

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(ⁱPr)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 CDCl₃ 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].

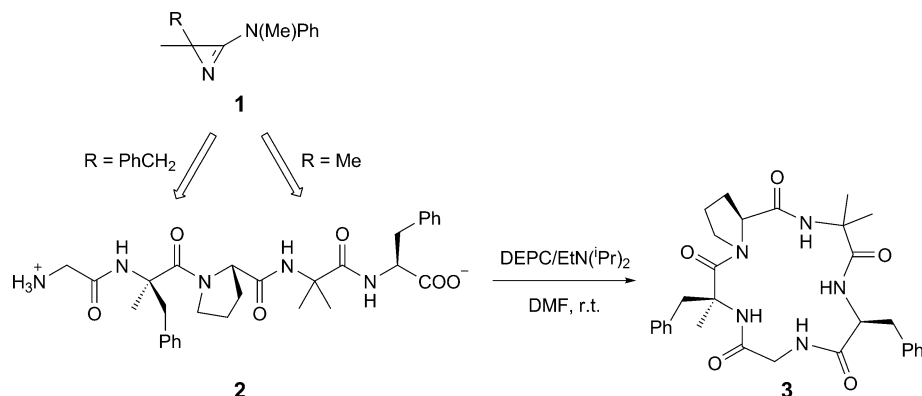
In our recent publication [1], we described the cyclization of several pentapeptides with 2-methylphenylalanine (Phe(2 Me)), and one or two Aib units, *e.g.* **2**, to give the corresponding cyclopentapeptides, *e.g.*, **3** (Scheme 1).

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 · H₂O in THF/H₂O/MeOH gave the peptide acid Z-Gly-Aib-Acb-Aib-Gly-OH (**5a**; 96%), and subsequent hydrogenolysis, either

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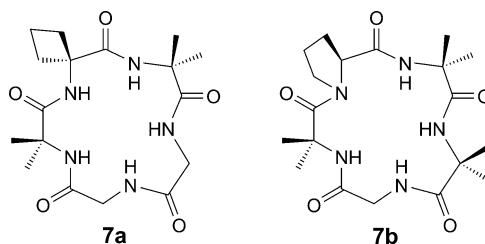
Scheme 1



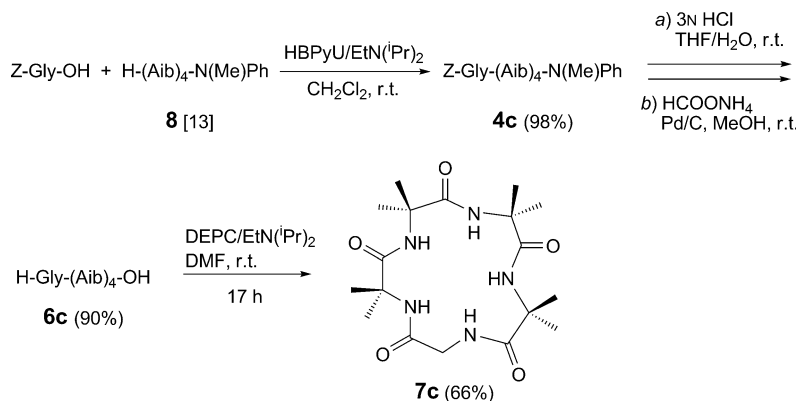
with $\text{H}_2/\text{Pd/C}$ in MeOH or with $\text{HCOONH}_4/\text{Pd/C}$ in MeOH, led to H-Gly-Aib-Aib-Gly-OH (**6a**) in quantitative and 95% yield, respectively. Selective hydrolysis of **4b** with 3N HCl in THF/ H_2O at room temperature yielded Z-Gly-Aib-Pro-Aib-Aib-OH (**5b**, 70%) [12]. The latter was deprotected at the N-terminus to furnish H-Gly-Aib-Pro-Aib-Aib-OH (**6b**) quantitatively ($\text{H}_2/\text{Pd/C}$) and in 67% yield ($\text{HCOONH}_4/\text{Pd/C}$), respectively.

The cyclization of the two pentapeptides was achieved smoothly by treatment with diethyl phosphorocyanidate (DEPC)/ $\text{EtN}(\text{iPr})_2$ (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 ($[M + \text{Na}]^+$) besides the $[M + 1]^+$ peak at m/z 382. The ^1H -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 (2s, 2 NH), and only two *singlets* 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.

