The "Azirine/Oxazolone Method" under Solid-Phase Conditions

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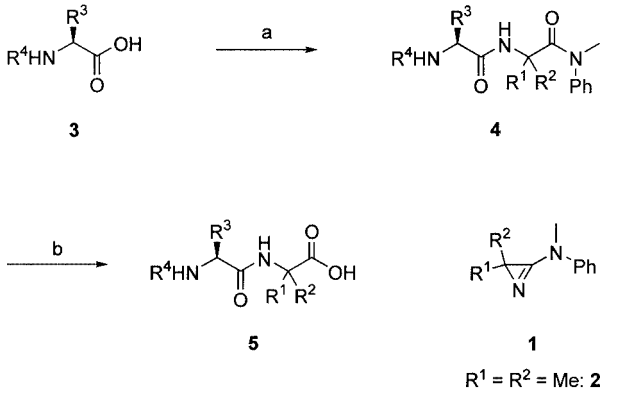
Aib-containing peptides have been synthesized from the *N*- to the *C*-terminus by the "azirine/oxazolone method" under solid-phase conditions. In this new and convenient method for the synthesis of sterically demanding peptides on solid phase, 2*H*-azirine-3-amines are used to introduce aminoisobutyric acid, an α,α-disubstituted α-amino acid, into the peptide without the need for further reagents. Segments of naturally occurring peptaibols have been prepared by this method.

Introduction

Solid-phase peptide synthesis (SPPS) allows rapid access to peptides and peptide conjugates.[1−3] Its importance is particularly evident in combinatorial chemistry, where it made the synthesis of compound libraries possible. SPPS is exclusively carried out from the *C*- to the *N*-terminus, attempts to reverse the strategy having largely failed because of incomplete couplings and significant epimerization during the coupling steps. The synthesis of *C*-terminal-modified peptides and peptide libraries is therefore not routine, but methods for the synthesis of peptides from the *N*- to the *C*-terminus have nevertheless been investigated.[4−9] Moreover, the introduction of sterically demanding α,α-disubstituted α-amino acids into peptides under solid-phase conditions still remains a challenge, although progress has been made in the case of aminoisobutyric acid (Aib) and isovaline (Iva).[10−12]

Peptides containing α,α-disubstituted α-amino acids are restricted in their conformational freedom.[13−16] As a consequence of the rigidity of the peptide backbone, secondary structures such as β-turns and helices are stabilized or even promoted.[17−20] The synthesis of such conformationally restricted peptides is a basic approach when searching for the biologically active conformation of a peptide. One useful method for the introduction of α,α-disubstituted α-amino acids into peptides is the "azirine/oxazolone method", in which 2*H*-azirin-3-amines **1** are used as amino acid synthons[21−23] (Scheme 1). Thus, the reaction between 2*H*-azirin-3-amines, such as the Aib synthon **2**, and amino or peptide acids **3** gives rise to peptide amides **4**, the terminal amide bonds of which can be hydrolyzed selectively to give extended peptide acids **5**. In solution-phase chemistry, the "azirine/oxazolone method" has proven successful for the introduction of a multitude of sterically demanding α,α-disubstituted α-amino acids into peptides and has found successful application in the synthesis of some antibiotic active peptaibols or segments of them.[24−28] A drawback of this method was that it was not yet applicable to solid-phase synthesis.



Scheme 1. a) **1**, CH_2Cl_2; b) HCl (3 M), THF/H_2O

Here we report a successful attempt to synthesize peptides by the "azirine/oxazolone method" on solid phase − a synthesis from the *N*- to the *C*-terminus, in which 2*H*-azirin-3-amines are used to introduce α,α-disubstituted α-amino acids into peptides without the need for further reagents.

Results and Discussion

The first questions we asked concerned chemical reactivity: do 2*H*-azirin-3-amines react in a similar way on a solid

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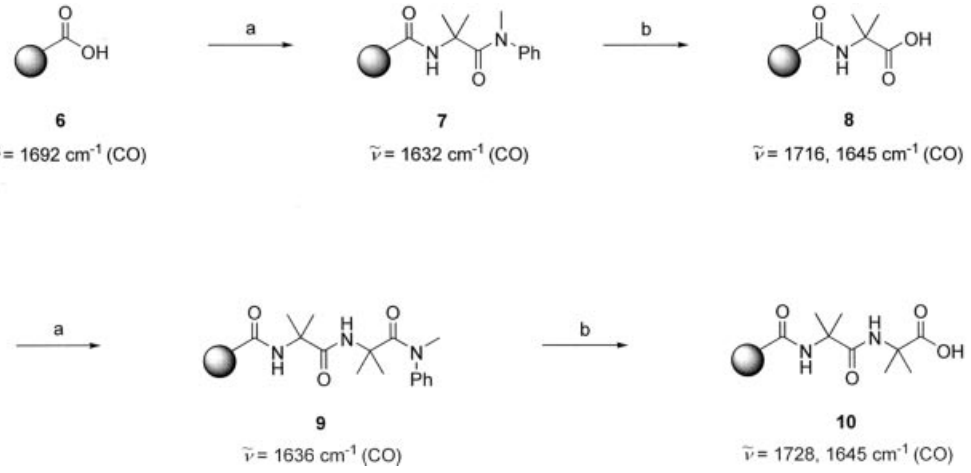
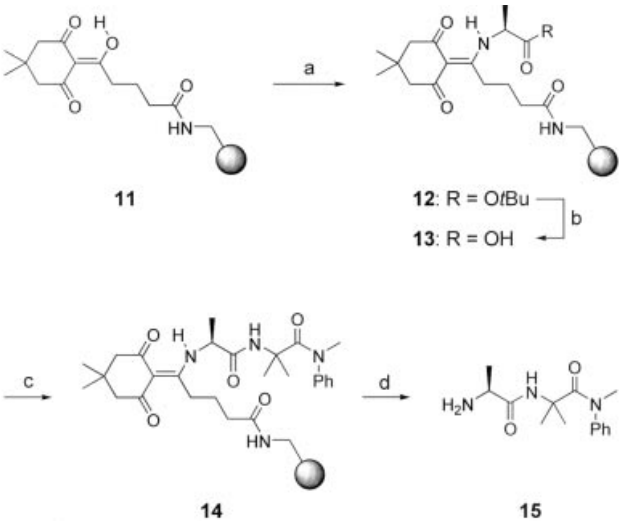


Scheme 2. a) **2**, CH_2Cl_2; b) HCl (3 M), THF/H_2O

support as they do in solution, that is to say, is a terminal amide formed by the reaction between a 2*H*-azirin-3-amine and a solid-phase-bound carboxylic acid? Is selective hydrolysis of the terminal amide possible? Attenuated total reflectance (ATR) FT-IR measurements of the corresponding intermediates **7**, **8**, **9**, and **10** (Scheme 2), as well as the synthesis of a resin-bound tripeptide and its hydrolysis,[29] confirmed our hopes.

