SYNTHESIS OF Aib-CONTAINING CYCLOPEPTIDES VIA THE 'AZIRINE/OXAZOLONE METHOD'

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Dedicated to Dr. Alfred Bader on the occasion of his 85th birthday.

The cyclic pentapeptide *cyclo*[Gly-Phe(2Me)-Aib-Aib-Gly], containing three α,α-disubstituted α-amino acids, was prepared by cyclization of H-Gly-Phe(2Me)-Aib-Aib-Gly-OH with diphenyl phosporazidate (DPPA) in 60% yield. The synthesis of the linear precursor was performed by using the 'azirine/oxazolone method', in which the α,α-disubstituted α-amino acids were introduced by coupling of the corresponding amino or peptide acid with a 2,2-disubstituted 3-amino-2*H*-azirine. Whereas the cyclization of an analogous hexapeptide afforded the cyclopeptide in 42% yield, the corresponding tetrapeptide yielded a 1:2 mixture of the monomeric and dimeric cyclopeptide. In the case of the tripeptide H-Gly-Phe(2Me)-Aib-OH, the cyclization with DPPA led to the dimeric cyclohexapeptide exclusively.

Keywords: α-Amino acids; 3-Amino-2*H*-azirines; Peptides; α,α-Disubstituted amino acids; Cyclizations; X-ray diffraction.

Cyclic peptides are of continuing interest, mainly because of their diverse biological activities[1–8]. This is documented by a large series of recent papers describing the isolation and activity of new naturally occurring cyclopeptides[9–14]. On the other hand, the development of efficient methods for the synthesis of natural and designed cyclic peptides is a challenging task[15–20], and an increasing number of papers dealing with syntheses of cyclic peptides are published year after year[21–29]. It has been shown that the yield of the cyclization is not only dependent on the ring size of the product but also on the sequence of the amino acids as well as on the position of the ring closure (e.g. refs.[30,31]). One of the main problems in the case of

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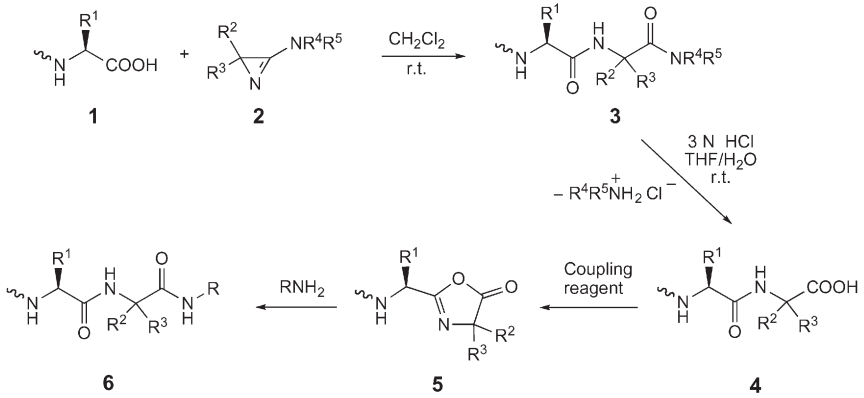
the cyclization of tri-, tetra-, and pentapeptides is the formation of dimeric cyclopeptides[30]. For example, Kondo et al.[32] showed that the cyclization of H-Val-Orn(Z)-Leu-D-Phe-Pro-ONp led to a ca. 2:1 mixture of gramicidine S and the monomeric cyclopentapeptide, whereas the corresponding pentapeptides with Val substituted by Gly or Aib (α-aminoisobutyric acid) yielded the cyclopentapeptide exclusively. Similarly, Hlaváček et al.[33] obtained a 1:1 mixture of the cyclopenta- and the dimeric cyclodecapeptide when H-Tyr(*t*-Bu)-Asp(O*t*-Bu)-Pro-Ala-Pro-OH was cyclized.

With the 'azirine/oxazolone method' we have elaborated a convenient and efficient access to peptides containing α,α-disubstituted α-amino acids[34] (Scheme 1).

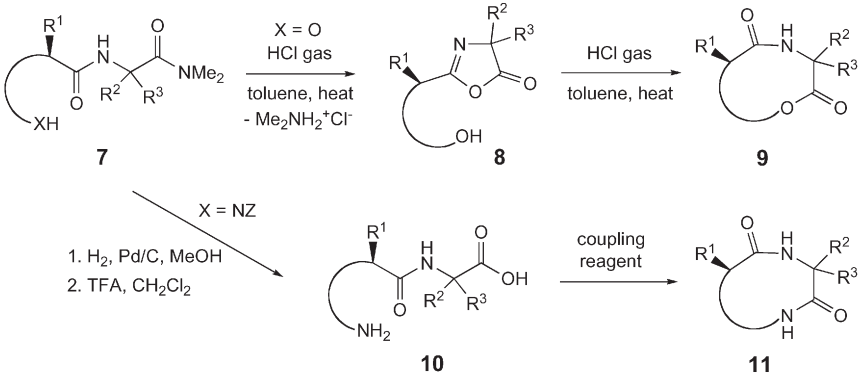


SCHEME 1

The coupling of an amino acid or a peptide acid **1** with a 3-amino-2*H*-azirine **2** yields a peptide amide **3**, which is then hydrolyzed selectively to give the peptide **4**. The latter can be coupled with another 2*H*-azirine **2** or with a C-protected amino acid or peptide. It has been shown that a 1,3-oxazol-5(4*H*)-one **5** is formed as an intermediate, which reacts with the amino component to give the new peptide **6**. The smooth ring closure to **5** has been explained as the result of the Thorpe–Ingold effect[35]. Using this methodology, we have prepared sterically congested model peptides[36–40] and peptaibols[41–43], i.e., natural Aib-containing peptide alcohols with antibiotic activity[44]. A characteristic feature of all these peptides is their restricted flexibility and preferred helical structure. Recently we have adopted the 'azirine/oxazolone method' to solid-phase peptide synthesis[45].

As an extension of the 'azirine/oxazolone method', we have synthesized cyclic depsipeptides containing α,α-disubstituted α-amino acids via the

so-called 'direct amide cyclization'[34,46–50] (Scheme 2). In these reactions, a suspension of a peptide containing Aib ($R^2 = R^3 = Me$) at the C-terminus of type **7** (X = O) with a terminal hydroxy group in toluene at 80–100 °C was treated with gaseous HCl leading to cyclodepsipeptides **9** via formation of the lactone bond. It has been shown that 1,3-oxazol-5(4*H*)-ones are intermediates. Therefore, in the case of an optically active penultimate α-amino acid ($R^1 \neq H$), partial epimerization has been observed under the conditions of the 'direct amide cyclization'. An analogous ring closure giving cyclopeptides **11** was achieved via sequential deprotection of **7** (X = NZ or NFmoc (for abbreviations, see Experimental)) and treatment with a coupling reagent[31,51,52]. A systematic study revealed that 18-membered cyclohexapeptides are formed in good to excellent yields[31,52a,52b], and also cyclohepta- and cyclooctapeptides (21- and 24-membered rings, respectively) could be obtained in reasonable to good yields[52c].



SCHEME 2

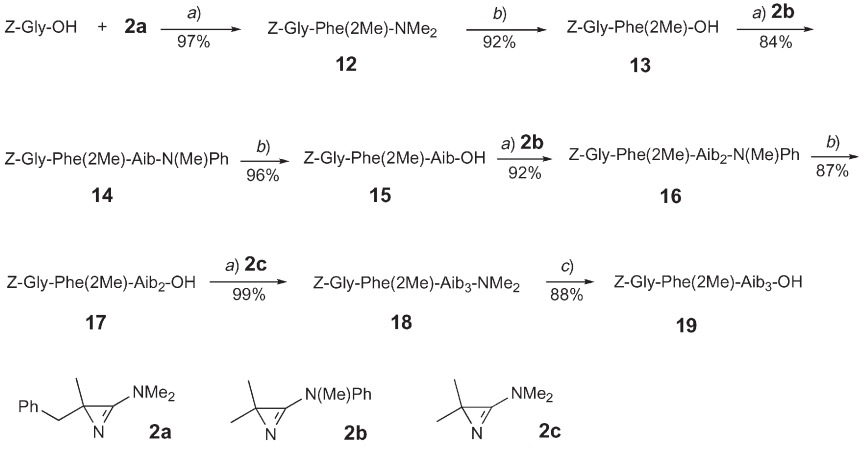
In the present study, the cyclization of tri-, tetra-, penta-, and hexapeptides with 2-methylphenylalanine (Phe(2Me)) and Aib in their backbone was investigated. Linear precursors with Gly at both termini were chosen with the aim of avoiding steric hindrance in the cyclization as well as the formation of an intermediate oxazolone.

RESULTS AND DISCUSSION

Synthesis of Linear Peptides Containing α,α-Disubstituted α-Amino Acids

Following the protocol of the 'azirine/oxazolone method', the peptides **15**, **17**, and **19**, containing two, three, and four α,α-disubstituted α-amino ac-

ids, were prepared (Scheme 3). For example, Z-protected glycine reacted with racemic 2-benzyl-3-(dimethylamino)-2-phenyl-2*H*-azirine (**2a**) in THF at 0 °C to give dipeptide amide **12** (97%), which was hydrolyzed with 6 M HCl/THF (1:1) at 35 °C leading to **13** in 92% yield. Repeating the 'azirine coupling' with **2b** gave tripeptide amide **14** (84%) and subsequent hydrolysis yielded **15** (96%). This coupling/hydrolysis sequence was repeated according to Scheme 3.

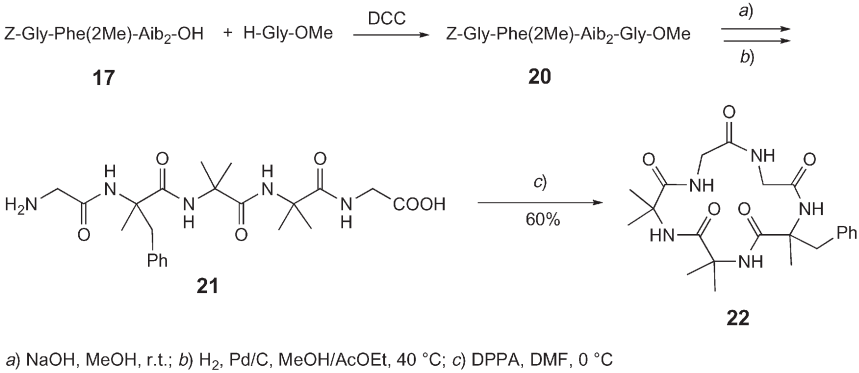


SCHEME 3

Synthesis of the Cyclopentapeptide cyclo[Gly-Phe(2Me)-Aib$_2$-Gly]

The first cyclization experiments were carried out with the pentapeptide H-Gly-Phe(2Me)-Aib-Aib-Gly-OH (**21**), which was obtained from **17** via DCC coupling with methyl glycinate, alkaline hydrolysis and hydrogenolytic deprotection of the N-terminus (Scheme 4). The structure of **21** was deduced from analytical and spectroscopic data and, finally, established by X-ray crystallography (Fig. 1a, Tables I and II). In the crystal structure of **21**, the asymmetric unit contains one peptide molecule and four molecules of water. Some of the water H atoms could not be located. The peptide molecule is protonated at the terminal amino group. The molecule is presumably a zwitterion with the terminal carboxylate function; a H atom could not be located here, but this terminal group has disordered O atoms, which might inhibit the detection of the hydroxy H atom, even if present. If the group was protonated, then one of the water molecules would have to be a hydroxy anion, which seems unlikely. Each N–H group of the molecule acts

as a donor for hydrogen bonds. Two intramolecular hydrogen bonds (N(4)–H of Aib(4) with O(1) of Gly(1) and N(5)–H of Gly(5) with O(2) of Phe(2Me)) form β-turns of type III′ and, therefore, keep the molecule in an incipient 3_{10}-helical conformation (for peptide conformations, see ref.[54]). Each of these interactions has a graph set motif[55] of S(10). The ammonium group forms intermolecular hydrogen bonds with an amide O atom and a carboxylate O atom of the same neighboring molecule, as well as with the other carboxylate O atom of a different neighboring molecule. These interactions link the peptide molecules into extended chains which run parallel to the [010] and [101] directions, respectively, and can be described by graph set motifs of C(11) and C(17). N(2)–H and N(3)–H form intermolecular hydrogen bonds with different water molecules, while the water molecules form hydrogen bonds between themselves and with amide O atoms of various neighboring peptide molecules. Where a water H atom could not be located, the interaction is inferred from suitable O···O distances. The combination of all intermolecular interactions forms a three-dimensional network.



