Synthesis of Cyclopentapeptides with Three to Five Aib Units

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Four new Aib-containing cyclopentapeptides have been synthesized by cyclization of the corresponding linear pentapeptides using the diethyl phosphorocyanidate (DEPC)/EtN(^{i}Pr)$_2$ method. The linear precursors were prepared *via* the 'azirine/oxazolone method', *i.e.*, the Aib units were introduced by the reaction of amino acids or peptide acids with a 2,2-dimethyl-2*H*-azirin-3-amine, followed by selective hydrolysis of the terminal amide function. Most remarkably, cyclo[(Aib)$_5$] exists in $CDCl_3$ solution in a symmetrical conformation, *i.e.*, no intramolecular H-bonds are detectable.

1. Introduction. – Although cyclopeptides have been known for many decades, their structures, syntheses and biological activities are of continuing interest (see refs. cit. in [1]). Regarding their structures, cyclopeptides containing α-aminoisobutyric acid (Aib) are of special relevance because of the conformation-determining properties of α,α-disubstituted α-amino acids. Besides a few natural Aib-containing cyclopeptides [2], several have been synthesized, *e.g.*, tetra- [3], penta- [4], and hexapeptides [5], as well as those with larger rings [6].

Our studies toward the use of 2,2-disubstituted 2*H*-azirin-3-amines **1** as building blocks in the synthesis of peptides containing α,α-disubstituted glycines [7] established that the 'azirine/oxazolone method' is a convenient and efficient approach [8]. Based on this method, we have also prepared a series of cyclopeptides with Aib or other α,α-disubstituted glycines in their backbone [1][9]. As expected on the basis of structural studies of Aib-containing peptides [10], the β-turn motif is also a preferred structure of cyclopeptides with α,α-disubstituted glycines in their skeleton [1][9][11].

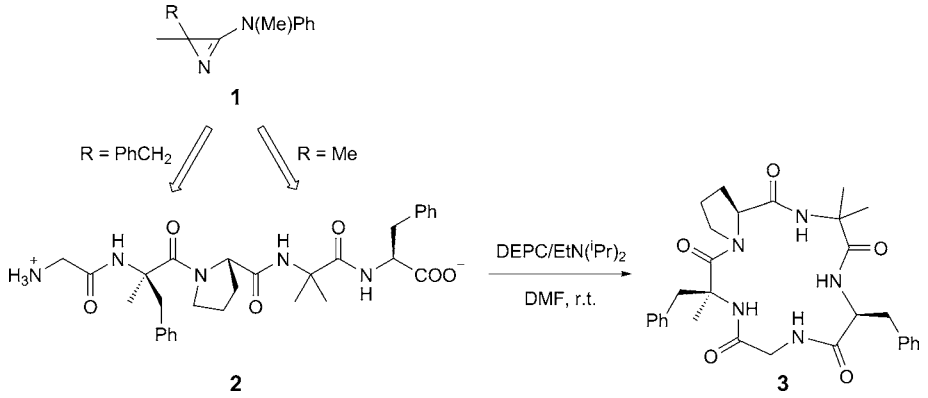
In our recent publication [1], we described the cyclization of several pentapeptides with 2-methylphenylalanine (Phe(2 Me)), and one or two Aib units, *e.g.* **2**, to give the corresponding cyclopentapeptides, *e.g.*, **3** (*Scheme 1*).

In the present study, cyclopentapeptides with three to five Aib units were prepared.

2. Results and Discussion. – The syntheses of the pentapeptides Z-Gly-Aib-Acb-Aib-Gly-OMe (**4a**; Acb = 1-aminocyclobutanecarboxylic acid) and Z-Gly-Aib-Pro-Aib-Aib-N(Me)Ph (**4b**) have been described in [12]. They were deprotected in the usual way: saponification of **4a** with $LiOH \cdot H_2O$ in THF/H_2O/MeOH gave the peptide acid Z-Gly-Aib-Acb-Aib-Gly-OH (**5a**; 96%), and subsequent hydrogenolysis, either

[1]) In part from the Ph.D. thesis of *F. S. A.*, Universität Zürich, 1997. Present address: *Bachem AG*, Hauptstrasse 144, CH-4416 Bubendorf

Scheme 1



with H_2/Pd/C in MeOH or with $HCOONH_4$/Pd/C in MeOH, led to H-Gly-Aib-Acb-Aib-Gly-OH (**6a**) in quantitative and 95% yield, respectively. Selective hydrolysis of **4b** with 3N HCl in THF/H_2O at room temperature yielded Z-Gly-Aib-Pro-Aib-Aib-OH (**5b**, 70%) [12]. The latter was deprotected at the N-terminus to furnish H-Gly-Aib-Pro-Aib-Aib-OH (**6b**) quantitatively (H_2/Pd/C) and in 67% yield ($HCOONH_4$/Pd/C), respectively.

The cyclization of the two pentapeptides was achieved smoothly by treatment with diethyl phosphorocyanidate (DEPC)/EtN(^{i}Pr)$_2$ (*Hünig* base) in DMF at room temperature to give the cyclopentapeptides **7a** and **7b** in 56 and 89% yield, respectively (*Fig. 1*). The structures were elucidated on the basis of the spectroscopic data compared with those of the previously reported analogs [1]. For example, the dominant peak in the ESI-MS of **7a** appeared at *m*/*z* 404 ($[M+Na]^+$) besides the $[M+1]^+$ peak at *m*/*z* 382. The ^{1}H-NMR spectrum in $CDCl_3$ showed four signals for NH groups at 7.21 (*s*, 2 NH), 7.08 (*t*-like, 1 NH), 6.57, and 6.23 ppm (2*s*, 2 NH), and only two *singlet*s for the Me groups of two Aib units. In the ^{13}C-NMR spectrum ($CDCl_3$), five signals of C=O groups were detected at 174.9, 174.8, 174.0, 171.8, and 171.4 ppm, and the signals of the Me groups of the two Aib units appeared at 25.2 and 24.7 ppm.

For the attempted synthesis of cyclo(Gly-Aib-Aib-Aib-Aib) (**7c**), two linear pentapeptide precursors were prepared as outlined in *Schemes 2* and *3*. Coupling of Z-Gly-OH with the known tetrapeptide amide **8** [13] using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylen)uronium hexafluorophosphate (HBPyU)/EtN(^{i}Pr)$_2$ gave

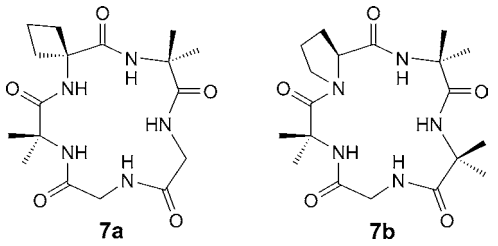
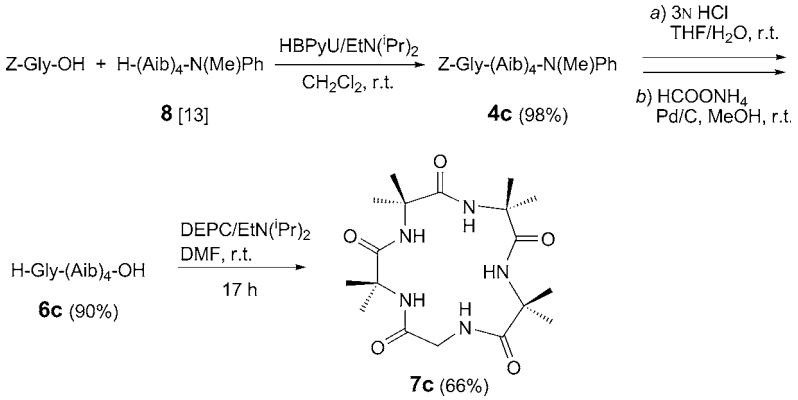
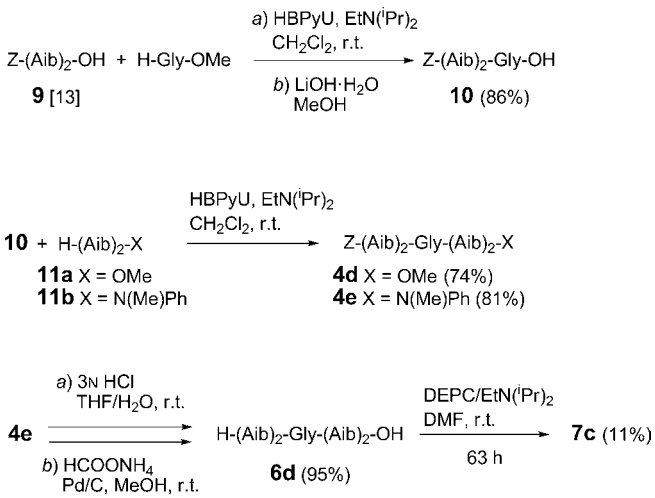


Fig. 1. *Structures of the cyclopentapeptides* **7a** *and* **7b**

Scheme 2



Scheme 3



4c in 98% yield (*Scheme 2*). Deprotection of the latter in the usual way yielded **6c** (90%), which was cyclized by treatment with DEPC/EtN(^{i}Pr)$_2$ at room temperature for 17 h to give **7c** in 66% yield.