The linker is of crucial importance in all variations of solid-phase synthesis, and so this was the second problem we dealt with. The 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) linker, developed by Bycroft et al.,[30] is stable in trifluoroacetic acid (TFA), but can be cleaved with 2% N_2H_4 in DMF. Its use resulted in the successful synthesis of H−Ala−Aib−N(Me)Ph (**15**, Scheme 3). However, the selective hydrolysis of the terminal amide group of **14** was not possible, since the Dde-linker was not stable in aqueous, acidic medium.

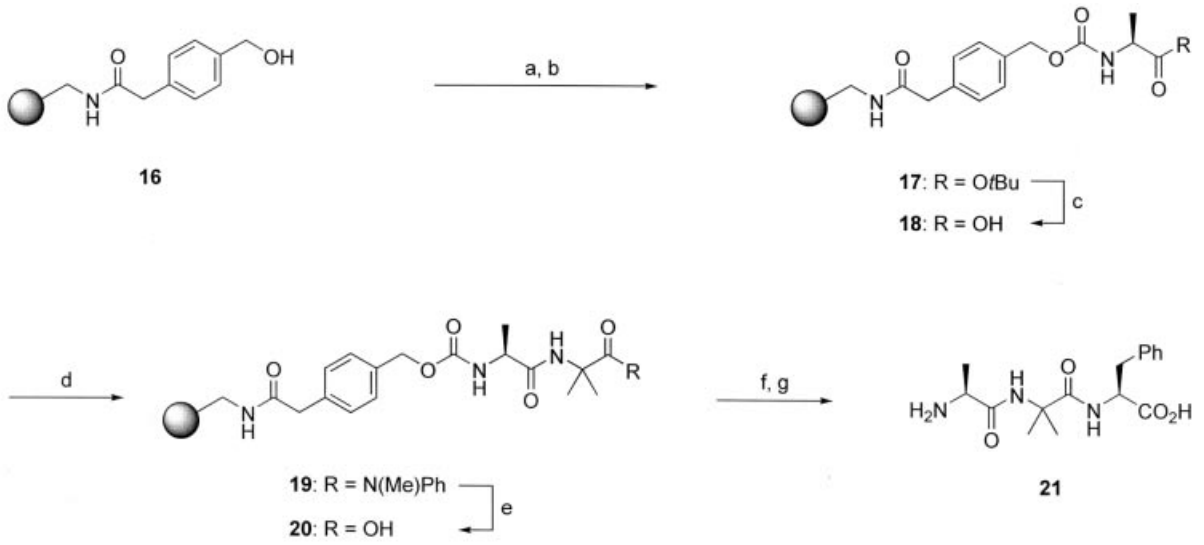


Scheme 3. a) H−Ala−O*t*Bu·HCl, CH_2Cl_2, DIPEA; b) TFA/CH_2Cl_2 (1:1), TIPS; c) **2**, CH_2Cl_2; d) N_2H_4 (2%), DMF. DIPEA = *N*,*N*-diisopropylethylamine, TFA = trifluoroacetic acid, TIPS = triisopropylsilane, DMF = *N*,*N*-dimethylformamide

A wide variety of amines have been immobilized on solid supports through carbamate linkers, synthesized variously with phosgene,[31−34] 1,1′-carbonyldiimidazole (CDI),[35] or 4-nitrophenyl chloroformate.[36−39] The use of a carbamate linker to attach amino acids through their *N*-termini was first reported by Letsinger.[5,31] We therefore also planned the use of a carbamate linker, but the stability of the carbamate had to be tuned carefully. The carbamate has to be stable during hydrolysis of the terminal amide with 3 M HCl and deprotection of the *t*Bu ester with TFA, but should finally be cleavable under conditions not affecting the peptide. It was shown that the carbamate linker formed from [4-(hydroxymethyl)phenyl]acetamidomethyl (PAM) resin **16** fitted these requirements the best.[40] (Scheme 4).

Phosgene in toluene (1.9 M) was added to PAM resin **16** to generate a chloroformate intermediate, and treatment with H−Ala−O*t*Bu afforded resin **17**. Amino acid *t*Bu esters have proven to be useful building blocks for inverse peptide synthesis,[4] because their chemistry is well known and they are readily available from commercial suppliers. Deprotection with TFA afforded resin **18**, which was treated with a solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 4 equiv.) in DCM (c_2 = 0.2 M). Unconsumed **2** can easily be recovered and used in further coupling steps. Hydrolysis of resin **19** with 3 M HCl in H_2O/THF afforded resin **20**, which was conventionally coupled to H−Phe−O*t*Bu with PyBOP as coupling reagent. Cleavage from the solid support was achieved with HBr (33%) in acetic acid. After HPLC purification and lyophilization, model peptide **21** was isolated in 50% yield. A second model peptide [H−Ala−Aib−Val−Aib−Phe−OH (**22**)] containing two Aib residues was synthesized analogously (Table 1) in 33% yield after HPLC and lyophilization.

A crucial point in the context of this strategy was the question of epimerization of the C_α center(s). In order to determine the extent of racemization/epimerization, the tripeptide **21** was hydrolyzed and the amino acids were analyzed by capillary gas chromatography with enantiomer labeling.[41] The results showed that alanine and phenylala-



Scheme 4. a) $COCl_2$ (1.9 M in PhMe), THF; b) H−Ala−O*t*Bu·HCl, DIPEA, CH_2Cl_2; c) TFA, CH_2Cl_2, TIPS; d) **2**, CH_2Cl_2; e) HCl (3 M), THF/H_2O; f) H−Phe−O*t*Bu·HCl, PyBOP, DIPEA, CH_2Cl_2; g) HBr (33%) in acetic acid. PyBOP = (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