SCHEME 4

The ring closure was achieved under different conditions by using various peptide-coupling reagents, e.g., with BOP and DMAP in methanol[56], with pentafluorophenol in DMF/CH_2Cl_2 at room temperature[56,57], or with DPPA in DMF [58,59]. In all cases, the solution of the coupling reagent was added very slowly by using a syringe. The highest yield of the crude cyclopentapeptide **22** (85%) was obtained with DPPA. After HPLC purification, 60% of pure **22** were isolated. The structure of this product was also determined by X-ray crystallography (Fig. 1b, Tables I and II).

The asymmetric unit of **22** contains one peptide molecule and one molecule of water. Each N–H group of the peptide molecule and the water molecule act as donors for hydrogen bonds. The molecule contains an intramolecular hydrogen bond across the macrocycle (N(4)–H···O(2)), which forms a β-turn of type I′ and a second one from N(1)–H to O(2), forming a five-membered loop (C_5-conformation, see ref.[60]), which is unusual for an Aib residue[61]. It has to be emphasized that O(2) is a double acceptor of

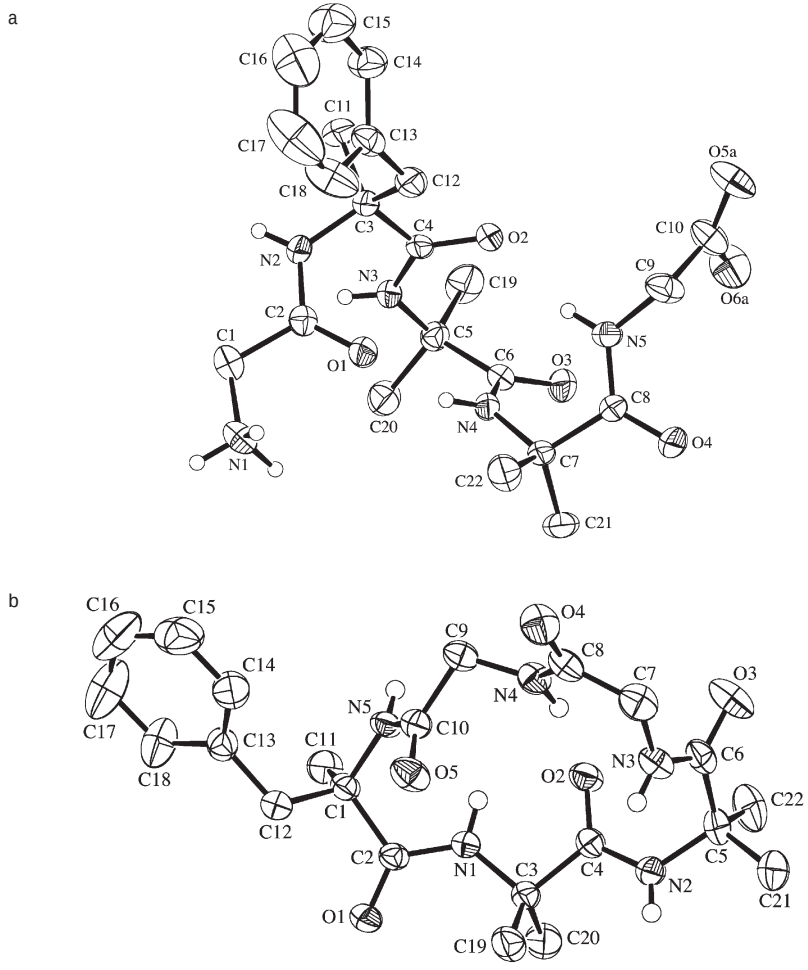


FIG. 1
ORTEP plots[53] of the molecular structure of compounds a **21** and b **22** (arbitrary numbering of atoms; 30 and 50% probability ellipsoids, respectively)

TABLE I
Torsion angles of the backbone of compounds **21** and **22** (atom numbering refers to Fig. 1)

Compound	Amino acid		Atoms	Torsion angle, °
21	Gly(1)	ω_1	C(1)–C(2)–N(2)–C(3)	–170.6(2)
	Phe(2Me)(2)	ϕ_2	C(2)–N(2)–C(3)–C(4)	51.2(3)
		ψ_2	N(2)–C(3)–C(4)–N(3)	32.5(3)
		ω_2	C(3)–C(4)–N(3)–C(5)	177.9(2)
	Aib(3)	ϕ_3	C(4)–N(3)–C(5)–C(6)	50.1(3)
		ψ_3	N(3)–C(5)–C(6)–N(4)	37.7(3)
		ω_3	C(5)–C(6)–N(4)–C(7)	174.3(2)
	Aib(4)	ϕ_4	C(6)–N(4)–C(7)–C(8)	63.2(3)
		ψ_4	N(4)–C(7)–C(8)–N(5)	28.0(3)
		ω_4	C(7)–C(8)–N(5)–C(9)	165.1(2)
22	Gly(1)	ϕ_1	C(8)–N(4)–C(9)–C(10)	–120.9(3)
		ψ_1	N(4)–C(9)–C(10)–N(5)	–122.2(3)
		ω_1	C(9)–C(10)–N(5)–C(1)	175.8(2)
	Phe(2Me)(2)	ϕ_2	C(10)–N(5)–C(1)–C(2)	–57.7(4)
		ψ_2	N(5)–C(1)–C(2)–N(1)	–25.3(3)
		ω_2	C(1)–C(2)–N(1)–C(3)	165.2(3)
	Aib(3)	ϕ_3	C(2)–N(1)–C(3)–C(4)	–159.7(3)
		ψ_3	N(1)–C(3)–C(4)–N(2)	166.4(2)
		ω_3	C(3)–C(4)–N(2)–C(5)	172.1(2)
	Aib(4)	ϕ_4	C(4)–N(2)–C(5)–C(6)	56.1(4)
		ψ_4	N(2)–C(5)–C(6)–N(3)	39.9(4)
		ω_4	C(5)–C(6)–N(3)–C(7)	177.3(3)
	Gly(5)	ϕ_5	C(6)–N(3)–C(7)–C(8)	100.0(4)
		ψ_5	N(3)–C(7)–C(8)–N(4)	–16.1(4)
		ω_5	C(7)–C(8)–N(4)–C(9)	170.8(3)

TABLE II
Intramolecular hydrogen bonds of compounds **21**, **22**, and **31a** (atom numbering refers to Figs. 1 and 2)

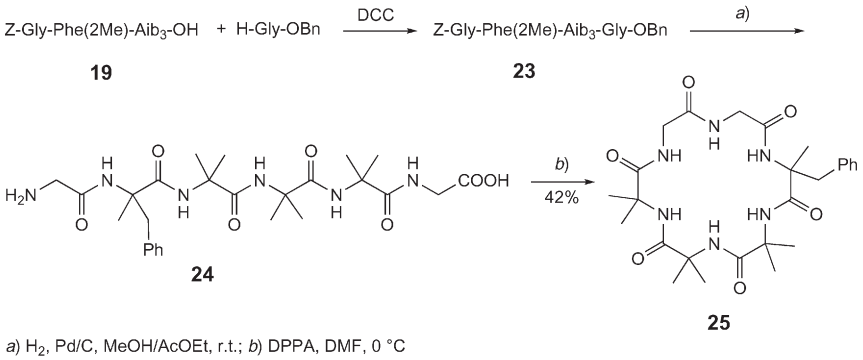
Compound	Hydrogen bond	N–H, Å	H···O, Å	N···O, Å	N–H···O, °
21	N(4)–H···O(1)	0.81(2)	2.30(2)	3.050(3)	155(2)
	N(5)–H···O(2)	0.86(3)	2.12(3)	2.960(3)	167(2)
22	N(1)–H···O(2)	0.84(3)	2.05(3)	2.538(3)	117(3)
	N(4)–H···O(2)	0.87(3)	2.20(3)	3.028(3)	160(2)
31a	N(10)–H···O(1)	0.88	2.05	2.895(8)	161
	N(22)–H···O(5)	0.88	2.24	2.999(9)	145

hydrogen bonds. The pattern of the intramolecular hydrogen bonds in **22** is surprising because it does not correspond with the usually observed conformations found for different cyclopentapeptides[62,63]. These interactions can be described by graph set motifs[55] of S(10) and S(5), respectively. N(3)–H forms an intermolecular hydrogen bond with the water O atom. N(2)–H forms an intermolecular hydrogen bond with the same amide O atom of a neighboring molecule that accepts the N(4)–H intramolecular interaction. This interaction links the molecules into extended chains which run parallel to the [0 $-\bar{1}$ 1] direction and which can be described by a graph set motif of C(8). N(5)–H forms an intermolecular hydrogen bond with another amide O atom in a different neighboring molecule and thereby links the molecules into extended chains which run parallel to the [001] direction and which can be described by a graph set motif of C(5). The water molecule donates hydrogen bonds to amide O atoms in two different peptide molecules. One of these is back to the same peptide molecule from which the water molecule accepts a hydrogen bond, which generates a ring with a graph set motif of $R_2^2(10)$. The other is with a different peptide molecule and thereby links water and peptide molecules alternately into extended chains which run parallel to the [011] direction and which can be described by a binary graph set motif of $C_2^2(6)$. The combination of all intermolecular interactions forms a three-dimensional network.

Synthesis of Cyclohexapeptide cyclo[Gly-Phe(2Me)-Aib$_3$-Gly]

The cyclohexapeptide **25** was prepared in an analogous manner to the above described synthesis of cyclopentapeptide **22** (Scheme 5). The cou-

pling of the pentapeptide **19** and benzyl glycinate in DMF with DCC in the presence of $ZnCl_2$, followed by hydrogenolytic deprotection, gave hexapeptide **24** in 83% yield. Cyclization of the latter with DPPA yielded, after HPLC purification, the cyclohexapeptide **25** (42%). The structure was supported by the ^1H and ^{13}C NMR spectra and the comparison of the data with those of **22** and by FAB-MS. Based on this experiment, a series of additional cyclohexapeptides with analogous structures have been prepared and their 3D-structure established by X-ray crystallography[31,52a,52b].

