Total Synthesis of Sequential Retro-Peptide Oligomers

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The total synthesis of sequential oligomers of retro-peptides of the general formula Boc(-*g*Gly-*m*Aib)$_n$-OBn, starting from dimethylmalonic acid, has been carried out in good overall yield. The key step is the formation of the *g*Gly unit >N–CH_2–N<, which is easily obtained in a one-pot/three-step reaction from BnO-*m*Aib-Gly-OH and diphenylphosphoryl azide. The synthesis of the sequential oligomers of RCO(-*g*Ala-*m*Aib)$_n$-OR′ was also attempted, but the oligomerization step failed because the *g*Ala moiety readily decomposes or racemizes.

Introduction

Backbone modifications of peptides were driven by their use as pharmaceuticals, since the physicochemical properties of most peptides preclude their exploitation as drugs. As the mammalian body presents many barriers to the entry of macromolecules, peptides are affected by poor absorption because they do not readily pass across biological membranes, while swift metabolism by proteolytic enzymes and rapid excretion through the liver and kidneys also take place.[1,2,3] These barriers result in peptides suffering from low bioavailability and short biological half-lives.

Goodman and Chorev[4,5,6] introduced the family of retro-peptides, regioisomers of a parent peptide in which the direction of the amino acid sequence is reversed (peptidomimetics), as a variation of the linear main chain (Figure 1). The nomenclature currently utilized for retro-peptides involves the prefixes *g* (geminal) and *m* (malonic) for the α,α-diamino residue and for the α,α-dicarboxy residue, respectively. For instance, the dipeptide unit shown in Figure 1 is termed *g*Gly-*m*Gly.

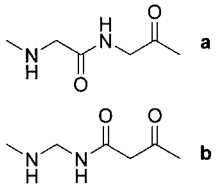

Figure 1. Chemical structures of the parent Gly-Gly (**a**) and retro-modified peptide *g*Gly-*m*Gly (**b**) dipeptide units

To the best of our knowledge, a variety of compounds incorporating single retro-peptide units have been prepared,[7,8,9,10,11,12] but no strictly sequential oligomeric chains of retro-peptides have ever been synthesized. Aleman and co-workers[13,14,15,16,17] calculated the conformational preferences of poly(retro-peptides), including poly(retro-Gly) and its fully C^{α}-methylated analogue poly(retro-Aib)$_n$ (Aib, α-aminoisobutyric acid). These authors concluded that the use of retro-modified peptide units might be useful for the stabilization of specific helical foldamers, the natures of which, however, do not always match those preferred by the parent polypeptides.

In this paper we describe an approach to the synthesis of the poly(retro-peptide) series Boc-(*g*Gly-*m*Aib)$_n$-OBn (Boc, *tert*-butyloxycarbonyl; OBn, benzyloxy) with $n = 1-4$ (Figure 2), which may provide information on the preferred conformations of these compounds.

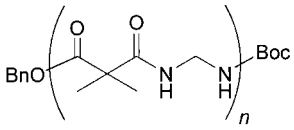

Figure 2. Chemical structure of the sequential Boc-(*g*Gly-*m*Aib)$_n$-OBn ($n = 1-4$; **4**, **7**, **9**, **11**) peptide oligomers

Results and Discussion

The synthesis of the retro-dipeptide unit Boc-*g*Gly-*m*Aib-OBn (**4**) was achieved by starting from 2,2-dimethylmalonic

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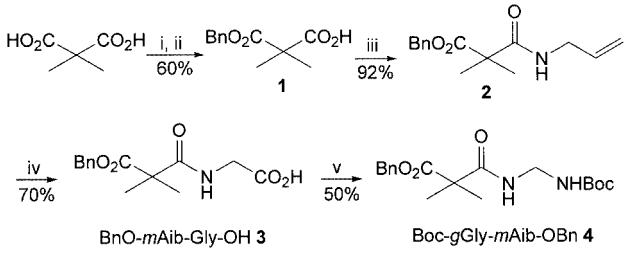
acid, which was transformed into the corresponding benzyl ester **1** by partial hydrolysis of the dibenzyl ester in a well known procedure (Scheme 1).[18] All attempts to obtain the benzyl ester **1** in good yield directly from dimethylmalonic acid failed. The easiest way to introduce a *g*Gly unit was envisaged as by Curtius rearrangement of the corresponding Gly unit, itself in turn obtained by oxidation of an allylamine moiety. To this end the benzyl ester **1** was transformed into the corresponding allylamide **2** in very high yield by treatment with allylamine in the presence of *O*-(6-chloro-1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HCTU)[19] and triethylamine (TEA). Compound **2** was transformed into BnO-*m*Aib-Gly-OH **3** by oxidation of the C=C double bond with potassium permanganate[20] in acetone. The product was obtained as a solid in good yield.



