM), pH 7.8. A portion (4 μL) of the relevant substrate stock solutions was subsequently diluted by addition of the appropriate reaction buffer (31 μL) to establish the desired conditions (160 μM substrate, 35 μL). With the exception of leucine aminopeptidase, the enzyme stock solutions (160 μM enzyme concentration) were diluted to a 50 μM working stock solution prior to use by addition of the relevant reaction buffer.

Enzyme activity verification assay: The diluted enzyme solution (2.5 μL of 50 μM working stock solution) was added to a solution of α-peptide **41** in the appropriate reaction buffer (160 μM substrate, 37.5 μL), and the reaction mixture was incubated at 37 °C for 1 h (final substrate and enzyme concentrations were 150 μM and 3.13 μM, respectively). Reactions were halted by addition of AcOH (4 μL, 25% *v/v* in H_2O), with the exception of pepsin where glycine–NaOH buffer was used (4 μL). The resulting mixtures were analyzed by reversed-phase HPLC.

*General assay procedure for β-peptides **1**–**10** (excluding reaction with leucine aminopeptidase)*: A portion of the working stock solution of enzyme (5 μL of 50 μM) was added to the appropriate reaction buffer containing a β-peptide (160 μM in 35 μL), and the reaction mixture was incubated at 37 °C for five (**1**–**5**) or eight (**6**–**10**) days.

The final substrate and enzyme concentrations were 140 μM and 6.25 μM, respectively. Reactions were halted by addition of AcOH (4 μL, 25% *v/v* in H_2O) with the exception of pepsin, here glycine–NaOH buffer (4 μL) was used. The resulting mixtures were analyzed by reversed-phase HPLC.

*Assay procedure for β-peptides **6**–**10** with leucine aminopeptidase*: Enzyme stock solution (5.6 μL, 45 μM) was added to the reaction buffer containing a β-peptide (163 μM in 34.4 μL), and the reaction mixture was incubated at 37 °C for eight days. The final substrate and enzyme concentrations were 140 μM and 6.25 μM, respectively. Reactions were halted by addition of AcOH (4 μL, 25% *v/v* in H_2O), and the resulting mixtures were analyzed by reversed-phase HPLC.

Residual activity assays: Before quenching the β-peptide assays, an aliquot of the reaction mixture (5 μL) was added to a solution of α-peptide **41** in the appropriate reaction buffer (10 μL, 160 μM substrate), and the solution was incubated at 37 °C for 13 h. The final α-peptide **41** and enzyme concentrations were 107 μM and 2.08 μM, respectively. Reactions were halted by addition of AcOH (4 μL, 25% *v/v* in H_2O) with the exception of pepsin, here glycine–NaOH buffer (4 μL) was used. The resulting mixtures were analyzed by reversed-phase HPLC.

Analytical reversed-phase HPLC conditions: Detection was achieved by measurement of the UV absorption at 220 nm. A mixture of MeCN/H_2O (containing 0.1% TFA) was used as eluent with a flow rate of 1 mL min^{-1}. The gradient used for β-peptides **1**–**5** was 20:80→60:40, for **6**–**10** 95:5→50:50, in both cases over 40 min.

Synthesis of β-peptides 1–10 and β-amino acids 11–15

Abbreviations: β-hAa (β-homoamino acid), Bn (benzyl), Boc (*tert*-butoxycarbonyl), Boc_2O (di-*tert*-butyl dicarbonate), FC (flash chromatography), HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), h.v. (high vacuum, 0.01–0.1 Torr), NMM (4-methylmorpholine), TFA (trifluoroacetic acid), TFE (2,2,2-trifluoroethanol).

Reagents and methods: DMSO and Et_3N were distilled over CaH_2 and stored over 4 Å molecular sieves. Solvents for FC and workup procedures were distilled over *Sikkon* (anhydrous $CaSO_4$; Fluka). All other reagents and solvents were used as received from Fluka or Aldrich. Saturated HCl/MeOH solution was prepared by bubbling anhydrous HCl gas into MeOH at 0 °C (ice bath). The aldehyde **16**,[34,35] cyanohydrins (*S*,*S*)-**17** and (*R*,*S*)-**17**,[36,52] the benzyl ester **23**,[53] and β-tetrapeptides **27**[46] and **35**[9] were synthesized according to published procedures. Thin-layer chromatography (TLC) analysis was performed on Merck silica gel 60 F_{254} plates; detection with UV light or by dipping the plates into a solution of ninhydrin (0.6 g), AcOH (2 mL), H_2O (13 mL), and BuOH (285 mL) or a solution of phosphomolybdic acid (25 g), $Ce(SO_4)_2{\cdot}H_2O$ (10 g), conc. H_2SO_4 (60 mL), and H_2O (940 mL), followed by heating. FC was carried out with Fluka silica gel 60 (40–63 μm) at a pressure of about 0.3 bar.

Instrumental analysis: Analytical reversed-phase HPLC (RP-HPLC) analyses were performed on a Knauer HPLC system K1000 (pump type 64, EuroChrom 2000 integration package, degaser, UV detector K2000 (variable-wavelength monitor)) on a Macherey–Nagel C_8 column (Nucleosil 100–5 C_8 (250×4 mm)) at 220 nm. Preparative RP-HPLC separations were performed on a Knauer HPLC system (pump type 64, programmer 50, UV detector (variable-wavelength monitor)) on a Macherey–Nagel C_8 column (Nucleosil 100–7 C_8 (250×21 mm)) at 220 nm. Melting points were measured on a Büchi 510 apparatus; the values reported are uncorrected. Optical rotations ($[\alpha]_D^{RT}$) were measured on a Perkin–Elmer 241 polarimeter (10 cm, 1 mL cell) at RT; the solvent and the concentration (g per 100 mL) are indicated in the text below. IR spectra were measured on a Perkin–Elmer 782 spectrophotometer. NMR spectra were recorded on a Bruker AMX600 (^{1}H 600 MHz, ^{13}C 150.9 MHz), AMX500 (^{1}H 500 MHz, ^{13}C 125 MHz), AMX400 (^{1}H 400 MHz, ^{13}C 100 MHz) or Varian Gemini 300 (^{1}H 300 MHz, ^{13}C 75 MHz, ^{19}F 282 MHz) spectrometer; chemical shifts (ppm) and coupling constants (Hz) are indicated in the text below. High-resolution mass spectrometry was performed on an IonSpec Ultima 4.7 (electrospray ionization (HR-ESI-MS) or matrix-assisted laser desorption ionization (HR-MALDI-MS) in a 2,5-dihydroxybenzoic acid matrix) spectrometer and used to measure *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

General procedures (GP)

Methanolysis of cyanohydrins (GP 1): A solution of the cyanohydrin (1 equiv) in anhydrous HCl/MeOH (10 mL mmol^{-1}) was stirred at RT for 12 h, concentrated under reduced pressure, poured into H_2O, cautiously neutralized by the addition of solid K_2CO_3, and extracted with AcOEt (3×). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by FC.

Benzyl hydrogenolysis (GP 2): A catalytic amount of Pd/C (10%, 30 mg per equivalent of benzyl group) was added to a solution of the Bn-protected compound in MeOH (10 mL mmol^{-1}). The apparatus was evacuated and flushed with H_2 (3×), and the mixture was vigorously stirred for 12 to 24 h. The catalyst was filtered off through celite, washed several times with MeOH, and the combined organic fractions evaporated. The crude product was used without further purification.

Boc-protection (GP 3): Boc_2O (1.5 equiv) and Et_3N (3–5 equiv) were added to a solution of the amine (1 equiv) in MeOH (3 mL mmol^{-1}). The mixture was stirred at RT for 12 h, concentrated under reduced pressure, dissolved in AcOEt, and washed successively with HCl (0.5 M), saturated K_2CO_3, and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated, and the crude product was purified by FC.

Methyl ester hydrolysis (GP 4): A mixture of the methyl ester (1 equiv) and $LiOH{\cdot}H_2O$ (3 equiv) in EtOH/H_2O (2:1, 4 mL mmol^{-1}) was well stirred for 1 h at RT, cooled to 0 °C (ice bath), and neutralized by the addition of HCl (1 M). The mixture was diluted with H_2O and extracted with AcOEt or CH_2Cl_2 (3×). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The crude product was used without further purification.

Boc-deprotection (GP 5a): A solution of the *N*-Boc-protected compound in CH_2Cl_2 (3 mL mmol^{-1}) was cooled to 0 °C (ice bath), treated with TFA (3 mL mmol^{-1}), and stirred at 0 °C for 1.5 h. After concentration under reduced pressure, the TFA salt was dried under h.v. for 2 h and used without further purification.

Boc-deprotection (GP 5b): After Boc deprotection according to GP 5a, the TFA salt was dissolved in AcOEt, washed with saturated K_2CO_3 (2×), dried over $MgSO_4$, filtered, and evaporated. The resulting amine was used without further purification.

Fluorination reactions with DAST (GP 6): All reactions were performed in PET flasks under an inert atmosphere of N_2. According to a published procedure,[39] a solution of the hydroxylated ester derivative (1 equiv) in CH_2Cl_2 or THF (2 mL mmol^{-1}) was treated dropwise with DAST (1.5–3 equiv) at 0 °C (ice bath) or RT. The mixture was stirred at this temperature for 0.5 to 4 h, poured into H_2O, cautiously neutralized by the addition of solid K_2CO_3, and extracted

with Et_2O (2×). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by FC.

Peptide coupling with HATU (GP 7a): A solution of the free amine or TFA salt (1 equiv) in DMF (6 mL mmol^{-1}) was cooled to 0 °C, treated successively with the appropriate acid (1 equiv), NMM (3–5 equiv), and HATU (1.2 equiv), and stirred at RT for 12 h. The mixture was diluted with AcOEt, washed with HCl (1 M, 3×), saturated K_2CO_3 (3×), and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated, and the crude product purified by FC.

Peptide coupling with HATU (GP 7b): The peptide coupling reaction was performed according to GP 7a, but during the reaction the formed peptide precipitates. The mixture was evaporated, and the residue was stirred in AcOEt for 10 min. The resulting suspension was separated in a centrifuge, and the solid stirred successively in AcOEt (2×) and MeOH/H_2O 1:1 (3×) for 10 min each. After the final centrifugation, the product was dried under h.v. for 12 h.

Preparation of α-hydroxy amino acids (*S,S*)-11 and (*R,S*)-11

*(2S,3S)-3-Dibenzylamino-2-hydroxybutanoic acid methyl ester (S,S)-**18***. The cyanohydrin (*S,S*)-**17** (1.90 g, 6.8 mmol) was treated with an anhydrous HCl/MeOH solution (68 mL) according to GP 1. FC (AcOEt/hexane 1:9→3:7) yielded the methyl ester (*S,S*)-**18** (1.63 g, 76%) as a light yellow oil. R_f = 0.43 (AcOEt/hexane 3:7); $[\alpha]_D^{RT}$ = +37.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.11 (d, *J* = 6.9, 3H; Me), 2.94 (br d, *J* = 5.8, 1H; OH), 3.13 (qd, *J* = 4.6, 6.9, 1H; NCH), 3.60 (d, *J* = 13.9, 2H; PhCH_2N), 3.70 (s, 3H; OMe), 3.71 (d, *J* = 13.9, 2H; PhCH_2N), 4.36 (m, 1H; C*H*OH), 7.20–7.38 (m, 10H; arom.);. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.8, 52.3 (CH_3), 54.4 (CH_2), 56.2, 72.1, 126.9, 128.2, 128.8 (CH), 139.8, 174.8 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3547 (w), 3064 (w), 3008 (m), 2954 (w), 2807 (w), 1731 (s), 1602 (w), 1494 (m), 1453 (m), 1375 (w), 1262 (m), 1120 (m), 1074 (m), 1028 (m), 983 (w), 954 (w), 909 (w); HR-MALDI-MS: *m/z* (%): 336.2 (19) [*M*+Na]$^+$, 314.2 (100) [*M*+H]$^+$, 224.1 (30) [*M*+H-90]$^+$; elemental analysis calcd (%) for $C_{19}H_{23}NO_3$ (313.40): C 72.82, H 7.40, N 4.47; found: C 72.88, H 7.25, N 4.53.

*(2R,3S)-3-Dibenzylamino-2-hydroxybutanoic acid methyl ester (R,S)-**18***. The cyanohydrin (*R,S*)-**17** (1.10 g, 3.94 mmol) was treated with an anhydrous HCl/MeOH solution (40 mL) according to GP 1. FC (pentane/Et_2O, 9:1) yielded the methyl ester (*R,S*)-**18** (1.08 g, 88%) as a light yellow oil. R_f = 0.33 (pentane/Et_2O, 3:2); $[\alpha]_D^{RT}$ = +77.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20 (d, *J* = 6.8, 3H; Me), 3.03–3.12 (m, 1H; NCH), 3.34 (d, *J* = 13.4, 2H; CH_2Ph), 3.59 (s, 3H; OMe), 3.85 (s, 1H; OH), 3.89 (d, *J* = 13.4, 2H; CH_2Ph), 4.04 (d, *J* = 6.5, 1H; C*H*OH), 7.20–7.34 (m, 10H; arom.); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 9.0, 52.3 (CH_3), 54.5 (CH_2), 55.5, 74.2, 127.1, 128.3, 129.0 (CH); 138.9, 173.7 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3518 (w), 3354 (w), 3015 (m), 2954 (w), 2931 (w), 1921 (w), 1954 (w), 1882 (w), 1810 (w), 1733 (s), 1600 (w), 1493 (m), 1451 (m), 1380 (m), 1272 (m), 1164 (m), 1108 (m), 1026 (m), 908 (w); HR-MALDI-MS: 336.2 (10) [*M*+Na]$^+$, 314.2 (100) [*M*+H]$^+$, 254.2 (6), 224.1 (14) [*M*+H-90]$^+$, 157 (6); elemental analysis calcd (%) for $C_{19}H_{23}NO_3$ (313.40): C 72.82, H 7.40, N 4.47, O 15.32; found: C 72.87, H 7.53, N 4.63.