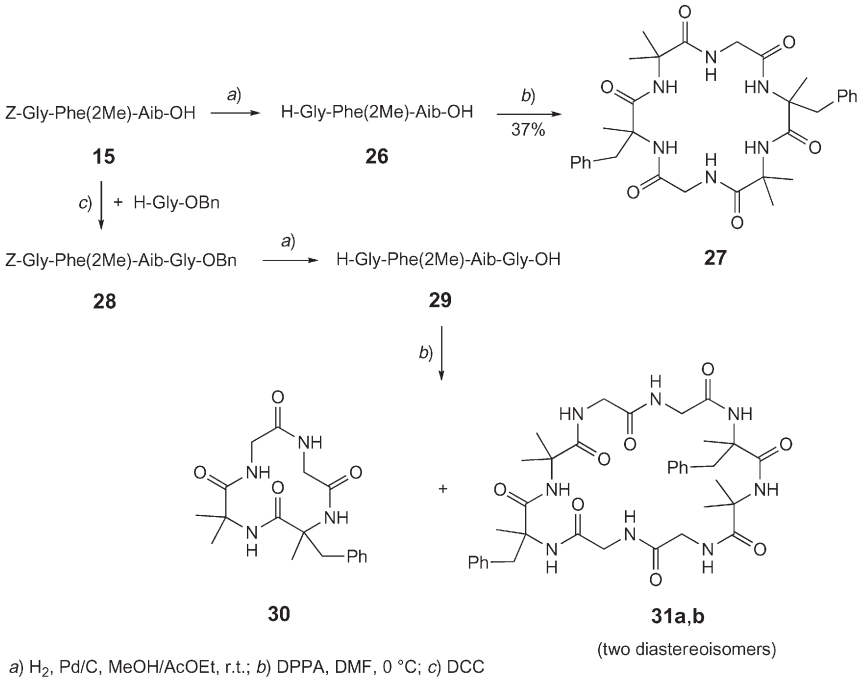


a) H_2, Pd/C, MeOH/AcOEt, r.t.; *b*) DPPA, DMF, 0 °C

SCHEME 5

Attempted Synthesis of Cyclotripeptide cyclo[Gly-Phe(2Me)-Aib] and Cyclotetrapeptide cyclo[Gly-Phe(2Me)-Aib-Gly]

Encouraged by the successful preparation of Phe(2Me) and Aib containing cyclopenta- and cyclohexapeptides, the more demanding synthesis of a strained cyclotripeptide, as well as a cyclotetrapeptide, was undertaken. As a tripeptide, H-Gly-Phe(2Me)-Aib-OH (**26**) was chosen, which was obtained by the hydrogenolytic deprotection of the terminal amino group of **15**. The cyclization with DPPA led, after purification by HPLC, to a product, which most likely is a mixture of diastereoisomeric cyclodimers, i.e., cyclohexapeptide **27** (Scheme 6). No decision concerning the relative configuration of the two Phe(2Me) units was possible on the basis of the NMR data, but the ^{13}C NMR spectrum and the ^1H NMR spectra at different temperatures indicate the presence of two diastereoisomers. All attempts to favor the formation of the monomeric cyclotripeptide by carring out the cyclization in more dilute solutions were in vain. The failure of the cyclization to the nine-membered cyclotripeptide can be explained kinetically by the increased steric hindrance in the case of a C-terminal Aib and thermodynamically by the high ring strain of nine-membered rings.



a) H_2, Pd/C, MeOH/AcOEt, r.t.; *b*) DPPA, DMF, 0 °C; *c*) DCC

SCHEME 6

The coupling of Z-protected tripeptide **15** and H-Gly-OBn with DCC/$ZnCl_2$ in DMF [34,64] gave the protected tetrapeptide **28** in 95% yield (Scheme 6). Simultaneous deprotection of both the amino and carboxyl group was achieved by hydrogenolysis, leading to **29** (85%). Treatment of the latter with DPPA under the usual conditions gave a complex mixture of products. Their separation by HPLC led to two fractions, which, according to FAB-MS, were still mixtures of cyclotetrapeptide **30**, cyclodimer and cyclotrimer.

With the aim of reducing the oligomerization, the cyclization was repeated in higher dilution (0.19 mmol of **29** in 200 ml of DMF instead of 0.21 mmol in 23 ml of DMF). After the usual workup, reversed-phase HPLC gave two fractions in a ratio of 1:2 (ca. 45% total yield). The first fraction contained a single product, which, according to the FAB-MS was the cyclotetrapeptide **30** (Scheme 6). The second fraction consisted of two diastereoisomeric cyclodimers **31a** and **31b**, which could be separated by another HPLC and were isolated as crystalline materials. The structures of these products were proposed on the basis of their FAB-MS and ^1H and ^{13}C NMR data, but it was not possible to assign the relative configurations of the two Phe(2Me) residues. Fortunately, one of the two isomers, **31a**, after crystalli-

zation from methanol, was obtained as single crystals suitable for an X-ray crystal-structure determination, which established the presence of the *cis*-isomer, that is the (*R*,*R*/*S*,*S*) diastereoisomer (Fig. 2, Tables II and III). The conformation of the 24-membered ring is stabilized by two intramolecular hydrogen bonds (N(10)–H···O(1) and N(22)–H···O(5)), which form two β-turns of type III′ including Phe(2Me) and Aib.

Since the space group of **31a** is centrosymmetric, the compound in the crystal is racemic. The asymmetric unit contains one molecule of the peptide plus five molecules of MeOH, one of which is disordered. As expected, the peptide is a cyclic dimeric molecule, which almost possesses pseudo two-fold symmetry, although the N(1)/O(8) and N(13)/O(4) regions deviate significantly from a two-fold relationship. The crystals were very weakly diffracting and gave limited observed intensities, probably partially due to a large amount of solvent in the crystal lattice. The crystals lost solvent and were destroyed immediately upon removal from the mother liquor; therefore it was necessary to mount a crystal in a capillary together with some mother liquor without the crystal ever leaving the solution. Each N–H group of the molecule acts as a donor for hydrogen bonds. Every second amide N–H group [N(1)–H, N(7)–H, N(13)–H, N(19)–H] forms an intermolecular hydrogen bond with the O atom of a different MeOH molecule. Two of the remaining amide N–H groups, N(10)–H and N(22)–H, related by

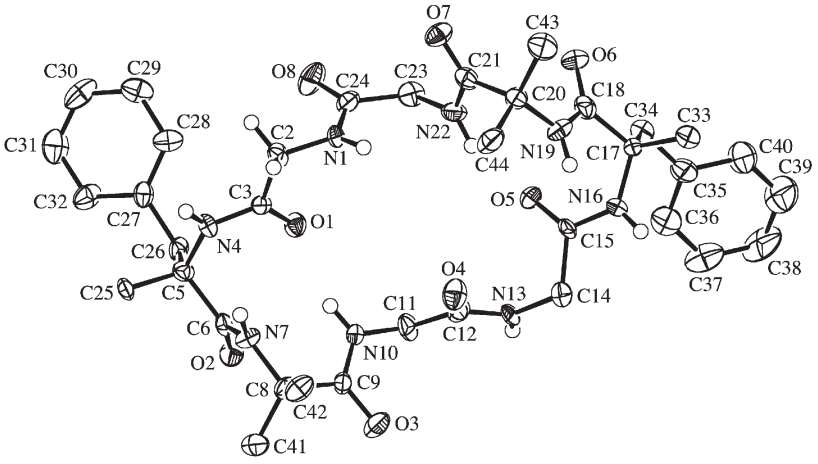


FIG. 2
ORTEP plot[53] of the molecular structure of compound **31a** (*cis*-isomer; arbitrary numbering of atoms; 50% probability ellipsoids)

the pseudo two-fold axis within the peptide molecule, form intramolecular hydrogen bonds with amide O atoms within the cyclic peptide molecule and thereby form loops which can be described by a graph set motif[55] of S(10). N(4)–H and N(16)–H, also related by the pseudo-symmetry, form intermolecular hydrogen bonds with other amide O atoms in two different neighboring peptide molecules and thereby link the molecules into ex-

TABLE III
Torsion angles of the backbone of compound **31a** (atom numbering refers to Fig. 2)

Amino acid		Atoms	Torsion angles, °
Gly(1)	ϕ_1	C(24)–N(1)–C(2)–C(3)	79.5(9)
	ψ_1	N(1)–C(2)–C(3)–N(4)	179.7(6)
	ω_1	C(2)–C(3)–N(4)–C(5)	175.5(6)
Phe(2Me)(2)	ϕ_2	C(3)–N(4)–C(5)–C(6)	54.3(9)
	ψ_2	N(4)–C(5)–C(6)–N(7)	39.4(9)
	ω_2	C(5)–C(6)–N(7)–C(8)	–177.0(7)
Aib(3)	ϕ_3	C(6)–N(7)–C(8)–C(9)	66.1(9)
	ψ_3	N(7)–C(8)–C(9)–N(10)	8(1)
	ω_3	C(8)–C(9)–N(10)–C(11)	–179.3(7)
Gly(4)	ϕ_5	C(9)–N(10)–C(11)–C(12)	–82.1(9)
	ψ_5	N(10)–C(11)–C(12)–N(13)	167.5(7)
	ω_5	C(11)–C(12)–N(13)–C(14)	172.7(7)
Gly(5)	ϕ_5	C(12)–N(13)–C(14)–C(15)	–78.9(9)
	ψ_5	N(13)–C(14)–C(15)–N(16)	–175.2(6)
	ω_5	C(14)–C(15)–N(16)–C(17)	173.9(7)
Phe(2Me)(6)	ϕ_6	C(15)–N(16)–C(17)–C(18)	55.1(9)
	ψ_6	N(16)–C(17)–C(18)–N(19)	37.3(9)
	ω_6	C(17)–C(18)–N(19)–C(20)	176.2(6)
Aib(7)	ϕ_7	C(18)–N(19)–C(20)–C(21)	55(1)
	ψ_7	N(19)–C(20)–C(21)–N(22)	31(1)
	ω_7	C(20)–C(21)–N(22)–C(23)	–173.6(7)
Gly(8)	ϕ_8	C(21)–N(22)–C(23)–C(24)	–79.6(9)
	ψ_8	N(22)–C(23)–C(24)–N(1)	–15(1)
	ω_8	C(23)–C(24)–N(1)–C(2)	–167.3(6)

tended chains. These chains run parallel to the [001] direction and both interactions can be described by a graph set motif of C(8). The MeOH molecules form intermolecular hydrogen bonds with amide O atoms in various neighboring peptide molecules. Although there are a large number of interactions, the total hydrogen bonding network serves only to link the molecules into extended zig-zag chains which run parallel to the [001] direction.