In the first attempt to prepare the alternative precursor **6d**, the dipeptide acid **9** [13] was coupled with H-Gly-OMe to give, after saponification with LiOH · H$_2$O in MeOH, Z-(Aib)$_2$-Gly-OH (**10**) in 86% yield (*Scheme 3*). Subsequent coupling of the latter with H-(Aib)$_2$-OMe (**11a**)[2]), yielded the protected pentapeptide **4d** (74%), the structure of which was established by X-ray crystallography (*Fig. 2*). Unexpectedly, all attempts to

[2]) Compound **11a** was prepared from **9** *via* esterification with MeOH/BF$_3$ · Et$_2$O, followed by hydrogenolysis with H$_2$/Pd/C at room temperature, in 86% yield. The product was contaminated with *ca.* 6% of 3,3,6,6-tetramethylpiperazine-2,5-dione.

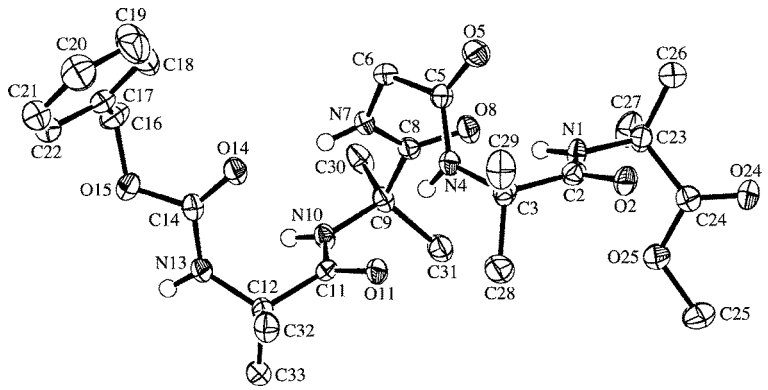


Fig. 2. *ORTEP Plot* [14] *of the molecular structure of the pentapeptide* **4d** (50% probability ellipsoids, arbitrary atom numbering, H-atoms bonded to C-atoms omitted for clarity)

cleave the ester group by treatment with $LiOH \cdot H_2O$ to obtain the pentapeptide acid failed. Therefore, the pentapeptide amide **4e** was prepared by condensation of **10** with H-$(Aib)_2$-N(Me)Ph (**11b**), which was obtained from Z-$(Aib)_2$-N(Me)Ph [13] by treatment with H_2/Pd/C in MeOH. Selective cleavage of the terminal amide bond by treatment with 3N HCl in THF/H_2O at room temperature and subsequent deprotection of the N-terminus *via* transfer hydrogenolysis afforded the desired pentapeptide **6d** in 95% yield. The cyclization of **6d** under the conditions used for the case of **6c** → **7c**, but for 63 h, gave the same cyclopentapeptide **7c**, but in the modest yield of 11%.

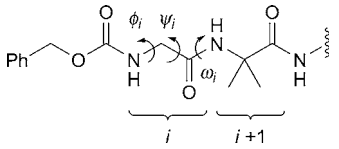
In the ESI-MS, characteristic for cyclopentapeptide **7c** were the $[M+1]^+$ peak at *m*/*z* 398 (100%) together with peaks for $[M+Na]^+$ (*m*/*z* 420) and $[M+K]^+$ (*m*/*z* 436). The ^{1}H-NMR spectrum in $CDCl_3$ showed a signal for the lactam NH of Gly at 7.31 ppm (*t*-like), and four *singlet*s of Aib-NH at 7.05, 6.65, 6.29, and 6.25 ppm. The signals of the lactam C=O groups appeared in the ^{13}C-NMR spectrum at 176.5, 174.7, 174.1, 173.9, and 172.4 ppm. The eight Me groups of the four Aib units resonated as four *singlet*s in the ^{1}H-NMR spectrum (1.56, 1.55, 1.53, and 1.51 ppm) and gave rise to three signals in the ^{13}C-NMR spectrum (25.5, 24.9, 24.8 ppm (*ca.* 1:2:1)). In addition, four *singlet*s for C(2) of four Aib and a *triplet* for CH_2 of Gly were detected in the ^{13}C-NMR spectrum.

Suitable crystals of **4d** for an X-ray crystal-structure determination were obtained from AcOEt/hexane by slow evaporation of the solvent. The molecule adopts an overall helical conformation (*Fig. 2*). Each NH group of **4d** acts as a donor for H-bonds (*Table 1*). Three of them are intramolecular ones, forming a regular pattern along the peptide chain: N(1)–H, N(4)–H, and N(7)–H interact with the amide O-atom that is seven atoms further along the peptide backbone. Each of these interactions has a graph set motif [15] of *S*(10), *i.e.*, three β-turns of type III are formed (*Table 2*) leading to an overall 3_{10}-helical conformation of the peptide. The remaining N(10)–H and N(13)–H groups, which are not able to form such intramolecular H-bonds, due to their positions in the backbone, form intermolecular H-bonds with the ester C=O and amide O-atoms, respectively, at the opposite end of the same neighboring molecule. These interactions link the molecules into extended chains running parallel to the $[10\bar{1}]$ direction; graph sets *C*(14) for each interaction.

Table 1. *Intra- and Intermolecular H-Bonds of Pentapeptide* **4d** (for atom numbering, see *Fig. 2*)

Donor···Acceptor	N···O [Å]	H···O [Å]	N–H···O [°]
N(1)–H···O(8)	2.952(3)	2.10(3)	167(2)
N(4)–H···O(11)	3.053(3)	2.21(3)	169(2)
N(7)–H···O(14)	2.879(3)	2.05(3)	158(2)
N(10)–H···O(24′)	3.053(3)	2.21(3)	164(2)
N(13)–H···O(2′)	2.860(3)	2.03(3)	168(2)

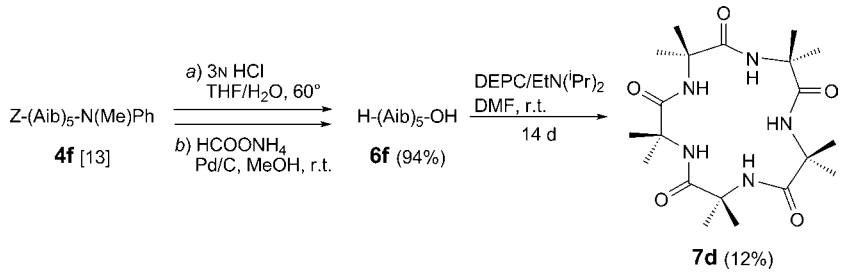
Table 2. *Torsion Angles* ϕ, ψ, *and* ω *[°] of the Backbone of* **4d** *in the Crystal*



$\phi_{(i)}$	– 54.1(3)	$\psi_{(i+2)}$	– 23.7(3)
$\psi_{(i)}$	– 42.1(3)	$\omega_{(i+2)}$	174.9(2)
$\omega_{(i)}$	– 177.3(2)	$\phi_{(i+3)}$	– 60.4(3)
$\phi_{(i+1)}$	– 55.2(3)	$\psi_{(i+3)}$	– 20.2(3)
$\psi_{(i+1)}$	– 27.5(3)	$\omega_{(i+3)}$	– 176.9(2)
$\omega_{(i+1)}$	– 179.3(2)	$\phi_{(i+4)}$	55.1(3)
$\phi_{(i+2)}$	– 58.4(3)	$\psi_{(i+4)}$	44.5(3)

Finally, the pentapeptide Z-$(Aib)_5$-N(Me)Ph (**4f**) [13], prepared by repeated azirine coupling and selective hydrolysis, was deprotected to give **6f** in 94% yield (*Scheme 4*). As **4f** was rather insoluble in THF/H_2O, the hydrolysis of the terminal amide group with 3N HCl had to be performed at 60°. The cyclization of **6f** proved to be very difficult. Under the usual conditions, with DEPC/EtN(^{i}Pr)$_2$ in DMF at room temperature as well as at 80°, mixtures of products were obtained, among them formylated pentapeptides, H-$(Aib)_5$-OMe, H-$(Aib)_{10}$-OMe (MS), *etc.* Also in CH_2Cl_2 or in MeCN at reflux, only traces of the desired cyclo[$(Aib)_5$] (**7d**) could be detected (MS). Finally, after treatment with DEPC/EtN(^{i}Pr)$_2$ in DMF at room temperature for 14 d and repeated chromatographic purification, **7d** was obtained in 12% yield.

Scheme 4



The ESI-MS of **7d** exhibited a dominant $[M+Na]^+$ peak (100%) at *m/z* 448. Surprisingly, the ^{1}H-NMR spectrum in $CDCl_3$ showed only two *singlet*s at 6.63 (NH) and 1.54 ppm (Me_2C) with an intensity ratio of 1:6. Similarly, only three signals appeared in the ^{13}C-NMR spectrum ($CDCl_3$): a *singlet* at 175.2 (C=O lactam), a *singlet* at 58.0 (C(2) of (Aib)), and a *quadruplet* at 25.0 ppm (*Me*$_2$C of Aib). From these data, we concluded that cyclo[(Aib)$_5$] (**7d**) in $CDCl_3$ solution exists in a perfectly symmetrical conformation, and no stable intramolecular H-bonds are formed.