Scheme 1. Synthetic routes for Boc-*g*Gly-*m*Aib-OBn **(4)**; reagents and conditions: (i) BnBr (2.5 equiv.), DIEA (3.5 equiv.), DMAP (0.13 equiv.), CH_2Cl_2, 24 h, r.t.; (ii) KOH (1.1 equiv.), BnOH, 72 h, r.t.; (iii) allylamine (1 equiv.), HCTU (1 equiv.), Et_3N (2 equiv.), CH_3CN, 20 min, r.t.; (iv) $KMnO_4$ (3.5 equiv.), acetone/water/AcOH 82:12:6; (v) DPPA (1 equiv.), Et_3N (1 equiv.), *t*BuOH, 4 h, reflux

Formation of the *g*Gly unit was achieved in a one-pot/three-step reaction between BnO-*m*Aib-Gly-OH **3** and diphenylphosphoryl azide (DPPA) (Figure 3), in an approach involving the formation of an acylazide, which spontaneously decomposes to afford N_2 and a nitrene, which in turn rearranges to the corresponding isocyanate.[21–23] Upon addition of *tert*-butyl alcohol, the isocyanate produces a carbamate unit. As *tert*-butyl alcohol behaves both as a reagent and as a solvent, a large excess is crucial for the production of **4** in a satisfactory yield. An alternative approach allowing **4** to be obtained from the rearrangement of the amide Boc-mAib-Gly−NH_2 by treatment with [bis-(trifluoroacetoxy)iodo]benzene (TIB)[24–27] was also examined, but it afforded the desired compound in an unsatisfactory yield.

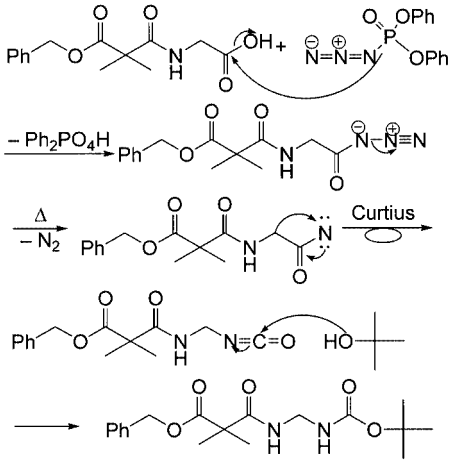
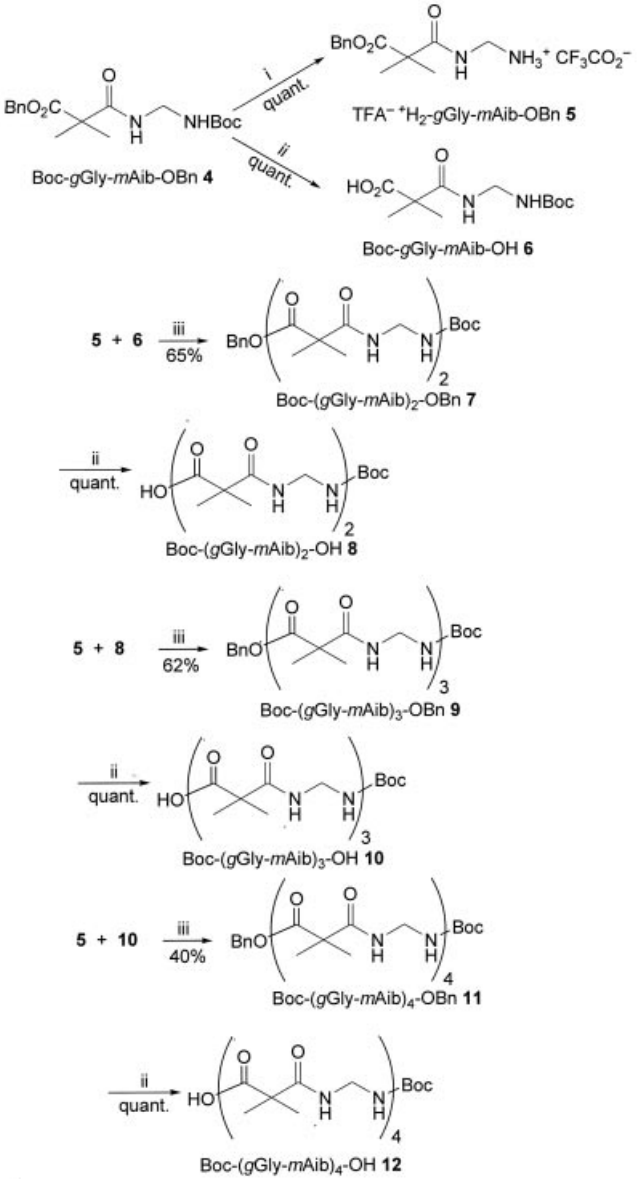