CONCLUSIONS

The presented results show that peptides, containing 2,2-disubstituted α-amino acids such as Phe(2Me) and Aib, can conveniently be prepared via the 'azirine/oxazolone method', in which the sterically congested amino acids are introduced by the coupling of an amino acid or peptide acid with a 3-amino-2*H*-azirine. For cyclization experiments, we have chosen tetra-, penta- and hexapeptides with two, three, and four 2,2-disubstitued α-amino acids and Gly residues at the termini with the aim of avoiding steric hindrance in the ring-closing step. The cyclizations to the cyclopenta- and cyclohexapeptide (15- and 18-membered ring, respectively) were achieved in good yields by using DPPA in DMF, whereas in the cases of the tetrapeptide a mixture of the monomeric cyclotetrapeptide and the dimer, i.e., a 24-membered cyclooctapeptide, in favor of the latter was obtained. Under analogous reaction conditions, the tripeptide H-Gly-Phe(2Me)-Aib-OH gave the dimeric, 18-membered cyclohexapeptide, as a mixture of two diastereoisomers exclusively.

The determination of the conformation of the linear pentapeptide H-Gly-Phe(2Me)-Aib-Aib-Gly-OH by X-ray crystallography confirms the expected helical structure stabilized by two intramolecular hydrogen bonds forming two consecutive β-turns. The crystal structure of the cyclopentapeptide also confirms the formation of a β-turn by an intramolecular hydrogen bond but, surprisingly, Aib and Gly are included, not Phe(2Me) and Aib. In the case of the dimeric cyclooctapeptide, two intramolecular hydrogen bonds form two β-turns, both including Phe(2Me) and Aib.

The conformational properties of cyclopeptides containing 2,2-disubstituted α-amino acids will be investigated in solution. The presented synthetic approach enables us to prepare such cyclopeptides of various ring sizes conveniently.

EXPERIMENTAL

Solvents were purified by standard procedures; tetrahydrofuran (THF) was distilled from sodium/benzophenone, acetonitrile (MeCN) from P_2O_5, diethyl ether (Et_2O) from sodium, and dichloromethane (CH_2Cl_2) from anhydrous calcium chloride; dimethylformamide (DMF, puriss.) was dried over molecular sieves. All other chemicals were of analytical grade and were used without purification. The 3-amino-2*H*-azirines **2a**, **2b**, and **2c** were prepared according to the references cited in ref.[33] Melting points (m.p.) were determined on a Mettler-FP-5 apparatus; uncorrected. IR absorption spectra were measured on a Perkin–Elmer-297 or 781 spectrophotometer; in KBr; absorptions in cm^{-1}. ^1H NMR spectra were recorded on a Varian XL-200, Bruker AC-300, Bruker AM-360 or Bruker AM-400 spectrometer. ^{13}C NMR spectra were recorded on a Varian XL-200 spectrometer at 50.4 MHz; indicated multiplicities of signals were determined by DEPT spectra. Solvents are indicated, TMS was used as an internal standard (δ in ppm, coupling constants *J* in Hz). Mass spectra were measured on a Varian MAT-711, Finnigan MAT-90 or Finnigan SSQ-700 instrument; EI spectra at 70 eV, CI-MS with 2-methylpropane or NH_3. Thin layer chromatography (TLC) on silica gel 60 F_{254} (Merck); column chromatography (CC, flash chromatography[65]) with silica gel Merck 60 (0.040–0.063 mm). High performance liquid chromatography (HPLC) with a Varian 2510 instrument and Varian 2550 UV detector (254 nm) or Waters 600 E instrument with Waters 484 UV detector (254 nm); Lichrosorb RP 18 or LichroCast RP 18 (reversed phase) columns. Elemental analyses were performed in our Institute.

Abbreviations: AcOEt, ethyl acetate; Aib, 2-aminoisobutyric acid; Bn, benzyl; BOP, (1*H*-benzo[*d*][1,2,3]triazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; CC, column chromatography; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)-pyridine; DMF, *N*,*N*-dimethylformamid; DMSO, dimethyl sulfoxide; DPPA, diphenyl phosphorazidate; Et_2O, diethyl ether; Fmoc, (9*H*-fluoren-9-yl)methyloxycarbonyl; HPLC, high performance liquid chromatography; MeCN, acetonitrile, MeOH, methanol, Phe(2Me), 2-methylphenylalanine, THF, tetrahydrofuran; TLC, thin layer chromatography; Tos, toluolsulfonyl (tosyl); Z, (benzyloxy)carbonyl.

Synthesis of Linear Peptides Containing α,α-Disubstituted α-Amino Acids

Z-Gly-Phe(2Me)-NMe$_2$ (**12**). To a solution of Z-Gly-OH (1.0 g, 4.78 mmol) in THF (10 ml) at 0 °C was added dropwise 2-benzyl-3-(dimethylamino)-2-methyl-2*H*-azirine (**2a**; 945 mg, 5.02 mmol). Then, the mixture was stirred at 0 °C for 15 min and at r.t. for 2 h. The white precipitate was filtered off and washed with Et_2O: 1.83 g (97%) of **12**; colorless crystals; m.p. 155.4 °C. IR (KBr): 3280m, 1730s, 1660s, 1615s, 1545m, 1535m, 1250s, 710m. ^1H NMR (200 MHz, $CDCl_3$): 7.45–6.90 (3m, 10 arom. H, NH); 5.35 (br s, NH); 5.11 (s, PhC**H**$_2$O); 3.79 (d, *J* = 5.5, CH_2(Gly)); 3.57, 3.26 (AB, *J* = 14, PhC**H**$_2$); 3.08 (br s, Me_2N); 1.68 (s, MeC(2)). ^{13}C NMR (50.4 MHz, $CDCl_3$): 171.6, 167.4 (2s, 2 CO(amide)); 156.4 (s, CO(urethane)); 136.4, 136.1 (2s, 2 arom. C); 130.0, 128.5, 128.2, 128.1, 128.0, 126.8 (6d, 10 arom. CH); 67.0 (t, PhC**H**$_2$O); 60.3 (s, C(2)(Phe(2Me))); 44.8 (t, CH_2(Gly)); 40.9 (t, PhC**H**$_2$); 38.3 (q, Me_2N); 22.7 (q, MeC(2)). CI-MS: 398 (100, $[M + 1]^+$). For $C_{22}H_{27}N_3O_4$ (397.48) calculated: 66.48% C, 6.58% H, 10.57% N; found: 66.64% C, 6.58% H, 10.71% N.

Z-Gly-Phe(2Me)-OH (**13**). A solution of **12** (1.70 g, 4.3 mmol) in THF/6 M HCl (1:1, 43 ml) was stirred at 35 °C for 15 h. Then, 2 M HCl (43 ml) was added and the mixture extracted with Et_2O (3 ×). The organic phase was dried with Na_2SO_4 and the solvent evaporated. The

oily residue was crystallized by addition of Et_2O: 1.46 g (92%) of **13**; colorless crystals; m.p. 130.0 °C. IR (KBr): 3350s, 1720s, 1635s, 1555m, 1530s, 1455m, 1280m, 1250s, 1225s, 1125s, 700m. ^{1}H NMR (200 MHz, DMSO-d_6): 12.61 (br s, COOH); 7.74 (br s, NH); 7.48 (t, J = 6, NH(Gly)); 7.44–7.00 (3m, 10 arom. H); 5.06 (s, PhC**H**$_2$O); 3.62 (d, J = 6, CH_2(Gly)); 3.26, 3.05 (AB, J = 13.5, PhC**H**$_2$); 1.26 (s, MeC(2)). ^{13}C NMR (50.4 MHz, DMSO-d_6): 174.8 (s, COOH); 168.5 (s, CO(amide)); 156.5 (s, CO(urethane)); 137.1, 136.7 (2s, 2 arom. C); 130.4, 128.3, 127.8, 127.76, 127.7, 126.4 (6d, 10 arom. CH); 65.4 (t, PhC**H**$_2$O); 58.6 (s, C(2)(Phe(2Me))); 43.5 (t, CH_2(Gly)); 40.0 (t, PhC**H**$_2$); 22.5 (q, MeC(2)). CI-MS: 371 (79, $[M + 1]^+$), 91 (100). For $C_{20}H_{22}N_2O_5$ (370.41) calculated: 64.85% C, 5.99% H, 7.56% N; found: 65.00% C, 5.93% H, 7.47% N.

Z-Gly-Phe(2Me)-Aib-N(Me)Ph (**14**). To a solution of **13** (1.39 g, 3.75 mmol) in THF (8 ml) at 0 °C was added dropwise 3-[(methyl)(phenyl)amino]-2,2-dimethyl-2*H*-azirine (**2b**; 687 mg, 3.94 mmol). Then, the mixture was stirred at 0 °C for 10 min and at r.t. for 15 h. The solvent was evaporated, the oily residue crystallized from Et_2O and dried under high vacuum: 1.71 g (84%) of **14**; colorless crystals; m.p. 113.0 °C. IR (KBr): 3280m, 1725m, 1675s, 1650s, 1545m, 1495m, 1270m, 1240m, 710m, 700m. ^{1}H NMR (400 MHz, $CDCl_3$): 7.78 (br s, NH); 7.45–7.05 (3m, 15 arom. H); 6.65, 5.47 (2br s, 2 NH); 5.10 (s, PhC**H**$_2$O); 3.80–3.75 (m, CH_2(Gly)); 3.37, 3.22 (AB, J = 13.5, PhC**H**$_2$); 3.30 (s, MeN); 1.59 (s, MeC(2)); 1.43, 1.40 (2s, Me_2C). ^{13}C NMR (50.4 MHz, $CDCl_3$): 173.9, 171.2, 168.2 (3s, 3 CO(amide)); 156.5 (s, CO(urethane)); 144.0, 136.2, 136.0 (3s, 3 arom. C); 130.2, 129.4, 128.5, 128.3, 128.2, 128.0, 126.9 (7d, 15 arom. CH); 67.1 (t, PhC**H**$_2$O); 61.0, 58.9 (2s, C(2)(Phe(2Me)), C(2)(Aib)); 45.1 (t, CH_2(Gly)); 41.8 (t, PhC**H**$_2$); 41.6 (q, MeN); 24.6, 23.7 (2q, Me_2C, MeC(2)). CI-MS: 545 (100, $[M + 1]^+$). For $C_{31}H_{36}N_4O_5$ (544.66) calculated: 68.36% C, 6.66% H, 10.29% N; found: 68.22% C, 6.61% H, 9.96% N.