Table 1. General procedures

Description	Reagents[a]	Duration and repetition
General procedure for the attachment of the first amino acid		
Chloroformate formation	10 equiv. $COCl_2$ (1.9 M) in toluene, THF	1 × 2 h
Washes	THF, CH_2Cl_2	2 ×, each
Coupling	4 equiv. H−AA−O*t*Bu·HCl, 8 equiv. DIPEA, CH_2Cl_2	1 ×
Washes	CH_2Cl_2, DMF, CH_2Cl_2	3 ×, each
General procedure for removal of the *t*Bu protecting group		
Hydrolysis	TFA/CH_2Cl_2, TIPS (5%)	1 × 5 s (25%), 1 × 30 min (50%)
Washes	CH_2Cl_2, DMF, CH_2Cl_2	3 ×, each
General procedure for peptide synthesis with 2*H*-azirin-3-amine 2		
Coupling	4 equiv. **2**, CH_2Cl_2	1 ×
Washes	CH_2Cl_2	3 ×
Hydrolysis	HCl (3 M) in THF/H_2O	1 ×
Washes	THF, DMF, CH_2Cl_2	3 ×, each
General procedure for peptide synthesis with coupling reagents by Method A, (removal of *t*Bu see above)		
Coupling	4 equiv. PyBOP, 4 equiv. H−AA−O*t*Bu·HCl, 12 equiv. DIPEA, CH_2Cl_2	1 ×
Washes	CH_2Cl_2, DMF, CH_2Cl_2	2 ×, each
General procedure for peptide synthesis with coupling reagents by Method B,[47] (removal of *t*Bu see above)		
Coupling	6 equiv. HOBt, 4 equiv. PyBOP, 4 equiv. H−AA−O*t*Bu·HCl, 11 equiv. NMM, DMF	1 ×
Washes	CH_2Cl_2, DMF, CH_2Cl_2	2 ×, each
General procedure for cleavage from the support		
Cleavage	HBr (33%) in acetic acid, 2 drops of H_2O	1 × 6 h
Washes	Acetic acid/CH_2Cl_2 (1:1), CH_3CN/CH_2Cl_2 (1:1)	3 ×, each

[a] DIPEA = *N*,*N*-diisopropylethylamine, HOAt = 1-hydroxy-7-azabenzotriazole, HOBt = 1-hydroxybenzotriazole, NMM = *N*-methylmorpholine, PyBOP = (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TIPS = triisopropylsilane.

nine had racemized by 0.6% and 2.8%, respectively. It was not possible to achieve resolution of the epimers by HPLC[42] on the crude product, the derivatized crude product,[43] or the purified product. We therefore assume that the extent of racemization/epimerization in the crude product is similar to that in the purified product, and hence that the synthesis had been carried out with an acceptable degree of racemization, similar to that seen in classical SPPS.

To examine the use of the "azirine/oxazolone method" under solid-phase conditions we attempted to synthesize a *Peptaibolin* derivative and two segments of other peptaibols.[44] The hexapeptide H−Ala−Aib−Ala−Gln−Aib−Val−OH (A4−9) (**23**) of the peptaibol antibiotic *Alamethicin*[45,46] was synthesized in 25% yield by the procedures listed in Table 1 (Table 2). Both Aib residues were introduced by the "azirine/oxazolone method", Ala (A3) by

Table 2. Synthesized peptides

Description	Sequence	Yield [%][a]
Model tripeptide	H−Ala−Aib−Phe−OH (**21**)	50
Model pentapeptide	H−Ala−Aib−Val−Aib−Phe−OH (**22**)	33
A4−9 of *Alamethicin*	H−Ala−Aib−Ala−Gln−Aib−Val−OH (**23**)	25
Peptaibolin derivative	H−Leu−Aib−Leu−Aib−Phe−OH (**24**)	20
A9−13 of *Stilboflavin A3*	H−Val−Aib−Gly−Aib−Ala−OH (**25**)	30

[a] Yield of product isolated after HPLC purification.

method A (Table 1) and both Gln and Val by method B.[47] Method B was applied to prevent nitrile formation, as the side chain of Gln was not protected.[48] The pentapeptide H−Leu−Aib−Leu−Aib−Phe−OH (**24**) is a derivative of *Peptaibolin*[49] (Ac−Leu−Aib−Leu−Aib−Pheol), and was also synthesized by the procedures listed in Table 1. Both Aib residues were introduced by the "azirine/oxazolone method", while method A was used for the coupling of the other amino acids. The lower yield (20%) is the result of incomplete coupling of phenylalanine, but a repeated coupling with additional pentafluorophenol did not increase the yield. The segment H−Val−Aib−Gly−Aib−Ala−OH (A9−13) (**25**) of *Stilboflavin A3*[50] was prepared in 30% yield by the "azirine/oxazolone method" and method A.

Conclusion

In conclusion, we have been able to adapt the "azirine/oxazolone method" under solid-phase conditions, and have consequently developed a new and convenient method for the synthesis of sterically demanding peptides on solid phase. The simple procedure may possibly be automatable. It has been shown that this approach for the synthesis of Aib-containing peptides from the *N*- to the *C*-terminus can be combined with conventional coupling methods. The method found a successful application in the synthesis of different peptaibol segments.

Experimental Section

General Remarks: Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Hydroxymethylpolystyrene, 1% divinylbenzene, 100−200 mesh, loading 0.98 mmol/g was from Novabiochem (Calbiochem−Novabiochem, Läufelfingen, Switzerland), [4-(hydroxymethyl)phenyl]acetamidomethylpolystyrene [4-(oxomethyl)phenylacetamidomethylpolystyrene], 1% divinylbenzene, 100−200 mesh, loading 0.62 mmol/g from Acros Organics (Acros Organics, Geel, Belgium), and carboxylpolystyrene, 1% divinylbenzene, 200−400 mesh, loading 4.0 mmol/g from Lipal Biochemicals (Lipal Biochemicals, Gundetswil, Switzerland). Aminomethylpolystyrene, 1% divinylbenzene, 100−200 mesh, loading 1.14 mmol/g, hydroxyethylpolystyrene, 1% divinylpolystyrene, 100−200 mesh, loading 1.30 mmol/g, and carboxylpolystyrene, 1% divinylbenzene, 100−200 mesh, loading 1.96 mmol/g were from Rapp Polymere (Rapp Polymere, Tübingen, Germany). *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine was synthesized by Villalgordo and Heimgartner's method.[51,52] Reaction vessels for solid-phase synthesis: single fritted (20 μm) PE reservoirs (15 mL) (Separtis, Grenzach-Wyhlen, Germany) were used on an Advanced ChemTech PLS 4 × 6 Shaker (Advanced ChemTech, Inc., Louisville, KY, USA) with a custom-made adapter. The original Advanced ChemTech reaction vessels were used for reactions under N_2. High-performance liquid chromatography (HPLC): instrument: Waters 600E multisolvent delivery system equipped with a Waters 996 PDA (Waters, Milford, CA, USA); column: Interchim Uptisphere ODB C18, 300 Å, 10 μm, 250 × 4.6 mm (Interchim, Montluçon, France), Interchim Uptisphere WOD C18, 300 Å, 10 μm, 250 × 21.2 mm (prep. HPLC), or Vydac 218TP C18, 300 Å, 10 μm, 250 × 22 mm (prep. HPLC) (Vydac, Hesperia, CA, USA). Column chromatography (CC): silica gel C-560 (0.04−0.063 mm, 230−400 mesh) from Chemie Uetikon (CU Chemie Uetikon GmbH, Uetikon, Switzerland). Prep. TLC: Merck TLC plates (glass), silica gel 60 F_{254}, 0.25 mm (Merck KGaA, Darmstadt, Germany). IR Spectra: Perkin−Elmer, Spectrum one FT-IR spectrophotometer (Perkin−Elmer, Wellesley, MA, USA); ATR-FT-IR with a Bio-Rad FTS-45 (Bio-Rad, Hercules, CA, USA) instrument equipped with a MKII Golden Gate single reflection ATR system from Specac (Specac Inc., Smyrna, GA, USA). NMR Spectra: Bruker ARX-300, Bruker DRX-500, or Bruker DRX-600 machines (Bruker Biospin, Karlsruhe, Germany). Chemical shifts are given in ppm relative to tetramethylsilane (TMS) as internal standard. 2D NMR experiments were performed for assignment of the signals. Some spin systems were simulated with NMR-Sim from Bruker. HPLC-MS: instrument: Hewlett−Packard HP 1100 HPLC system (Hewlett−Packard, Palo Alto, CA, USA) equipped with a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), connected to a Bruker ESQUIRE-LC quadrupole ion trap instrument (Bruker Daltonik GmbH, Bremen, Germany) equipped with a Hewlett−Packard Electrospray Ionization (ESI) source (Hewlett−Packard Co., Palo Alto, CA, USA); column: Interchim Uptisphere HDO C18, 120 Å, 3 μm, 200 × 2.0 mm column (Interchim, Montluçon, France); eluents: A = H_2O/HCOOH (99.95:0.05), B = CH_3CN/HCOOH (99.95:0.05); flow rate: 0.18 mL/min, gradient (A:B): 0−10 min: 95:5−50:50, 10−20 min: 50:50−0:100, 20−30 min: 0:100. MS: Bruker ESQUIRE-LC quadrupole ion trap instrument (Bruker Daltonik GmbH, Bremen, Germany) or Finnigan TSQ-700 triple quadrupole instrument (Finnigan MAT, San Jose, CA, USA). Direct infusion ESI-MS were performed with a syringe infusion pump at a flow rate of 5 μL/min.