*(2S,3S)-3-*tert*-Butoxycarbonylamino-2-hydroxybutanoic acid methyl ester (S,S)-(**19**)*: Hydrogenolysis of the ester (*S,S*)-**18** (3.23 g, 10.31 mmol) was performed in the presence of TFA (790 μL, 10.3 mmol) according to GP 2. The resulting TFA salt was dissolved in MeOH (30 mL) and treated with Boc_2O (2.7 g, 12.4 mmol) and Et_3N (7.2 mL, 51.6 mmol) according to GP 3. FC (CH_2Cl_2/AcOEt, 9:1) yielded the methyl ester (*S,S*)-**19** (1.74 g, 72%) as a colorless solid. M.p. 94–95 °C; R_f = 0.20 (pentane/AcOEt, 2:1); $[\alpha]_D^{RT}$ = +14.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05 (d, *J* = 6.9, 3H; Me), 1.45 (s, 9H; CMe_3), 3.15 (br d, *J* = 5.1, 1H; OH), 3.81 (s, 3H; OMe), 4.04–4.16 (m, 1H; NCH), 4.33 (dd, *J* = 2.9, 5.6, 1H; C*H*OH), 4.85 (br s, 1H; NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.6, 28.4 (CH_3); 48.8 (CH), 52.8 (CH_3), 73.1 (CH), 79.7, 155.4, 173.4 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3539 (w), 3446 (m), 3005 (w), 2985 (m), 1733 (s), 1708 (s), 1503 (s), 1456 (m), 1441 (m), 1390 (s), 1369 (m), 1318 (m), 1164 (s), 1133 (m), 1056 (m), 1015 (m), 985 (w), 877 (w); HR-MALDI-MS: 256.1 (9) [*M*+Na]$^+$, 199.0 (17), 198.0 (8), 136.5 (7); elemental analysis calcd (%) for $C_{10}H_{19}NO_5$ (233.26): C 51.49, H 8.21, N 6.00; found: C 51.47, H 8.06, N 5.96.

*(2R,3S)-3-*tert*-Butoxycarbonylamino-2-hydroxybutanoic acid methyl ester (R,S)-(**19**)*: Hydrogenolysis of the ester (*R,S*)-**18** (1.68 g, 5.35 mmol) was performed in the presence of TFA (450 μL, 5.9 mmol) according to GP 2. The resulting TFA salt was dissolved in MeOH (15 mL) and treated with Boc_2O (1.75 g, 8.02 mmol) and Et_3N (3.7 mL, 26.7 mmol) according to GP 3. FC (pentane/Et_2O, 4:1→3:2) yielded the methyl ester (*R,S*)-**19** (1.15 g, 92%) as a colorless oil. R_f = 0.10 (pentane/Et_2O, 2:1); $[\alpha]_D^{RT}$ = −52.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.21 (d, *J* = 6.9, 3H; CH*Me*), 1.37 (d, *J* = 5.3, 1H; OH), 1.41 (s, 9H; CMe_3), 3.75 (s, 3H; OMe), 3.99–4.14 (m, 1H; CHN), 4.04–4.07 (m, 1H; C*H*OH), 4.80 (br d, *J* = 5.0, 1H; NH);. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.1, 28.3 (CH_3), 48.7 (CH), 52.8, (CH_3), 73.2, (CH), 79.5, 155.0, 173.8 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3530 (w), 3442 (w), 3008 (w), 2981 (m), 1734 (s), 1708 (s), 1501 (s), 1456 (m), 1392 (m), 1368 (m), 1348 (m), 1163 (s), 1129 (m), 1055 (m), 1033 (w), 989 (w), 882 (w), 850 (w); HR-MALDI-MS: 256.1 (100) [*M*+Na]$^+$, 200.1 (45), [*M*+Na−isobutylene]$^+$, 199.0 (16), 156.1 (10) [*M*+Na-Boc]$^+$, 136.5 (11); elemental analysis calcd (%) for $C_{10}H_{19}NO_5$ (233.26): C 51.49, H 8.21, N 6.00; found: C 51.65, H 8.06, N 5.95.

*(2S,3S)-3-*tert*-Butoxycarbonylamino-2-hydroxybutanoic acid (S,S)-**11***: The ester (*S,S*)-**19** (1.16 g, 4.97 mmol) was hydrolyzed with LiOH·H_2O (626 mg, 14.91 mmol) according to GP 4. The crude carboxylic acid (*S,S*)-**11** (708 mg, 65%, colorless solid) was used without further purification. M.p. 99–101 °C; $[\alpha]_D^{RT}$ = +1.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): δ = 1.07 (d, *J* = 6.9, 3H; Me), 1.44 (s, 9H; CMe_3), 3.95–4.05 (m, 1H; NCH), 4.20 (d, *J* = 3.7, 1H; C*H*OH); ^{13}C NMR (75 MHz, CD_3OD): δ = 13.5, 27.5 (CH_3); 48.9, 72.6 (CH); 79.0, 156.4, 174.5 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3442 (m), 2981 (s), 2935 (w), 1709 (s), 1505 (s), 1456 (w), 1393 (m), 1369 (m), 1344 (w), 1163 (s), 1130 (w), 1060 (m), 1010 (w), 877 (w), 846 (w); HR-ESI-MS: 517.3 (8), 483.2 (25), 477.2 (27), [2*M*+K]$^+$, 461.2 (100) [2*M*+Na]$^+$, 298.2 (11), 256.1 (18), 242.1 (76), [*M*+Na]$^+$, 186.0 (9); elemental analysis calcd (%) for $C_9H_{17}NO_5$ (219.24): C 49.31, H 7.82, N 6.39; found: C 49.20, H 7.89, N 6.42.

*(2R,3S)-3-*tert*-Butoxycarbonylamino-2-hydroxybutanoic acid (R,S)-**11***: The ester (*R,S*)-**19** (553 mg, 2.37 mmol) was hydrolyzed with LiOH·H_2O (298 mg, 7.11 mmol) according to GP 4. The crude carboxylic acid (*R,S*)-**11** (490 mg, 94%, colorless solid) was used without further purification. M.p. 111–122 °C; $[\alpha]_D^{RT}$ = −19.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.22 (d, 3H; *J* = 6.9, Me), 1.38 (s, 9H; CMe_3), 4.05–4.19 (m, 1H; CHN), 4.12 (s, 1H; C*H*OH), 5.17 (d, 1H; *J* = 9.0, NH), 7.59–8.09 (m, 2H; OH+COOH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.9, 28.3 (CH_3); 48.8, 73.0 (CH); 80.4, 155.8, 175.1 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3497 (w), 3437 (m), 2981 (m), 2935 (m), 1711 (s), 1506 (s), 1456 (m), 1393 (m), 1368 (s), 1162 (s), 1128 (m), 1057 (m), 1010 (w), 880 (w); HR-MALDI-MS: 243.1 (100, [*M*+Na]$^+$), 199.0 (30), 186.0 (22).

Preparation of α-fluoro amino acids (*S,S*)-12, (*R,S*)-12 and 14

*(2S,3S)-3-Dibenzylamino-2-fluorobutanoic acid methyl ester (S,S)-**20***: A solution of ester (*S,S*)-**18** (3.04 g, 9.70 mmol) in CH_2Cl_2 (20 mL) was fluorinated with DAST (1.9 mL, 14.54 mmol) at 0 °C for 3 h

according to GP 6. FC (pentane/Et_2O, 9:1) yielded (*S,S*)-**20** (2.59 g, 85%) as a yellow oil. R_f = 0.41 (pentane/Et_2O, 7:1); $[\alpha]_D^{RT}$ = +25.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18 (dd, *J* = 1.6, 6.9, *3*H; Me), 3.28 (qdd, *J* = 4.4, 6.9, 24.5, 1H; NCH), 3.66 (s, 4H; 2CH_2Ph), 3.70 (s, 3H; OMe), 5.14 (dd, *J* = 4.4, 50.0, 1H; CHF), 7.20–7.34 (m, 10H; arom.); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.8 (d, *J* = 4.5, CH_3), 52.2 (CH_3), 54.3 (CH_2), 54.8 (d, *J* = 21.0, CH), 90.4 (d, *J* = 189.0, CHF), 127.0, 128.3, 128.7 (CH); 139.5 (C), 169.6 (d, *J* = 23.4, CO); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −198.6 (dd, *J* = 24.5, 49.1, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3067 (w), 3036 (w), 2954 (w), 2841 (w), 1754 (s), 1492 (m), 1451 (m), 1436 (w), 1287 (m), 1138 (w), 1113 (w), 1072 (w), 1026 (m); HR-MALDI-MS: 338.2 (11) [*M*+Na]$^+$, 316.2 (100) [*M*+H]$^+$, 296.2 (4), 268.2 (5), 224.1 (9), 158.1 (8); elemental analysis calcd (%) for $C_{19}H_{22}FNO_2$ (315.39): C 72.36, H 7.03, N 4.44; found: C 72.42, H 7.04, N 4.53.

*(2S,3R)-3-Hydroxy-2-dibenzylaminobutanoic acid benzyl ester (**21**)*: A solution of L-threonine (11.9 g, 100 mmol), K_2CO_3 (27.6 g, 200 mmol), and NaOH (8.0 g, 200 mmol) in H_2O (200 mL) was heated to reflux and treated with BnBr (36.8 mL, 310 mmol). After 1 h under reflux, the mixture was cooled to RT and extracted with Et_2O (3×). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. FC (hexane/AcOEt, 9:1) yielded the benzyl ester **21** (16.12 g, 41%) as a colorless oil. R_f = 0.45 (hexane/AcOEt, 8:2); $[\alpha]_D^{RT}$ = −154.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06 (d, *J* = 6.0, 3H; Me), 3.09 (d, *J* = 9.6, 1H; NCH), 3.37 (d, *J* = 13.4, 2H; 2C*H*H′Ph), 3.45 (s, 1H; OH), 3.98 (d, *J* = 13.4, 2H; 2CH*H*′Ph), 4.05 (dq, *J* = 6.0, 9.6, 1H; C*H*OH), 5.20 (d, *J* = 12.1, 1H; C*H*H′Ph), 5.32 (d, *J* = 12.1, 1H; CH*H*′Ph), 7.21–7.47 (m, 15H arom.); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.1 (CH_3), 54.8 (CH_2), 63.2 (CH), 66.4 (CH_2), 67.3, 127.5, 128.5, 128.6, 128.7, 128.7, 129.1 (CH); 135.7, 138.1, 170.2 (C); IR ($CHCl_3$): $\tilde{\nu}$ = 3479 (w), 3068 (w), 3032 (m), 2938 (w), 2851 (w), 1726 (s), 1603 (w), 1496 (m), 1455 (m), 1403 (w), 1377 (m), 1280 (m), 1174 (s), 1104 (s), 1083 (m), 1028 (w), 970 (m), 937 (w), 912 (w), 822 (w); HR-MALDI-MS: 390.2 (100) [*M*+H]$^+$, 368.2 (7), 344.2 (6), 282.2 (19), elemental analysis calcd (%) for $C_{25}H_{27}NO_3$ (389.49): C 77.09, H 6.99, N 3.60; found: C 76.96, H 6.92, N 3.57.

*(2R,3S)-3-Dibenzylamino-2-fluorobutanoic acid benzyl ester (**22**)*: A solution of ester **21** (15.71 g, 40.35 mmol) in THF (80 mL) was fluorinated with DAST (6.3 mL, 48.4 mmol) at RT for 30 min according to GP 6. FC (hexane/AcOEt, 95:5) yielded **22** (9.45 g, 60%) as a light yellow oil. R_f = 0.42 (hexane/AcOEt, 9:1); $[\alpha]_D^{RT}$ = −11.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24 (d, *J* = 7.0, 3H; Me), 3.35 (qdd, *J* = 3.9, 7.0, 30.9, 1H; NCH), 3.36 (d, *J* = 13.5, 2H; 2C*H*H′Ph), 3.91 (d, *J* = 13.5, 2H; 2CH*H*′Ph), 4.85 (d, *J* = 12.2, 1H; C*H*H′Ph), 4.87 (dd, *J* = 3.9, 49.1, 1H; CHF), 5.35 (d, *J* = 12.2, 1H; CH*H*′Ph), 7.17–7.35 (m, 15H; arom.); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 8.5 (d, *J* = 4.4, CH_3), 54.2 (d, *J* = 18.4, CH), 55.0, 55.0, 66.8 (CH_2); 94.2 (d, *J* = 189.8, CH), 126.9, 128.1, 128.4, 128.5, 129.1 (CH); 135.1, 139.5 (C); 168.4 (d, *J* = 25.4, C); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −199.5 (dd, *J* = 30.9, 48.0, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3034 (w), 2970 (w), 2940 (w), 2809 (w), 1760 (s), 1495 (m), 1454 (m), 1384 (m), 1350 (w), 1293 (m), 1170 (m), 1136 (w), 1106 (w), 1074 (w), 1023 (w), 910 (w); HR-MALDI-MS: 414.2 (10) [*M*+Na]$^+$, 392.2 (100) [*M*+H]$^+$, 372.2 (8), 300.1 (8), 224.1 (12), 196.1 (6); elemental analysis calcd (%) for $C_{25}H_{26}FNO_2$ (391.48): C 76.70, H 6.69, N 3.58; found: C 76.76, H 6.61, N 3.60.