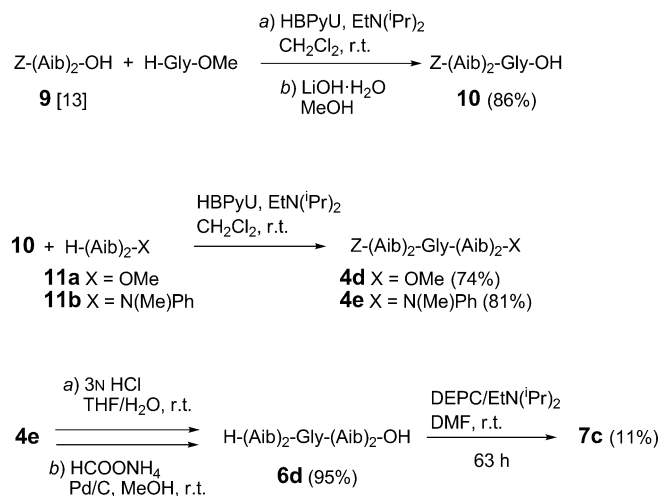
For the attempted synthesis of cyclo(Gly-Aib-Aib-Aib-Aib) (**7c**), two linear pentapeptide precursors were prepared as outlined in Schemes 2 and 3. Coupling of Z-Gly-OH with the known tetrapeptide amide **8** [13] using *O*-(benzotriazol-1-yl)-*N,N,N',N'*-bis(tetramethylen)uronium hexafluorophosphate (HBTU)/ $\text{EtN}(\text{iPr})_2$ 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(iPr)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 · H₂O 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/BF₃ · Et₂O, followed by hydrogenolysis with H₂/Pd/C at room temperature, in 86% yield. The product was contaminated with ca. 6% of 3,3,6,6-tetramethylpiperazine-2,5-dione.

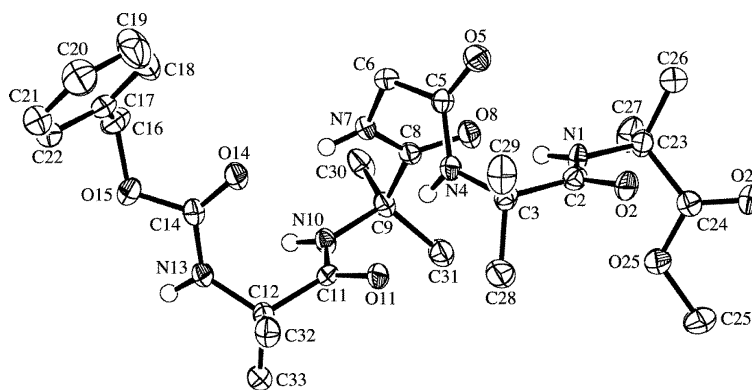


Fig. 2. ORTEP Plot [14] of the molecular structure of the pentapeptide **4d** (50% probability ellipsoids, arbitrary atom numbering, H-atoms bonded to C-atoms omitted for clarity)

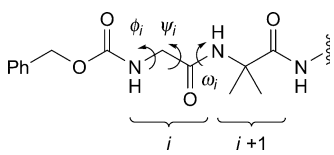
cleave the ester group by treatment with $\text{LiOH} \cdot \text{H}_2\text{O}$ to obtain the pentapeptide acid failed. Therefore, the pentapeptide amide **4e** was prepared by condensation of **10** with $\text{H}(\text{Aib})_2\text{-N}(\text{Me})\text{Ph}$ (**11b**), which was obtained from $\text{Z}(\text{Aib})_2\text{-N}(\text{Me})\text{Ph}$ [13] by treatment with $\text{H}_2/\text{Pd/C}$ 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** \rightarrow **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 $[M+\text{Na}]^+$ (m/z 420) and $[M+\text{K}]^+$ (m/z 436). The $^1\text{H-NMR}$ spectrum in CDCl_3 showed a signal for the lactam NH of Gly at 7.31 ppm (*t*-like), and four *singlets* 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 $^{13}\text{C-NMR}$ 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 *singlets* in the $^1\text{H-NMR}$ spectrum (1.56, 1.55, 1.53, and 1.51 ppm) and gave rise to three signals in the $^{13}\text{C-NMR}$ spectrum (25.5, 24.9, 24.8 ppm (*ca.* 1 : 2 : 1)). In addition, four *singlets* for C(2) of four Aib and a *triplet* for CH_2 of Gly were detected in the $^{13}\text{C-NMR}$ 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)