3. Conclusions. – Three cyclopentapeptides containing three, four, and five Aib units, as well as cyclo(Gly-Aib-Acb-Aib-Gly) were prepared by cyclization of the corresponding linear precursors by treatment with DEPC/EtN(^{i}Pr)$_2$ in DMF at room temperature. The syntheses of the linear pentapeptides were achieved by applying the 'azirine/oxazolone method' and segment condensation. The results of the cyclization reactions evidence once more (see *Introduction*) that the efficiency of the ring closure strongly depends on steric factors, especially at the N-terminal amino acid, *i.e.*, the nucleophile in the cyclization. Whereas cyclo[Gly-(Aib)$_4$] was obtained in 66% yield from H-Gly-(Aib)$_4$-OH, the yield dropped to 11% in the case of H-(Aib)$_2$-Gly-(Aib)$_2$-OH. Also, the ring closure of penta-Aib to give cyclo[(Aib)$_5$] occurred only sluggishly.

Most remarkable are the NMR spectra of cyclo[(Aib)$_5$], as they indicate a symmetrical conformation, excluding the presence of stable intramolecular H-bonds.

We thank the analytical sections of our institute for spectra and analyses, and the *Stipendienfonds der Basler Chemischen Industrie* and *F. Hoffmann-La Roche AG*, Basel, for financial support.

Experimental Part

1. *Abbreviations.* Aib, 2-aminoisobutyric acid (2-methylalanin); DEPC, diethyl phosphorocyanidate; EtN(^{i}Pr)$_2$, ethyl(diisopropyl)amine (*Hünig* base); HBPyU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylen)uronium hexafluorophosphate; Z, (benzyloxy)carbonyl.

2. *General.* See [1][12]. Solvents were purified by standard procedures. TLC: *Merck* glass plates, silica gel *60 F*$_{254}$. Column chromatography (CC): *Uetikon-Chemie*, silica gel *C-560* (0.04–0.063 mm) or *Merck 60*, 0.040–0.063 mm. M.p.: *Mettler-FP-5* apparatus; uncorrected. $[\alpha]_D$ Values: *Perkin-Elmer-241* polarimeter at 21°. IR Spectra: *Perkin-Elmer-781* spectrometer, in KBr. ^{1}H- and ^{13}C-NMR spectra: *Bruker AC-300*, *Bruker ARX-300*, or *Bruker AMX-600* spectrometer (at 300 or 600 (^{1}H) and 75.5 or 150 MHz, resp.); in $CDCl_3$, CD_3OD or (D_6)DMSO; multiplicities of ^{13}C signals determined by the DEPT technique. ESI- and CI-MS: *Finnigan TSQ-700* and *Finnigan SSQ-700* instrument, respectively; in *m/z* (rel. %).

General Procedure 1 (*GP 1; Saponification of Peptide Methyl Esters*). To a soln. of a peptide methyl ester (1 mmol) in 10 ml of THF/MeOH/H_2O (3:1:1) at 0° was added LiOH·H_2O (2.5 mmol). The mixture was stirred at 0° for 1 h. Then, it was neutralized by addition of aq. 2N HCl, and the org. solvents were evaporated (rotavapor). The residue was dissolved in AcOEt, und the mixture was washed with aq. 0.5N HCl. The org. phase was dried (Na_2SO_4), and the solvent was evaporated.

General Procedure 2 (*GP 2; Hydrogenolysis*). A mixture of Z-protected peptide in MeOH and *ca.* 10% Pd/C (10%) at r.t. was stirred under H_2 (balloon) overnight. The mixture was filtered through a *Celite* pad, and the solvent of the filtrate was evaporated to dryness.

General Procedure 3 (*GP 3; Transfer Hydrogenolysis*). To a mixture of Z-protected peptide (1 mmol) and the same amount of Pd/C (10%) in MeOH was added $HCOONH_4$ (5 mmol). The mixture was heated at reflux for 10 min, and the hot mixture was filtered through a *Celite* pad and washed with MeOH. The solvent of the filtrate was evaporated to dryness.

General Procedure 4 (*GP 4; Hydrolysis of Peptide Amides*). A soln. of Z-protected peptide amide (1 mmol) in 3N HCl (THF/H_2O 1:1) was stirred at r.t. for 1–4.5 h. Then, 2N HCl was added, and the mixture was extracted with Et_2O. The org. phase was dried (Na_2SO_4), and the solvent was evaporated.

General Procedure 5 (*GP 5; Cyclization with DEPC*). To a *ca.* 1.5×10^{-3} M soln. of a deprotected pentapeptide (0.1 mmol) in DMF (67 ml) at 0° were added dropwise DEPC (0.2–0.4 mmol) and EtN(^{i}Pr)$_2$ (1% (*v*/*v*)), and the mixture was stirred overnight at r.t. Then, DMF was evaporated, and the residue was purified chromatographically and crystallized.

General Procedure 6 (*GP 6, Segment Condensation*). To a mixture of a *N*-protected peptide (1 mmol), *C*-protected amino acid (1.1 mmol), and HBPyU (1 mmol) in CH_2Cl_2 (1 ml) at r.t. was added EtN(^{i}Pr)$_2$ (2 mmol; 3 mmol in the case of an amino acid chloride), and the mixture was stirred for 1 h. Then, the solvent was evaporated, and the residue was dissolved in AcOEt (20 ml), washed with aq. $KHSO_4$ (5%, 3×), aq. $NaHCO_3$ (5%, 3×), and aq. NaCl, and purified by CC.

3. *Synthesis of Cyclo(Gly-Aib-Acb-Aib-Gly)* (**7a**). 3.1. *Z-Gly-Aib-Acb-Aib-Gly-OH* (**5a**). Hydrolysis of *Z-Gly-Aib-Acb-Aib-Gly-OMe* (**4a** [12]; 215 mg, 0.339 mmol) was performed with LiOH·H_2O (43 mg, 1.025 mmol) in THF/H_2O/MeOH (3:1:1, 4 ml) according to *GP 1:* 201 mg (96%) of **5a**. Colorless solid. M.p. 89.6–91.4°. IR (KBr): 3300*s* (br), 3060*m*, 2990*m*, 2940*m*, 1755*s*, 1750*s*, 1740*s*, 1730*s*, 1715*s*, 1705*s*, 1695*s*, 1670*s*, 1660*s*, 1650*s*, 1645*s*, 1555*s*, 1540*s*, 1535*s*, 1525*s*, 1505*m*, 1470*m*, 1465*m*, 1455*m*, 1430*m*, 1415*m*, 1390*m*, 1365*m*, 1340*m*, 1310*m*, 1265*m*, 1240*m*, 1185*m*, 1050*m*, 1015*m*, 740*m*, 695*m*. ^{1}H-NMR ((D$_6$)DMSO): 8.41, 8.10 (2*s*, 2 NH); 7.53 (*t*-like, NH); 7.35–7.3 (*m*, 5 arom. H, NH); 7.25 (*s*, NH); 5.02 (*s*, PhCH_2); 3.7–3.6 (*m*, 2 CH_2(Gly)); 2.55–2.4, 2.1–2.0, 1.9–1.75 (3*m*, 3 CH_2(Acb)); 1.36, 1.34 (2*s*, 2 Me_2C). ^{13}C-NMR ((D$_6$)DMSO): 174.7, 174.5, 172.5, 170.9, 169.8 (5*s*, 4 CO(amide), COOH); 156.7 (*s*, CO(urethane)); 136.8 (*s*, 1 arom. C); 128.3, 127.7, 127.5 (3*d*, 5 arom. CH); 65.5 (*t*, PhCH_2); 58.6, 55.9, 55.6 (3*s*, 2 C(2)(Aib), C(2)(Acb)); 43.7, 40.8 (2*t*, 2 CH_2(Gly)); 30.4 (*t*, 2 CH_2(Acb)); 25.0, 24.7 (2*q*, 2 Me_2C); 15.0 (*t*, CH_2(Acb)). ESI-MS: 556 (100, $[M+Na]^+$), 459 (3, $[M-Gly]^+$), 374 (10, $[M-Aib\text{-}Gly]^+$).

3.2. *H-Gly-Aib-Acb-Aib-Gly-OH* (**6a**). *a*) Hydrogenolysis of **5a** (233 mg, 0.437 mmol) in MeOH (10 ml) with Pd/C (24 mg) according to *GP 2*; addition of H_2O (10 ml), and filtration through *Celite*: 177 mg (quant.) of **6a**.

b) Hydrogenolysis of **5a** (114 mg, 0.214 mmol) in MeOH according to *GP 3* with Pd/C (114 mg): 81 mg (95%) of **6a**. Colorless solid. M.p. 239.2–240.7°. ^{1}H-NMR (D_2O): 3.83, 3.75 (2*s*, 2 CH_2(Gly)); 2.65–2.5, 2.25–2.1, 2.05–1.9 (3*m*, 3 CH_2(Acb)); 1.52, 1.50 (2*s*, 2 Me_2C). ^{13}C-NMR (D_2O): 179.7, 179.0, 177.9, 169.4 (4*s*, 4 CO(amide), COOH); 62.1, 59.5, 59.4 (3*s*, 2 C(2)(Aib), C(2)(Acb)); 46.3, 43.3 (2*t*, 2 CH_2(Gly)); 33.3 (*t*, 2 CH_2(Acb)); 27.1, 26.7 (2*q*, 2 Me_2C); 17.7 (*t*, CH_2(Acb)). ESI-MS: 422 (100, $[M+Na]^+$).