*(2R)-3-Dibenzylamino-2-fluoropropanoic acid benzyl ester (**24**)*: A solution of benzyl ester **23** (7.83 g, 20.87 mmol) in THF (40 mL) was fluorinated with DAST (3.3 mL, 25.1 mmol) at RT for 30 min according to GP 6. FC (hexane/AcOEt, 95:5) yielded **24** (7.11 g, 90%) as a yellow oil. R_f = 0.40 (hexane/AcOEt, 9:1); $[\alpha]_D^{RT}$ = +2.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.95–3.12 (m, 2H; NCH_2), 3.55 (d, *J* = 13.7, 2H; 2C*H*H′Ph), 3.80 (d, *J* = 13.7, 2H; 2CH*H*′Ph), 5.05 (d, *J* = 12.2, 1H; C*H*H′Ph), 5.08 (ddd, *J* = 3.3, 5.9, 49.5, 1H; CHF), 5.21 (d, *J* = 12.2, 1H; CH*H*′Ph), 7.20–7.36 (m, 15H; arom.); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 54.4 (d, *J* = 20.3, CH_2), 58.7, 58.7, 67.0 (CH_2); 89.2 (d, *J* = 186.7, CH), 127.1, 128.3, 128.4, 128.5, 128.6, 129.0 (CH); 135.0, 138.7 (C); 168.7 (d, *J* = 24.4, C); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −189.3 (dt, *J* = 24.5, 50.2, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3064 (w), 3033 (m), 2950 (w), 2806 (m), 1758 (s), 1602 (w), 1495 (m), 1454 (m), 1375 (w), 1279 (w), 1136 (w), 1068 (w), 1028 (w), 974 (w); HR-MALDI-MS: 400.2 (16) [*M*+Na]$^+$, 378.2 (100) [*M*+H]$^+$, 358.2 (6), 286.1 (5), 210.1 (4), 189.1 (5); elemental analysis calcd (%) for $C_{24}H_{24}FNO_2$ (377.46): C 76.37, H 6.41, N 3.71; found: C 76.32, H 6.39, N 3.79.

*(2S,3S)-3-*tert*-Butoxycarbonylamino-2-fluorobutanoic acid ((S,S)-**12**)*: Ester (*S,S*)-**20** (2.41 g, 7.64 mmol) was hydrolyzed according to GP 4, the resulting acid was debenzylated according to GP 2, and finally Boc protected according to GP 3. FC (CH_2Cl_2/MeOH/AcOH, 100:3:1→100:5:1) yielded the carboxylic acid (*S,S*)-**12** (1.47 g, 87% over 3 steps) as a colorless solid. M.p. 115–117 °C; R_f = 0.43 (CH_2Cl_2/MeOH/AcOH, 100:5:1); $[\alpha]_D^{RT}$ = +2.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ = 1.13 (d, *J* = 7.0, 3H; Me), 1.45 (s, 9H; CMe_3), 4.06–4.12 (m, 1H; NCH), 4.95 (dd, *J* = 2.7, 49.9, 1H; CHF); ^{13}C NMR (125 MHz, CD_3OD): δ = 14.2 (d, *J* = 5.3, CH_3), 28.8 (CH_3), 49.0 (d, NCH), 80.5 (C), 91.3 (d, *J* = 186.9, CHF), 157.5 (C), 171.6 (d, *J* = 23.7, C); ^{19}F NMR (282 MHz, [D_6]DMSO): δ = −204.8 (dd, *J* = 27.8, 49.1, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3445 (m), 3323 (w), 3032 (w), 2982 (m), 2933 (w), 1749 (m), 1710 (s), 1504 (s), 1456 (w), 1393 (w), 1369 (m), 1339 (w), 1272 (w), 1163 (s), 1113 (w), 1063 (m), 1003 (w), 877 (w), 847 (w); HR-ESI-MS: 509.2 (40), 487.2 (58), 481.2 (18) [2*M*+K]$^+$, 465.2 (15) [2*M*+Na]$^+$, 266.1 (49), 244.1 (100) [*M*+Na]$^+$; elemental analysis calcd (%) for $C_9H_{16}FNO_4$ (221.23): C 48.86, H 7.29, N 6.33; found: C 48.90, H 7.21, N 6.29.

*(2R,3S)-3-*tert*-Butoxycarbonylamino-2-fluorobutanoic acid ((R,S)-**12**)*: Hydrogenolysis of benzyl ester **22** (2.92 g, 7.46 mmol) was performed according to GP 2, and the resulting amino acid was Boc protected according to GP 3. FC (CH_2Cl_2/MeOH/AcOH, 100:5:1) yielded the carboxylic acid (*R,S*)-**12** (1.07 g, 65% over 2 steps) as a colorless solid. M.p. 97–100 °C; R_f = 0.33 (CH_2Cl_2/MeOH/AcOH, 100:5:1); $[\alpha]_D^{RT}$ = −22.8 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ = 1.24 (d, *J* = 7.0, 3H; Me), 1.42 (s, 9H; CMe_3), 4.18 (ddq, *J* = 2.9, 7.0, 24.3, 1H; NCH), 4.87 (dd, *J* = 2.7, 48.0, 1H; CHF); ^{13}C NMR (100 MHz, CD_3OD): δ = 17.1, 28.7 (CH_3); ca. 49.0 (d, CH), 80.4 (C), 91.7 (d, *J* = 185.9, CH), 157.5 (C), 171.7 (d, *J* = 24.3, C); ^{19}F NMR (282 MHz, CD_3OD): δ = −204.2 (dd, *J* = 23.5, 47.0, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3438 (m), 3011 (w), 2982 (m), 2933 (w), 1749 (m), 1711 (s), 1506 (s), 1455 (m), 1393 (w), 1369 (m), 1343 (w), 1165 (s), 1107 (w), 1058 (m), 1005 (w), 848 (w); HR-MALDI-MS: 244.1 (7) [*M*+Na]$^+$, 214.1 (9), 193.0 (8), 144.7 (4) [*M*+Na−Boc]$^+$; elemental analysis calcd (%) for $C_9H_{16}FNO_4$ (221.23): C 48.86, H 7.29, N 6.33; found: C 48.84, H 7.16, N 6.19.

*(2R)-3-*tert*-Butoxycarbonylamino-2-fluoropropanoic acid (**14**)*: Hydrogenolysis of benzyl ester **24** (1.82 g, 4.82 mmol) was performed according to GP 2, and the resulting amino acid was Boc protected according to GP 3. FC (hexane/AcOEt, 2:1 + 2% AcOH) yielded carboxylic acid **14** (924 mg, 93% over 2 steps) as a colorless solid. M.p. 72–76 °C; R_f = 0.12 (hexane/AcOEt, 1:1 + 2% AcOH); $[\alpha]_D^{RT}$ = −3.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): δ = 1.43 (s, 9H; CMe_3), 3.39–3.67 (m, 2H; NCH_2), 4.94 (ddd, *J* = 3.4, 6.5, 48.9, 1H; CHF); ^{13}C NMR (75 MHz, CD_3OD): δ = 27.5 (CH_3), 42.1 (d, *J* = 21.4, CH_2), 79.3 (C), 87.8 (d, *J* = 183.7, CH), 157.0, 170.4 (d, *J* = 23.8, C); ^{19}F NMR (282 MHz, CD_3OD): δ = −194.5 (dt, *J* = 24.5, 48.0, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3455 (m), 3011 (w), 2982 (m), 2933 (w), 1749 (m), 1715 (s), 1510 (s), 1455 (w), 1394 (w), 1369 (m), 1164 (s), 1107 (s), 1040

(w), 875 (w), 851 (w); HR-ESI-MS: 495.4 (6), 481.1 (30), 459.2 (100), 437.2 (78) $[2M+Na]^+$, 252.1 (32), 230.1 (67) $[M+Na]^+$; elemental analysis calcd (%) for $C_8H_{14}FNO_4$ (207.20): C 46.37, H 6.81, N 6.76; found: C 46.62, H 6.86, N 6.74.

Preparation of α,α-difluoro amino acid 13

*(2R/S,3S)-3-Dibenzylamino-2-hydroxybutanoic acid methyl ester ((R/S,S)-**18**)*: According to a published procedure,[54] a vigorously stirred, biphasic solution of the freshly prepared aldehyde **16** (15.4 g, 60.8 mmol) in hexane/H_2O (120 mL, 3:1) was treated with acetone cyanohydrin (8.5 mL, 91.2 mmol) at RT. After the mixture had been stirred for 5 min, catalytic amounts of KCN (127.5 mg, 1.8 mmol) and Bu_4NI (150.5 mg, 0.6 mmol) were added. The mixture was stirred at RT for 2 h, poured into H_2O, and extracted with Et_2O (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The resulting epimeric mixture of cyanohydrins was treated with anhydrous HCl/MeOH according to GP 1 without further purification. FC (hexane/AcOEt, 95:5) yielded methyl ester (*R/S,S*)-**18** (14.75 g, 80% over 2 steps) as a yellow oil (mixture of epimers). $R_f = 0.43$ (hexane/AcOEt, 7:3). The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of (*R/S,S*)-**18** was in accordance with that described above for (*S,S*)-**18** and (*R,S*)-**18**.

*(3S)-3-Dibenzylamino-2,2-difluorobutanoic acid methyl ester (**26**)*: A dry three-necked round-bottomed flask, equipped with a magnetic stirrer and a dropping funnel, was charged with anhydrous CH_2Cl_2 (33 mL) under an atmosphere of N_2. After the mixture had been cooled to −78 °C (dry ice/acetone bath), oxalyl chloride (0.5 mL, 5.26 mmol) and anhydrous DMSO (0.7 mL, 8.76 mmol) were added dropwise, so that the temperature did not exceed −65 °C. The mixture was stirred at −78 °C for 10 min, treated dropwise with a solution of (*R/S,S*)-**18** (1.37 g, 4.38 mmol) in CH_2Cl_2 (5 mL), and stirred for an additional 1.5 h at −78 °C. After addition of dry Et_3N (2.7 mL, 17.52 mmol), the mixture was allowed to warm to RT over 0.5 h, whereupon H_2O (20 mL) was added. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic phases were washed with 1% HCl, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and evaporated. The resulting crude ketoester **25** in CH_2Cl_2 (8 mL) was fluorinated with DAST (1.8 mL, 13.4 mmol) at RT for 3 h, according to GP 6. FC (pentane/CH_2Cl_2, 85:15) yielded the methyl ester **26** (1.2 g, 81% over 2 steps) as a colorless solid. $R_f = 0.26$ (pentane/CH_2Cl_2, 85:15). M.p. 62–63 °C; $[\alpha]_D^{RT} = +19.9$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.25$ (d, $J = 6.9$, 3H; Me); 3.33 (d, $J = 13.4$, 2H; 2C*H*H′Ph), 3.40–3.49 (m, 1H; NCH), 3.64 (s, 3H; OMe), 3.86 (d, $J = 13.4$, 2H; 2CH*H*′Ph), 7.22–7.32 (m, 10H; arom.); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 4.6$, 52.8 (CH_3); 54.4, 54.5 (CH_2); 55.4 (dd, $J = 21.2$, 28.8, NCH), 117.4 (dd, $J = 252.9$, 258.2, CF_2), 127.2, 128.2, 129.2 (CH); 138.8 (C), 164.7 (dd, $J = 29.8$, 35.1, CO); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -103.96$ (d, $J = 256.1$, C*F*F′), −120.4 (d, $J = 258.2$, CF*F*′); IR ($CHCl_3$): $\tilde{\nu} = 3066$ (w), 3011 (w), 2956 (w), 2844 (w), 2814 (w), 1953 (w), 1770 (s), 1603 (w), 1496 (m), 1453 (m), 1440 (m), 1384 (w), 1369 (w), 1339 (w), 1311 (m), 1117 (s), 1069 (s), 1044 (m), 1029 (s), 983 (w), 959 (w), 912 (w), 829*w*; HR-ESI-MS: 356.1 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{19}H_{21}F_2NO_2$ (333.38): C 68.45, H 6.35, N 4.20; found: C 68.39, H 6.51, N 4.11.