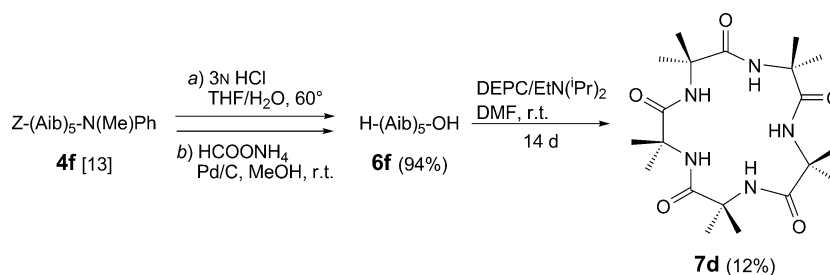
| Donor... Acceptor | N...O [Å] | H...O [Å] | N–H...O [°] |
|-------------------|-----------|-----------|-------------|
| N(1)–H...O(8) | 2.952(3) | 2.10(3) | 167(2) |
| N(4)–H...O(11) | 3.053(3) | 2.21(3) | 169(2) |
| N(7)–H...O(14) | 2.879(3) | 2.05(3) | 158(2) |
| N(10)–H...O(24') | 3.053(3) | 2.21(3) | 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/H₂O, 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(ⁱPr)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 CH₂Cl₂ or in MeCN at reflux, only traces of the desired cyclo[(Aib)₅] (**7d**) could be detected (MS). Finally, after treatment with DEPC/EtN(ⁱPr)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 ^1H -NMR spectrum in CDCl_3 showed only two *singlets* 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_2C 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-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.