Z-Gly-Phe(2Me)-Aib-OH (**15**). 11.50 g (21.0 mmol) of **14** were dissolved in THF/6 M HCl (1:1, 212 ml) at 50 °C and the solution stirred at r.t. for 6 h. The colorless precipitate was filtered off and washed with cold Et_2O, and the filtrate was extracted with Et_2O (3 ×). The combined organic phase was dried with Na_2SO_4 and the solvent evaporated. The crystalline residue was dried under high vacuum: 9.26 g (96%) of **15**; colorless crystals; m.p. 203.0 °C. IR (KBr): 3360s, 3320m, 1725s, 1715s, 1660s, 1640s, 1560m, 1530m, 1255m, 1165m. ^{1}H NMR (400 MHz, DMSO-d_6): 7.66 (br s, 2 NH); 7.60 (t, J = 5.8, NH(Gly)); 7.40–7.05 (3m, 10 arom. H); 5.05 (s, PhC**H**$_2$O); 3.59 (d, J = 5.7, CH_2(Gly)); 3.32, 3.18 (AB, J = 13.5, PhC**H**$_2$); 1.35, 1.32, 1.31 (3s, MeC(2), Me_2C). ^{13}C NMR (50.4 MHz, DMSO-d_6): 175.5 (s, COOH); 172.3, 168.8 (2s, 2 CO(amide)); 156.6 (s, CO(urethane)); 137.0, 136.9 (2s, 2 arom. C); 130.4, 128.3, 127.7, 127.6, 126.2 (5d, 10 arom. CH); 65.4 (t, PhC**H**$_2$O); 59.3, 55.2 (2s, C(2)(Phe(2Me)), C(2)(Aib)); 44.2 (t, CH_2(Gly)); 38.9 (t, PhC**H**$_2$); 24.6 (q, **Me**$_2$C); 23.3 (q, MeC(2)). CI-MS: 456 (100, $[M + 1]^+$). For $C_{24}H_{29}N_3O_6$ (455.52) calculated: 63.28% C, 6.42% H, 9.22% N; found: 63.47% C, 6.55% H, 9.09% N.

Z-Gly-Phe(2Me)-Aib-Aib-N(Me)Ph (**16**). To a solution of **15** (1.0 g, 2.20 mmol) in THF (20 ml) and DMF (1 ml) at 0 °C was added dropwise **2b** (402 mg, 2.31 mmol). Then, the mixture was stirred at 0 °C for 10 min and at r.t. for 15 h. The solvent was evaporated and the oily residue was dried under high vacuum: 1.27 g (92%) of **16**; white foam. IR (KBr): 3300m (br), 2990m, 1670s (br), 1595m, 1530s, 1495m, 1455m, 1390m, 1380m, 1365m, 1270m, 1235m, 1095m, 745m, 705m. ^{1}H NMR (400 MHz, CD_3OD): 7.40–7.05 (m, 15 arom. H); 5.11, 5.04 (AB, J = 12.5, PhC**H**$_2$O); 3.77, 3.66 (AB, J = 16.5, CH_2(Gly)); 3.35 (s, MeN); 3.32, 2.98 (AB, J = 13.5, PhC**H**$_2$); 1.51, 1.47, 1.43 (3s, 2 Me_2C); 1.32 (s, MeC(2)). ^{13}C NMR (50.4 MHz, CD_3OD): 176.9, 176.1, 175.3, 172.8 (4s, 4 CO(amide)); 159.6 (s, CO(urethane)); 147.4, 138.3, 137.9

(3s, 3 arom. C); 132.2, 130.6, 129.8, 129.5, 129.4, 129.1, 128.5, 128.3, 128.2 (9d, 15 arom. CH); 68.1 (t, Ph**C**H_2O); 61.2 (s, C(2)(Phe(2Me))); 58.8, 58.5 (2s, 2 C(2)(Aib)); 45.7 (t, CH_2(Gly)); 41.7 (t, Ph**C**H_2); 41.3 (q, MeN); 27.0, 26.7, 26.5, 25.2, 23.8 (5q, 2 **Me**$_2$C, MeC(2)). CI-MS: 523 (100, $[M + 1 - Ph(Me)N]^+$). For $C_{35}H_{43}N_5O_6$ (629.76) calculated: 66.75% C, 6.88% H, 11.12% N; found: 66.63% C, 7.12% H, 11.06% N.

Z-Gly-Phe(2Me)-Aib-Aib-OH (**17**). 11.44 g (18.2 mmol) of **16** were dissolved in THF/6 M HCl (1:1, 182 ml) and the solution was stirred at r.t. for 6 h. The workup as for compound **15** gave 8.6 g (87%) of **17**; colorless solid; m.p. 138.5 °C. IR (KBr): 3310m (br), 1730m (br), 1665s (br), 1530s (br), 1455m, 1280m, 1235m, 1160m. ^{1}H NMR (400 MHz, acetone-d_6): 10.52 (br s, COOH); 7.59, 7.53, 7.44 (3s, 3 NH); 7.40–7.10 (3m, 10 arom. H); 6.95 (X part of ABX, J = 5.0, NH(Gly)); 5.13, 5.04 (AB, J = 12.5, PhC**H**$_2$O); 3.81, 3.80 (AB of ABX, J = 16.5, 5.5, CH_2(Gly)); 3.37, 3.05 (AB, J = 13.5, PhC**H**$_2$); 1.48, 1.38 (2s, 2 Me_2C); 1.30 (s, MeC(2)). ^{13}C NMR (50.4 MHz, acetone-d_6): 175.8, 174.9, 174.4, 171.7 (4s, COOH, 3 CO(amide)); 158.3 (s, CO(urethane)); 137.9, 137.4 (2s, 2 arom. C); 131.6, 129.2, 128.9, 128.7, 128.6, 127.4 (6d, 10 arom. CH); 67.3 (t, Ph**C**H_2O); 60.5 (s, C(2)(Phe(2Me))); 57.4, 57.0 (2s, 2 C(2)(Aib)); 45.6 (t, CH_2(Gly)); 40.8 (t, Ph**C**H_2); 26.3, 25.5, 25.4, 24.5, 23.4 (5q, 2 **Me**$_2$C, MeC(2)). CI-MS: 541 (42, $[M + 1]^+$), 523 (100, $[M + 1 - H_2O]^+$). For $C_{28}H_{36}N_4O_7$ (540.62) calculated: 62.21% C, 6.71% H, 10.36% N; found: 62.20% C, 6.58% H, 10.19% N.

Z-Gly-Phe(2Me)-Aib-Aib-Aib-NMe$_2$ (**18**). To a solution of **17** (9.68 g, 17.9 mmol) in THF (40 ml) and DMF (8 ml) at 0 °C was added dropwise 3-(dimethylamino)-2,2-dimethyl-2H-azirine (**2c**; 2.35 g, 20.9 mmol). Then, the mixture was stirred at 0 °C for 15 h. The workup as for compound **14** gave 11.52 g (99%) of **18**; colorless solid; m.p. 203–212 °C. IR (KBr): 3320m, 3280m, 1705m, 1685s, 1655s, 1625s, 1550m, 1525m, 1270m. ^{1}H NMR (400 MHz, DMSO-d_6): 8.23, 7.87, 7.44 (3s, 3 NH); 7.57 (t, J = 6.0, NH(Gly)); 7.40–7.00 (2m, 10 arom. H, NH); 5.06, 4.99 (AB, J = 12.6, PhC**H**$_2$O); 3.80–3.50 (2m, CH_2(Gly)); 3.31 (s, Me_2N); 3.29, 2.96 (AB, J = 13.3, PhC**H**$_2$); 1.34, 1.33, 1.32, 1.30, 1.28 (5s, 3 Me_2C); 1.19 (s, MeC(2)). ^{13}C NMR (50.4 MHz, DMSO-d_6): 176.6, 173.7, 173.3, 172.2, 170.6 (5s, 5 CO(amide)); 156.9 (s, CO(urethane)); 137.1, 136.9 (2s, 2 arom. C); 131.0, 128.5, 128.04, 128.00, 127.7, 126.5 (6d, 10 arom. CH); 65.7 (t, Ph**C**H_2O); 58.9 (s, C(2)(Phe(2Me))); 56.4, 56.2, 55.7 (3s, 3 C(2)(Aib)); 43.8 (t, CH_2(Gly)); 39.1 (t, Ph**C**H_2); 37.4 (br q, **Me**$_2$N); 26.8, 26.1, 25.7, 24.0, 23.6, 22.5 (6q, 3 Me_2C, MeC(2)). EI-MS: 608 (100, $[M - 45]^+$), 523 (26), 518 (12), 474 (11), 438 (24), 185 (14), 134 (70), 93 (31), 91 (75). For $C_{34}H_{48}N_6O_7$ (652.80) calculated: 62.56% C, 7.41% H, 12.87% N; found: 62.37% C, 7.21% H, 12.78% N.

Z-Gly-Phe(2Me)-Aib-Aib-Aib-OH (**19**). 1.68 g (2.35 mmol) of **18** were dissolved in THF/6 M HCl (1:1, 182 ml) and the solution was stirred at r.t. for 6 h. After this time, the reaction was not complete. The workup as for compound **15** gave 0.92 mg (63%) of **19**. The analogous hydrolysis of 200 mg (0.31 mmol) of **18** under reflux (16 h) gave 168 mg (88%) of **19**; colorless solid; m.p. 223–225 °C. IR (KBr): 3350m, 3310m, 1725m, 1680m, 1670m, 1650s, 1565m, 1555m, 1455w, 1280w, 1255w, 1150w. ^{1}H NMR (400 MHz, DMSO-d_6): 11.13 (br s, COOH); 7.60–7.45 (m, NH); 7.42, 7.38 (2s, 2 NH); 7.30–7.00 (m, 10 arom. H); 5.02, 4.91 (AB, J = 12.3, PhC**H**$_2$O); 3.75–3.50 (m, CH_2(Gly)); 3.26, 2.90 (AB, J = 13.0, PhC**H**$_2$); 1.43, 1.37, 1.36, 1.31 (4s, 3 Me_2C); 1.23 (s, MeC(2)). ^{13}C NMR (50.4 MHz, DMSO-d_6): 178.0 (s, COOH); 176.6, 176.3, 176.0, 172.4 (4s, 4 CO(amide)); 159.3 (s, CO(urethane)); 138.4, 138.0 (2s, 2 arom. C); 132.2, 129.9, 129.5, 129.4, 129.1, 128.1 (6d, 10 arom. CH); 67.9 (t, Ph**C**H_2O); 60.8 (s, C(2)(Phe(2Me))); 58.1, 58.0, 57.1 (3s, 3 C(2)(Aib)); 45.6 (t, Ph**C**H_2); 41.3 (t, CH_2(Gly)); 27.1, 26.8, 26.2, 25.3, 25.1, 24.8, 23.9 (7q, 3 **Me**$_2$C, MeC(2)). A doubling of the signals at 176.6, 176.3, and 58.0 was observed, which disappeared at 60 °C. CI-MS: 626 (25, $[M + 1]^+$), 608

(100, $[M + 1 - H_2O]^+$). For $C_{32}H_{43}N_5O_8$ (625.73) calculated: 61.43% C, 6.93% H, 11.19% N; found: 61.13% C, 7.14% H, 11.04% N.