*(3S)-3-*tert*-Butoxycarbonylamino-2,2-difluorobutanoic acid (**13**)*: After hydrolysis of methyl ester **26** (3.5 g, 10.4 mmol) according to GP 4 and hydrogenolysis according to GP 2, the resulting amino acid was Boc protected according to GP 3. FC (CH_2Cl_2/MeOH/AcOH, 200:5:2) yielded the carboxylic acid **13** (2.0 g, 80% over 3 steps) as a light yellow solid. M.p. 187 °C; $[\alpha]_D^{RT} = +12.5$ ($c = 1.0$, MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 1.18$ (d, $J = 6.9$, 3H; Me), 1.43 (s, 9H; CMe_3), 4.24–4.30 (m, $J = 6.9$, 1H; NCH); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 14.6$, 28.8 (CH_3), 49.8–50.2 (m, NCH), 80.7 (C), 117.3 (CF_2), 158.0, 170.6 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta = -114.5$ (m, CF_2); IR ($CHCl_3$): $\tilde{\nu} = 3405$ (w), 3007 (w), 1710 (s), 1617 (w), 1417 (w), 1363 (s), 1090 (w), 1010 (w), 902.6 (w); HR-MALDI-MS: 262.1 (20), $[M+Na]^+$, 162.0 (3) $[M\text{-Boc}+Na]^+$.

Synthesis of protected β-tetrapeptides 28–34 and β-heptapeptides 36–40

*Boc-(2S,3S)-β^{2,3}-hAla(α-OH)-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-OBn (**28**)*: The β-tripeptide **27** (267 mg, 0.50 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in DMF (3 mL) and treated with acid (*S,S*)-**11** (110 mg, 0.50 mmol), NMM (165 μL, 1.50 mmol), and HATU (228 mg, 0.60 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 95:5) yielded β-tetrapeptide **28** (243 mg, 76%) as a colorless solid. M.p. 190–192 °C; $R_f = 0.34$ (CH_2Cl_2/MeOH, 92.5:7.5); $[\alpha]_D^{RT} = -42.7$ ($c = 1.0$, MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.86$–0.93 (m, 12H; 4Me), 1.03 (d, $J = 6.8$, 3H; Me), 1.09 (d, $J = 6.7$, 3H; Me), 1.25 (ddd, $J = 4.6$, 9.2, 13.8, 1H; C*H*H′CH), 1.41–1.46 (m, 1H; CH*H*′CH), 1.43 (s, 9H; CMe_3), 1.56–1.64 (m, 1H; C*H*Me_2), 1.79–1.86 (m, 1H; C*H*Me_2), 2.16 (dd, $J = 7.5$, 13.8, 1H; C*H*H′CO), 2.31 (dd, $J = 8.9$, 14.2, 1H; C*H*H′CO), 2.37 (dd, $J = 6.1$, 13.8, 1H; CH*H*′CO), 2.40 (dd, $J = 4.6$, 14.3, 1H; CH*H*′CO), 2.48 (dd, $J = 7.0$, 14.9, 1H; C*H*H′CO), 2.52 (dd, $J = 6.3$, 14.9, 1H; CH*H*′CO), 3.99–4.15 (m, 4H; C*H*OH + 3NHC*H*), 4.27–4.33 (m, 1H; NHC*H*), 5.06 (dd, $J = 12.3$, 1H; C*H*H′Ph), 5.14 (dd, $J = 12.3$, 1H; CH*H*′Ph), 7.29–7.38 (m, 5H; arom.); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 14.6$, 18.9, 19.7, 20.1, 22.2, 23.6 (CH_3); 26.0 (CH), 28.8 (CH_3), 33.2 (CH), 39.4, 41.2, 43.5, 44.5 (CH_2); 44.6, 46.1, 50.1, 53.3 (CH); 67.4 (CH_2), 75.0 (CH), 80.2 (C), 129.3, 129.5, 129.6 (CH); 137.6, 157.5, 172.6, 172.7, 172.8, 174.0 (C); IR ($CHCl_3$): $\tilde{\nu} = 3399$ (m), 3007 (w), 2966 (m), 2874 (w), 1708 (m), 1661 (s), 1503 (s), 1456 (m), 1391 (w), 1368 (m), 1172 (s), 1052 (m), 1003 (w), 882 (w); HR-MALDI-MS: 673.3 (2) $[M+K]^+$, 657.4 (47) $[M+Na]^+$, 573.3 (6) $[M+K-Boc]$, 557.3 (100) $[M+Na-Boc]^+$, 535.3 (42) $[M+H-Boc]^+$, 419.2 (20), 321.2 (24), 300.2 (6), 272.2 (8); elemental analysis calcd (%) for $C_{33}H_{54}N_4O_8$ (634.81): C 62.44, H 8.57, N 8.83; found: C 62.59, H 8.36, N 8.84.

*Boc-(2R,3S)-β^{2,3}-hAla(α-OH)-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-OBn (**29**)*: The β-tripeptide **27** (251 mg, 0.47 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in DMF (5 mL) and treated with acid (*R,S*)-**11** (104 mg, 0.47 mmol), NMM (157 μL, 1.43 mmol), and HATU (217 mg, 0.57 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 95:5) yielded β-tetrapeptide **29** (281 mg, 93%) as a colorless solid. M.p. 134–136 °C; $R_f = 0.28$ (CH_2Cl_2/MeOH, 92.5:7.5); $[\alpha]_D^{RT} = -10.8$ ($c = 1.0$, MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.87$–0.93 (m, 12H; 4Me), 1.12 (d, $J = 6.7$, 3H; Me), 1.17 (d, $J = 6.6$, 3H; Me), 1.26 (ddd, $J = 4.6$, 9.1, 13.8, 1H; C*H*H′CH), 1.41 (s, 9H; CMe_3), 1.41–1.47 (m, 1H; CH*H*′CH), 1.56–1.64 (m, 1H; C*H*Me_2), 1.79–1.86 (m, 1H; C*H*Me_2), 2.20 (dd, $J = 7.6$, 13.9, 1H; C*H*H′CO), 2.33–2.42 (m, 3H; CH*H*′CO + C*H*$_2$CO), 2.48–2.54 (m, 2H; C*H*$_2$CO), 3.95–4.04 (m, 3H; 2NHC*H* + C*H*OH), 4.15–4.19 (m, 1H; NHC*H*), 4.29–4.34 (m, 1H; NHC*H*), 5.07 (d, $J = 12.3$, 1H; C*H*H′Ph), 5.14 (d, $J = 12.3$, 1H; CH*H*′Ph), 7.29–7.34 (m, 5H; arom.); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 18.0$, 18.8, 20.0, 20.2, 22.2, 23.6 (CH_3); 26.0 (CH), 28.8 (CH_3), 32.9 (CH), 39.7, 41.1, 43.6 (CH_2); 44.5 (CH), 44.6 (CH_2), 46.0, 50.5, 53.4 (CH); 67.5 (CH_2), 75.0 (CH), 80.4 (C), 129.3, 129.4, 129.6 (CH); 137.5, 157.7, 172.5, 172.7, 172.8, 174.8 (C); IR ($CHCl_3$): $\tilde{\nu} = 3690$ (w), 3405 (w), 3322 (m), 2963 (m), 2923 (w), 2872 (w), 1709 (m), 1658 (s), 1540 (m), 1500 (m), 1455 (m), 1392 (w), 1368 (m), 1292 (w), 1175 (m), 1123 (w), 1055 (m), 848 (m); HR-MALDI-MS: 834.5 (7), 657.4 (40) $[M+Na]^+$, 599.3 (7), 557.3 (63), $[M+Na-Boc]^+$, 535.3 (100) $[M+H-Boc]^+$, 427.3 (5), 384.2 (5), 321.2 (26), 282.2 (8), 267.7 (7), 257.2 (6).

Boc-(2S,3S)-β²,³-hAla(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OBn (***30***): The β-tripeptide **27** (275 mg, 0.52 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in CH_2Cl_2 (4 mL) and treated with acid (*S*,*S*)-**12** (115 mg, 0.52 mmol), NMM (170 μL, 1.6 mmol), and HATU (237 mg, 0.62 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 95:5) yielded the β-tetrapeptide **30** (190 mg, 58%) as a colorless solid. R_f = 0.50 (CH_2Cl_2/MeOH, 92.5:7.5). M.p. 193–195 °C; $[\alpha]_D^{RT} = -37.7$ (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 0.86–0.95 (m, 12H; 4Me), 1.10 (d, *J* = 6.7, 3H; Me), 1.11 (d, *J* = 6.9, 3H; Me), 1.25 (ddd, *J* = 4.7, 9.1, 13.8, 1H; C*H*H′CH), 1.41–1.47 (m, 1H; CH*H*′CH), 1.44 (s, 9H; CMe_3), 1.56–1.62 (m, 1H; C*H*Me_2), 1.80–1.87 (m, 1H; C*H*Me_2), 2.15 (dd, *J* = 7.8, 13.8, 1H; C*H*H′CO), 2.31 (dd, *J* = 8.8, 14.4, 1H; C*H*H′CO), 2.38 (dd, *J* = 5.9, 13.7, 1H; CH*H*′CO), 2.44 (dd, *J* = 4.6, 14.4, 1H; CH*H*′CO), 2.48 (dd, *J* = 2.1, 10.2, 1H; C*H*H′CO), 2.52 (dd, *J* = 1.6, 10.1, 1H; CH*H*′CO), 4.07–4.19 (m, 3H; 3NCH), 4.28–4.33 (m, 1H; NCH), 4.92 (dd, *J* = 2.6, 49.8, 1H; CHF), 5.06 (d, *J* = 12.3, 1H; C*H*H′Ph), 5.14 (d, *J* = 12.2, 1H; CH*H*′Ph), 7.28–7.38 (m, 5H; arom.); ^{13}C NMR (125 MHz, CD_3OD): δ = 14.2 (d, *J* = 5.8, CH_3), 19.1, 19.8, 20.1, 22.2, 23.6 (CH_3); 26.0 (CH), 28.8 (CH_3), 33.2 (CH), 39.2, 41.2, 43.5, 44.5 (CH_2); 44.6, 46.1 (CH); ca. 49.0 (d, CH), 53.6 (CH), 67.4 (CH_2), 80.4 (C), 93.8 (d, *J* = 190.7, CH), 129.3, 129.5, 129.6 (CH); 137.6, 157.4 (C); 169.6 (d, *J* = 19.8, C), 172.5, 172.6, 172.7 (C); ^{19}F NMR (282 MHz, CD_3OD): δ = −204.1 (dd, *J* = 28.8, 49.1, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3432 (m), 3007 (w), 2965 (m), 2874 (w), 1713 (s), 1666 (s), 1502 (s), 1456 (w), 1390 (w), 1368 (m), 1170 (s), 1097 (w), 1059 (m), 988 (w), 882 (w); HR-MALDI-MS: 659.4 (22) $[M+Na]^+$, 559.3 (16) $[M+Na-Boc]^+$, 537.3 (100) $[M+H-Boc]^+$, 429.3 (27), 321.2 (19), 302.2 (27); elemental analysis calcd (%) for $C_{33}H_{53}FN_4O_7$ (636.80): C 62.24, H 8.39, N 8.80; found: C 62.12, H 8.22, N 8.66.

Boc-(2R,3S)-β²,³-hAla(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OBn (***31***): The β-tripeptide **27** (267 mg, 0.50 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in CH_2Cl_2 (2.5 mL) and treated with acid (*R*,*S*)-**12** (111 mg, 0.50 mmol), NMM (165 μL, 1.5 mmol), and HATU (228 mg, 0.6 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 95:5) yielded the β-tetrapeptide **31** (290 mg, 91%) as a colorless solid. R_f = 0.25 (CH_2Cl_2/MeOH, 95:5). M.p. 196–198 °C; $[\alpha]_D^{RT} = -12.9$ (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 0.86–0.93 (m, 12H; 4Me), 1.11 (d, *J* = 6.7, 3H; Me), 1.20 (d, *J* = 6.9, 3H; Me), 1.25 (ddd, *J* = 4.6, 9.2, 13.8, 1H; C*H*H′CH), 1.41 (s, 9H; CMe_3), 1.41–1.47 (m, 1H; CH*H*′CH), 1.56–1.63 (m, 1H; C*H*Me_2), 1.80–1.87 (m, 1H; C*H*Me_2), 2.18 (dd, *J* = 7.6, 13.8, 1H; C*H*H′CO), 2.34–2.43 (m, 3H; CH*H*′CO + CH_2CO), 2.49 (dd, *J* = 1.2, 9.3, 1H; C*H*H′CO), 2.52 (dd, *J* = 0.6, 9.2, 1H; CH*H*′CO), 4.03–4.18 (m, 3H; 3NCH), 4.28–4.34 (m, 1H; NCH), 4.80 (dd, *J* = 3.7, 47.9, 1H; CHF), 5.06 (d, *J* = 12.3, 1H; C*H*H′Ph), 5.14 (d, *J* = 12.3, 1H; CH*H*′Ph), 7.29–7.38 (m, 5H; arom.); ^{13}C NMR (125 MHz, CD_3OD): δ = 17.3, 18.9, 19.9, 20.1, 22.2, 23.6 (CH_3); 26.0 (CH), 28.8 (CH_3), 32.9 (CH), 39.3, 41.2, 43.5, 44.5 (CH_2); 44.6, 46.0 (CH); ca. 49.0 (d, CH), 53.6 (CH), 67.4 (CH_2), 80.4 (C), 94.0 (d, *J* = 189.4, CH), 129.3, 129.5, 129.6 (CH); 137.6, 157.5 (C); 169.9 (d, *J* = 21.0, C), 172.5, 172.6, 172.6 (C); ^{19}F NMR (282 MHz, CD_3OD): δ = −197.7 (dd, *J* = 23.5, 47.0, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3429 (m), 3004 (w), 2966 (m), 2873 (w), 1709 (s), 1664 (s), 1503 (s), 1455 (w), 1390 (w), 1368 (m), 1342 (w), 1303 (w), 1171 (s), 1092 (w), 1056 (w), 1026 (w), 990 (w), 848 (w); HR-MALDI-MS: 659.4 (27) $[M+Na]^+$, 587.4 (12), 559.3 (17) $[M+Na-Boc]^+$, 537.3 (100), $[M+H-Boc]^+$, 429.3 (11), 321.2 (7), 302.2 (6), 284.2 (11); elemental analysis calcd (%) for $C_{33}H_{53}FN_4O_7$ (636.80): C 62.24, H 8.39, N 8.80; found: C 62.31, H 8.25, N 8.78.