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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'*-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. ^1H - and ^{13}C -NMR spectra: *Bruker AC-300*, *Bruker ARX-300*, or *Bruker AMX-600* spectrometer (at 300 or 600 (^1H) 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/H₂O (3:1:1) at 0° was added LiOH·H₂O (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, and 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 H₂ (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 HCOONH₄ (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/H₂O 1:1) was stirred at r.t. for 1–4.5 h. Then, 2N HCl was added, and the mixture was extracted with Et₂O. The org. phase was dried (Na₂SO₄), 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(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 CH₂Cl₂ (1 ml) at r.t. was added EtN(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. KHSO₄ (5%, 3 ×), aq. NaHCO₃ (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·H₂O (43 mg, 1.025 mmol) in THF/H₂O/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): 3300s (br), 3060m, 2990m, 2940m, 1755s, 1750s, 1740s, 1730s, 1715s, 1705s, 1695s, 1670s, 1660s, 1650s, 1645s, 1555s, 1540s, 1535s, 1525s, 1505m, 1470m, 1465m, 1455m, 1430m, 1415m, 1390m, 1365m, 1340m, 1310m, 1265m, 1240m, 1185m, 1050m, 1015m, 740m, 695m. ¹H-NMR ((D₆)DMSO): 8.41, 8.10 (2s, 2 NH); 7.53 (t-like, NH); 7.35–7.3 (m, 5 arom. H, NH); 7.25 (s, NH); 5.02 (s, PhCH₂); 3.7–3.6 (m, 2 CH₂(Gly)); 2.55–2.4, 2.1–2.0, 1.9–1.75 (3m, 3 CH₂(Acb)); 1.36, 1.34 (2s, 2 Me₂C). ¹³C-NMR ((D₆)DMSO): 174.7, 174.5, 172.5, 170.9, 169.8 (5s, 4 CO(amide), COOH); 156.7 (s, CO(urethane)); 136.8 (s, 1 arom. C); 128.3, 127.7, 127.5 (3d, 5 arom. CH); 65.5 (t, PhCH₂); 58.6, 55.9, 55.6 (3s, 2 C(2)(Aib), C(2)(Acb)); 43.7, 40.8 (2t, 2 CH₂(Gly)); 30.4 (t, 2 CH₂(Acb)); 25.0, 24.7 (2q, 2 Me₂C); 15.0 (t, CH₂(Acb)). ESI-MS: 556 (100, [M + Na]⁺), 459 (3, [M – Gly]⁺), 374 (10, [M – Aib-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 H₂O (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°. ¹H-NMR (D₂O): 3.83, 3.75 (2s, 2 CH₂(Gly)); 2.65–2.5, 2.25–2.1, 2.05–1.9 (3m, 3 CH₂(Acb)); 1.52, 1.50 (2s, 2 Me₂C). ¹³C-NMR (D₂O): 179.7, 179.0, 177.9, 169.4 (4s, 4 CO(amide), COOH); 62.1, 59.5, 59.4 (3s, 2 C(2)(Aib), C(2)(Acb)); 46.3, 43.3 (2t, 2 CH₂(Gly)); 33.3 (t, 2 CH₂(Acb)); 27.1, 26.7 (2q, 2 Me₂C); 17.7 (t, CH₂(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(Pr)₂ (0.45 ml) according to GP 5. After CC (CH₂Cl₂/MeOH/NH₃ 10:1:0.1) and crystallization from CHCl₃/hexane, 14.5 mg (56%) of **7a** were obtained. Colorless solid. M.p. 135.3–136.4°. ¹H-NMR (CDCl₃): 7.21 (s, 2 NH); 7.08 (t-like, NH); 6.57, 6.23 (2s, 2 NH); 3.99 (d, J = 6.3, CH₂(Gly)); 3.85 (d, J = 6.4, CH₂(Gly)); 2.6–2.45, 2.4–2.3, 2.15–2.0, 2.0–1.8 (4m, 2:2:1:1, 3 CH₂(Acb)); 1.58, 1.55 (2s, 2 Me₂C). ¹³C-NMR (CDCl₃): 174.9, 174.8, 174.0, 171.8, 171.4 (5s, 5 CO(amide)); 60.1, 57.7, 57.4 (3s, 2 C(2)(Aib), C(2)(Acb)); 44.7, 44.0 (2t, 2 CH₂(Gly)); 31.1 (t, 2 CH₂(Acb)); 25.2, 24.7 (2q, 2 Me₂C); 15.7 (t, CH₂(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 HCOONH₄ (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): 3320m, 3050m, 2980m, 2930m, 2870m, 1670s, 1620s, 1550s, 1470m, 1450m, 1415m, 1390m, 1360m, 1280m, 1210m, 1190m, 1170m. ¹H-NMR (D₂O): 4.33 (t-like, CH(2)(Pro)); 3.89 (s, CH₂(Gly)); 3.8–3.65, 3.6–3.5 (2m, CH₂(5)(Pro)); 2.3–2.15, 2.1–1.8 (2m, 1:3, CH₂(3), CH₂(4)(Pro)); 1.51, 1.48, 1.43, 1.42 (4s, 1:3:1:1, 3 Me₂C). ¹³C-NMR (D₂O): 184.5, 178.2, 176.7, 176.4, 168.5 (5s,