Abbreviations: Aib: α-aminoisobutyric acid; ATR-FT-IR: attenuated total reflectance Fourier transform infrared spectroscopy; CC: column chromatography; DCM: dichloromethane; Dde: *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl; DIPCDI: diisopropylcarbodiimide; DIPEA: *N*,*N*-diisopropylethylamine; HM: hydroxymethyl; HOAt: 1-hydroxy-7-azabenzotriazole; HOBt: 1-hydroxybenzotriazole; NMM: *N*-methylmorpholine; PAM: [4-(hydroxymethyl)phenyl]acetamidomethyl; PfpOH: pentafluorophenol;

PyBOP: (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TIPS: triisopropylsilane.

Synthesis of Resin 10, IR Analysis. Resin 7: Carboxylpolystyrene (50 mg, 0.20 mmol) was swollen in DCM. A solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 178 mg, 1.02 mmol) in DCM (1.5 mL) was added, and the resin was agitated at room temp. for 15 h. The resin was separated by filtration, washed with DCM (3 ×) and Et_2O (3 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1632 cm^{-1} (carboxylpolystyrene, beads: $\tilde{\nu}$ = 1692 cm^{-1}). **Resin 8:** Resin **7** was swollen in THF. A solution of HCl (2 mL, 3 M in THF/H_2O, prepared from concd. HCl and THF) was added, and the resin was agitated at room temp. for 16 h. The resin was separated by filtration, washed with THF (3 ×), DCM (3 ×), and Et_2O (3 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1716, 1645 cm^{-1}. **Resin 9:** Remaining resin **8** (ca. 5 mg) was swollen in DCM. A solution of **2** (13 mg, 0.08 mmol) in DCM (1.5 mL) was added, and the resin was agitated at room temp. for 16 h. The resin was separated by filtration, washed with DCM (3 ×) and Et_2O (3 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1636 cm^{-1}. **Resin 10:** Resin **9** was swollen in THF. A solution of HCl (2 mL, 3 M in THF/H_2O, prepared from concd. HCl and THF) was added, and the resin was agitated at room temp. for 7 h. The resin was separated by filtration, washed with THF (3 ×), DCM (3 ×), and Et_2O (3 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1728, 1645 cm^{-1}.

Synthesis of Resin-bound Ala−Aib−Phe−O*t*Bu and its Hydrolysis: Carboxylpolystyrene (103 mg, 0.20 mmol) was swollen in DCM. A solution of H−Ala−O*t*Bu·HCl (76 mg, 0.42 mmol) in DCM (1 mL), a solution of PyBOP (208 mg, 0.40 mmol) in DCM (1 mL), and DIPEA (0.2 mL, 1.17 mmol) were added, and the resin was agitated at room temp. overnight. The resin was separated by filtration, washed with DMF (4 ×), DCM (3 ×), and Et_2O (2 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1729, 1663, 1652 cm^{-1} (carboxylpolystyrene, beads: $\tilde{\nu}$ = 1685 cm^{-1}). The new resin was swollen in DCM. TFA/DCM (3 mL, 1:1) and TIPS (0.2 mL) were added, and the resin was agitated for 2 h. The resin was separated by filtration, washed with DMF (3 ×), DCM (3 ×), and Et_2O (2 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1733, 1641 cm^{-1}. Half of the obtained resin was swollen in DCM. A solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 40 mg, 0.23 mmol) in DCM (2 mL) was added, and the resin was agitated at room temp. overnight. The resin was separated by filtration, washed with DCM (3 ×) and Et_2O (2 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1637 cm^{-1}. The resulted resin was swollen in THF. A solution of HCl (3 mL, 3 M in THF/H_2O, prepared from concd. HCl and THF) was added, and the resin was agitated at room temp. overnight. The resin was separated by filtration, washed with THF (3 ×), DCM (3 ×), and Et_2O (2 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1719, 1636 cm^{-1}. The resin was swollen in DCM. A solution of PyBOP (103 mg, 0.20 mmol) in DCM (1 mL), H−Phe−O*t*Bu·HCl (51 mg, 0.20 mmol) in DCM (1 mL), and DIPEA (0.1 mL, 0.58 mmol) were added, and the resin was agitated at room temp. overnight. The resin was separated by filtration, washed with DMF (3 ×), DCM (3 ×), and Et_2O (2 ×), and dried in vacuo. ATR-FT-IR (beads): $\tilde{\nu}$ = 1730, 1652, 1646 cm^{-1}. The resin-bound peptide was hydrolyzed by the method of Westall et al.[53] (propionic acid/HCl, 1:1, at 130 °C, sealed tube). The mixture was filtered, and the filtrate was concentrated and dried in vacuo to yield a colorless powder. MS (ESI): *m*/*z* (%) = 90 (2) $[Ala + H]^+$, 104 (17) $[Aib + H]^+$, 120 (60),[54] 166 (100) $[Phe + H]^+$. ATR-FT-IR (beads): $\tilde{\nu}$ = 1686 cm^{-1}.