3.3. *Cyclo(Gly-Aib-Acb-Aib-Gly)* (**7a**). The cyclization of **6a** (27.3 mg, 0.068 mmol) in DMF (45 ml) was carried out with DEPC (125.5 mg, 0.77 mmol) and EtN(^{i}Pr)$_2$ (0.45 ml) according to *GP 5*. After CC (CH_2Cl_2/MeOH/NH_3 10:1:0.1) and crystallization from $CHCl_3$/hexane, 14.5 mg (56%) of **7a** were obtained. Colorless solid. M.p. 135.3–136.4°. ^{1}H-NMR ($CDCl_3$): 7.21 (*s*, 2 NH); 7.08 (*t*-like, NH); 6.57, 6.23 (2*s*, 2 NH); 3.99 (*d*, $J = 6.3$, CH_2(Gly)); 3.85 (*d*, $J = 6.4$, CH_2(Gly)); 2.6–2.45, 2.4–2.3, 2.15–2.0, 2.0–1.8 (4*m*, 2:2:1:1, 3 CH_2(Acb)); 1.58, 1.55 (2*s*, 2 Me_2C). ^{13}C-NMR ($CDCl_3$): 174.9, 174.8, 174.0, 171.8, 171.4 (5*s*, 5 CO(amide)); 60.1, 57.7, 57.4 (3*s*, 2 C(2)(Aib), C(2)(Acb)); 44.7, 44.0 (2*t*, 2 CH_2(Gly)); 31.1 (*t*, 2 CH_2(Acb)); 25.2, 24.7 (2*q*, 2 Me_2C); 15.7 (*t*, CH_2(Acb)). ESI-MS: 404 (100, $[M+Na]^+$), 382 (7, $[M+1]^+$).

4. *Synthesis of Cyclo(Gly-Aib-Pro-Aib-Aib)* (**7b**). 4.1. *H-Gly-Aib-Pro-Aib-Aib-OH* (**6b**). *a*) Hydrogenolysis of **5b** [12] (86 mg, 0.153 mmol) in MeOH (3 ml) with Pd/C (8.7 mg) was carried out according to *GP 2*. After 10 min, the reaction was complete (TLC), and a precipitate formed, which dissolved again during stirring overnight: 66 mg (quant.) of **6b**.

b) Hydrogenolysis of **5b** (330 mg, 0.588 mmol) in MeOH (3 ml) according to *GP 3* with $HCOONH_4$ (183 mg, 2.902 mmol) and Pd/C (331 mg) afforded 81 mg (95%) of **6b**. Colorless solid. M.p. 173.6–175.3°. IR (KBr): 3320*m*, 3050*m*, 2980*m*, 2930*m*, 2870*m*, 1670*s*, 1620*s*, 1550*s*, 1470*m*, 1450*m*, 1415*m*, 1390*m*, 1360*m*, 1280*m*, 1210*m*, 1190*m*, 1170*m*. ^{1}H-NMR (D_2O): 4.33 (*t*-like, CH(2)(Pro)); 3.89 (*s*, CH_2(Gly)); 3.8–3.65, 3.6–3.5 (2*m*, CH_2(5)(Pro)); 2.3–2.15, 2.1–1.8 (2*m*, 1:3, CH_2(3), CH_2(4)(Pro)); 1.51, 1.48, 1.43, 1.42 (4*s*, 1:3:1:1, 3 Me_2C). ^{13}C-NMR (D_2O): 184.5, 178.2, 176.7, 176.4, 168.5 (5*s*,

4 CO(amide), COOH); 65.7 (*d*, CH(2)(Pro)); 60.5, 59.6 (2*s*, 1:2, 3 C(2)(Aib)); 51.7 (*t*, CH_2(5)(Pro)); 42.8 (*t*, CH_2(Gly)); 31.0, 28.3 (2*t*, CH_2(3), CH_2(4)(Pro)); 27.2, 27.04, 26.97, 26.4 (4*q*, 3 *Me*$_2$C). ESI-MS: 466 (23, $[M+K]^+$, 450 (100, $[M+Na]^+$), 428 (52, $[M+1]^+$), 325 (16, $[M-Aib]^+$), 286 (20, [Pro-Aib-Aib]$^+$).

4.2. *Cyclo(Gly-Aib-Pro-Aib-Aib)* (**7b**). The cyclization of **6b** (48.2 mg, 0.113 mmol) in DMF (75 ml) was carried out with DEPC (67.0 mg, 0.411 mmol) and EtN(i-Pr)$_2$ (0.75 ml) according to *GP 5*. After CC (CH_2Cl_2/MeOH 10:1) and crystallization from AcOEt/hexane, 41.2 mg (89%) of **7b** were obtained. Colorless solid. M.p. 139.1–140.5°. $[\alpha]_D^{20} = -35.6$ (c = 0.95, EtOH). IR (KBr): 3300*s*, 3040*m*, 2980*m*, 2940*m*, 1695*s*, 1680*s*, 1670*s*, 1660*s*, 1650*s*, 1645*s*, 1635*s*, 1565*m*, 1555*s*, 1550*s*, 1540*s*, 1520*s*, 1505*s*, 1480*m*, 1470*m*, 1460*m*, 1455*m*, 1445*m*, 1390*s*, 1365*s*, 1270*m*, 1245*m*, 1215*m*, 1195*m*, 1175*m*, 1075*m*, 1050*m*, 1030*m*. ^{1}H-NMR ((D$_6$)DMSO): 8.7 (very br. *s*, NH); 7.83 (br. *s*, NH); 7.30 (br. *s*, NH); 6.8 (very br. *s*, NH); 4.5–4.35 (*m*, CH(2)(Pro)); 4.15–3.8 (*m*, 1 H of CH_2(Gly)); 3.55–3.3 (*m*, CH_2(5)(Pro), 1 H of CH_2(Gly)); 2.0–1.75 (*m*, CH_2(3), CH_2(4)(Pro)); 1.49, 1.44, 1.40, 1.31 (4*s*, 1:2:2:1, 3 Me$_2$C). ESI-MS: 432 (100, $[M+Na]^+$), 410 (20, $[M+1]^+$).

5. *Synthesis of Cyclo(Gly-Aib-Aib-Aib-Aib)* (**7c**). 5.1. Via *Cyclization of H-Gly-Aib-Aib-Aib-Aib-OH* (**6c**). 5.1.1. *Z-Gly-Aib-Aib-Aib-Aib-N(Me)Ph* (**4c**). According to *GP 6*, to a mixture of H-Aib-Aib-Aib-Aib-N(Me)Ph (**8** [13], 202.6 mg, 0.453 mmol), Z-Gly-OH (98.2 mg, 0.469 mmol), and HBPyU (202.7 mg, 0.470 mmol) in CH_2Cl_2 (4 ml) at r.t. was added EtN(i-Pr)$_2$ (119.7 mg, 0.926 mmol). After stirring for 21 h, the precipitate was filtered and washed with a little CH_2Cl_2 to give **4c** (243.7 mg) as a white powder. The filtrate was evaporated, and CC (CH_2Cl_2/MeOH) gave an additional 40.7 mg of **4c**. Total yield: 284.4 mg (98%). Colorless powder. M.p. 222.3–224.4°. IR (KBr): 3310*m*, 3280*m*, 2980*m*, 2940*m*, 1700*m*, 1680*s*, 1670*s*, 1660*s*, 1645*s*, 1635*s*, 1590*m*, 1530*s*, 1495*m*, 1465*m*, 1450*m*, 1395*m*, 1380*m*, 1360*m*, 1270*m*, 1230*m*, 1170*m*, 1090*m*, 710*m*. ^{1}H-NMR (CD$_3$OD): 7.4–7.2 (*m*, 10 arom. H); 5.10 (*s*, PhC*H*$_2$); 3.71 (*s*, CH_2(Gly)); 3.38 (*s*, MeN); 1.55, 1.48, 1.41, 1.38 (4*s*, 4 Me$_2$C). ^{13}C-NMR (CD$_3$OD): 177.1, 176.7, 176.4, 175.9, 172.1 (5*s*, 5 CO(amide)); 159.4 (*s*, CO(urethane)); 147.2, 138.2 (2*s*, 2 arom. C); 130.3, 129.6, 129.1, 128.8, 128.2, 128.1 (6*d*, 10 arom. CH); 67.8 (*t*, PhC*H*$_2$); 58.4, 58.2, 57.9, 57.6 (4*s*, 4 C(2)(Aib)); 45.2 (*t*, CH_2(Gly)); 41.1 (*q*, MeN); 26.3, 26.0, 25.34, 25.27 (4*q*, 4 *Me*$_2$C). ESI-MS: 661 (16, $[M+Na]^+$), 532 (100, $[M-N(Me)Ph]^+$), 447 (49, $[M-Aib-N(Me)Ph]^+$), 362 (18, $[M-Aib-Aib-N(Me)Ph]^+$).