Figure 3. Reaction mechanism for the synthesis of Boc-*g*Gly-*m*Aib-OBn (**4**) by a one-pot/three-step mechanism

Formation of the sequential oligomers Boc-(*g*Gly-*m*Aib)$_n$-OBn (**7**, **9** and **11**) was achieved by consecutive couplings of Boc-(*g*Gly-*m*Aib-)$_n$-OH (n = 1−3) (**6**, **8** and **10**) with TFA (trifluoroacetate)·$^+H_2$-*g*Gly-*m*Aib-OBn (**5**) in acetonitrile in the presence of HCTU and TEA (Scheme 2). Yields were generally good, although Boc-(*g*Gly-*m*Aib)$_4$-



Scheme 2. Synthetic routes for Boc-(*g*Gly-*m*Aib)$_n$-OBn sequential oligomers; reagents and conditions: (i) TFA (18 equiv.), CH_2Cl_2, 4 h, r.t.; (ii) H_2 (3 atm), C/Pd (10%), MeOH, 12 h, r.t.; (iii) TEA (3.5 equiv.), HCTU (1 equiv.), acetonitrile, 20 min, r.t.

OBn (**11**) was obtained only in moderate yield (40%), probably due to the low solubility of Boc-(*g*Gly-*m*Aib)$_3$-OH (**10**) in acetonitrile. All oligomers show decreasing solubility in organic solvents as the main-chain length increases.

Initial investigation of the preferred conformations adopted by the terminally protected oligomers **4**, **7**, **9** and **11** was carried out by IR absorption spectroscopy in the N−H stretching region in chloroform solution (Figure 4). As C=O···H−N-mediated intermolecular aggregation is of minor significance for **4**, **7**, and **9** at 1 mM concentrations (results not shown), only intramolecular hydrogen bonds should need to be considered for these oligomers. Non-hydrogen-bonded N−H bands are typically seen above 3400 cm^{-1}, while hydrogen-bonded N−H bands fall in the 3390−3330 cm^{-1} region.[28,29,30] The figure also shows a remarkable concentration effect taking place above 1 mM concentration for the longest oligomer **11**. Two additional bands at 3300 ± 20 cm^{-1}, indicative of the occurrence of strongly H-bonded NH groups, stand out clearly. In any case, the IR absorption spectra suggest an increasing amount of intramolecularly hydrogen-bonded NH groups with increasing main-chain length.

Analogously, no clearcut results were obtained from ^{1}H NMR titrations of the NH protons by addition of the perturbing agent DMSO (dimethyl sulfoxide) to the $CDCl_3$ solution.[31,32] Figure 5 highlights modest variations for most of the NH proton chemical shifts, which suggest that most of the NH groups are intramolecularly hydrogen bonded, but only weakly.

The above results suggest that the introduction of a substituent on the flexible *g*Gly unit could restrict the conformational space available to the retro-peptides and favour the formation of a helix, as proposed by Aleman and coworkers.[13−17] Unfortunately, insertion of an alkyl moiety into the *g*Gly unit is not a simple task, because an alkyl moiety in an aminal function makes it much less stable. Nonetheless, the preparation of oligomers containing the *g*Ala-*m*Aib dipeptide unit was attempted, by an approach similar to that utilized for the preparation of the oligomers