Synthesis of H−Ala−Aib−N(Me)Ph (15) with Use of the Dde-Linker: The immobilized linker system **11** was synthesized by the method of Chhabra et al.[55] with aminomethylpolystyrene (500 mg, 0.57 mmol), 5-(4,4-dimethyl-2,6-dioxocyclohexylidene)-5-hydroxypentanoic acid (291 mg, 1.14 mmol), DIPCDI (177 μL, 1.14 mmol), and HOAt (2.28 mL, 1.14 mmol of a 0.5 M solution in DMF). **Resin 12:** Resin **11** was swollen in DMF, H−Ala−O*t*Bu·HCl (416 mg, 2.29 mmol) and DIPEA (390 μL, 2.28 mmol) in DMF (5 mL) were then added, and the resin was agitated at room temp. for 1 d. The resin was separated by filtration and washed with DMF (3 ×) and DCM (3 ×). **Resin 13:** Resin **12** was swollen in DCM. TFA/DCM (5 mL, 1:1) and TIPS (230 μL) were added, and the resin was agitated for 2 h. The resin was separated by filtration and washed with DCM (3 ×), DMF (3 ×), and DCM (2 ×). **Resin 14:** Resin **13** was swollen in DCM. A solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 204 mg, 0.17 mmol) in DCM (4 mL) was added, and the resin was agitated at room temp. overnight. The resin was separated by filtration and washed with DCM (3 ×). **H−Ala−Aib−N(Me)Ph (15):** Resin **14** was swollen in DMF. A solution of $N_2H_4·H_2O$ (100 μL) in DMF (5 mL) was added, and the resin was agitated for 20 min. The resin was separated by filtration and the cleavage was repeated. Finally, the resin was washed with DMF (3 ×), and the filtrate was concentrated. CC (DCM/MeOH, 40:1, 1% Et_3N) yielded **15** (91 mg, 61%). ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.19 [d, *J* = 7.0 Hz, 3 H, CH_3 (Ala)], 1.51, 1.54 [2s, 6 H, 2 CH_3 (Aib)], 3.13 [q, *J* = 7.0 Hz, 1 H, $CH_α$ (Ala)], 3.26 [s, 3 H, CH_3 of $N(CH_3)Ph$], 7.41−7.23 (m, 5 arom. H) ppm. NH and NH_2 could not be detected. MS (ESI): *m*/*z* (%) = 157 (46) oxazolone of $[M - HN(CH_3)Ph + H]^+$, 264 (100) $[M + H]^+$, 286 (6) $[M + Na]^+$, 527 (5) $[2M + H]^+$.

General Procedures

a) Attachment of the First Amino Acid: All manipulations were carried out under N_2. PAM or HM resin was swollen in THF. After filtration, a solution of $COCl_2$ in toluene (1.9 M, 10 equiv.) and THF (ca. 2.5 mL/1 g resin) were added to the resin, which was agitated at room temp. for 2 h, and then washed with THF (2 ×) and DCM (2 ×). In a separate vial, H−AA−O*t*Bu·HCl (4 equiv.) was dissolved in DIPEA (8 equiv.) and DCM [c_{AA} = 0.2 M]. This mixture was added to the resin, any possibly occurring ammonium salt being removed by filtration. The resin was agitated at room temp. overnight and was then washed with DMF (3 ×) and DCM (3 ×).

b) Removal of the *t*Bu Protecting Group: The resin was swollen in DCM. TFA in DCM (1 × 5 s, 25%; 1 × 30 min, 50%) and TIPS (5%, in each case) were added, and the resin was agitated at room temp. Afterwards, the resin was washed with DCM (3 ×), DMF (2 ×), and DCM (3 ×).

c) Coupling with *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine (2): The resin was swollen in DCM. A solution of **2** (4 equiv.) in DCM (c_2 = 0.2 M) was added, and the resin was agitated at room temp. overnight, and then washed with DCM (3 ×). Unconsumed **2** can easily be recovered.

d) Hydrolysis of the Terminal Amide: The resin was swollen in THF. HCl (ca. 3−4 mL/200 mg resin, 3 M in THF/H_2O, prepared from concd. HCl and THF) was added, and the resin was agitated at room temp. overnight, and then washed with THF (3 ×), DMF (3 ×), and DCM (3 ×).

e) Coupling with H−AA−O*t*Bu·HCl (Method A): The resin was swollen in DCM. PyBOP (4 equiv.) in DCM was added, followed by H−AA−O*t*Bu·HCl (4 equiv.) in DCM and DIPEA (12 equiv.)

(c_{AA} = 0.2 M), and the resin was agitated at room temp. overnight, and then washed with DCM (2 ×), DMF (2 ×), and DCM (3 ×).

f) Coupling with H−AA−O*t*Bu·HCl) (Method B, according to Gausepohl et al.):[47] The resin was swollen in DMF. HOBt (6 equiv.) in DMF, PyBOP (4 equiv.) in DMF, NMM (2.3 equiv.), and then H−AA−O*t*Bu·HCl (4 equiv.) in DMF and NMM (4 equiv.) were added (c_{AA} = 0.2 M). The resin was agitated at room temp. and, after 10 and 20 min, additional NMM (each 2.3 equiv.) was added. The resin was agitated at room temp. for 1 h (5 h for coupling to Aib). The resin was washed with DMF (3 ×) and DCM (3 ×).

g) Coupling with H−AA−O*t*Bu·HCl (Method C): The resin was swollen in DCM. PfpOH (4 equiv.) in DCM, PyBOP (4 equiv.) in DCM, and then H−AA−O*t*Bu·HCl (4 equiv.) in DCM and DIPEA (12 equiv.) were added (c_{AA} = 0.2 M). The resin was agitated at room temp. overnight, and then washed with DCM (2 ×), DMF (2 ×), and DCM (3 ×).

h) Cleavage: The resin was swollen in DCM. HBr in AcOH (33%, 1 mL/100 mg resin) and two drops of water were added, and the resin was agitated for 5 to 6 h. The resin was separated by filtration and washed with AcOH/DCM (1:1, 3 ×) and MeCN/DCM (1:1, 3 ×). The solvents were evaporated under reduced pressure and the crude product was purified by HPLC. The purified product was lyophilized.

H−Ala−Aib−Phe−OH (21): PAM resin (202 mg, 0.125 mmol) was treated as in General Procedures a), b), c), d), e), and h) to yield **21** (32 mg, 50%) as a colorless powder after prep. HPLC purification and lyophilization.