5.1.2. *Z-Gly-Aib-Aib-Aib-Aib-OH* (**5c**). According to *GP 4*, **4c** (255.8 mg, 0.400 mmol) was hydrolyzed in 3N HCl (THF/H_2O 1:1, 10 ml, 1 h). Extraction with AcOEt gave 213.4 mg (97%) of **5c**. A sample was recrystallized from boiling AcOEt. Colorless needles. M.p. 201.8–203.0°. IR (KBr): 3340*s*, 3310*s*, 3300*s*, 3080*m*, 3070*m*, 3060*m*, 3040*m*, 2980*m*, 2940*m*, 2930*m*, 1730*s*, 1715*s*, 1710*s*, 1695*s*, 1680*s*, 1670*s*, 1660*s*, 1650*s*, 1645*s*, 1550*s*, 1540*s*, 1520*s*, 1510*s*, 1470*m*, 1455*s*, 1385*s*, 1365*m*, 1280*s*, 1240*s*, 1230*s*, 1170*s*, 1160*s*, 1155*s*, 1050*m*, 735*m*, 700*m*. ^{1}H-NMR (CD$_3$OD): 7.7–7.6 (*m*, 2 arom. H); 7.4–7.3 (*m*, 3 arom. H); 5.10 (*s*, PhC*H*$_2$); 3.72 (*s*, CH_2(Gly)); 1.49, 1.42, 1.41, 1.39 (4*s*, 4 Me$_2$C). ^{13}C-NMR (CD$_3$OD): 178.3, 176.9, 176.6, 172.1 (4*s*, 4 CO(amide), COOH); 159.8 (*s*, CO(urethane)); 138.2 (*s*, arom. C); 129.6, 129.1, 128.8 (3*d*, 5 arom. CH); 67.9 (*t*, PhC*H*$_2$); 57.9, 57.7, 57.1 (3*s*, 4 C(2)(Aib)); 45.3 (*t*, CH_2(Gly)); 25.7, 25.41, 25.36, 25.3 (4*q*, 4 *Me*$_2$C). CI-MS: 551 (23), 550 (79, $[M+1]^+$), 533 (26), 532 (93, $[M-OH]^+$), 448 (24), 447 (100, $[M-Aib-OH]^+$), 442 (32), 424 (10), 416 (18, $[M-BnCOO]^+$), 398 (12), 362 (32, $[M-Aib-Aib-OH]^+$), 104 (73, [H-Aib-OH + 1]$^+$). Anal. calc. for $C_{24}H_{36}N_4O_7 \cdot 0.5\ H_2O$ (558.63): C 55.90, H 7.22, N 12.54; found: C 55.80, H 7.14, N 12.44.

5.1.3. *H-Gly-Aib-Aib-Aib-Aib-OH* (**6c**). According to *GP 3*, **5c** (187.7 mg, 0.342 mmol) was deprotected by treatment with $HCOONH_4$ (109.8 mg, 1.741 mmol) and Pd/C (188.0 mg) in MeOH (7 ml) to furnish 132.3 mg (93%) of **6c**. Colorless solid. ^{1}H-NMR (D_2O): 3.84 (*s*, CH_2(Gly)); 1.48, 1.47, 1.43, 1.42 (4*s*, 4 Me$_2$C). ^{13}C-NMR (D_2O): 179.0, 178.8, 178.5, 169.3 (4*s*, 4 CO(amide), COOH); 60.6, 59.7 (2*s*, 4 C(2)(Aib)); 43.3 (*t*, CH_2(Gly)); 27.2, 27.1, 26.9, 26.8 (4*q*, 4 *Me*$_2$C). CI-MS: 417 (29), 416 (100, $[M+1]^+$), 398 (14, $[M-OH]^+$).

5.1.4. *Cyclo*(*Gly-Aib-Aib-Aib-Aib)* (**7c**). According to *GP 5*, to a soln. of **6c** (38.6 mg, 0.093 mmol) in DMF (63 ml) were added DEPC (46.6 mg, 0.286 mmol) and EtN(i-Pr)$_2$ (0.6 ml) at r.t. After stirring for 17 h, DMF was evaporated, and the residue was purified by CC (CH_2Cl_2/MeOH 10:1): 24.5 mg (66%) of **7c**. Colorless solid. M.p. 126.3–127.7°. IR (KBr): 3330*m*, 2980*w*, 2940*w*, 1690*s*, 1680*s*, 1670*s*, 1660*s*, 1650*s*, 1645*s*, 1635*s*, 1550*s*, 1540*s*, 1530*s*, 1515*s*, 1505*s*, 1470*m*, 1460*m*, 1390*m*, 1375*m*, 1225*m*. ^{1}H-NMR ($CDCl_3$): 7.31 (*t*-like, NH); 7.05, 6.65, 6.29, 6.25 (4*s*, 4 NH); 3.39 (*d*, *J* = 6.1, CH_2(Gly)); 1.56, 1.55, 1.53, 1.51 (4*s*,

4 Me_2C). ^{13}C-NMR ($CDCl_3$): 176.5, 174.7, 174.1, 173.9, 172.4 (5*s*, 5 CO(amide)); 59.1, 57.6, 57.4, 56.9 (4*s*, 4 C(2)(Aib)); 45.1 (*t*, CH_2(Gly)); 25.5, 24.9, 24.8 (3*q*, 4 *Me*$_2$C). ESI-MS: 436 (12, $[M+K]^+$), 420 (19, $[M+Na]^+$), 398 (100, $[M+1]^+$).

5.2. Via *Cyclization of H-Aib-Aib-Gly-Aib-Aib-OH* (**6d**). 5.2.1. *Z-Aib-Aib-Gly-OMe*. According to *GP 6*, with *Z-Aib-Aib-OH* (**9** [13], 797.1 mg, 2.473 mmol), H-Gly-OMe · HCl (343.1 mg, 2.733 mmol), HBPyU (1.070 g, 2.480 mmol), and EtN(^{i}Pr)$_2$ (1.3 ml, 7.594 mmol) in CH_2Cl_2 (7 ml); reaction time 2.5 h. The precipitate was filtered to give 550.3 mg of tripeptide; the filtrate was evaporated, and CC (AcOEt) gave an additional portion of tripeptide. Total yield: 906.8 mg (93%). Colorless solid. M.p. 151.5–152.2°. IR (KBr): 3350*s*, 3280*s*, 3040*m*, 2980*m*, 2950*m*, 1755*m*, 1700*s*, 1670*s*, 1660*s*, 1535*s*, 1520*s*, 1455*m*, 1435*m*, 1410*m*, 1385*m*, 1370*m*, 1265*m*, 1215*s*, 1210*s*, 1195*s*, 1175*s*, 1090*m*, 1080*s*, 985*m*, 965*m*, 755*m*, 700*m*. ^{1}H-NMR ($CDCl_3$): 7.42 (br. *s*, NH); 7.4–7.3 (*m*, 5 arom. H); 6.46, 5.32 (2*s*, 2 NH); 5.10 (*s*, PhC*H*$_2$); 3.99 (*d*, *J* = 5.6, CH_2(Gly)); 3.72 (*s*, MeO); 1.48 (*s*, 2 Me_2C). ^{13}C-NMR ($CDCl_3$): 174.6, 173.1, 170.5 (3*s*, 2 CO(amide), COOMe); 155.7 (*s*, CO(urethane)); 136.0 (*s*, arom. C); 128.7, 128.5, 128.4 (3*d*, 5 arom. CH); 67.1 (*t*, PhC*H*$_2$); 57.3, 57.1 (2*s*, 2 C(2)(Aib)); 51.9 (*q*, MeO); 41.4 (*t*, CH_2(Gly)); 25.43, 25.38 (2*q*, 2 *Me*$_2$C). CI-MS: 412 (6), 411 (30, $[M+NH_4]^+$), 395 (21), 394 (100, $[M+1]^+$), 331 (14), 306 (22), 305 (8). Anal. calc. for $C_{19}H_{27}N_3O_6$ (393.44): C 58.00, H 6.92, N 10.68; found: C 57.88, H 7.04, N 10.62.

5.2.2. *Z-Aib-Aib-Gly-OH* (**10**). According to *GP 1*, Z-Aib-Aib-Gly-OMe (906.8 mg, 2.305 mmol) was saponified with LiOH · H_2O (243.0 mg, 5.791 mmol) in THF/MeOH/H_2O 3:1:1 (7 ml): 809 mg (93%) of **10**. Colorless solid. IR (KBr): 3320*s*, 3300*s*, 3085*m*, 3030*m*, 3000*m*, 2940*m*, 2910*m*, 2900*m*, 1765*m*, 1750*m*, 1702*s*, 1665*s*, 1620*s*, 1615*s*, 1565*s*, 1515*s*, 1470*m*, 1450*m*, 1410*m*, 1385*m*, 1370*m*, 1335*m*, 1265*s*, 1220*m*, 1175*s*, 1085*s*, 900*m*, 740*m*, 695*m*. ^{1}H-NMR (CD_3OD): 7.90 (br. *s*, NH); 7.74 (*s*, NH); 7.4–7.25 (*m*, 5 arom. H); 5.09 (*s*, PhC*H*$_2$); 3.89–3.87 (*m*, CH_2(Gly)); 1.42, 1.39 (2*s*, 2 Me_2C). ^{13}C-NMR (CD_3OD): 177.6, 176.7, 173.0 (3*s*, 2 CO(amide), COOH); 158.1 (*s*, CO(urethane)); 138.2 (*s*, arom. C); 129.6, 129.24, 129.18 (3*d*, 5 arom. CH); 67.8 (*t*, PhC*H*$_2$); 58.0, 57.8 (2*s*, 2 C(2)(Aib)); 42.0 (*t*, CH_2(Gly)); 25.4 (*q*, 2 *Me*$_2$C). CI-MS: 398 (6), 397 (36, $[M+NH_4]^+$), 381 (19), 380 (100, $[M+1]^+$), 305 (15, $[M-Gly]^+$), 289 (17), 272 (16), 246 (30, $[M-BnOCO]^+$.