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*, CH₂(5)(Pro)); 42.8 (*t*, CH₂(Gly)); 31.0, 28.3 (2*t*, CH₂(3), CH₂(4)(Pro)); 27.2, 27.04, 26.97, 26.4 (4*q*, 3 Me₂C). 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(ⁱPr)₂ (0.75 ml) according to GP 5. After CC (CH₂Cl₂/MeOH 10:1) and crystallization from AcOEt/hexane, 41.2 mg (89%) of **7b** were obtained. Colorless solid. M.p. 139.1–140.5°. [α]_D²⁰ = –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*. ¹H-NMR ((D₆)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 CH₂(Gly)); 3.55–3.3 (*m*, CH₂(5)(Pro), 1 H of CH₂(Gly)); 2.0–1.75 (*m*, CH₂(3), CH₂(4)(Pro)); 1.49, 1.44, 1.40, 1.31 (4*s*, 1:2:2:1, 3 Me₂C). 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 CH₂Cl₂ (4 ml) at r.t. was added EtN(ⁱPr)₂ (119.7 mg, 0.926 mmol). After stirring for 21 h, the precipitate was filtered and washed with a little CH₂Cl₂ to give **4c** (243.7 mg) as a white powder. The filtrate was evaporated, and CC (CH₂Cl₂/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*. ¹H-NMR (CD₃OD): 7.4–7.2 (*m*, 10 arom. H); 5.10 (*s*, PhCH₂); 3.71 (*s*, CH₂(Gly)); 3.38 (*s*, MeN); 1.55, 1.48, 1.41, 1.38 (4*s*, 4 Me₂C). ¹³C-NMR (CD₃OD): 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*, PhCH₂); 58.4, 58.2, 57.9, 57.6 (4*s*, 4 C(2)(Aib)); 45.2 (*t*, CH₂(Gly)); 41.1 (*q*, MeN); 26.3, 26.0, 25.34, 25.27 (4*q*, 4 Me₂C). 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 3*N* HCl (THF/H₂O 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*. ¹H-NMR (CD₃OD): 7.7–7.6 (*m*, 2 arom. H); 7.4–7.3 (*m*, 3 arom. H); 5.10 (*s*, PhCH₂); 3.72 (*s*, CH₂(Gly)); 1.49, 1.42, 1.41, 1.39 (4*s*, 4 Me₂C). ¹³C-NMR (CD₃OD): 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*, PhCH₂); 57.9, 57.7, 57.1 (3*s*, 4 C(2)(Aib)); 45.3 (*t*, CH₂(Gly)); 25.7, 25.41, 25.36, 25.3 (4*q*, 4 Me₂C). 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 C₂₄H₃₆N₄O₇ · 0.5 H₂O (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 HCOONH₄ (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. ¹H-NMR (D₂O): 3.84 (*s*, CH₂(Gly)); 1.48, 1.47, 1.43, 1.42 (4*s*, 4 Me₂C). ¹³C-NMR (D₂O): 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*, CH₂(Gly)); 27.2, 27.1, 26.9, 26.8 (4*q*, 4 Me₂C). 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(ⁱPr)₂ (0.6 ml) at r.t. After stirring for 17 h, DMF was evaporated, and the residue was purified by CC (CH₂Cl₂/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*. ¹H-NMR (CDCl₃): 7.31 (*t*-like, NH); 7.05, 6.65, 6.29, 6.25 (4*s*, 4 NH); 3.39 (*d*, *J* = 6.1, CH₂(Gly)); 1.56, 1.55, 1.53, 1.51 (4*s*,