Boc-(3S)-β²,²,³-hAla(α,α-F₂)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OBn (***32***): The β-tripeptide **27** (246 mg, 0.46 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in DMF (3 mL) and treated with acid **13** (110 mg, 0.46 mmol), NMM (150 μL, 1.59 mmol), and HATU (210 mg, 0.55 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 98:2) yielded β-tetrapeptide **32** (185 mg, 61%) as a colorless solid. M.p. 189 °C; R_f = 0.30 (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{RT} = -9.0$ (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 0.86–0.95 (m, 12H; 4Me), 1.10 (d, *J* = 6.7, 3H; Me), 1.19 (d, *J* = 7.0, 3H; Me), 1.26 (ddd, *J* = 4.7, 9.1, 13.8, 1H; C*H*H′CH), 1.41–1.47 (m, 1H; CH*H*′CH), 1.42 (s, 9H; CMe_3), 1.56–1.64 (m, 1H; C*H*Me_2), 1.80–1.90 (m, 1H; C*H*Me_2), 2.17 (dd, *J* = 7.7, 13.8, 1H; C*H*H′CO), 2.32–2.54 (m, 5H; CH*H*′CO + 2CH_2O), 4.04–4.16 (m, 2H; 2NCH), 4.27–4.34 (m, 2H; 2NCH), 5.06 (d, *J* = 12.3, 1H; C*H*H′Ph), 5.14 (d, *J* = 12.3, 1H; CH*H*′Ph), 7.28–7.37 (m, 5H; arom.); ^{13}C NMR (125 MHz, CD_3OD): δ = 14.5, 19.1, 19.8, 20.0, 22.2, 23.6 (CH_3); 26.1 (CH), 28.7 (CH_3), 33.1 (CH), 39.1, 41.2, 43.5, 44.6 (CH_2); 44.6, 46.1, 54.3 (CH); 67.4 (CH_2), 80.7 (C), 117.7 (*t*, *J* = 25.5, CF_2), 129.3, 129.5, 129.6 (CH); 137.6, 157.6 (C); 165.1 (*t*, *J* = 28.5, C), 172.5, 172.6, 172.7 (C); ^{19}F NMR (282 MHz, CD_3OD): δ = −116.6 (dd, *J* = 12.8, 251.8, C*F*′), −119.1 (dd, *J* = 13.9, 251.8, CF*F*′); IR ($CHCl_3$): $\tilde{\nu}$ = 3429 (m), 3005 (w), 2965 (m), 2868 (w), 1713 (s), 1659 (s), 1504 (s), 1455 (m), 1388 (w), 1368 (m), 1324 (w), 1171 (s), 1051 (w), 1029 (w), 1002 (w), 912 (w), 857 (w); HR-MALDI-MS: 677.4 (21) $[M+Na]^+$, 577.3 (100) $[M+Na-Boc]^+$, 555.3 (16) $[M+H-Boc]^+$, 447.3 (17), 320.2 (8), 288.7 (7); elemental analysis calcd (%) for $C_{33}H_{52}F_2N_4O_7$ (654.79): C 60.53, H 8.00, N 8.56, F 5.80; found: C 60.46, H 7.90, N 8.56, F 5.91.

Boc-(2R)-β²-hGly(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OBn (***33***): The β-tripeptide **27** (138 mg, 0.26 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in DMF (2 mL), treated with acid **14** (54 mg, 0.26 mmol), NMM (140 μL, 1.3 mmol), and HATU (118 mg, 0.3 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 95:5) yielded β-tetrapeptide **33** (112 mg, 70%) as a colorless solid. M.p. 212–213 °C; R_f = 0.20 (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{RT} = +3.4$ (*c* = 1.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 0.86–0.94 (m, 12H; 4Me), 1.11 (d, *J* = 6.7, 3H; Me), 1.25 (ddd, *J* = 4.6, 9.1, 13.8, 1H; C*H*H′CH), 1.41–1.47 (m, 1H; CH*H*′CH), 1.43 (s, 9H; CMe_3), 1.56–1.64 (m, 1H; C*H*Me_2), 1.81 (qd, *J* = 6.8, 13.5, 1H; C*H*Me_2), 2.18 (dd, *J* = 7.6, 13.8, 1H; C*H*H′CO), 2.32 (dd, *J* = 8.7, 14.3, 1H; C*H*H′CO), 2.39 (dd, *J* = 6.0, 13.8, 1H; CH*H*′CO), 2.44 (dd, *J* = 4.7, 14.4, 1H; CH*H*′CO), 2.48 (dd, *J* = 6.9, 15.0, 1H; C*H*H′CO), 2.52 (dd, *J* = 6.4, 15.0, 1H; CH*H*′CO), 3.41 (ddd, *J* = 7.1, 14.8, 21.9, 1H; BocHNC*H*H′), 3.55–3.63 (m, 1H; BocHNCH*H*′), 4.04–4.08 (m, 1H; NCH), 4.10–4.17 (m, 1H; NCH), 4.28–4.34 (m, 1H; NCH), 4.90 (ddd, *J* = 3.5, 7.1, 49.5, 1H; CHF), 5.07 (d, *J* = 12.3, 1H; C*H*H′Ph), 5.14 (d, *J* = 12.3, 1H; CH*H*′Ph), 7.28–7.38 (m, 5H; arom.); ^{13}C NMR (125 MHz, CD_3OD): δ = 18.9, 19.8, 20.1, 22.2, 23.6 (CH_3); 26.1 (CH), 28.8 (CH_3), 33.2 (CH), 39.5, 41.2, 43.6, 43.8 (CH_2); 44.6, 46.1, 53.7 (CH); 67.5 (CH_2), 80.5 (C), 91.3 (d, *J* = 187.5), 129.3, 129.5, 129.6 (CH); 137.6, 158.3, 169.9 (d, *J* = 19.8); 172.6, 172.7, 172.7 (C); ^{19}F NMR (282 MHz, CD_3OD): δ = −193.3 (ddd, *J* = 21.4, 27.8, 49.1, CHF); IR ($CHCl_3$): $\tilde{\nu}$ = 3427 (w), 2963 (m), 2873 (w), 1715 (s), 1662 (s), 1505 (s), 1456 (w), 1368 (m), 1272 (w), 1173 (s), 1087 (w), 923 (w), 853 (w); HR-MALDI-MS: 661.3 (4) $[M+K]^+$, 645.4 (53) $[M+Na]^+$, 561.3 (6) $[M+K-Boc]^+$, 545.3 (88), $[M+Na-Boc]^+$, 523.3 (100) $[M+H-Boc]^+$, 456.3 (4), 415.3 (22), 397.3 (5), 321.2 (11), 288.2 (13), 270.2 (24), 261.7 (4); elemental analysis calcd (%) for $C_{32}H_{51}FN_4O_7$ (622.78): C 61.72, H 8.25, N 9.00; found: C 61.76, H 8.22, N 8.98.

Boc-β-hGly-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OBn (***34***): The β-tripeptide **27** (136 mg, 0.25 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in DMF (2 mL), treated with acid **15** (48 mg, 0.25 mmol), NMM (140 μL, 1.3 mmol), and HATU (116 mg, 0.3 mmol) according to GP 7a. FC (CH_2Cl_2/MeOH, 95:5) yielded β-tetrapeptide **34** (139 mg, 90%) as a colorless solid. R_f = 0.51 (CH_2Cl_2/MeOH, 9:1). M.p. 188–189 °C; $[\alpha]_D^{RT} = -16.6$ (*c* = 1.0,

MeOH); ^{1}H NMR (600 MHz, CD_3OD): $\delta = 0.86$–0.93 (m, 12H; 4Me), 1.11 (d, $J = 6.7$, 3H; Me), 1.25 (ddd, $J = 4.6$, 9.2, 13.8, 1H; C*H*H′CH), 1.42 (s, 9H; CMe_3), 1.42–1.46 (m, 1H; CH*H*′CH), 1.56–1.63 (m, 1H; C*H*Me_2), 1.74–1.80 (m, 1H; C*H*Me_2), 2.17 (dd, $J = 7.5$, 13.8, 1H; C*H*H′CO), 2.21 (dd, $J = 9.3$, 14.2, 1H; C*H*H′CO), 2.31–2.43 (m, 4H; 2CH*H*′CO + CH_2CO), 2.49 (dd, $J = 6.9$, 15.0, 1H; C*H*H′CO), 2.51 (dd, $J = 6.4$, 15.0, 1H; CH*H*′CO), 3.28–3.31 (m, 2H; BocHNCH_2), 4.06–4.10 (m, 1H; NCH), 4.11–4.16 (m, 1H; NCH), 4.29–4.34 (m, 1H; NCH), 5.07 (d, $J = 12.3$, 1H; C*H*H′Ph), 5.14 (d, $J = 12.3$, 1H; CH*H*′Ph), 7.29–7.37 (m, 5H; arom.); ^{13}C NMR (150.9 MHz, CD_3OD): $\delta = 18.6$, 19.8, 20.2, 22.2, 23.6 (CH_3); 26.1 (CH), 28.8 (CH_3), 33.3 (CH), 37.5, 38.2, 40.1, 41.2, 43.5 (CH_2); 44.6 (CH), 44.6 (CH_2), 46.1, 53.5 (CH); 67.5 (CH_2), 80.2 (C), 129.3, 129.5, 129.6 (CH); 137.6, 158.3, 172.6, 172.6, 172.8, 173.5 (C); IR ($CHCl_3$): $\tilde{\nu} = 3428$ (w), 3007 (w), 2981 (m), 2872 (w), 1707 (s), 1659 (s), 1501 (s), 1456 (w), 1368 (m), 1308 (w), 1272 (w), 1172 (s), 984 (w); HR-MALDI-MS: 643.3 (2) $[M+K]^+$, 627.4 (32) $[M+Na]^+$, 527.3 (21) $[M+Na-Boc]^+$, 505.3 (100) $[M+H-Boc]^+$, 397.3 (5), 321.2 (25), 270.2 (9), 252.7 (6); elemental analysis calcd (%) for $C_{32}H_{52}N_4O_7$ (604.79): C 63.55, H 8.67, N 9.26; found: C 63.47, H 8.65, N 9.21.

Boc-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-(2S,3S)-$\beta^{2,3}$-hAla(α-OH)-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-OBn (***36***): The β-tetrapeptide **28** (91 mg, 0.14 mmol) was Boc deprotected according to GP 5a, the resulting TFA salt was dissolved in DMF (4 mL), treated with acid **35** (64 mg, 0.14 mmol), NMM (80 μL, 0.73 mmol), and HATU (66 mg, 0.17 mmol) according to GP 7b. Drying under h.v. yielded the β-heptapeptide **36** (79 mg, 58%) as a light yellow solid. M.p. 258–260 °C (dec.); ^{1}H NMR (500 MHz, $[D_6]$DMSO): $\delta = 0.78$–0.84 (m, 24H; 8Me), 0.88 (d, $J = 6.8$, 3H; Me), 0.97 (*t*, $J = 6.4$, 6H; 2Me), 1.10–1.17 (m, 2H; 2C*H*H′CH), 1.31–1.39 (m, 2H; 2CH*H*′CH), 1.36 (s, 9H; CMe_3), 1.54–1.61 (m, 2H; 2C*H*Me_2), 1.62–1.70 (m, 1H; C*H*Me_2), 1.71–1.75 (m, 1H; C*H*Me_2), 2.01–2.30 (m, 10H; 5CH_2CO), 2.40 (dd, $J = 6.8$, 14.8, 1H; C*H*H′CO), 2.45 (dd, $J = 6.9$, 14.8, 1H; CH*H*′CO), 3.60–3.70 (m, 1H; BocNHC*H*), 3.85–3.90 (m, 1H; NHC*H*), 3.95 (dd, $J = 2.8$, 5.4, 1H; C*H*OH), 4.04–4.17 (m, 5H; 5NHC*H*), 5.03 (d, $J = 12.4$, 1H; C*H*H′Ph), 5.09 (d, $J = 12.4$, 1H; CH*H*′Ph), 5.71 (d, $J = 5.3$, 1H; OH), 6.52 (d, $J = 9.2$, 1H; BocN*H*), 7.30–7.39 (m, 5H; arom.), 7.62–7.68 (m, 3H; 3NH), 7.73–7.79 (m, 3H; 3NH); ^{13}C NMR (125 MHz, $[D_6]$DMSO): $\delta = 13.4$, 17.8, 18.6, 19.1, 19.2, 19.5, 19.7, 21.4, 21.5, 23.1, 23.4 (CH_3); 24.2, 24.3 (CH); 28.2 (CH_3); 31.1, 31.7 (CH); 37.3, 38.4, 40.1, 41.8 (CH_2); 42.0, 42.1 (CH); 42.2, 42.4, 42.9, 43.0 (CH_2); 43.8, 44.1, 47.0, 50.9, 52.6 (CH); 65.5 (CH_2); 72.8 (CH); 77.2 (C); 127.9, 128.0, 128.4 (CH); 136.1, 155.2, 169.2, 169.3, 169.4, 169.5, 169.6, 170.6, 170.7 (C); HR-MALDI-MS: 983 (5) $[M+Na]^+$, 883 (12) $[M+Na-Boc]$, 861 (100) $[M+H-Boc]^+$, 847 (6), 788 (11), 599 (12), 554 (10), 535 (8), 469 (6).