5.2.3. *Z-Aib-Aib-OMe* [16]. To a soln. of Z-Aib-Aib-OH [13] (1.070 g, 3.317 mmol) in MeOH (30 ml) was added $BF_3 \cdot Et_2O$ (0.42 ml), and the mixture was stirred for 18 h. The solvent was evaporated, the residue was dissolved in CH_2Cl_2 (150 ml), and the mixture was extracted with aq. 2N HCl (2×), aq. 1N NaOH (2×), and brine, dried ($MgSO_4$), and the solvent was evaporated. Crystallization from AcOEt/hexane yielded Z-Aib-Aib-OMe (1.075 g, 97%). Colorless solid. M.p. 107.4–108.6°. IR (KBr): 3380*m*, 3360*m*, 3320*s*, 3280*s*, 3030*m*, 2980*m*, 2950*m*, 1730*s*, 1715*s*, 1660*s*, 1545*m*, 1535*s*, 1520*s*, 1470*m*, 1465*m*, 1450*m*, 1435*m*, 1385*m*, 1360*m*, 1300*m*, 1255*s*, 1225*m*, 1215*m*, 1190*m*, 1170*m*, 1155*m*, 1085*m*, 1070*s*, 960*m*, 745*m*, 695*m*. ^{1}H-NMR ($CDCl_3$): 7.4–7.3 (*m*, 5 arom. H); 6.90 (br. *s*, 1 NH); 5.31 (br. *s*, 1 NH); 5.09 (*s*, PhC*H*$_2$); 3.72 (*s*, MeO); 1.51, 1.50 (2*s*, 2 Me_2C). ^{13}C-NMR ($CDCl_3$): 175.0, 173.5 (2*s*, CO(amide), COOMe); 155.1 (*s*, CO(urethane)); 136.3 (*s*, arom. C); 128.5, 128.2, 128.1 (3*d*, 5 arom. CH); 66.7 (*t*, PhC*H*$_2$); 57.0, 56.5 (2*s*, 2 C(2)(Aib)); 52.2 (*q*, MeO); 25.4, 24.5 (2*q*, 2 *Me*$_2$C). CI-MS: 338 (17), 337 (100, $[M+1]^+$), 230 (5), 229 (59). Anal. calc. for $C_{17}H_{24}N_2O_5$ (336.39): C 60.70, H 7.19, N 8.33; found: C 60.92, H 7.38, N 8.36.

5.2.4. *H-Aib-Aib-OMe* [16] (**11a**). According to *GP2*, Z-Aib-Aib-OMe (204.8 mg, 0.609 mmol) in MeOH (3 ml) was deprotected (19.6 mg Pd/C; 30 min): 109.5 mg (89%) of **11a** (contaminated with *ca.* 6% of 3,3,6,6-tetramethylpiperazine-2,5-dione [17]). ^{1}H-NMR ((D$_6$)DMSO): 8.03 (*s*, NH); 3.57 (*s*, MeO); 2.45–1.7 (br. signal, NH_2); 1.38, 1.15 (2*s*, 2 Me_2C). ^{13}C-NMR ((D$_6$)DMSO): 177.0, 174.4 (2*s*, CO(amide), COOMe); 54.7, 54.0 (2*s*, 2 C(2)(Aib)); 51.7 (*q*, MeO); 28.3, 24.6 (2*q*, 2 *Me*$_2$C). CI-MS: 405 (23, $[2M+1]^+$), 204 (9), 203 (100, $[M+1]^+$).

5.2.5. *H-Aib-Aib-N(Me)Ph* (**11b**). According to *GP2*, Z-Aib-Aib-N(Me)Ph [13] (613 mg, 1.490 mmol) in MeOH (2 ml) was deprotected (10 mg Pd/C, 30 min): 413 mg (quant.) of **11b**. Colorless oil. ^{1}H-NMR ($CDCl_3$): 7.68 (br. *s*, NH); 7.45–7.25 (*m*, 5 arom. H); 3.27 (*s*, MeN); 1.48 (br. *s*, NH_2); 1.49, 1.25 (2*s*, 2 Me_2C). ^{13}C-NMR ($CDCl_3$): 175.9, 173.4 (2*s*, 2 CO(amide)); 145.2 (*s*, arom. C); 129.3, 128.0, 127.6 (3*d*, 5 arom. CH); 57.4, 54.9 (2*s*, 2 C(2)(Aib)); 41.4 (*q*, MeN); 28.8, 26.6 (2*q*, 2 *Me*$_2$C).

5.2.6. *Z-Aib-Aib-Gly-Aib-Aib-OMe* (**4d**). According to *GP 6*, with **10** (138.8 mg, 0.366 mmol), freshly prepared **11a** (82.3 mg, 0.407 mmol), HBPyU (158.2 mg, 0.367 mmol), and EtN(^{i}Pr)$_2$ (131 mg, 1.014 mmol) in CH_2Cl_2 (3 ml); reaction time 2 h. The precipitate formed during evaporation was

filtered to give 103.2 mg **4d**; the filtrate was evaporated, and CC (AcOEt) gave an additional portion of **4d**. Total yield: 151.9 mg (74%). Colorless solid. M.p. 175.3–176.1°. IR (KBr): 3350*s*, 3320*s*, 3300*s*, 3260*s*, 3040*m*, 2980*m*, 2940*m*, 1760*s*, 1700*s*, 1680*s*, 1665*s*, 1660*s*, 1650*s*, 1540*s*, 1515*s*, 1470*m*, 1465*m*, 1455*m*, 1410*m*, 1385*m*, 1360*m*, 1305*m*, 1265*s*, 1230*m*, 1215*m*, 1195*m*, 1160*m*, 1080*s*, 700*m*. ^{1}H-NMR ($CDCl_3$): 7.74 (*t*-like, NH); 7.33 (br. *s*, 5 arom. H, NH); 7.08, 6.55, 6.05 (3*s*, 3 NH); 5.09 (*s*, PhCH_2); 3.70 (*d*, $J = 5.8$, CH_2(Gly)); 3.66 (*s*, MeO); 1.53, 1.51, 1.48, 1.39 (4*s*, 4 Me_2C). ^{13}C-NMR ($CDCl_3$): 175.5, 175.3, 174.5, 174.3, 169.0 (5*s*, 4 CO(amide), COOMe); 156.5 (*s*, CO(urethane)); 136.0 (*s*, arom. C); 128.7, 128.6, 128.1 (3*d*, 5 arom. CH); 67.5 (*t*, Ph*C*H_2); 57.1, 56.7, 55.8 (3*s*, 4 C(2)(Aib)); 52.1 (*q*, MeO); 44.7 (*t*, CH_2(Gly)); 25.4, 25.13, 25.08, 25.0 (4*q*, 4 *Me*$_2$C). ESI-MS: 586 (100, $[M + Na]^+$). Anal. calc. for $C_{27}H_{41}N_5O_8$ (563.65): C 57.54, H 7.43, N 12.42; found: C 57.54, H 7.20, N 12.21.

Crystals suitable for an X-ray crystal-structure determination were grown from AcOEt/hexane by slow evaporation of the solvent.

5.2.7. *Z-Aib-Aib-Gly-Aib-Aib-N(Me)Ph* (**4e**). According to *GP 6*, with **10** (386.5 mg, 1.019 mmol), **11b** (338.2 mg, 1.219 mmol), HBPyU (442 mg, 1.025 mmol), and EtN(^{i}Pr)$_2$ (309 mg, 2.391 mmol) in CH_2Cl_2 (5 ml); reaction time 16 h. Purification by CC (CH_2Cl_2/MeOH 20:1) gave 529.8 mg (81%) of **4e**. A sample was recrystallized from CH_2Cl_2/hexane. Colorless crystals. M.p. 199.7–201.4°. IR (KBr): 3310*s*, 3250*m*, 2980*m*, 1690*s*, 1680*s*, 1660*s*, 1645*s*, 1595*m*, 1495*m*, 1470*m*, 1455*m*, 1435*m*, 1395*m*, 1385*m*, 1360*m*, 1270*s*, 1215*m*, 1195*m*, 1175*m*, 1090*m*, 1070*m*, 700*m*. ^{1}H-NMR (CD_3OD): 7.45–7.25 (*m*, 10 arom. H); 5.11 (*s*, PhCH_2); 3.62 (*s*, CH_2(Gly)); 3.36 (*s*, MeN); 1.53, 1.51, 1.41, 1.35 (4*s*, 4 Me_2C). ^{13}C-NMR (CD_3OD): 178.5, 177.4, 176.5, 175.6, 171.8 (5*s*, 5 CO(amide)); 158.5 (*s*, CO(urethane)); 147.0, 138.5 (2*s*, 2 arom. C); 130.3, 129.6, 129.2, 128.9, 128.4 (5*d*, 10 arom. CH); 68.0 (*t*, Ph*C*H_2); 58.5, 57.2 (2*s*, 4 C(2)(Aib)); 45.3 (*t*, CH_2(Gly)); 41.1 (*q*, MeN); 26.4, 26.0, 25.4, 25.3 (4*q*, 4 *Me*$_2$C). ESI-MS: 661 (100, $[M + Na]^+$), 532 (6, $[M - N(Me)Ph]^+$).