Z-Gly-Phe(2Me)-Aib-Aib-Gly-OMe (**20**). To a solution of **17** (7.0 g, 12.9 mmol) in DMF (26 ml) at 0 °C was added DCC (2.67 g, 1.29 mmol). After 3 min, $ZnCl_2$ (3.52 g, 25.8 mmol), H-Gly-OMe·HCl (1.86 g, 14.8 mmol), and Et_3N (1.50 g, 14.8 mmol) in DMF (10 ml) were added and the mixture stirred at 0 °C to r.t. for 15 h. Filtration and evaporation of the solvent gave a colorless oil, which was dissolved in CH_2Cl_2, washed with 1 M NaOH, 2 M HCl, and saturated NaCl solution (2 × each). The organic phase was dried over Na_2SO_4, the solvent evaporated, and the residue purified by CC ($MeOH/CH_2Cl_2$ 5:95): 7.21 g (91%) of **20**; colorless foam. IR (KBr): 3320m (br), 1755m, 1710m, 1665s (br), 1545s, 1530s, 1455m, 1385m, 1275m (br), 1230m, 1195m, 1180m. ^{1}H NMR (400 MHz, $CDCl_3$): 7.72 (br s, NH); 7.45–7.05 (2m, 10 arom. H, NH); 6.93, 6.68, 6.08 (3br s, 3 NH); 5.09, 4.96 (AB, *J* = 12.5, PhC**H**$_2$O); 4.02 (d, *J* = 5.0, CH_2(Gly)); 3.80–3.65 (m, CH_2(Gly-OMe)); 3.62 (s, MeO); 3.22, 2.99 (AB, *J* = 13.7, PhC**H**$_2$); 1.54, 1.45 (2s, 2 Me_2C); 1.38 (s, MeC(2)). ^{13}C NMR (50.4 MHz, $CHCl_3$): 176.1 (s, CO(ester)); 173.9, 173.6, 170.6, 170.2 (4s, 4 CO(amide)); 157.5 (s, CO(urethane)); 136.0, 135.3 (2s, 2 arom. C); 130.5, 128.6, 128.4, 128.3, 127.9, 127.2 (6d, 10 arom. CH); 67.3 (t, PhC**H**$_2$O); 59.8 (s, C(2)(Phe(2Me))); 57.2, 57.1 (2s, 2 C(2)(Aib)); 51.8 (q, MeO); 46.0 (t, PhC**H**$_2$); 42.3, 41.3 (2t, 2 CH_2(Gly)); 25.6, 25.4, 25.2, 25.0 (4q, 2 **Me**$_2$C); 23.0 (q, MeC(2)). CI-MS: 612 (4, $[M + 1]^+$), 91 (100). For $C_{31}H_{41}N_5O_8$ (611.70) calculated: 60.87% C, 6.76% H, 11.45% N; found: 60.93% C, 6.93% H, 11.20% N.

Z-Gly-Phe(2Me)-Aib-Aib-Gly-OH. To a solution of **20** (2.0 g, 3.27 mmol) in MeOH (25 ml) at r.t., 2 M NaOH (11 ml) was slowly added. The reaction was complete after 25 min (TLC), 2 M HCl was added until pH 1 was reached, and the mixture was extracted with Et_2O (3 ×) and CH_2Cl_2 (2 ×). The combined organic phase was dried over Na_2SO_4, filtered, and the solvent evaporated. The oily residue was dried under high vacuum: 1.60 g (82%) of Z-Gly-Phe(2Me)-Aib-Aib-Gly-OH; colorless foam. IR (KBr): 3310s, 3270s, 1705m, 1680s, 1670s, 1645m, 1625m, 1540s, 1385m, 1265m, 1230w, 1195w, 1165w. ^{1}H NMR (400 MHz, CD_3OD): 7.70 (s, NH); 7.40–7.0 (m, 10 arom. H); 5.11, 5.06 (AB, *J* = 12.5, PhC**H**$_2$O); 4.00, 3.84 (AB, *J* = 17.7, CH_2(Gly)); 3.84, 3.67 (AB, *J* = 16.7, CH_2(Gly)); 3.37, 3.01 (AB, *J* = 13.6, PhC**H**$_2$); 1.50, 1.49, 1.39, 1.38 (4s, 2 Me_2C); 1.33 (s, MeC(2)). ^{13}C NMR (50.4 MHz, CD_3OD): 178.1 (s, COOH); 176.7, 176.4, 173.1, 172.4 (4s, 4 CO(amide)); 159.5 (s, CO(urethane)); 138.1, 137.6 (2s, 2 arom. C); 132.0, 129.7, 129.4, 129.3, 128.9, 128.1 (6d, 10 arom. CH); 68.1 (t, PhC**H**$_2$O); 60.9 (s, C(2)(Phe(2Me))); 58.3 (s, 2 C(2)(Aib)); 45.6 (t, PhC**H**$_2$); 42.2, 41.5 (2t, 2 CH_2(Gly)); 26.7, 26.4, 25.3, 24.9 (4q, 2 **Me**$_2$C); 23.7 (q, MeC(2)). CI-MS: 580 (100, $[M + 1 - H_2O]^+$). For $C_{30}H_{39}N_5O_8$ (597.67) calculated: 60.29% C, 6.58% H, 11.72% N; found: 60.15% C, 6.85% H, 11.63% N.

H-Gly-Phe(2Me)-Aib-Aib-Gly-OH (**21**). To a solution of Z-Gly-Phe(2Me)-Aib-Aib-Gly-OH (447 mg, 0.75 mmol) in MeOH (2 ml) and AcOEt (1 ml) was added 66 mg Pd/C (10%). Then, H_2 was bubbled through the stirred mixture at 40 °C for 9 h. Filtration and evaporation of the solvent gave a oily residue, which was dried under high vacuum: 334 mg (96%) of **21**; colorless foam. IR (KBr): 3380m, 3270m, 1685s, 1660s, 1590s, 1540s, 1390w, 1285w, 1230w, 1195w. ^{1}H NMR (360 MHz, DMSO-d_6): 8.36 (br s, NH); 7.85, 7.46 (2s, 2 NH); 7.30–7.20, 7.10–7.05 (2m, 5 arom. H); 7.17 (X of ABX, *J* = 6.0, NH(Gly)); 3.52, 3.36 (AB of ABX, *J* = 18.0, 6.0, CH_2(Gly)); 3.37, 3.31 (AB, *J* = 14.5, CH_2(Gly)); 3.27, 3.02 (AB, *J* = 13.4, PhC**H**$_2$); 1.38, 1.37 (2s, 2 Me_2C); 1.20 (s, MeC(2)). ^{13}C NMR (50.4 MHz, D_2O): 177.4, 176.9, 176.8, 175.2 (4s, 4 CO(amide)); 166.9 (s, COOH); 138.5 (s, 1 arom. C); 131.1, 128.8, 127.7 (3d, 5 arom. CH); 60.6 (s, C(2)(Phe(2Me))); 57.5, 57.2 (2s, 2 C(2)(Aib)); 43.9 (t, PhC**H**$_2$); 40.9,

40.8 (2t, 2 CH_2(Gly)); 24.9, 24.7, 24.6, 24.0 (4q, 2 **Me**$_2$C); 22.4 (q, MeC(2)). FAB-MS: 464 (83, $[M + 1]^+$), 304 (100).

Suitable crystals for X-ray crystal-structure determination were obtained from a solution of **21** in MeOH/H_2O by slow evaporation of the solvent.

Z-Gly-Phe(2Me)-Aib-Aib-Aib-Gly-OBn (**23**). To a stirred solution of **19** (677 mg, 1.08 mmol) in DMF (2 ml) at 0 °C was added DCC (223 mg, 1.08 mmol). After 3 min, a solution of H-Gly-OBn·TsOH (420 mg, 1.24 mmol) and Et_3N (114 mg, 1.13 mmol) in DMF (1 ml) was added, then $ZnCl_2$ (301 mg, 2.21 mmol), and the mixture was stirred at 0 °C to r.t. for 2 days. Filtration and evaporation of the solvent gave a colorless oil, which was dissolved in CH_2Cl_2 and washed with 1 M NaOH and 2 M HCl (2 ×). The organic phase was dried over Na_2SO_4 and filtered, the solvent evaporated, and the residue crystallized by addition of Et_2O: 690 mg (83%) of **23**; colorless crystals; m.p. 194.5–199.5 °C. IR (KBr): 3310s (br), 1710m, 1660s (br), 1550s, 1540s, 1530s, 1455m, 1385m, 1265m (br), 1235m (br), 1185m, 1170m. ^{1}H NMR (400 MHz, DMSO-d_6): 8.21, 7.90 (2s, 2 NH); 7.74 (X of ABX, J = 6.0, NH(Gly)); 7.64 (s, NH); 7.57 (X of ABX, J = 5.8, NH(Gly)); 7.40–7.00 (2m, 15 arom. H, NH); 5.08 (s, PhC**H**$_2$O); 5.09, 5.00 (AB, J = 12.6, PhC**H**$_2$O); 3.94, 3.62 (AB of ABX, J = 17.0, 6.0, CH_2(Gly)); 3.93, 3.84 (AB of ABX, J = 17.3, 5.8, CH_2(Gly)); 3.30, 2.94 (AB, J = 13.0, PhC**H**$_2$); 1.37, 1.33, 1.291, 1.286 (4s, 3 Me_2C); 1.19 (s, MeC(2)). ^{13}C NMR (50.4 MHz, CD_3OD, 49.8 °C): 178.1, 177.4, 176.9, 175.7, 172.4, 171.1 (6s, CO(ester), 5 CO(amide)); 158.6 (s, CO(urethane)); 138.1, 137.5, 137.3 (3s, 3 arom. C); 131.9, 129.50, 129.46, 129.3, 129.2, 128.7, 127.9 (7d, 15 arom. CH); 68.0, 67.7 (2t, 2 PhC**H**$_2$O); 60.8 (s, C(2)(Phe(2Me))); 58.3, 58.2, 58.0 (3s, 3 C(2)(Aib)); 45.6 (t, PhC**H**$_2$); 42.4, 41.7 (2t, 2 CH_2(Gly)); 26.6, 26.3, 26.1, 25.2, 24.8, 24.6 (6q, 3 **Me**$_2$C); 23.5 (q, MeC(2)). FAB-MS: 795 (5, $[M + Na]^+$), 773 (10, $[M + 1]^+$), 134 (100). For $C_{41}H_{52}N_6O_9$ (772.91) calculated: 63.72% C, 6.78% H, 10.87% N; found: 63.49% C, 6.68% H, 10.56% N.

H-Gly-Phe(2Me)-Aib-Aib-Aib-Gly-OH (**24**). To a solution of **23** (500 mg, 0.65 mmol) in MeOH (2 ml) and DMF (1 ml) under argon was added 50 mg Pd/C (10%). Then, H_2 was bubbled through the mixture at r.t. for 9 h. The precipitate was dissolved by addition of DMF, then, the solution was filtered through Celite and the solvent evaporated. The residue was crystallized from Et_2O/MeOH: 271 mg (76%) of **24**; colorless crystals; m.p. 211–213 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.16, 7.63, 7.45 (3s, 3 NH); 7.40 (t, J = 5.5, NH(Gly)); 7.35–7.05 (2m, 5 arom. H, NH); 3.63, 3.49 (AB of ABX, J = 17.1, 5.3, CH_2(Gly)); 3.34, 3.26 (AB, J = 16.5, CH_2(Gly)); 3.25, 2.99 (AB, J = 13.5, PhC**H**$_2$); 1.37, 1.36, 1.33, 1.31, 1.29 (5s, 3 Me_2C); 1.21 (s, MeC(2)). CI-MS: 531 (63, $[M + 1 - H_2O]^+$), 353 (100). FAB-MS: 549 (17, $[M + 1]^+$), 474 (31, $[M + 1 - Gly]^+$), 389 (41, $[M + 1 - Gly - Aib]^+$), 304 (100, $[M + 1 - Gly - Aib - Aib]^+$), 191 (37, $[M + 1 - Gly - Aib - Aib - Aib - CO]^+$).