Boc-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-(2R,3S)-$\beta^{2,3}$-hAla(α-OH)-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-OBn (***37***): The β-tetrapeptide **29** (124 mg, 0.19 mmol) was Boc deprotected according to GP 5a, the resulting TFA salt was dissolved in DMF (4 mL), treated with acid **35** (84 mg, 0.19 mmol), NMM (105 μL, 0.95 mmol), and HATU (87 mg, 0.23 mmol) according to GP 7b. Drying under h.v. yielded the β-heptapeptide **37** (136 mg, 74%) as a colorless solid. M.p. 247–249 °C (dec.); ^{1}H NMR (500 MHz, $[D_6]$DMSO): $\delta = 0.78$–0.83 (m, 24H; 8Me), 0.96–0.99 (m, 9H; 3Me), 1.11–1.17 (m, 2H; 2C*H*H′CH), 1.29–1.39 (m, 2H; 2CH*H*′CH), 1.36 (s, 9H; CMe_3), 1.53–1.61 (m, 2H; 2C*H*Me_2), 1.62–1.67 (m, 1H; C*H*Me_2), 1.69–1.73 (m, 1H; C*H*Me_2), 2.02–2.26 (m, 10H; 5CH_2CO), 2.40 (dd, $J = 6.9$, 14.7, 1H; C*H*H′CO), 2.45 (dd, $J = 6.9$, 14.8, 1H; CH*H*′CO), 3.56–3.65 (m, 1H; BocNHC*H*), 3.82 (dd, $J = 3.8$, 5.4, 1H; C*H*OH), 3.88–3.92 (m, 1H; NHC*H*), 4.01–4.17 (m, 5H; 5NHC*H*), 5.06 (q, $J = 12.4$, 2H; CH_2Ph), 5.70 (d, $J = 5.5$, 1H; OH), 6.50 (d, $J = 9.2$, 1H; BocN*H*), 7.30–7.39 (m, 6H; NH + 5H; arom.), 7.60–7.63 (m, 3H; 3NH), 7.75–7.77 (m, 2H; 2NH); ^{13}C NMR (125 MHz, $[D_6]$DMSO): $\delta = 16.5$, 17.7, 18.0, 19.0, 19.1, 19.5, 19.6, 21.3, 21.4, 23.1 (CH_3); 23.2 (CH), 24.1, 28.1 (CH_3); 30.8, 31.6 (CH); 37.5 (CH_2), 41.7, 42.0 (CH); 42.9 (CH_2), 43.7, 44.0, 47.3, 50.7, 52.5 (CH); 65.4 (CH_2), 72.8 (CH), 77.1 (C), 127.9, 128.0, 128.3 (CH); 136.0, 155.1, 169.0, 169.1, 169.2, 169.3, 169.4, 170.5, 171.2 (C); HR-MALDI-MS: 983 (72) $[M+Na]^+$, 883 (100), $[M+Na-Boc]$, 861 (21) $[M+H-Boc]^+$, 790 (10), 627 (7), 554 (14), 535 (8), 469 (6).

Boc-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-(2S,3S)-$\beta^{2,3}$-hAla(α-F)-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-OBn (***38***): The β-tetrapeptide **30** (35 mg, 56 μmol) was Boc deprotected according to GP 5b, the resulting free amine (30 mg, 56 μmol) was dissolved in DMF (1 mL), treated with acid **35** (25 mg, 56 μmol), NMM (20 μL, 0.17 mmol), and HATU (25.5 mg, 67 μmol) according to GP 7b. Drying under h.v. yielded the β-heptapeptide **38** (43 mg, 80%) as a colorless solid. M.p. 257–259 °C (dec.); ^{1}H NMR (500 MHz, $[D_3]$TFE): $\delta = 0.88$–0.97 (m, 24H; 8Me), 1.12 (d, $J = 6.7$, 3H; Me), 1.18–1.21 (m, 6H; 2Me), 1.26–1.35 (m, 2H; 2C*H*H′CH), 1.41–1.53 (m, 2H; 2CH*H*′CH), 1.45 (s, 9H; CMe_3), 1.58–1.65 (m, 2H; 2C*H*Me_2), 1.74–1.77 (m, 1H; C*H*Me_2), 1.85–1.89 (m, 1H; C*H*Me_2), 2.21–2.57 (m, 12H; 6CH_2CO), 3.71–3.76 (m, 1H; BocHNC*H*), 4.08–4.12 (m, 2H; 2NCH), 4.22–4.38 (m, 3H; 3NCH), 4.49–4.56 (m, 1H; NCH), 4.99 (dd, $J = 2.2$, 49.9, 1H; CHF), 5.10 (d, $J = 12.2$, 1H; C*H*H′Ph), 5.18 (d, $J = 12.2$, 1H; CH*H*′Ph), 7.34–7.39 (m, 5H; arom.); ^{13}C NMR (125 MHz, $[D_3]$TFE): $\delta = 13.7$ (d, $J = 5.7$, CH_3), 18.4, 19.2, 19.8, 19.9, 20.2, 20.5, 22.3, 22.4, 23.5, 23.6 (CH_3); 26.5 (CH), 29.0 (CH_3), 33.5, 34.4 (CH); 39.7, 41.2, 41.5, 43.6, 43.9, 44.3, 44.7, 44.8 (CH_2); 45.5, 45.6, 47.1, 47.7 (CH); 48.7 (d, $J = 19.3$, CH), 54.5, 55.7 (CH); 69.0 (CH_2), 82.6 (C), 93.9 (d, $J = 191.1$, CH), 130.1, 130.3, 130.4 (CH); 137.5, 159.4 (C); 170.4 (d, $J = 19.0$, C), 173.9, 174.1, 174.4, 174.9, 175.0 (C); ^{19}F NMR (282 MHz, $[D_6]$DMSO): $\delta = -203.4$ (dd, $J = 31.0$, 49.1, CHF); HR-MALDI-MS: 984.6 (40) $[M+Na]^+$, 884.6 (31) $[M+Na-Boc]^+$, 862.6 (100) $[M+H-Boc]^+$, 842.6 (14), 791.5 (13), 683.5 (14), 664.4 (20), 627.4 (20), 556.3 (30), 537.3 (28).

Boc-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-(2R,3S)-$\beta^{2,3}$-hAla(α-F)-(3S)-β^3-hVal-(3S)-β^3-hAla-(3S)-β^3-hLeu-OBn (***39***): The β-tetrapeptide **31** (182 mg, 0.286 mmol) was Boc deprotected according to GP 5b, the resulting free amine (154 mg, 0.286 mmol) was dissolved in DMF (6 mL), treated with acid **35** (126 mg, 0.286 mmol), NMM (100 μL, 0.87 mmol), and HATU (130 mg, 0.34 mmol) according to GP 7b. Drying under h.v. yielded the β-heptapeptide **39** (244 mg, 88%) as a colorless solid. M.p. 254–255 °C; ^{1}H NMR (500 MHz, $[D_3]$TFE): $\delta = 0.89$–0.96 (m, 24H; 8Me), 1.14 (d, $J = 6.7$, 3H; Me), 1.20 (d, $J = 6.6$, 3H; Me), 1.25 (d, $J = 7.0$, 3H; Me), 1.28–1.34 (m, 2H; 2C*H*H′CH), 1.41–1.51 (m, 2H; 2CH*H*′CH), 1.45 (s, 9H; CMe_3), 1.55–1.63 (m, 2H; 2C*H*Me_2), 1.73–1.80 (m, 1H; C*H*Me_2), 1.82–1.89 (m, 1H; C*H*Me_2), 2.25–2.50 (m, 10H; 5CH_2CO), 2.56 (dd, $J = 7.2$, 15.5, 1H; C*H*H′CO), 2.61 (dd, $J = 5.7$, 15.4, 1H; CH*H*′CO), 3.69–3.78 (m, 1H; BocHNC*H*), 4.09–4.11 (m, 1H; NCH), 4.18–4.28 (m, 2H; 2NCH), 4.33–4.44 (m, 2H; 2NCH), 4.48–4.55 (m, 1H; NCH), 4.84 (dd, $J = 3.2$, 47.1, 1H; CHF), 5.11 (d, $J = 12.3$, 1H; C*H*H′Ph), 5.20 (d, $J = 12.3$, 1H; CH*H*′Ph), 7.34–7.42 (m, 5H; arom.); ^{13}C NMR (125 MHz, $[D_3]$TFE): $\delta = 16.6$, 18.5, 19.2, 19.7, 19.8, 20.4, 20.7, 22.6, 22.6, 23.4, 23.6 (CH_3), 26.5, 26.6 (CH); 29.1 (CH_3), 33.7, 34.4 (CH); 39.2, 40.9, 41.3, 43.1, 43.7, 44.1, 45.1 (CH_2); 45.3, 45.4 (CH); 45.5 (CH_2), 47.0, 47.4 (CH); 48.1 (d, $J = 21.8$, CH), 54.4, 55.7 (CH); 68.8 (CH_2), 82.4 (C), 93.8 (d, $J = 191.1$, CH), 129.9, 130.3, 130.4 (CH); 137.6, 159.3 (C); 170.6 (d, $J = 20.2$, C), 173.6, 173.8, 174.0, 174.2, 174.8, 175.0 (C); ^{19}F NMR (282 MHz, $[D_6]$DMSO): $\delta = -193.0$ (dd, $J = 22.4$, 47.0, CHF); HR-MALDI-MS: 984.6 (33) $[M+Na]^+$, 884.6 (20) $[M+Na-Boc]^+$, 862.6 (100) $[M+H-Boc]^+$, 791.5 (34), 683.5 (17), 664.4 (12), 556.4 (74), 537.3 (48), 471.3 (9).

Boc-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-(3S)-β²,²,³-hAla(α,α-F₂)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OBn (***40***): The β-tetrapeptide **32** (149 mg, 0.23 mmol) was Boc deprotected according to GP 5b, the resulting free amine was dissolved in DMF (3 mL), treated with acid **35** (101 mg, 0.23 mmol), NMM (70 μL, 0.66 mmol), and HATU (100 mg, 0.26 mmol) according to GP 7b. Drying under h.v. yielded the β-heptapeptide **40** (165 mg, 74%) as a colorless solid. M.p. > 260 °C (dec.); $[\alpha]_D^{RT} = -33.3$ ($c = 1.0$, hexafluoro-*iso*-propanol); ^{1}H NMR (500 MHz, [D$_3$]TFE): $\delta = 0.89$–0.98 (m, 24H; 8Me), 1.12 (d, $J = 6.7$, 3H; Me), 1.20 (d, $J = 6.6$, 3H; Me), 1.24 (d, $J = 7.0$, 3H; Me), 1.28–1.36 (m, 2H; 2CHH′CH), 1.41–1.51 (m, 2H; 2CHH′CH), 1.45 (s, 9H; CMe$_3$), 1.56–1.60 (m, 2H; 2CHMe$_2$), 1.74–1.78 (m, 1H; CHMe$_2$), 1.83–1.88 (m, 1H; CHMe$_2$), 2.25–2.63 (m, 12H; 6CH$_2$CO), 3.68–3.77 (m, 1H; BocHNCH), 4.11–4.39 (m, 5H; 5NCH), 4.71–4.77 (m, 1H; NCHCF$_2$), 5.10 (d, 1H; $J = 12.3$, CHH′Ph), 5.20 (d, $J = 12.3$, 1H; CHH′Ph), 7.35–7.41 (m, 5H; arom.); ^{13}C NMR (125 MHz, [D$_3$]TFE): $\delta = 14.4$, 18.4, 19.3, 19.7, 19.8, 20.3, 20.7, 22.4, 22.5, 23.4, 23.6 (CH$_3$); 26.5 (CH), 29.1 (CH$_3$), 33.7, 34.4 (CH); 39.2, 41.0, 41.4, 43.3, 43.8, 44.2, 44.9, 45.0 (CH$_2$); 45.4, 47.0, 47.6 (CH); 48.9 (*t*, $J = 26.4$, CHCF$_2$), 55.0, 55.7 (CH); 68.9 (CH$_2$), 82.5 (C), 118.1 (*t*, $J = 255.9$, CF$_2$), 129.9, 130.3, 130.4 (CH); 137.5, 159.4 (C); 165.8 (*t*, $J = 28.3$, CF$_2$CO), 173.7, 174.0, 174.2, 174.6, 174.9, 175.0 (C); ^{19}F NMR (282 MHz, [D$_6$]DMSO): $\delta = -109.7$ (dd, $J = 9.6$, 247.6, CFF′), −117.4 (dd, $J = 18.1$, 244.7, CFF′); HR-MALDI-MS: 1019 (3) $[M+K]^+$, 1003 (60) $[M+Na]^+$, 903 (100), $[M+Na-Boc]^+$, 881 (30), $[M+H-Boc]^+$, 809 (12), 701 (10), 682 (13), 574 (25), 555 (20).