5.2.8. *Z-Aib-Aib-Gly-Aib-Aib-OH*. According to *GP 4*, **4e** (448.5 mg, 0.702 mmol) was hydrolyzed in 3N HCl (THF/H_2O 1:1; 7 ml): 370.2 mg (96%) of Z-Aib-Aib-Gly-Aib-Aib-OH. Colorless solid. M.p. 215.4–216.8°. IR (KBr): 3300*s*, 3060*m*, 3040*m*, 2990*m*, 2940*m*, 1720*s*, 1695*s*, 1670*s*, 1660*s*, 1550*m*, 1540*s*, 1530*s*, 1525*s*, 1470*m*, 1460*m*, 1455*m*, 1385*m*, 1365*m*, 1270*s*, 1220*m*, 1170*m*, 1090*m*, 1080*m*, 700*m*. ^{1}H-NMR (CD_3OD): 7.45–7.3 (*m*, 5 arom. H); 5.11 (*s*, PhCH_2); 3.64 (*s*, CH_2(Gly)); 1.49, 1.48, 1.41, 1.36 (4*s*, 4 Me_2C). ^{13}C-NMR (CD_3OD): 178.3, 178.0, 177.4, 176.2, 171.8 (5*s*, 4 CO(amide), COOH); 158.5 (*s*, CO(urethane)); 138.5 (*s*, arom. C); 129.6, 129.2, 128.9 (3*d*, 5 arom. CH); 68.0 (*t*, Ph*C*H_2); 58.2, 57.8, 57.7, 57.1 (4*s*, 4 C(2)(Aib)); 45.0 (*t*, CH_2(Gly)); 25.7, 25.4, 25.30, 25.27 (4*q*, 4 *Me*$_2$C). ESI-MS: 572 (100, $[M + Na]^+$).

5.2.9. *H-Aib-Aib-Gly-Aib-Aib-OH* (**6d**). According to *GP 3*, Z-Aib-Aib-Gly-Aib-Aib-OH (281.5 mg, 0.513 mmol) was deprotected in MeOH (9 ml) by treatment with $HCOONH_4$ (163 mg, 2.585 mmol) and Pd/C (280.3 mg): 214.0 mg (quant.) of **6d**. Colorless solid. ^{1}H-NMR (D_2O): 3.85 (*s*, CH_2(Gly)); 1.64, 1.51, 1.49, 1.43 (4*s*, 4 Me_2C). ^{13}C-NMR (CD_3OD): 184.7, 179.9, 177.8, 175.2, 173.2 (5*s*, 4 CO(amide), COOH); 60.8, 60.0, 59.3 (3*s*, 4 C(2)(Aib)); 46.0 (*t*, CH_2(Gly)); 27.1, 26.9, 26.7, 25.9 (4*q*, 4 *Me*$_2$C). ESI-MS: 416 (100, $[M + 1]^+$).

5.2.10. *Cyclo*(*Gly-Aib-Aib-Aib-Aib)* (**7c**). According to *GP 5*, to a soln. of **6d** (27.2 mg, 0.066 mmol) in DMF (45 ml) were added DEPC (34.0 mg, 0.21 mmol) and EtN(^{i}Pr)$_2$ (0.4 ml) at r.t. After stirring for 63 h, DMF was evaporated, and the residue was purified by CC (CH_2Cl_2/MeOH 20:1 → 10:1): 2.8 mg (11%) of **7c**.

6. *Synthesis of Cyclo(Aib-Aib-Aib-Aib-Aib)* (**7d**). 6.1. *Z-Aib-Aib-Aib-Aib-Aib-OH*. According to *GP 4*, *Z-Aib-Aib-Aib-Aib-Aib-N(Me)Ph* (**4f** [13]; 881.1 mg, 1.321 mmol) was hydrolyzed at 60° for 1 h. The precipitate was filtered and washed with Et_2O to give 726.8 mg of Z-Aib-Aib-Aib-Aib-Aib-OH. Extraction of the aq. phase with Et_2O (4 ×) gave additional 34.0 mg of the product. Total yield: 760.8 mg (quant.). Colorless solid. M.p. 226.2–227.3°. IR (KBr): 3410*s*, 3320*s*, 3290*s*, 3250*s*, 3040*s*, 2980*s*, 2940*s*, 2920*m*, 2900*m*, 1745*s*, 1730*s*, 1710*s*, 1705*s*, 1695*s*, 1680*s*, 1675*s*, 1665*s*, 1645*s*, 1635*s*, 1580*m*, 1565*m*, 1550*s*, 1530*s*, 1515*s*, 1465*s*, 1455*s*, 1445*s*, 1385*s*, 1365*s*, 1320*s*, 1315*s*, 1280*s*, 1270*s*, 1265*s*, 1225*s*, 1210*s*, 1185*s*, 1180*s*, 1170*s*, 1165*s*, 1090*s*, 1080*s*, 955*m*, 945*m*, 750*m*, 700*m*. ^{1}H-NMR ((D$_6$)DMSO): 11.83 (br. *s*, COOH); 8.23, 7.85, 7.46 (3*s*, 3 NH); 7.4–7.3 (*m*, 5 arom. H, 1 NH); 7.28 (*s*, NH); 5.09 (*s*, PhCH_2); 1.33, 1.32, 1.21 (3*s*, 3:1:1, 5 Me_2C). ^{13}C-NMR ((D$_6$)DMSO): 175.5, 174.9, 174.7, 173.42, 173.38 (5*s*, 4 CO(amide), COOH); 155.7 (*s*, CO(urethane)); 137.1 (*s*, arom. C); 128.3, 127.6, 127.1 (3*d*, 5 arom. CH); 65.4 (*t*, Ph*C*H_2); 55.9,

55.81, 55.76, 55.6, 54.5 (5*s*, 5 C(2)(Aib)); 25.0, 24.6, 24.5, 24.3 (4*q*, 5 *Me*$_2$C). ESI-MS: 660 (100, $[M+Na]^+$), 578 (80, $[M+1]^+$), 560 (8, $[M-OH]^+$), 475 (19, $[M-$Aib-OH$]^+$). Anal. calc. for $C_{28}H_{43}N_5O_8$ (577.68): C 58.22, H 7.50, N 12.12; found: C 58.36, H 7.30, N 11.93.

6.2. *H-Aib-Aib-Aib-Aib-Aib-OH* (**6f**). According to *GP 3*, Z-Aib-Aib-Aib-Aib-Aib-OH (233.2 mg, 0.404 mmol) in MeOH (7 ml) was deprotected by treatment with $HCOONH_4$ (129.5 mg, 2.054 mmol) and Pd/C (240 mg). The crude material was dissolved in MeOH and precipitated with Et_2O to give 169.8 mg (95%) of **6f**. Colorless powder. ^{1}H-NMR (D_2O): 7.54, 7.52 (2*s*, 2 NH); 1.65, 1.48, 1.43, 1.42 (4*s*, 1:2:1:1, 5 Me_2C). ^{13}C-NMR (D_2O): 178.9, 178.6, 178.4, 175.3 (4*s*, 4 CO(amide), COOH); 60.1, 59.9, 59.7, 59.6 (4*s*, 5 C(2)(Aib)); 27.2, 27.1, 26.9, 26.5, 25.9 (5*q*, 5 *Me*$_2$C). ESI-MS: 466 (7, $[M+Na]^+$), 444 (100, $[M+1]^+$).

6.3. *Cyclo(Aib-Aib-Aib-Aib-Aib)* (**7d**). To a soln. of **6f** (171.3 mg, 0.386 mmol) in DMF (260 ml) were added DEPC (294.8 mg, 1.807 mmol) and EtN(^{i}Pr)$_2$ (2.6 ml) at r.t. After stirring at r.t. for 2 weeks, DMF was removed in high vacuum, and subsequent CC (CH_2Cl_2/MeOH 15:1, 3 ×) yielded 30.7 mg of a mixture of **7d** and an unknown compound. Additional CC (AcOEt/MeOH 20:1, 2 ×) gave 19.7 mg (12%) **7d**. Colorless powder. M.p. $>260°$. IR (KBr): 3420*m*, 3320*s*, 3120*m*, 2990*m*, 2980*m*, 2940*m*, 1690*s*, 1670*s*, 1650*s*, 1570*s*, 1520*s*, 1480*m*, 1450*m*, 1385*s*, 1360*s*, 1310*m*, 1280*s*, 1220*s*, 1210*s*, 1185*m*, 1170*m*. ^{1}H-NMR ($CDCl_3$): 6.63 (*s*, 5 NH); 1.54 (*s*, 5 Me_2C). ^{13}C-NMR ($CDCl_3$): 175.2 (*s*, 5 CO(lactam)); 58.0 (*s*, 5 C(2)(Aib)); 25.0 (*q*, 5 *Me*$_2$C). ESI-MS: 448 (100, $[M+Na]^+$).