HM resin (201 mg, 0.197 mmol) was treated as in General Procedures a), b), c), d), e), and h) to yield **21** (33 mg, 40%) as a colorless powder after precipitation in ether or prep. HPLC purification and lyophilization. HPLC-MS: t_R = 11.6 min, *m/z* (%) = 129 (31), 157 (47) oxazolone of [M − Phe + H]$^+$, 251 (28) [M − Ala + H]$^+$, 322 (100) [M + H]$^+$, 344 (8) [M + Na]$^+$. MS (ESI): *m/z* (%) = 322 (100) [M + H]$^+$, 344 (60) [M + Na]$^+$. IR (KBr): $\tilde{\nu}$ = 3422 s, 3575 s, 3237 s, 3068 s, 3034 s, 2992 s, 2945 s, 2617 w, 1724 vs, 1671 vs, 1534 vs, 1500 s, 1467 m, 1457 m, 1443 m, 1392 m, 1369 m, 1332 w, 1266 s, 1201 vs, 1142 vs, 1031 m, 1003 w, 839 w, 800 m, 723 m, 702 m, 641 m, 518 w cm^{-1}. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 1.32 [d, *J* = 7.0 Hz, 3 H, CH$_3$ (Ala)], 1.34, 1.35 [2s, 6 H, CH$_3$ (Aib)], 2.95 [dd, *J* (CH$_A$*H*$_B$,CH$_\alpha$) = 8.3, ^{2}*J* = 13.7 Hz, 1 H, CH$_A$*H*$_B$ (Phe)], 3.08 [dd, *J* (C*H*$_A$H$_B$,CH$_\alpha$) = 5.3, ^{2}*J* = 13.7 Hz, 1 H, C*H*$_A$H$_B$ (Phe)], 3.82−3.83 [m, 1 H, CH$_\alpha$ (Ala)], 4.44 [ddd, *J* (CH$_\alpha$,CH$_A$*H*$_B$) = 8.3, *J* (CH$_\alpha$,NH) = 8.0, *J* (CH$_\alpha$,C*H*$_A$H$_B$) = 5.3 Hz, 1 H, CH$_\alpha$ (Phe)], 7.18−7.28 (m, 5 arom. H), 7.53 [d, *J* (NH, CH$_\alpha$) = 8.0 Hz, 1 H, NH (Phe)], 8.04 [br. s, 3 H, NH$_3^+$ (Ala)], 8.35 [s, 1 H, NH (Aib)], ca. 12.0−13.5 (br. s, 1 H, COOH) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 17.1 [q, CH$_3$ (Ala)], 24.4, 25.0 [2q, 2 CH$_3$ (Aib)], 36.7 (t, CH$_2$), 48.3 [d, CH$_\alpha$ (Ala)], 53.5 [d, CH$_\alpha$ (Phe)], 56.3 [s, C$_\alpha$ (Aib)], 126.4 (d, arom. CH$_p$), 128.1 (d, arom. CH$_m$), 129.3 (d, arom. CH$_o$), 137.5 (s, arom. C), 168.9 [s, CO (Ala)], 172.7 [s, CO (Phe)], 172.9 [s, CO (Aib)] ppm.

Ac−Ala−Aib−Phe−OMe (26) (Derivatization of 21): The crude product **21** (from a 0.062 mmol batch) was dissolved in (NH$_4$)$_2$CO$_3$ (2.4 mL, 0.1 M), Ac$_2$O/MeOH (6 mL, 1:3) was then added, and the solution was stirred at room temp. for 1 h. After lyophilization, the residue was dissolved in MeOH/H$_2$O (2 mL, 10:1), and a solution of CH$_2$N$_2$ in Et$_2$O (ca. 2 mL, ca. 1 M) was added. The solution was stirred at room temp. and then concentrated in vacuo. HPLC-MS: t_R = 14.8 min, *m/z* (%) = 120 (45), 180 (92) [H − Phe − OMe + H]$^+$, 199 (84) oxazolone of [M − (Phe−OMe) + H]$^+$, 265 (100) [M − (Ac−Ala) + H]$^+$, 378 (52) [M + H]$^+$, 400 (71) [M + Na]$^+$. Prep. TLC (DCM/MeOH, 20:1) yielded **26** (9 mg, 38%) as a colorless powder. HPLC-MS: t_R = 14.8 min, *m/z* (%) = 120 (37), 180 (100) [H − Phe − OMe + H]$^+$, 199 (63) oxazolone of [M − (Phe−OMe) + H]$^+$, 265 (82) [M − (Ac−Ala) + H]$^+$, 378 (48) [M + H]$^+$, 400 (89) [M + Na]$^+$.

H−Ala−Aib−Val−Aib−Phe−OH (22): PAM resin (402 mg, 0.249 mmol) was treated as in General Procedure a) and dried in vacuo. The synthesis was continued with half of the resin as in General Procedures b), c), d), e), b), c), d), e), and h) to yield **22** (25 mg, 33%) as a colorless powder after prep. HPLC purification and lyophilization.

HM resin (501 mg, 0.491 mmol) was treated as in General Procedure a) and dried in vacuo. The synthesis was continued with 25% of the resin as described in General Procedures b), c), d), e), b), c), d), e), and h) to yield **22** (16 mg, 21%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS: t_R = 10.4 min, *m/z* (%) = 256 (11), 341 (48) oxazolone of [M − Phe + H]$^+$, 506 (100) [M + H]$^+$. MS (ESI): *m/z* (%) = 506 (100) [M + H]$^+$, 528 (31) [M + Na]$^+$, 550 (9) [MNa + Na]$^+$. IR (KBr): $\tilde{\nu}$ = 3428 m, 3308 s, 3065 s, 2976 s, 2940 s, 2622 w, 1722 s, 1668 vs, 1529 vs, 1468 m, 1458 m, 1389 m, 1367 m, 1322 w, 1263 m, 1202 vs, 1139 s, 1004 w, 929 w, 837 w, 800 w, 722 m, 701 w, 598 w cm^{-1}. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.81, 0.85 [2d, *J* = 6.8 Hz, 6 H, 2 CH$_3$ (Val)], 1.34 [d, *J* = 6.9 Hz, 3 H, CH$_3$ (Ala)], 1.29, 1.35, 1.38, 1.41 [4s, 12 H, 4 CH$_3$ (Aib)], 2.01 [oct., *J* = 6.8 Hz, 1 H, CH$_\beta$ (Val)], 2.94 [dd, *J* (CH$_A$*H*$_B$,CH$_\alpha$) = 8.1, ^{2}*J* = 13.8 Hz, 1 H, CH$_A$*H*$_B$ (Phe)], 3.03 [dd, *J* (C*H*$_A$H$_B$,CH$_\alpha$) = 5.7, ^{2}*J* = 13.8 Hz, 1 H, C*H*$_A$H$_B$ (Phe)], 3.85 [q, *J* = 6.9 Hz, 1 H, CH$_\alpha$ (Ala)], 4.02 [t, *J* = 7.3 Hz, 1 H, CH$_\alpha$ (Val)], 4.41 [ddd, *J* (CH$_\alpha$,CH$_A$*H*$_B$) = 8.1, *J* (CH$_\alpha$,NH) = 7.9, *J* (CH$_\alpha$,C*H*$_A$H$_B$) = 5.7 Hz, 1 H, CH$_\alpha$ (Phe)], 7.18−7.27 (m, 5 arom. H), 7.30 [d, *J* = 7.7 Hz, 1 H, NH (Val)], 7.48 [d, *J* (NH,CH$_\alpha$) = 7.9 Hz, 1 H, NH (Phe)], 7.5−8.5 [br. s, 3 H, NH$_3^+$ (Ala)], 7.91, 8.48 [2s, 2 H, 2 NH (Aib)], ca. 11.0−13.5 (br. s, 1 H, COOH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 17.0 [q, CH$_3$ (Ala)], 18.4, 19.3 [2q, 2 CH$_3$ (Val)], 24.3, 24.9, 25.0, 25.1 [4q, 4 CH$_3$ (Aib)], 30.1 [d, CH$_\beta$ (Val)], 36.8 [t, CH$_2$ (Phe)], 48.4 [d, CH$_\alpha$ (Ala)], 53.6 [d, CH$_\alpha$ (Phe)], 56.1, 56.5 [2s, 2 C$_\alpha$ (Aib)], 58.4 [d, CH$_\alpha$ (Val)], 126.4 (d, arom. CH$_p$), 128.1 (d, arom. CH$_m$), 129.2 (d, arom. CH$_o$), 137.5 (s, arom. C), 169.1 [s, CO (Ala)], 170.4 [s, CO (Val)], 172.6 [s, CO (Phe)], 173.3, 173.5 [2s, 2 CO (Aib)] ppm.