H-Gly-Phe(2Me)-Aib-OH (**26**). As a minor product in the hydrogenolysis of Z-Gly-Phe(2Me)-Aib-Gly-OBn (**28**), 80 mg of **26** were obtained from the HPLC purification (as for compound **29**). ^{1}H NMR (400 MHz, DMSO-d_6): 8.22, 8.20 (2s, 2 NH); 7.30–7.00 (2m, 5 arom. H); 3.60–3.35 (m, CH_2(Gly)); 3.27, 3.18 (AB, J = 14.0, PhC**H**$_2$); 1.33, 1.32 (2s, Me_2C); 1.28 (s, MeC(2)). FAB-MS: 322 (100, $[M + 1]^+$).

Z-Gly-Phe(2Me)-Aib-Gly-OBn (**28**). To a stirred solution of **15** (280 mg, 0.62 mmol) in DMF (1.3 ml) at 0 °C was added DCC (127 mg, 0.62 mmol). After 3 min, $ZnCl_2$ (170 mg, 1.23 mmol) and a solution of H-Gly-OBn·TsOH (239 mg, 0.71 mmol) and Et_3N (62 mg, 0.62 mmol) in DMF (1 ml) were added, and the mixture stirred at 0 °C to r.t. for 2 days. Filtration through Celite and evaporation of the solvent at 70 °C gave a yellowish oil, which was dissolved in CH_2Cl_2 and washed with 1 M NaOH and 1 M HCl, and saturated NaCl solu-

tion (2 × each). The organic phase was dried over Na_2SO_4, filtered, and the solvent evaporated: 350 mg (95%) of crude **28**; colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6): 8.03 (s, NH); 7.76 (X of ABX, J = 5.8, NH(Gly)); 7.58 (X of ABX, J = 6.0, NH(Gly)); 7.43 (s, NH); 7.40–7.00 (m, 15 arom. H); 5.08 (s, PhC**H**$_2$O); 5.05-4.95 (m, PhC**H**$_2$O); 3.82, 3.78 (AB of ABX, J = 17.5, 5.8, CH_2(Gly)); 3.66, 3.40 (AB of ABX, J = 17.0, 6.0, CH_2(Gly)); 3.24, 3.01 (AB, J = 13.4, PhC**H**$_2$); 1.35, 1.32 (2s, Me_2C); 1.21 (s, MeC(2)).

H-Gly-Phe(2Me)-Aib-Gly-OH (**29**). To a suspension of **28** (1.98 g, 3.53 mmol) in MeOH (7 ml) and AcOEt (30 ml) was added 200 mg Pd/C (10%). Then, H_2 was bubbled through the mixture at r.t. for 24 h. The mixture was filtered through Celite, the residue washed with MeOH, and the filtrate evaporated. The yellowish, solid residue was dissolved in 1 M NaOH and washed with Et_2O (3 ×) and AcOEt (3 ×). The aqueous phase was neutralized with 2 M HCl, washed with Et_2O, and evaporated under high vacuum. The residue was dissolved in H_2O and passed through a XAD resin column. After the elution of NaCl with H_2O, the peptide with eluted with MeOH. The solvent was evaporated and the residue dried under high vacuum: 1.09 g (85%) of crude **29**. Purification by HPLC (Lichrosorb RP 18, MeOH/H_2O) gave **29** as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): 8.39 (br s, NH); 7.82 (s, NH); 7.35–7.05 (m, 5 arom. H, NH); 4.00–3.55 (br m, NH_3^+); 3.55–3.30 (m, 2 CH_2(Gly)); 3.27, 3.00 (AB, J = 13.4, PhC**H**$_2$); 1.38, 1.32, 1.23 (3s, MeC(2), Me_2C). FAB-MS: 401 (14, $[M + Na]^+$), 379 (5, $[M + 1]^+$), 134 (100).

Cyclization of Peptides Containing α,α-Disubstituted α-Amino Acids

Cyclo[Gly-Phe(2Me)-Aib-Aib-Gly] (**22**). In analogy to ref.[57], argon was bubbled through a solution of **21** (150 mg, 0.32 mmol) in DMF (30 ml) and the mixture cooled to 0 °C. A cooled solution of DPPA (110 mg, 0.40 mmol) in DMF (9 ml) was added very slowly by means of an infusion pump within 3.5 h. After the addition of $NaHCO_3$ (138 mg, 1.64 mmol), the mixture was stirred vigorously at 0 °C under argon for 6 days. Then, 2 M HCl was added until pH 7 was reached, the mixture was diluted to 100 ml with distilled H_2O, the salts removed by treatment with XAD resin (analytical grade, type 2, 100–200 μm) and H_2O (100 ml), and the organic product was eluted with MeOH/H_2O (1:1) and MeOH. The combined MeOH phase was evaporated and the white residue (powder; 122 mg, 85%) purified by HPLC (Lichrosorb RP 18, H_2O/MeCN 95:1): 86 mg (60%) of **22**. ^{1}H NMR (400 MHz, DMSO-d_6): 8.69, 8.63 (2s, 2 NH); 8.05–7.90 (m, 2 NH); 7.35–7.00 (2m, 5 arom. H, NH); 4.05–3.90 (m, CH_2(Gly)); 3.55–3.40 (m, CH_2(Gly)); 3.34, 2.88 (AB, J = 13.5, PhC**H**$_2$); 1.60, 1.45, 1.33, 1.30 (4s, 2 Me_2C); 1.11 (s, MeC(2)). ^{13}C NMR (50.4 MHz, CD_3OD): 178.4, 176.8, 174.5, 172.1, 171.2 (5s, 5 CO(amide)); 137.9 (s, 1 arom. C); 131.8, 129.1, 127.8 (3d, 5 arom. CH); 62.7 (s, C(2)(Phe(2Me))); 58.8, 58.0 (2s, 2 C(2)(Aib)); 43.8, 43.5 (2t, 2 CH_2(Gly)); 40.7 (t, Ph**C**H$_2$); 25.6, 25.0, 24.1, 23.4, 22.8 (5q, 2 **Me**$_2$C, MeC(2)). FAB-MS: 446 (62, $[M + 1]^+$), 134 (100).

Suitable crystals for X-ray crystal-structure determination were obtained from a solution of **22** in MeOH/H_2O by slow evaporation of the solvent.

Cyclo[Gly-Phe(2Me)-Aib-Aib-Aib-Gly] (**25**). In analogy to compound **22**, to a solution of **24** (70 mg, 0.13 mmol) in DMF (135 ml) at 0 °C, a solution of DPPA (42 mg, 0.15 mmol) in DMF (10 ml) was added slowly. After the addition of $NaHCO_3$ (54 mg, 0.64 mmol), the mixture was stirred vigorously at 0 °C under argon for 4 days. Then, DMF was evaporated, the residue was dissolved in MeCN/H_2O (2:1) and purified by HPLC (Lichrosorb RP 18, H_2O/MeCN): 28 mg (42%) of **25**. ^{1}H NMR (300 MHz, $CDCl_3$): 8.63 (s, NH); 7.55 (X of ABX, J = 7.1, 5.7, NH(Gly2)); 7.35–7.05 (2m, 5 arom. H); 7.19 (s, NH); 6.98 (X of ABX, J = 8.2, 3.5,

NH(Gly1)); 6.83, 6.00 (2s, 2 NH); 4.59, 3.82 (AB of ABX, *J* = 17.0, 8.4, 3.6 CH_2(Gly1)); 4.17, 3.43 (AB of ABX, *J* = 13.3, 7.7, 5.3 CH_2(Gly2)); 3.34, 2.88 (AB, *J* = 13.4, PhC**H**$_2$); 1.65, 1.62, 1.54, 1.52, 1.50, 1.42, 1.39 (7s, 3 Me_2C, MeC(2)). ^{13}C NMR (50.4 MHz, $CDCl_3$): 175.7, 174.5, 174.1, 173.8, 173.5, 172.1 (6s, 6 CO(amide)); 134.1 (s, 1 arom. C); 130.7, 128.4, 127.4 (3d, 5 arom. CH); 60.5 (s, C(2)(Phe(2Me))); 57.5, 56.5 (2s, 3 C(2)(Aib)); 44.7, 44.2, 43.4 (3t, 2 CH_2(Gly), Ph**C**H$_2$); 27.0, 26.6, 25.2, 24.9, 24.0, 23.9 (6q, 3 **Me**$_2$C); 20.9 (q, MeC(2)). FAB-MS: 553 (58, $[M + Na]^+$), 531 (40, $[M + 1]^+$).

Cyclo[Gly-Phe(2Me)-Aib-Gly-Phe(2Me)-Aib] (**27**). In analogy to the preparation of compound **22**, to a suspension of **26** (40 mg, 0.13 mmol) in DMF (124 ml) at 0 °C, a solution of DPPA (39 mg, 0.14 mmol) in DMF (9 ml) was added very slowly by means of an infusion pump within 4 h. After the addition of $NaHCO_3$ (52 mg, 0.62 mmol), the mixture was stirred vigorously at 0 °C under argon for 15 days. Then, DMF was evaporated under high vacuum (55 °C), the residue was dissolved in MeCN/H_2O/MeOH and purified by HPLC (Lichrosorb RP 18, H_2O/MeCN 95:1): 14 mg (37%) of **27**. No cyclotripeptide could be detected. ^{1}H NMR (400 MHz, DMSO-d_6, 23 °C): 8.23, 8.21, 7.63, 7.56 (4s, 4 NH); 7.52 (t, *J* = 5.3, NH(Gly1)); 7.47 (X of ABX, *J* = 6.5, 4.1, NH(Gly2)); 7.30–7.00 (2m, 10 arom. H); 3.91, 3.69 (AB of ABX, *J* = 17.1, 6.7, 4.0, CH_2(Gly2)); 3.80 (d, *J* = 5.3, CH_2(Gly1)); 3.21, 2.97 (AB, *J* = 13.3, PhC**H**$_2$); 3.12, 3.03 (AB, *J* = 13.2, PhC**H**$_2$); 1.42, 1.41, 1.35, 1.33 (4s, 2 Me_2C); 1.20, 1.19 (2s, 2 MeC(2)). ^{1}H NMR (400 MHz, DMSO-d_6, 65 °C): 7.98, 7.97 (2s, 2 NH); 7.45 (t, *J* = 5.4, NH(Gly1)); 7.40 (t, *J* = 5.7, NH(Gly2)); 7.35, 7.29 (2s, 2 NH); 7.30–7.05 (2m, 10 arom. H); 3.88, 3.72 (AB of ABX, *J* = 16.8, 6.3, 4.5, CH_2(Gly2)); 3.80 (d, *J* = 5.4, CH_2(Gly1)); 3.22, 3.04 (AB, *J* = 13.5, PhC**H**$_2$); 3.18, 3.07 (AB, *J* = 13.5, PhC**H**$_2$); 1.431, 1.425, 1.39, 1.38 (4s, 2 Me_2C); 1.253, 1.247 (2s, 2 MeC(2)). ^{1}H NMR (300 MHz, DMSO-d_6, 123 °C): 7.78 (br s, 2 NH); 7.45–7.30 (m, 2 NH); 7.30–7.00 (2m, 10 arom. H, 2 NH); 3.95–3.65 (m, 2 CH_2(Gly)); 3.30–3.05 (m, 2 PhC**H**$_2$); 1.44, 1.42 (2s, 2 Me_2C); 1.30, 1.29 (2s, 2 MeC(2)). ^{13}C NMR (50.4 MHz, DMSO-d_6): 174.2, 172.7, 172.4, 169.7, 169.6 (5s, 6 CO(amide)); 136.9, 136.6 (2s, 2 arom. C); 130.5, 127.8, 126.4, 126.3 (4d, 10 arom. CH); 59.4, 59.3, 57.0, 56.9 (4s, 2 C(2)(Phe(2Me)), 2 C(2)(Aib)); 41.8, 40.7, 40.1, 40.0 (4t, 2 CH_2(Gly), 2 Ph**C**H$_2$); 26.3, 26.0, 24.8, 24.6 (4q, 2 Me_2C); 22.1, 21.9 (2q, 2 **Me**C(2)). FAB-MS: 629 (41, $[M + Na]^+$), 607 (100, $[M + 1]^+$).