7. *X-Ray Crystal-Structure Determination of* **4d** (see *Table 3* and *Fig. 2*)[3]). The measurements were conducted on a *Rigaku AFC5R* diffractometer using graphite-monochromated MoK_α radiation (λ 0.71073 Å) and a 12-kW rotating anode generator. The intensities were corrected for *Lorentz* and polarization effects. Azimuthal scans of several reflections indicated no need for an absorption correction. Equivalent reflections were merged. The data collection and refinement parameters are given in *Table 3*, and a view of the molecule is shown in *Fig. 2*. The structure was solved by direct methods using SHELXS86 [18], which revealed the positions of all non-H-atoms. The non-H-atoms were refined anisotropically. The amide H-atoms were placed in the positions indicated by a difference electron-

Table 3. *Crystallographic Data for Compound* **4d**

Crystallized from		AcOEt/hexane	D_x [g cm^{-3}]	1.215
Empirical formula		$C_{27}H_{41}N_5O_8$	μ(MoK_α) [mm^{-1}]	0.090
Formula weight		563.65	Scan type	$\omega/2\theta$
Crystal color, habit		colorless, needle	$2\theta_{(max)}$ [°]	55
Crystal dimensions [mm]		0.20 × 0.25 × 0.45	Total reflections measured	7404
Temp. [K]		173(1)	Symmetry-independent reflections	7068
Crystal system		monoclinic	Reflections with $I>2\sigma(I)$	3881
Space group		$P2_1/n$	Reflections used in refinement	7068
Z		4	Parameters refined	391
Reflections for cell determination		25	Final $R(F)$ [$I>2\sigma(I)$ reflections]	0.0530
2θ Range for cell determination [°]		20–37	$wR(F^2)$ (all data)	0.1412
Unit cell parameters	a [Å]	11.674(2)	Weighting parameter (a)[a])	0.0513
	b [Å]	16.174(2)	Goodness-of-fit	1.003
	c [Å]	16.774(2)	Secondary extinction coefficient	0.0018(5)
	β [°]	103.29(1)	Final Δ_{max}/σ	0.000
V [Å^3]		3082.1(8)	$\Delta\rho$ (max; min) [e Å^{-3}]	0.25; −0.23

[a]) $w^{-1}=\sigma^2(F_o^2)+(aP)^2$, where $P=(F_o^2+2F_c^2)/3$

[3]) CCDC-1034190 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from *The Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

density map, and their positions were allowed to refine together with individual isotropic displacement parameters. All remaining H-atoms were placed in geometrically calculated positions and refined by using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 U_{eq} of its parent C-atom (1.5 U_{eq} for the Me groups). The refinement of the structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function $\Sigma w(F_o^2 - F_c^2)^2$. A correction for secondary extinction was applied. Neutral atom scattering factors for non-H-atoms were taken from [19], and the scattering factors for H-atoms were taken from [20]. Anomalous dispersion effects were included in F_c [21]; the values for f' and f'' were those of [22]. The values of the mass attenuation coefficients are those of [23]. The SHELXL-2014 program [24] was used for all calculations.

REFERENCES

[1] F. S. Arnhold, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2015**, *98*, in press; doi: 10.1002/hlca.201400323.

[2] T. Degenkolb, W. Gams, H. Brückner, *Chem. Biodiversity* **2008**, *5*, 693.

[3] J. Pastuszak, J. H. Gardner, J. Singh, D. H. Rich, *J. Org. Chem.* **1982**, *47*, 2982; T. Suga, S. Osada, H. Kodama, 'Contribution of Hydrophobic Side-chain in Cyclic Tetrapeptide Forming Ion Channel', in 'Peptide Science Symposium', 47th Japanese peptide symposium, Japanese Peptide Society, 2010, p. 130; X.-H. Li, Y.-D. Wei, S.-M. Wang, M.-N. Wang, D.-W. Huang, Z.-L. Xiu, *Chem. Res. Chin. Univ.* **2012**, *28*, 1011.

[4] F. Rossi, M. Saviano, P. Di Talia, B. Di Blasio, C. Pedone, G. Zanotti, M. Mosca, G. Saviano, T. Tancredi, K. Ziegler, E. Benedetti, *Pept. Sci.* **1996**, *40*, 465; G. Zanotti, M. Saviano, G. Saviano, T. Tancredi, F. Rossi, C. Pedone, E. Benedetti, *J. Pept. Res.* **1998**, *51*, 460.

[5] E. Escudero, X. Vidal, X. Solans, E. Peggion, J. A. Subirana, *J. Pept. Sci.* **1996**, *2*, 59; J. Wang, S. Osada, H. Kodama, M. Kondo, *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1221; Y. Demizu, S. Nagoya, M. Doi, Y. Sato, M. Tanaka, M. Kurihara, *J. Org. Chem.* **2012**, *77*, 9361.

[6] C. Cabrele, M. Langer, A. G. Beck-Sickinger, *J. Org. Chem.* **1999**, *64*, 4353.

[7] H. Heimgartner, *Israel J. Chem.* **1981**, *21*, 151; H. Heimgartner, *Israel J. Chem.* **1986**, *27*, 3; H. Heimgartner, in 'Amino Acids: Chemistry, Biology and Medicine', Eds. G. Lubec, G. A. Rosenthal, ESCOM, Leiden 1990, p. 29; H. Heimgartner, *Angew. Chem., Int. Ed.* **1991**, *30*, 238.

[8] D. Obrecht, H. Heimgartner, *Helv. Chim. Acta* **1981**, *64*, 482; P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1986**, *69*, 1153; D. Obrecht, H. Heimgartner, *Helv. Chim. Acta* **1987**, *70*, 102; P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1987**, *70*, 354; P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1988**, *71*, 140; f) P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1990**, *73*, 13.

[9] I. Dannecker-Dörig, A. Linden, H. Heimgartner, *Collect. Czech. Chem. Commun.* **2009**, *74*, 901; T. Jeremic, A. Linden, H. Heimgartner, *Chem. Biodiversity* **2004**, *1*, 1730; T. Jeremic, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2004**, *87*, 3056; T. Jeremic, A. Linden, K. Moehle, H. Heimgartner, *Tetrahedron* **2005**, *61*, 1871; T. Jeremic, A. Linden, H. Heimgartner, *J. Pept. Sci.* **2008**, *14*, 1051.

[10] B. V. V. Prasad, P. Balaram, E. Benedetti, *Crit. Rev. Biochem. Mol. Biol.* **1984**, *16*, 307; C. Toniolo, E. Benedetti, *ISI Atlas Sci.: Biochem.* **1988**, *1*, 225; C. Toniolo, E. Benedetti, *Macromolecules* **1991**, *24*, 4004; C. Toniolo, *Biopolymers* **1989**, *28*, 247.

[11] I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747; E. Benedetti, M. Saviano, R. Iacovino, C. Pedone, A. Santini, M. Crisma, F. Formaggio, C. Toniolo, Q. B. Broxterman, J. Kamphuis, *Biopolymers* **1998**, *46*, 433.

[12] F. S. Arnhold, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2014**, *97*, 619.

[13] I. Dannecker-Dörig, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2011**, *94*, 993; I. Dannecker-Dörig, Ph. D. thesis, University of Zürich, 1995.

[14] C. K. Johnson, ORTEP II, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1976.

[15] J. Bernstein, R. E. Davis, L. Shimoni, N.-L. Chang, *Angew. Chem., Int. Ed.* **1995**, *34*, 1555.

[16] M. T. Leplawy, D. S. Jones, G. W. Kenner, R. C. Sheppard, *Tetrahedron* **1960**, *11*, 39.

[17] R. Nagaraj, P. Balaram, *Heterocycles* **1977**, *7*, 885.
[18] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **1990**, *46*, 467.
[19] E. N. Maslen, A. G. Fox, M. A. O'Keefe, 'International Tables for Crystallography', Ed. A. J. C. Wilson, Kluwer Academic Publishers, Dordrecht, 1992, Vol. C, Table 6.1.1.1, p. 477.
[20] R. F. Stewart, E. R. Davidson, W. T. Simpson, *J. Chem. Phys.* **1965**, *42*, 3175.
[21] J. A. Ibers, W. C. Hamilton, *Acta Crystallogr.* **1964**, *17*, 781.
[22] D. C. Creagh, W. J. McAuley, 'International Tables for Crystallography', Ed. A. J. C. Wilson, Kluwer Academic Publishers, Dordrecht, 1992, Vol. C, Table 4.2.6.8, p. 219.
[23] D. C. Creagh, J. H. Hubbell, 'International Tables for Crystallography', Ed. A. J. C. Wilson, Kluwer Academic Publishers, Dordrecht, 1992, Vol. C, Table 4.2.4.3, p. 200.
[24] G. M. Sheldrick, *Acta Crystallogr., Sect. C* **2015**, *71*, 3.

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