4 Me₂C). ¹³C-NMR (CDCl₃): 176.5, 174.7, 174.1, 173.9, 172.4 (5s, 5 CO(amide)); 59.1, 57.6, 57.4, 56.9 (4s, 4 C(2)(Aib)); 45.1 (t, CH₂(Gly)); 25.5, 24.9, 24.8 (3q, 4 Me₂C). 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(ⁱPr)₂ (1.3 ml, 7.594 mmol) in CH₂Cl₂ (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): 3350s, 3280s, 3040m, 2980m, 2950m, 1755m, 1700s, 1670s, 1660s, 1535s, 1520s, 1455m, 1435m, 1410m, 1385m, 1370m, 1265m, 1215s, 1210s, 1195s, 1175s, 1090m, 1080s, 985m, 965m, 755m, 700m. ¹H-NMR (CDCl₃): 7.42 (br. s, NH); 7.4–7.3 (m, 5 arom. H); 6.46, 5.32 (2s, 2 NH); 5.10 (s, PhCH₂); 3.99 (d, *J* = 5.6, CH₂(Gly)); 3.72 (s, MeO); 1.48 (s, 2 Me₂C). ¹³C-NMR (CDCl₃): 174.6, 173.1, 170.5 (3s, 2 CO(amide), COOMe); 155.7 (s, CO(urethane)); 136.0 (s, arom. C); 128.7, 128.5, 128.4 (3d, 5 arom. CH); 67.1 (t, PhCH₂); 57.3, 57.1 (2s, 2 C(2)(Aib)); 51.9 (q, MeO); 41.4 (t, CH₂(Gly)); 25.43, 25.38 (2q, 2 Me₂C). CI-MS: 412 (6), 411 (30, [M + NH₄]⁺), 395 (21), 394 (100, [M + 1]⁺), 331 (14), 306 (22), 305 (8). Anal. calc. for C₁₉H₂₇N₃O₆ (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·H₂O (243.0 mg, 5.791 mmol) in THF/MeOH/H₂O 3:1:1 (7 ml): 809 mg (93%) of **10**. Colorless solid. IR (KBr): 3320s, 3300s, 3085m, 3030m, 3000m, 2940m, 2910m, 2900m, 1765m, 1750m, 1702s, 1665s, 1620s, 1615s, 1565s, 1515s, 1470m, 1450m, 1410m, 1385m, 1370m, 1335m, 1265s, 1220m, 1175s, 1085s, 900m, 740m, 695m. ¹H-NMR (CD₃OD): 7.90 (br. s, NH); 7.74 (s, NH); 7.4–7.25 (m, 5 arom. H); 5.09 (s, PhCH₂); 3.89–3.87 (m, CH₂(Gly)); 1.42, 1.39 (2s, 2 Me₂C). ¹³C-NMR (CD₃OD): 177.6, 176.7, 173.0 (3s, 2 CO(amide), COOH); 158.1 (s, CO(urethane)); 138.2 (s, arom. C); 129.6, 129.24, 129.18 (3d, 5 arom. CH); 67.8 (t, PhCH₂); 58.0, 57.8 (2s, 2 C(2)(Aib)); 42.0 (t, CH₂(Gly)); 25.4 (q, 2 Me₂C). CI-MS: 398 (6), 397 (36, [M + NH₄]⁺), 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 BF₃·Et₂O (0.42 ml), and the mixture was stirred for 18 h. The solvent was evaporated, the residue was dissolved in CH₂Cl₂ (150 ml), and the mixture was extracted with aq. 2N HCl (2 ×), aq. 1N NaOH (2 ×), and brine, dried (MgSO₄), 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): 3380m, 3360m, 3320s, 3280s, 3030m, 2980m, 2950m, 1730s, 1715s, 1660s, 1545m, 1535s, 1520s, 1470m, 1465m, 1450m, 1435m, 1385m, 1360m, 1300m, 1255s, 1225m, 1215m, 1190m, 1170m, 1155m, 1085m, 1070s, 960m, 745m, 695m. ¹H-NMR (CDCl₃): 7.4–7.3 (m, 5 arom. H); 6.90 (br. s, 1 NH); 5.31 (br. s, 1 NH); 5.09 (s, PhCH₂); 3.72 (s, MeO); 1.51, 1.50 (2s, 2 Me₂C). ¹³C-NMR (CDCl₃): 175.0, 173.5 (2s, CO(amide), COOMe); 155.1 (s, CO(urethane)); 136.3 (s, arom. C); 128.5, 128.2, 128.1 (3d, 5 arom. CH); 66.7 (t, PhCH₂); 57.0, 56.5 (2s, 2 C(2)(Aib)); 52.2 (q, MeO); 25.4, 24.5 (2q, 2 Me₂C). CI-MS: 338 (17), 337 (100, [M + 1]⁺), 230 (5), 229 (59). Anal. calc. for C₁₇H₂₄N₂O₅ (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]). ¹H-NMR ((D₆)DMSO): 8.03 (s, NH); 3.57 (s, MeO); 2.45–1.7 (br. signal, NH₂); 1.38, 1.15 (2s, 2 Me₂C). ¹³C-NMR ((D₆)DMSO): 177.0, 174.4 (2s, CO(amide), COOMe); 54.7, 54.0 (2s, 2 C(2)(Aib)); 51.7 (q, MeO); 28.3, 24.6 (2q, 2 Me₂C). CI-MS: 405 (23, [2 M + 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. ¹H-NMR (CDCl₃): 7.68 (br. s, NH); 7.45–7.25 (m, 5 arom. H); 3.27 (s, MeN); 1.48 (br. s, NH₂); 1.49, 1.25 (2s, 2 Me₂C). ¹³C-NMR (CDCl₃): 175.9, 173.4 (2s, 2 CO(amide)); 145.2 (s, arom. C); 129.3, 128.0, 127.6 (3d, 5 arom. CH); 57.4, 54.9 (2s, 2 C(2)(Aib)); 41.4 (q, MeN); 28.8, 26.6 (2q, 2 Me₂C).