Preparation of β-heptapeptides 1–5 and β-tetrapeptides 6–10

TFA·H-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-(2S,3S)-β²,³-hAla(α-OH)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***1***): Hydrogenolysis of the β-heptapeptide **36** (48 mg, 50 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 10 to 50% B over 40 min) at a flow rate of 20 mL min^{-1}. Lyophilization yielded the β-heptapeptide **1** (31 mg, 69%) as a colorless foam. ^{1}H NMR (500 MHz, CD$_3$OH): 0.90–0.96 (m, 18H; 6Me), 1.05 (d, $J = 6.9$, 3H; Me), 1.06 (d, $J = 6.9$, 3H; Me), 1.12 (d, $J = 6.9$, 3H; Me), 1.14 (d, $J = 6.9$, 3H; Me), 1.21 (d, $J = 6.7$, 3H; Me), 1.25–1.34 (m, 2H; 2CHH′CH), 1.40–1.46 (m, 2H; 2CHH′CH), 1.57–1.63 (m, 2H; 2CHMe$_2$), 1.79–1.85 (m, 1H; CHMe$_2$), 1.99–2.05 (m, 1H; CHMe$_2$), 2.28–2.71 (m, 12H; 6CH$_2$CO), 3.45–3.51 (m, 1H; H$_2$NCH), 4.07 (d, $J = 6.7$, 1H; CHOH), 4.13–4.19 (m, 1H; NCH), 4.21–4.27 (m, 1H; NCH), 4.30–4.40 (m, 3H; 3NCH), 4.41–4.48 (m, 1H; NCH), 7.73 (d, $J = 10.4$, 1H; NH), 7.75 (d, $J = 8.9$, 1H; NH), 7.82 (d, $J = 9.1$, 1H; NH), 8.12 (d, $J = 7.9$, 1H; NH), 8.14 (d, $J = 8.5$, 1H; NH), 8.20 (d, $J = 8.6$, 1H; NH); ^{13}C NMR (125 MHz, CD$_3$OH): 16.0, 17.8, 18.9, 19.3, 19.7, 20.6, 21.1, 22.6, 22.7, 23.6, 23.7 (CH$_3$); 26.0, 26.1, 32.0, 33.9 (CH); 36.1, 39.1, 41.1, 42.8, 43.3, 43.4 (CH$_2$); 44.0, 44.1 (CH); 45.3 (CH$_2$), 45.8 (CH), 46.1 (CH$_2$), 46.3, 48.7, 53.4, 56.2, 75.6 (CH); 171.6, 172.0, 172.3, 172.5, 173.0, 173.7, 175.2 (C); HR-MALDI-MS: 814.5 (7), 792.5 (32) $[M+Na]^+$, 770.5 (100) $[M+H]^+$, 699.5 (6), 681.5 (9), 572.4 (5), 554.4 (8), 537.4 (6), 469.3 (6), 445.3 (10), 385.3 (5), 344.3 (5), 328.2 (5).

TFA·H-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-(2R,3S)-β²,³-hAla(α-OH)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***2***): Hydrogenolysis of the β-heptapeptide **37** (55 mg, 57 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 10 to 50% B over 40 min) at a flow rate of 20 mL min^{-1}. Lyophilization yielded the β-heptapeptide **2** (22 mg, 44%) as a colorless foam. ^{1}H NMR (500 MHz, CD$_3$OH): 0.89–0.95 (m, 18H; 6Me), 1.07 (d, $J = 6.9$, 6H; 2Me), 1.14 (d, $J = 6.7$, 3H; Me), 1.18 (d, $J = 6.9$, 3H; Me), 1.23 (d, $J = 6.7$, 3H; Me), 1.25–1.33 (m, 2H; 2CHH′CH), 1.36–1.47 (m, 2H; 2CHH′CH), 1.55–1.65 (m, 2H; 2CHMe$_2$), 1.77–1.84 (m, 1H; CHMe$_2$), 1.99–2.09 (m, 1H; CHMe$_2$), 2.34–2.56 (m, 8H; 2CHH′CO + 3CH$_2$CO), 2.60 (dd, $J = 3.1$, 15.5, 1H; CHH′CO), 2.65 (dd, $J = 11.9$, 14.8, 1H; CHH′CO), 2.70 (dd, $J = 11.5$, 15.5, 1H; CHH′CO), 2.78 (dd, $J = 11.8$, 14.9, 1H; CHH′CO), 3.55–3.63 (m, 1H; H$_2$NCH), 4.08 (d, $J = 2.7$, 1H; CHOH), 4.20–4.27 (m, 1H; NCH), 4.35–4.48 (m, 3H; 3NCH), 4.54–4.60 (m, 2H; 2NCH), 7.64 (d, $J = 8.3$, 1H; NH), 7.70 (d, $J = 9.4$, 1H; NH), 7.83 (d, $J = 9.1$, 1H; NH), 7.99 (d, $J = 9.0$, 1H; NH), 8.16 (d, $J = 9.1$, 1H; NH), 8.38 (d, $J = 9.2$, 1H; NH); ^{13}C NMR (125 MHz, CD$_3$OH): 17.6, 17.6, 18.9, 19.3, 19.6, 20.9, 21.3, 22.6, 23.0, 23.5, 23.7 (CH$_3$); 26.0, 26.1, 32.1, 34.1 (CH); 36.1, 38.2, 40.6, 42.1, 43.0, 43.3 (CH$_2$); 43.6, 43.8 (CH); 45.3 (CH$_2$), 45.6, 45.9 (CH); 46.7 (CH$_2$), 48.3, 53.0, 56.2, 75.4 (CH); 171.4, 172.0, 172.1, 172.7, 173.2, 173.9, 174.9 (C); HR-MALDI-MS: 814.5 (20), 792.5 (100) $[M+Na]^+$, 775.5 (52), 770.5 (56) $[M+H]^+$, 752.5 (25), 699.5 (19), 681.5 (20), 559.3 (10), 554.4 (25), 537.4 (27), 469.3 (19), 445.3 (20).

TFA·H-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-(2S,3S)-β²,³-hAla(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***3***): Hydrogenolysis of the β-heptapeptide **38** (38.7 mg, 40 μmol) was performed in TFE (10 mL) according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 10 to 50% B over 40 min) at a flow rate of 20 mL min^{-1}. Lyophilization yielded the β-heptapeptide **3** (26.6 mg, 75%) as a colorless foam. ^{1}H NMR (500 MHz, CD$_3$OH): 0.91–0.95 (m, 18H; 6Me), 1.04 (d, $J = 6.8$, 3H; Me), 1.05 (d, $J = 6.8$, 3H; Me), 1.14 (d, $J = 6.7$, 3H; Me), 1.18 (d, $J = 6.8$,3H; Me), 1.21 (d, $J = 6.7$, 3H; Me), 1.25–1.34 (m, 2H; 2CHH′CH), 1.39–1.47 (m, 2H; 2CHH′CH), 1.57–1.65 (m, 2H; 2CHMe$_2$), 1.79–1.86 (m, 1H; CHMe$_2$), 1.97–2.04 (m, 1H; CHMe$_2$), 2.27–2.66 (m, 12H; 6CH$_2$CO), 3.47–3.57 (m, 1H; H$_2$NCH), 4.13–4.19 (m, 1H; NCH), 4.28–4.48 (m, 5H; 5NCH), 4.88 (dd, $J = 5.8$, 49.1, 1H; CHF), 7.81 (d, $J = 8.3$, 1H; NH), 7.85 (d, $J = 9.0$, 1H; NH), 7.99 (d, $J = 8.8$, 1H; NH), 8.09 (d, $J = 8.2$, 1H; NH), 8.10 (d, $J = 8.8$, 1H; NH), 8.33 (d, $J = 8.5$, 1H; NH); ^{13}C NMR (125 MHz, CD$_3$OH): 15.3 (d, $J = 2.7$), 17.9, 18.8, 19.3, 19.8, 20.5, 21.1, 22.5, 22.6, 23.5, 23.6 (CH$_3$); 26.0, 26.1, 32.0, 33.7 (CH); 35.8, 39.1, 41.1, 42.7, 43.3, 43.4 (CH$_2$); 44.1, 44.2 (CH); 45.1, 45.8 (CH$_2$); 45.8, 46.4, 47.2 (d, $J = 24.1$); 53.8, 56.1, 93.7 (d, $J = 183.9$) (CH); 169.0 (d, $J = 19.5$), 171.6, 172.1, 172.2, 172.7, 172.9, 175.2 (C); ^{19}F NMR (282 MHz, CD$_3$OD): $\delta = -75.1$ (s, TFA), −199.2 (dd, $J = 30.9$, 48.0, CHF); HR-MALDI-MS: 816.5 (20), 794.5 (95) $[M+Na]^+$, 772.5 (100) $[M+H]^+$, 757.5 (41), 752.5 (22), 701.5 (11), 683.5 (11), 574.4 (26), 559.4 (59), 556.4 (29), 537.4 (27), 471.3 (10), 447.3 (35).

TFA·H-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-(2R,3S)-β²,³-hAla(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***4***): Hydrogenolysis of the β-heptapeptide **39** (66 mg, 69 μmol) was performed in TFE (20 mL) according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 10 to 50% B over 40 min) at a flow rate of 20 mL min^{-1}. Lyophilization yielded the β-heptapeptide **4** (32 mg, 52%) as a colorless foam. ^{1}H NMR (500 MHz, CD$_3$OH): 0.90–0.96 (m, 18H; 6Me), 1.07 (d, $J = 6.9$, 6H; 2Me), 1.14 (d, $J = 6.7$, 3H; Me), 1.23 (d, $J = 6.7$, 3H; Me), 1.25 (d, $J = 7.1$, 3H; Me), 1.27–1.35 (m, 2H; 2CHH′CH), 1.37–1.44 (m, 2H; 2CHH′CH), 1.55–1.65 (m, 2H; 2CHMe$_2$), 1.77–1.84 (m, 1H; CHMe$_2$), 2.02–2.09 (m, 1H; CHMe$_2$), 2.32 (dd, $J = 10.3$, 15.1, 1H; CHH′CO), 2.39–2.54 (m, 7H; CHH′CO + 3CH$_2$CO), 2.59 (dd, $J = 3.3$, 15.5, 1H; CHH′CO), 2.66 (dd, $J = 11.2$, 15.5, 1H; CHH′CO), 2.76 (dd, $J = 7.3$, 14.8, 1H; CHH′CO), 2.78 (dd, $J = 7.4$, 15.0, 1H; CHH′CO), 3.57–3.64 (m, 1H; H$_2$NCH), 4.25–4.31 (m, 1H; NCH), 4.36–4.51 (m,

3H; 3NCH), 4.52–4.61 (m, 1H; NCH), 4.64–4.66 (m, 1H; NCH), 5.07 (dd, $J=2.5$, 47.6, 1H; CHF), 7.59 (d, $J=8.6$, 1H; NH), 7.72 (d, $J=9.0$, 1H; NH), 7.76–7.78 (m, 2H; 2NH), 8.00 (d, $J=9.2$, 1H; NH), 8.48 (d, $J=8.0$, 2H; 2NH); ^{13}C NMR (125 MHz, CD_3OH): 16.9 (d, $J=5.5$), 17.6, 19.0, 19.5, 19.7, 21.0, 21.3, 22.8, 23.0, 23.5, 23.6 (CH_3); 26.0, 26.1, 32.0, 34.1 (CH); 36.1, 38.1, 40.5, 41.8, 43.2, 43.3 (CH_2); 43.6, 43.7, 45.4 (CH); 45.5 (CH_2), 45.8 (CH), 46.7 (CH_2), 47.0 (d, $J=18.9$), 53.3, 56.3, 94.8 (d, $J=192.3$) (CH); 169.5 (d, $J=20.4$), 171.3, 171.6, 171.7, 172.9, 173.2, 174.8 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta=-75.2$ (s, TFA), -203.5 (dd, $J=34.1$, 47.0, CHF); HR-MALDI-MS: 816.5 (2), 794.5 (24) $[M+Na]^+$, 772.5 (100) $[M+H]^+$, 701.5 (28), 683.5 (6), 574.4 (7), 556.4 (32), 447.3 (23), 386.3 (6).