Cyclo[Gly-Phe(2Me)-Aib-Gly] (**30**) *and cyclo[Gly-Phe(2Me)-Aib-Gly-Gly-Phe(2Me)-Aib-Gly]* (**31**). In analogy to the preparation of compound **22**, to a solution of **29** (70 mg, 0.19 mmol) in DMF (200 ml) at 0 °C, a solution of DPPA (67 mg, 0.24 mmol) in DMF (9 ml) was added very slowly by means of an infusion pump within 3 h. After the addition of $NaHCO_3$ (80 mg, 0.95 mmol), the mixture was stirred vigorously at 4 °C under argon for 5 days. Then, DMF was evaporated, the residue was dissolved in H_2O, the pH was adjusted to 7 by addition of 2 M HCl, the salts were removed with XAD resin and the organic material eluted with H_2O, MeOH/H_2O (1:1) and MeOH. The solvents were evaporated and the residue purified by HPLC (Lichrosorb RP 18, H_2O/MeCN 95:5 to MeCN 100): Fraction 1: 9 mg (13.5%) of **30**; fraction 2: 21 mg (31.5%) of **31** as a mixture of two diastereoisomers.

Data of **30**: FAB-MS: 383 (100, $[M + Na]^+$), 361 (55, $[M + 1]^+$).

Data of **31**: FAB-MS: 743 (50, $[M + Na]^+$), 721 (100, $[M + 1]^+$). The two diastereoisomers of **31** were separated by HPLC.

Data of **31a**: ^{1}H NMR (400 MHz, $CDCl_3$): 8.25 (br s, NH(Gly1), NH(Gly5)); 7.91 (br s, 2 NH); 7.76 (t, *J* = 5.8, NH(Gly2), NH(Gly8)); 7.40–7.05 (2m, 10 arom. H); 6.20 (br s, 2 NH); 4.18, 3.56 (AB of ABX, *J* = 15.5, 6.0, 5.3, CH_2(Gly2), CH_2(Gly8)); 4.07, 3.77 (AB of ABX, *J* = 17.2, 7.0, 5.4, CH_2(Gly1), CH_2(Gly5)); 3.23, 2.99 (AB, *J* = 13.0, 2 PhC**H**$_2$); 1.48, 1.46 (2s,

2 Me_2C); 1.37 (s, 2 MeC(2)). ^{13}C NMR (50.4 MHz, CD_3OD): 178.8, 176.5, 173.2, 171.8 (4s, 8 CO(amide)); 137.6 (s, 2 arom. C); 132.2, 129.5, 128.3 (3d, 10 arom. CH); 61.2, 58.7 (2s, 2 C(2)(Phe(2Me)), 2 C(2)(Aib)); 44.2, 43.9 (2t, 4 CH_2(Gly)); 42.0 (t, 2 Ph**C**H_2); 26.1, 25.6 (2q, 2 **Me**$_2$C); 23.1 (q, 2 MeC(2)). FAB-MS: 743 (62, $[M + Na]^+$), 721 (100, $[M + 1]^+$).

Suitable crystals for X-ray crystal-structure determination were obtained from a solution of **31a** in MeOH by slow evaporation of the solvent.

Data of **31b**: ^1H NMR (400 MHz, $CDCl_3$): 8.27 (br s, NH(Gly1), NH(Gly5)); 7.71 (t, J = 5.8, NH(Gly2), NH(Gly8)); 7.40-7.10 (2m, 10 arom. H, 2 NH); 6.33 (br s, 2 NH); 3.98, 3.82 (AB of ABX, J = 17.3, 6.2, 5.8, CH_2(Gly1), CH_2(Gly5)); 4.00–3.70 (2m, CH_2(Gly2), CH_2(Gly8)); 3.17, 3.12 (AB, J = 13.3, 2 Ph**C**$\mathbf{H}_2$); 1.46, 1.44, 1.43 (3s, 2 Me_2C, 2 MeC(2)). ^{13}C NMR (50.4 MHz, CD_3OD): 177.7, 176.3, 172.9, 171.5 (4s, 8 CO(amide)); 137.5 (s, 2 arom. C); 132.0, 129.2, 127.9 (3d, 10 arom. CH); 60.8, 58.3 (2s, 2 C(2)(Phe(2Me)), 2 C(2)(Aib)); 43.8, 43.5 (2t, 4 CH_2(Gly)); 41.4 (t, 2 Ph**C**H_2); 27.3 (q, 2 **Me**C(2)); 24.0, 22.3 (2q, 2 Me_2C). FAB-MS: 721 (100, $[M + 1]^+$). ESI-MS: 743 (100, $[M + Na]^+$).

X-ray Crystal-Structure Determination of **21**, **22**, and **31a**

The measurements for **21** and **22** were performed on a Nicolet R3 diffractometer, those for **31a** on a Rigaku AFC5R diffractometer using graphite-monochromatized MoKα radiation (λ = 0.71073 Å) and, in the case of **31a**, a 12-kW rotating anode generator. The data collection and refinement parameters are given in Table IV, and views of the molecules are shown in Figs. 1 and 2. The intensities were corrected for Lorentz and polarization effects, but not for absorption. Equivalent reflections were merged. The structures were solved by direct methods using SHELXS86 [66], which revealed the positions of all non-H atoms. In the case of **21**, the asymmetric unit contains one molecule of the peptide plus four molecules of water. The peptide molecule is protonated at the terminal amino group. Presumably the molecule is a zwitterion with the terminal hydroxy group instead of carboxylate function; an H atom could not be located here, but this terminal group shows disorder of the O atoms, which might inhibit the detection of the hydroxy H atom, even if present. Two sets of overlapping positions were defined for the O atoms of the terminal carboxylate group and the site occupation factor of the major conformation refined to 0.529(13). Similarity restraints were applied to the chemically equivalent bond lengths and angles involving the disordered O atoms and one O–H bond length for one of the water molecules [O(7)] was also restrained. In the case of **22**, the asymmetric unit contains one peptide molecule plus one molecule of water; in the case of **31a**, the asymmetric unit contains one peptide molecule plus five molecules of MeOH, one of which is disordered. Two positions were defined for the atoms of the disordered MeOH molecule and the site occupation factor of the major orientation refined to 0.760(8). The C–O bond lengths of the two orientations were restrained to 1.45(1) Å, while these atoms were restrained to have similar atomic displacement parameters. Atom C(6) was also restrained to have pseudo-isotropic atomic displacement parameters. The non-H atoms in each structure were refined anisotropically. For **21**, the amide and most water H atoms were placed in the positions indicated by a difference electron density map and their positions were refined together with individual isotropic displacement parameters. One H atom could not be located for each of the water molecules O(7) and O(9), while none could be located for water molecule O(10). For **22**, the amide and water H atoms were placed in the positions indicated by a difference electron density map and their positions were refined together with individual isotropic displacement parameters.

TABLE IV
Crystallographic data of compounds **21**, **22**, and **31a**

Parameter	**21**	**22**	**31a**
Crystallized from	MeOH/H_2O	MeOH/H_2O	MeOH
Empirical formula	$C_{22}H_{33}N_5O_6 \cdot 4H_2O$	$C_{22}H_{31}N_5O_5 \cdot H_2O$	$C_{36}H_{48}N_8O_8 \cdot 5MeOH$
Formula weight, g mol^{-1}	535.59	463.53	881.03
Crystal color, habit	colorless, prism	colorless, prism	colorless, prism
Temperature, K	294(1)	294(1)	173(1)
Crystal system	monoclinic	orthorhombic	monoclinic
Space group	$P2_1/n$	$Pna2_1$	$P2_1/c$
Z	4	4	4
Reflections for cell determination	25	25	22
2θ range for cell determination, °	20–28	20–26	37–42
Unit cell parameters			
a, Å	11.091(4)	21.972(3)	29.064(10)
b, Å	17.068(5)	9.498(1)	10.395(5)
c, Å	15.883(4)	11.684(1)	16.108(9)
β, °	104.12(2)	90	96.76(6)
V, $Å^3$	2915(1)	2438.3(5)	4832(4)
D_x, g cm^{-3}	1.220	1.263	1.211
μ(Mo$K\alpha$), mm^{-1}	0.0961	0.0929	0.0904
Scan type	ω	ω	ω
$2\theta_{max}$, °	46	55	40
Total reflections measured	4885	3197	4585
Symmetry independent reflections	4071	2939	4169
Reflections with $I > 2\sigma(I)$	3048	2188	2318
Reflections used in refinement	4071	2939	4169
Parameters refined; restraints	399; 7	332; 1	593; 38
Final $R(F)$ ($I > 2\sigma(I)$ reflections)	0.0474	0.0435	0.0712
$wR(F^2)$ (all data)	0.1283	0.0905	0.2181
Weights[a]: a; b	0.0647; 0.7960	0.0357; 0.1956	0.1235; 0
Goodness of fit	1.032	1.053	1.033
Secondary extinction coefficient	0.0070(9)	0.0040(8)	0.005(1)
Final Δ_{max}/σ	0.001	0.001	0.001
Δρ (max; min), e $Å^{-3}$	0.19; –0.14	0.16; –0.16	0.54; –0.58

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

All remaining H atoms of **21** and **22** and all H atoms of **31a** 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 $1.2U_{eq}$ of its parent C atom ($1.5U_{eq}$ for the methyl groups). The refinement of each structure was carried out on F^2 by using full-matrix least-squares procedures, which minimised the function $\Sigma w(F_o^2 - F_c^2)^2$. A correction for secondary extinction was applied in all cases. Neutral atom scattering factors for non-H atoms were taken from ref.[67a], and the scattering factors for H atoms from ref.[68]. Anomalous dispersion effects were included in F_c [69]; the values for f' and f'' were those of ref.[67b]. The values of the mass attenuation coefficients are those of ref.[67c]. All calculations were performed using the SHELXL97 [70] program.

CCDC 720132–720134 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre* via http://www.ccdc.cam.ac.uk/data_request/cif.

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