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(ⁱPr)₂ (131 mg, 1.014 mmol) in CH₂Cl₂ (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): 3350s, 3320s, 3300s, 3260s, 3040m, 2980m, 2940m, 1760s, 1700s, 1680s, 1665s, 1660s, 1650s, 1540s, 1515s, 1470m, 1465m, 1455m, 1410m, 1385m, 1360m, 1305m, 1265s, 1230m, 1215m, 1195m, 1160m, 1080s, 700m. ¹H-NMR (CDCl₃): 7.74 (*t*-like, NH); 7.33 (br. *s*, 5 arom. H, NH); 7.08, 6.55, 6.05 (3s, 3 NH); 5.09 (*s*, PhCH₂); 3.70 (*d*, *J* = 5.8, CH₂(Gly)); 3.66 (*s*, MeO); 1.53, 1.51, 1.48, 1.39 (4s, 4 Me₂C). ¹³C-NMR (CDCl₃): 175.5, 175.3, 174.5, 174.3, 169.0 (5s, 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*, PhCH₂); 57.1, 56.7, 55.8 (3s, 4 C(2)(Aib)); 52.1 (*q*, MeO); 44.7 (*t*, CH₂(Gly)); 25.4, 25.13, 25.08, 25.0 (4*q*, 4 Me₂C). ESI-MS: 586 (100, [M + Na]⁺). Anal. calc. for C₂₇H₄₁N₅O₈ (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 CH₂Cl₂ (5 ml); reaction time 16 h. Purification by CC (CH₂Cl₂/MeOH 20 : 1) gave 529.8 mg (81%) of **4e**. A sample was recrystallized from CH₂Cl₂/hexane. Colorless crystals. M.p. 199.7–201.4°. IR (KBr): 3310s, 3250m, 2980m, 1690s, 1680s, 1660s, 1645s, 1595m, 1495m, 1470m, 1455m, 1435m, 1395m, 1385m, 1360m, 1270s, 1215m, 1195m, 1175m, 1090m, 1070m, 700m. ¹H-NMR (CD₃OD): 7.45–7.25 (*m*, 10 arom. H); 5.11 (*s*, PhCH₂); 3.62 (*s*, CH₂(Gly)); 3.36 (*s*, MeN); 1.53, 1.51, 1.41, 1.35 (4s, 4 Me₂C). ¹³C-NMR (CD₃OD): 178.5, 177.4, 176.5, 175.6, 171.8 (5s, 5 CO(amide)); 158.5 (*s*, CO(urethane)); 147.0, 138.5 (2s, 2 arom. C); 130.3, 129.6, 129.2, 128.9, 128.4 (5*d*, 10 arom. CH); 68.0 (*t*, PhCH₂); 58.5, 57.2 (2s, 4 C(2)(Aib)); 45.3 (*t*, CH₂(Gly)); 41.1 (*q*, MeN); 26.4, 26.0, 25.4, 25.3 (4*q*, 4 Me₂C). 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 3*N* HCl (THF/H₂O 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): 3300s, 3060m, 3040m, 2990m, 2940m, 1720s, 1695s, 1670s, 1660s, 1550m, 1540s, 1530s, 1525s, 1470m, 1460m, 1455m, 1385m, 1365m, 1270s, 1220m, 1170m, 1090m, 1080m, 700m. ¹H-NMR (CD₃OD): 7.45–7.3 (*m*, 5 arom. H); 5.11 (*s*, PhCH₂); 3.64 (*s*, CH₂(Gly)); 1.49, 1.48, 1.41, 1.36 (4s, 4 Me₂C). ¹³C-NMR (CD₃OD): 178.3, 178.0, 177.4, 176.2, 171.8 (5s, 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*, PhCH₂); 58.2, 57.8, 57.7, 57.1 (4s, 4 C(2)(Aib)); 45.0 (*t*, CH₂(Gly)); 25.7, 25.4, 25.30, 25.27 (4*q*, 4 Me₂C). 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 HCOONH₄ (163 mg, 2.585 mmol) and Pd/C (280.3 mg): 214.0 mg (quant.) of **6d**. Colorless solid. ¹H-NMR (D₂O): 3.85 (*s*, CH₂(Gly)); 1.64, 1.51, 1.49, 1.43 (4s, 4 Me₂C). ¹³C-NMR (CD₃OD): 184.7, 179.9, 177.8, 175.2, 173.2 (5s, 4 CO(amide), COOH); 60.8, 60.0, 59.3 (3s, 4 C(2)(Aib)); 46.0 (*t*, CH₂(Gly)); 27.1, 26.9, 26.7, 25.9 (4*q*, 4 Me₂C). 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 (CH₂Cl₂/MeOH 20 : 1 → 10 : 1): 2.8 mg (11%) of **7c**.