TFA·H-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-(3S)-β²,²,³-hAla(α,α-F₂)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***5***): Hydrogenolysis of the β-heptapeptide **40** (51.2 mg, 52 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 10 to 50% B over 40 min) at a flow rate of 20 mL min^{-1}. Lyophilization yielded the β-heptapeptide **5** (36 mg, 77%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OH): 0.90–0.97 (m, 18H; 6Me), 1.05 (d, $J=6.8$, 3H; Me), 1.06 (d, $J=6.9$, 3H; Me), 1.13 (d, $J=6.7$, 3H; Me), 1.22 (d, $J=6.6$, 3H; Me), 1.23 (d, $J=6.9$, 3H; Me), 1.26–1.34 (m, 2H; 2CHH′CH), 1.36–1.44 (m, 2H; 2CHH′CH), 1.55–1.65 (m, 2H; 2C*H*Me$_2$), 1.78–1.85 (m, 1H; C*H*Me$_2$), 2.01–2.07 (m, 1H; C*H*Me$_2$), 2.33 (dd, $J=9.9$, 15.1, 1H; C*H*H′C(O), 2.41–2.75 (m, 11H; 5C*H*$_2$CO + CH*H*′CO), 3.51–3.59 (m, 1H; H$_2$NC*H*), 4.19–4.25 (m, 1H; NCH), 4.36–4.48 (m, 3H; 3NCH), 4.50–4.57 (m, 1H; NCH), 4.85–4.96 (m, 1H; NCHCF$_2$), 7.65 (d, $J=8.6$,1H; NH), 7.77 (d, $J=9.0$, 1H; NH), 7.87 (d, $J=9.0$, 1H; NH), 8.36 (d, $J=8.8$, 1H; NH), 8.43 (d, $J=9.1$, 1H; NH), 8.55 (d, $J=9.7$, 1H; NH); ^{13}C NMR (125 MHz, CD_3OH): 13.1 (d, $J=5.1$), 17.5, 18.9, 19.4, 19.9, 20.9, 21.5, 22.7, 23.0, 23.5, 23.6 (CH_3); 26.0, 26.1, 32.1, 34.0 (CH); 35.7, 38.4, 40.6, 41.7, 43.2, 43.3 (CH_2); 43.7, 43.8 (CH); 45.5 (CH_2), 45.5, 45.8 (CH); 46.6 (CH_2), 47.4 (dd, $J=23.1$, 32.9), 54.1, 56.1, 118.3 (dd, $J=251.5$, 263.3) (CH); 164.7 (*t*, $J=26.4$), 171.4, 171.6, 171.8, 173.0, 173.1, 174.9 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta=-75.2$ (s, TFA), -104.7 (d, $J=258.2$, CFF′), -123.1 (dd, $J=25.6$, 258.2, CFF′); HR-MALDI-MS: 812 (17) $[M+Na]^+$, 791 (100) $[M+H]^+$, 719 (20), 701 (7), 592 (11), 574 (22), 465 (17), 395 (6).

TFA·H-(2S,3S)-β²,³-hAla(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***6***): Hydrogenolysis of the β-tetrapeptide **30** (10 mg, 15.7 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 5 to 50% B over 45 min) at a flow rate of 18 mL min^{-1}. Lyophilization yielded the β-tetrapeptide **6** (7.7 mg, 87%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.91$–0.96 (m, 12H; 4Me), 1.14 (d, $J=6.7$, 3H; Me), 1.29–1.34 (m, 1H; CHH′CH), 1.31 (d, $J=7.0$, 3H; Me), 1.47 (ddd, $J=4.9$, 10.0, 13.9, 1H; CH*H*′CH), 1.58–1.66 (m, 1H; C*H*Me$_2$), 1.80–1.87 (m, 1H; C*H*Me$_2$), 2.23 (dd, $J=7.1$, 13.8, 1H; C*H*H′CO), 2.34 (dd, $J=9.3$, 14.6, 1H; C*H*H′CO), 2.39 (dd, $J=6.6$, 13.8, 1H; CH*H*′CO), 2.44 (d, $J=6.6$, 2H; C*H*$_2$CO), 2.48 (dd, $J=4.3$, 14.5, 1H; CH*H*′CO), 3.87 (ddq, $J=2.9$, 6.8, 25.0, 1H; BocHNC*H*), 4.09–4.12 (m, 1H; NCH), 4.13–4.21 (m, 1H; NCH), 4.26–4.32 (m, 1H; NCH), 5.15 (dd, $J=2.9$, 49.2, 1H; CHF); ^{13}C NMR (125 MHz, CD_3OD): $\delta=12.7$ (d, $J=5.1$), 19.1, 19.7, 20.2, 22.3, 23.6 (CH_3); 26.1, 33.3 (CH); 39.0, 41.0, 43.5 (CH_2); 44.5 (CH), 44.7 (CH_2), 46.0, 49.8 (d, $J=20.9$), 54.0, 91.1 (d, $J=191.2$) (CH); 167.5 (d, $J=19.3$), 172.5, 172.7, 175.0 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta=-75.2$ (s, TFA), -203.3 (dd, $J=24.5$, 49.1, CHF); HR-MALDI-MS: 469.3 (11) $[M+Na]^+$, 447.3 (100) $[M+H]^+$, 429.3 (6), 302.2 (11), 231.2 (7).

TFA·H-(2R,3S)-β²,³-hAla(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***7***): Hydrogenolysis of the β-tetrapeptide **31** (12 mg, 18.8 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a.The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 5 to 50% B over 45 min) at a flow rate of 18 mL min^{-1}. Lyophilization yielded the β-tetrapeptide **7** (4 mg, 38%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.91$–0.98 (m, 12H; 4Me), 1.14 (d, $J=6.7$, 3H; Me), 1.31 (ddd, $J=4.7$, 9.1, 13.8, 1H; C*H*H′CH), 1.42–1.48 (m, 1H; CH*H*′CH), 1.46 (d, $J=7.0$, 3H; Me), 1.57–1.65 (m, 1H; C*H*Me$_2$), 1.77–1.84 (m, 1H; C*H*Me$_2$), 2.23–2.29 (m, 2H; 2C*H*H′CO), 2.36 (dd, $J=7.5$, 14.1, 1H; CH*H*′CO), 2.40–2.48 (m, 2H; C*H*$_2$CO), 2.53 (dd, $J=3.5$, 14.4, 1H; CH*H*′CO), 3.87 (ddq, $J=2.8$, 7.0, 24.6, 1H; BocHNC*H*), 4.07–4.11 (m, 1H; NCH), 4.15–4.22 (m, 1H; NCH), 4.29–4.34 (m, 1H; NCH), 5.10 (dd, $J=2.8$, 46.8, 1H; CHF); ^{13}C NMR (125 MHz, CD_3OD): $\delta=15.3$ (d, $J=3.6$), 19.1, 19.8, 20.3, 22.3, 23.6 (CH_3); 26.1, 33.5 (CH); 39.1, 40.8, 43.4 (CH_2); 44.3 (CH), 44.8 (CH_2), 45.9, 50.0 (d, $J=19.9$), 54.1, 91.9 (d, $J=193.9$) (CH); 168.2 (d, $J=20.3$), 172.4, 173.3, 174.9 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta=-75.2$ (s, TFA), -203.8 (dd, $J=23.5$, 45.9, CHF); HR-MALDI-MS: 491.3 (7), 469.3 (28) $[M+Na]^+$, 447.3 (100) $[M+H]^+$, 383.1 (4), 302.2 (4), 284.2 (6), 223.6 (5).

TFA·H-(3S)-β²,²,³-hAla(α,α-F₂)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***8***): Hydrogenolysis of β-tetrapeptide **32** (53 mg, 83 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 5 to 50% B over 45 min) at a flow rate of 18 mL min^{-1}. Lyophilization yielded β-tetrapeptide **8** (37.9 mg, 79%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.91$–0.97 (m, 12H; 4Me), 1.14 (d, $J=6.7$, 3H; Me), 1.31 (ddd, $J=4.6$, 9.1, 13.7, 1H; C*H*H′CH), 1.40 (d, $J=6.8$, 3H; Me), 1.46 (ddd, $J=4.9$, 10.0, 13.8, 1H; CH*H*′CH), 1.57–1.65 (m, 1H; C*H*Me$_2$), 1.80–1.87 (m, 1H; C*H*Me$_2$), 2.24 (dd, $J=7.1$, 13.9, 1H; C*H*H′CO), 2.32–2.44 (m, 4H; C*H*$_2$CO + C*H*H′CO + CH*H*′CO), 2.53 (dd, $J=4.1$, 14.7, 1H; CH*H*′CO), 3.98–4.08 (m, 1H; BocHNC*H*), 4.09–4.13 (m, 1H; NCH), 4.15–4.20 (m, 1H; NCH), 4.27–4.33 (m, 1H; NCH); ^{13}C NMR (125 MHz, CD_3OD): $\delta=12.6$, 19.1, 19.7, 20.2, 22.2, 23.6 (CH_3); 26.1, 33.4 (CH); 38.8, 41.0, 43.4 (CH_2); 44.4 (CH), 44.7 (CH_2), 45.9, 50.6 (*t*, $J=27.2$), 54.5 (CH); 115.8 (*t*, $J=257.1$), 163.1 (*t*, $J=27.4$), 172.4, 172.6, 174.9 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta=-75.2$ (s, TFA), -114.4 (dd, $J=11.7$, 260.4, CFF′), -116.1 (dd, $J=11.7$, 259.3, CFF′); HR-MALDI-MS: 509.3 (8), 487.3 (37) $[M+Na]^+$, 465.3 (100) $[M+H]^+$, 447.3 (15), 391.3 (5), 320.2 (13), 302.2 (5).

TFA·H-(2R)-β²-hGly(α-F)-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (***9***): Hydrogenolysis of β-tetrapeptide **33** (22.8 mg, 36.6 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 5 to 50% B over 45 min) at a flow rate of 18 mL min^{-1}. Lyophilization yielded β-tetrapeptide **9** (12.5 mg, 63%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.91$–0.97 (m, 12H; 4Me), 1.14 (d, $J=6.7$, 3H; Me), 1.30 (ddd, $J=4.6$, 9.1, 13.7, 1H; C*H*H′CH), 1.46 (ddd, $J=5.0$, 10.0, 13.9, 1H; CH*H*′CH), 1.57–1.65 (m, 1H; C*H*Me$_2$), 1.77–1.84 (m, 1H; C*H*Me$_2$), 2.24–2.30 (m, 2H; 2C*H*H′CO), 2.38 (dd, $J=7.1$, 14.0, 1H; CH*H*′CO), 2.44 (d, $J=6.4$, 2H; C*H*$_2$CO), 2.51 (dd, $J=3.6$, 14.3, 1H; CH*H*′CO), 3.41–3.53 (m, 1H; BocHNC*H*$_2$), 4.06–4.10 (m, 1H; NCH), 4.16–4.22 (m, 1H; NCH), 4.27–4.33 (m, 1H; NCH), 5.27 (td, $J=4.6$, 47.6, 1H; CHF); ^{13}C NMR (125 MHz, CD_3OD): $\delta=19.1$, 19.8, 20.3, 22.2, 23.6 (CH_3); 26.1, 33.5 (CH); 39.1, 41.0, 42.2 (d, $J=20.7$), 43.5 (CH_2); 44.3 (CH), 44.7 (CH_2), 45.9, 54.2, 88.9 (d, $J=190.5$) (CH); 168.1 (d, $J=19.6$), 172.5, 173.1,

175.0 (C); ^{19}F NMR (282 MHz, CD_3OD): $\delta = -75.2$ (s, TFA), −195.4 (ddd, $J = 21.3$, 24.5, 47.0, CHF); HR-MALDI-MS: 477.2 (5), 455.3 (31) $[M+Na]^+$, 433.3 (100) $[M+H]^+$, 415.3 (6), 288.2 (8), 270.2 (9), 231.2 (5).

*TFA·H-β-hGly-(3S)-β³-hVal-(3S)-β³-hAla-(3S)-β³-hLeu-OH (**10**)*: Hydrogenolysis of β-tetrapeptide **34** (19.5 mg, 32.2 μmol) was performed according to GP 2, and the resulting carboxylic acid was Boc deprotected according to GP 5a. The crude product was purified by preparative RP-HPLC with a gradient of A (0.1% TFA in H_2O) and B (MeCN, 5 to 50% B over 45 min) at a flow rate of 18 mL min^{-1}. Lyophilization yielded β-tetrapeptide **10** (10.7 mg, 63%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.91$–0.94 (m, 12H; 4Me), 1.14 (d, $J = 6.7$, 3H; Me), 1.31 (ddd, $J = 4.7$, 9.1, 13.8, 1H; C*H*H′CH), 1.46 (ddd, $J = 5.0$, 10.0, 13.8, 1H; CH*H*′CH), 1.58–1.66 (m, 1H; C*H*Me$_2$), 1.73–1.82 (m, 1H; C*H*Me$_2$), 2.21 (dd, $J = 10.2$, 14.2, 1H; C*H*H′CO), 2.27 (dd, $J = 6.6$, 14.1, 1H; C*H*H′CO), 2.38 (dd, $J = 7.1$, 14.1, 1H; CH*H*′CO), 2.42–2.45 (m, 3H; CH_2CO + CH*H*′CO), 2.57 (ddd, $J = 5.3$, 6.6, 16.2, 1H; C*H*H′CO), 2.65 (ddd, $J = 5.2$, 8.0, 16.2, 1H; CH*H*′CO), 3.16 (ddd, $J = 5.4$, 6.7, 18.2, 1H; BocHNC*H*H′), 3.22 (ddd, $J = 5.3$, 8.0, 18.2, 1H; BocHNCH*H*′), 4.08–4.12 (m, 1H; NCH), 4.15–4.22 (m, 1H; NCH), 4.27–4.33 (m, 1H; NCH); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 18.7$, 19.6, 20.3, 22.3, 23.6 (CH_3); 26.1 (CH), 33.2 (CH_2), 33.4 (CH), 37.5, 39.6, 41.0, 43.4 (CH_2); 44.4 (CH), 44.8 (CH_2), 46.0, 53.8 (CH); 171.9, 172.5, 173.0, 175.0 (C); HR-MALDI-MS: 459.3 (7), 437.3 (38) $[M+Na]^+$, 415.3 (100) $[M+H]^+$, 398.3 (15), 270.2 (5), 231.2 (14), 207.6 (5).

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