H−Ala−Aib−Ala−Gln−Aib−Val−OH (23): PAM resin (202 mg, 0.125 mmol) was treated as described in General Procedures a), b), c), d), e), b), f), b), c), d), f) and h) to yield **23** (21 mg, 25%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS: t_R = 8.3 min, *m/z* (%) = 441 (10) oxazolone of [M − Val + H]$^+$, 558 (100) [M + H]$^+$. MS (ESI): *m/z* (%) = 558 (29) [M + H]$^+$, 580 (100) [M + Na]$^+$, 602 (74) [MNa + Na]$^+$. IR (KBr): $\tilde{\nu}$ = 3315 s, 3065 m, 2984 m, 2942 m, 2614 w, 1665 vs, 1533 s, 1467 m, 1455 m, 1424 w, 1390 m, 1368 w, 1332 w, 1267 m, 1201 s, 1140 s, 1042 w, 1004 w, 930 w, 836 w, 800 w, 722 w, 598 w, 518 w cm^{-1}. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.82, 0.85 [2d, *J* = 6.8 Hz, 6 H, 2 CH$_3$ (Val)], 1.23 [d, *J* = 7.0 Hz, 3 H, CH$_3$ (Ala)], 1.36, 1.38 [2s, 6 H, 2 CH$_3$ (Aib)], 1.38 [d, *J* = 6.9 Hz, 3 H, CH$_3$ (Ala)], 1.40 [s, 6 H, 2 CH$_3$ (Aib)], 1.84−1.93 [m, 2 H, CH$_2$ (Gln)], 2.01−2.08 [m, 1 H, CH$_\beta$ (Val)], 2.10−2.14 [m, 2 H, CH$_2$ (Gln)], 3.84 [q, *J* = 7.0 Hz, 1 H, CH$_\alpha$ (Ala)], 4.06−4.10 [m, 2 H, CH$_\alpha$ (Val), CH$_\alpha$ (Gln)], 4.15 [quint., *J* = 6.9 Hz,1 H, CH$_\alpha$ (Ala)], 6.89 [s, 1 H, NH$_2$ (Gln)], 7.12 [d, *J* = 8.5 Hz, 1 H, NH (Val)], 7.40 (s, 1 H, NH$_2$ (Gln)], 7.66 [d, *J* = 6.3 Hz, 1 H, NH (Ala)], ca. 7.7−8.4 [br. s, 3 H, NH$_3^+$ (Ala)], 7.84 [d, *J* = 7.1 Hz, 1 H, NH

(Gln)], 7.86 [s, 1 H, NH (Aib)], 8.63 [s, 1 H, NH (Aib)], ca. 11.8−13.1 (br. s, 1 H, COOH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 17.0, 17.5 [2q, 2 CH$_3$ (Ala)], 18.0, 19.1, [2q, 2 CH$_3$ (Val)], 24.2, 24.6, 24.9, 25.7 [4q, 4 CH$_3$ (Aib)], 27.4 [t, CH$_2$ (Gln)], 29.9 [d, CH$_\beta$ (Val)], 31.8 [t, CH$_2$ (Gln)], 48.3, 48.9 [2d, 2 CH$_\alpha$ (Ala)], 53.1 [d, CH$_\alpha$ (Gln)], 56.2, 56.2 [2s, 2 C$_\alpha$ (Aib)], 57.3 [d, CH$_\alpha$ (Val)], 169.3 [s, CO (Ala)], 171.0 [s, CO (Gln)], 172.3 [s, CO (Ala)], 172.8 [s, CO (Val)], 173.4, 173.7 [2s, 2 CO (Aib)], 174.2 (s, CONH$_2$) ppm.