6. *Synthesis of Cyclo*(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 Et₂O to give 726.8 mg of *Z*-Aib-Aib-Aib-Aib-Aib-OH. Extraction of the aq. phase with Et₂O (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): 3410s, 3320s, 3290s, 3250s, 3040s, 2980s, 2940s, 2920m, 2900m, 1745s, 1730s, 1710s, 1705s, 1695s, 1680s, 1675s, 1665s, 1645s, 1635s, 1580m, 1565m, 1550s, 1530s, 1515s, 1465s, 1455s, 1445s, 1385s, 1365s, 1320s, 1315s, 1280s, 1270s, 1265s, 1225s, 1210s, 1185s, 1180s, 1170s, 1165s, 1090s, 1080s, 955m, 945m, 750m, 700m. ¹H-NMR ((D₆)DMSO): 11.83 (br. *s*, COOH); 8.23, 7.85, 7.46 (3s, 3 NH); 7.4–7.3 (*m*, 5 arom. H, 1 NH); 7.28 (*s*, NH); 5.09 (*s*, PhCH₂); 1.33, 1.32, 1.21 (3s, 3 : 1 : 1, 5 Me₂C). ¹³C-NMR ((D₆)DMSO): 175.5, 174.9, 174.7, 173.42, 173.38 (5s, 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*, PhCH₂); 55.9,

55.81, 55.76, 55.6, 54.5 (5s, 5 C(2)(Aib)); 25.0, 24.6, 24.5, 24.3 (4q, 5 Me₂C). ESI-MS: 660 (100, [M + Na]⁺), 578 (80, [M + 1]⁺), 560 (8, [M – OH]⁺), 475 (19, [M – Aib-OH]⁺). Anal. calc. for C₂₈H₄₃N₅O₈ (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 HCOONH₄ (129.5 mg, 2.054 mmol) and Pd/C (240 mg). The crude material was dissolved in MeOH and precipitated with Et₂O to give 169.8 mg (95%) of **6f**. Colorless powder. ¹H-NMR (D₂O): 7.54, 7.52 (2s, 2 NH); 1.65, 1.48, 1.43, 1.42 (4s, 1:2:1:1, 5 Me₂C). ¹³C-NMR (D₂O): 178.9, 178.6, 178.4, 175.3 (4s, 4 CO(amide), COOH); 60.1, 59.9, 59.7, 59.6 (4s, 5 C(2)(Aib)); 27.2, 27.1, 26.9, 26.5, 25.9 (5q, 5 Me₂C). 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(ⁱPr)₂ (2.6 ml) at r.t. After stirring at r.t. for 2 weeks, DMF was removed in high vacuum, and subsequent CC (CH₂Cl₂/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): 3420m, 3320s, 3120m, 2990m, 2980m, 2940m, 1690s, 1670s, 1650s, 1570s, 1520s, 1480m, 1450m, 1385s, 1360s, 1310m, 1280s, 1220s, 1210s, 1185m, 1170m. ¹H-NMR (CDCl₃): 6.63 (s, 5 NH); 1.54 (s, 5 Me₂C). ¹³C-NMR (CDCl₃): 175.2 (s, 5 CO(lactam)); 58.0 (s, 5 C(2)(Aib)); 25.0 (q, 5 Me₂C). 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 | <i>D</i> _x [g cm ^{−3}] | 1.215 |
| Empirical formula | C ₂₇ H ₄₁ N ₅ O ₈ | μ(MoK _α) [mm ^{−1}] | 0.090 |
| Formula weight | 563.65 | Scan type | ω/2θ |
| Crystal color, habit | colorless, needle | 2θ _(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>I</i> > 2σ(<i>I</i>) | 3881 |
| Space group | <i>P</i> 2 ₁ / <i>n</i> | Reflections used in refinement | 7068 |
| <i>Z</i> | 4 | Parameters refined | 391 |
| Reflections for cell determination | 25 | Final <i>R</i> (<i>F</i>) [<i>I</i> > 2σ(<i>I</i>) reflections] | 0.0530 |
| 2θ Range for cell determination [°] | 20–37 | <i>wR</i> (<i>F</i> ²) (all data) | 0.1412 |
| Unit cell parameters | <i>a</i> [Å] | Weighting parameter (<i>a</i>) ^{a)} | 0.0513 |
| | <i>b</i> [Å] | Goodness-of-fit | 1.003 |
| | <i>c</i> [Å] | Secondary extinction coefficient | 0.0018(5) |
| | β [°] | Final Δ _{max} /σ | 0.000 |
| <i>V</i> [Å ³] | 3082.1(8) | Δρ (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.

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