H−Leu−Aib−Leu−Aib−Phe−OH (24): PAM resin (201 mg, 0.125 mmol) was treated as described in General Procedures a), b), c), d), e), b), c), d), and e) and dried in vacuo. One part (14%) was cleaved as described in General Procedure h), and the crude product was analyzed by HPLC-MS. With the other part (86%) a second coupling was performed as described in General Procedure g). Cleavage as described in General Procedure h) yielded **24** (15 mg, 20%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS: t_R = 12.0 min, *m/z* (%) = 397 (15) oxazolone of [M − Phe + H]$^+$, 562 (100) [M + H]$^+$. IR (KBr): $\tilde{\nu}$ = 3423 m, 3320 s, 3064 m, 3034 m, 2962 s, 2939 s, 2874 m, 1723 *sh*, 1668 vs, 1529 vs, 1468 m, 1441 m, 1388 m, 1367 m, 1269 m, 1202 vs, 1140 s, 1081 w, 1031 w, 941 w, 878 w, 837 w, 800 w, 722 w, 700 w, 598 w, 518 w, 490 w cm^{-1}. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.83, 0.86, 0.90, 0.92 [4d, *J* = 6.3 Hz, 12 H, 4 CH$_3$ (Leu)], 1.30, 1.36, 1.38, 1.39 [4s, 12 H, 4 CH$_3$ (Aib)], 1.40−1.65 [m, 6 H, 2 CH$_2$ (Leu), 2 CH$_\gamma$ (Leu)], 2.95 [dd, *J* (CH$_A$*H*$_B$,CH$_\alpha$) = 8.3, ^{2}*J* = 13.8 Hz, 1 H, CH$_A$*H*$_B$ (Phe)], 3.04 [dd, *J* (C*H*$_A$H$_B$,CH$_\alpha$) = 5.4, ^{2}*J* = 13.8 Hz, 1 H, C*H*$_A$H$_B$ (Phe)], 3.77 [br. s, 1 H, CH$_\alpha$ (Leu)], 4.15 [m, 1 H, CH$_\alpha$ (Leu)], 5.41 [ddd, *J* (CH$_\alpha$,CH$_A$*H*$_B$) = 8.3, *J* (CH$_\alpha$,NH) = 7.8, *J* CH$_\alpha$,C*H*$_A$H$_B$) = 5.4 Hz, 1 H, CH$_\alpha$ (Phe)], 7.18−7.26 (m, 5 arom. H), 7.51 [d, *J* (NH,CH$_\alpha$) = 7.8 Hz, 1 H, NH (Phe)], 7.59 [d, *J* = 7.4 Hz, 1 H, NH (Leu)], 7.80 [s, 1 H, NH (Aib)], 8.08 [br. s, 3 H, NH$_3^+$ (Leu)], 8.58 [s, 1 H, NH (Aib)], ca. 12.4−13.0 (br. s, 1 H, COOH) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 21.5, 21.9, 22.5, 23.1 [4q, 4 CH$_3$ (Leu)], 23.6, 24.0 [2d, 2 CH$_\gamma$ (Leu)], 24.0, 24.2, 25.0, 25.5 [4q, 4 CH$_3$ (Aib)], 36.7 [t, CH$_2$ (Phe)], 39.5, 39.7 [2t, 2 CH$_2$(Leu)], 51.1, 51.8 [2d, 2 CH$_\alpha$ (Leu)], 53.6 [d, CH$_\alpha$ (Phe)], 55.9, 56.4 [2s, 2 C$_\alpha$ (Aib)], 126.3 (d, arom. CH$_p$), 128.0 (d, arom. CH$_m$), 129.1 (d, arom. CH$_o$), 137.5 (s, arom. C), 168.4, 171.3 [2s, 2 CO (Leu)], 172.6 [s, CO (Phe)], 173.3, 173.8 [2s, 2 CO (Aib)] ppm.

H−Val−Aib−Gly−Aib−Ala−OH (25): PAM resin (200 mg, 0.124 mmol) was treated as described in General Procedures a), b), c), d), e), b), c), d), e) and h) to yield **25** (20 mg, 31%) as a colorless powder, after prep. HPLC purification and lyophilization. HPLC-MS: t_R = 9.8 min, *m/z* (%) = 327 (51) oxazolone of [M − Ala + H]$^+$, 416 (100) [M + H]$^+$. IR (KBr): $\tilde{\nu}$ = 3315 s, 3066 s, 2985 s, 2943 s, 2642 w, 1667 vs, 1537 vs, 1468 m, 1387 m, 1367 m, 1335 w, 1296 m, 1248 m, 1202 vs, 1140 s, 1018 w, 980 w, 946 w, 837 w, 800 w, 722 m, 662 w, 598 w, 562 w, 518 w cm^{-1}. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.94, 0.95 [2d, *J* = 6.7 Hz, 6 H, 2 CH$_3$ (Val)], 1.27 [d, *J* = 7.3 Hz, 3 H, CH$_3$ (Ala)], 1.38, 1.39, 1.41 [3s, 12 H, 4 CH$_3$ (Aib)], 2.10 [oct., *J* = 6.7 Hz, 1 H, CH$_\beta$ (Val)], 3.52 [dd, *J* (NH,CH$_A$*H*$_B$) = 5.6, ^{2}*J* = −16.3 Hz, 1 H, CH$_A$*H*$_B$ (Gly)], 3.60 [d, *J* = 6.2 Hz, 1 H, CH$_\alpha$ (Val)], 3.65 [dd, *J* (C*H*$_A$H$_B$,NH) = 5.7, ^{2}*J* = −16.3 Hz, 1 H, C*H*$_A$H$_B$ (Gly)], 4.16 [quint., *J* = 7.3 Hz, 1 H, CH$_\alpha$ (Ala)], 7.47 [d, *J* = 7.3 Hz, 1 H, NH (Ala)], ca. 7.6−8.5 [br. s, 3 H, NH$_3^+$ (Val)], 7.70 [s, 1 H, NH (Aib)], 8.01 [dd, *J* (NH,C*H*$_A$H$_B$) = 5.7, *J* (NH,CH$_A$*H*$_B$) = 5.6 Hz, 1 H, NH (Gly)], 8.70 [s, 1 H, NH (Aib)], ca. 11.8−13.0 (br. s, 1 H, COOH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 17.0 [q, CH$_3$ (Ala)], 17.6, 18.4, [2q, 2 CH$_3$ (Val)], 23.7, 23.9, 25.8, 25.8 [4q, 4 CH$_3$ (Aib)], 29.6 [d, CH$_\beta$ (Val)], 43.4 [t, CH$_{2\alpha}$ (Gly)], 47.7 [d, CH$_\alpha$ (Ala)], 55.9, 56.3 [2s, 2 C$_\alpha$ (Aib)], 57.6 [d, CH$_\alpha$ (Val)], 167.8 [s, CO (Val)], 168.3 [s, CO (Gly)], 173.7 [s, CO (Aib)], 174.0 [s, CO (Ala)], 174.1 [s, CO (Aib)] ppm.

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[29] After PyBOP-mediated coupling of carboxylpolystyrene and H−Ala−O*t*Bu, the *t*Bu ester was hydrolyzed in TFA/DCM. The resin was treated with *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-

3-amine (**2**), the terminal amide was then hydrolyzed with 3 M HCl, and PyBOP-mediated coupling with H−Phe−O*t*Bu finally yielded the resin-bound tripeptide Ala−Aib−Phe−O*t*Bu. After every reaction, the resin was examined by ATR-FT-IR spectroscopy. Acidic hydrolysis (propionic acid/HCl, 130 °C) of the resin-bound tripeptide gave Ala, Aib and Phe, which were detected by ESI-MS.

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[41] Performed by C. A. T. GmbH & Co., Tübingen, Germany.

[42] *Interchim Uptisphere HDO C18*, 120 Å, 3 μm, 200 × 2.0 mm.

[43] The crude product was dissolved in aq. $(NH_4)_2CO_3$ (0.1 M), Ac_2O/MeOH (1:3) was then added, and the solution was stirred at room temp. for 1 h. After lyophilization, the residue was dissolved in MeOH/H_2O (10:1) and a solution of CH_2N_2 in Et_2O was added. The solution was stirred at room temp. and then concentrated in vacuo.

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