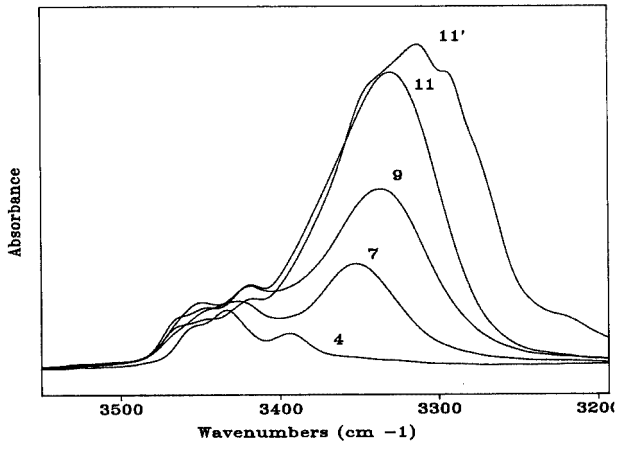


Figure 4. FT-IR absorption spectra in the 3550−3200 cm^{-1} region of the oligomers Boc-(*g*Gly-*m*Aib)$_n$-OBn (**4**, **7**, **9** and **11**) in $CDCl_3$ solution (peptide concentration: 1 mM); the spectrum of **11** at 0.1 mM concentration is also shown (**11′**)

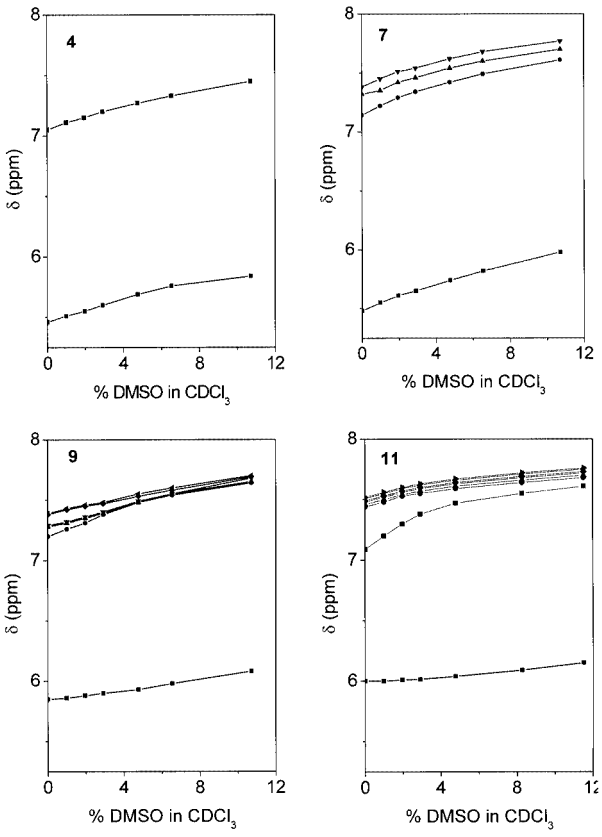
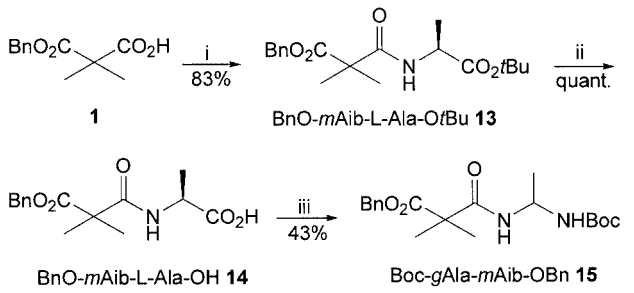


Figure 5. Plot of NH chemical shifts in the ^{1}H NMR spectra of oligomers Boc-(*g*Gly-*m*Aib)$_n$-OBn **4**, **7**, **9** and **11** as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (*v*/*v*); peptide concentration: 1 mM for **4**, **7**, and **9**; 0.1 M for **11**

based on the *g*Gly-*m*Aib unit (Scheme 3). The benzyl ester **1** was coupled with a commercial sample of HCl·H-L-Ala-O*t*Bu (O*t*Bu, *tert*-butyl) in acetonitrile in the presence of HCTU and TEA. The product BnO-*m*Aib-L-Ala-O*t*Bu (**13**) was achieved in high yield, and this was quantitatively hydrolysed to the corresponding carboxylic acid **14**. The synthesis of the *g*Ala unit was achieved by treatment of **14** with DPPA in the one-pot/three-step procedure[21−23] successfully used for the synthesis of Boc-*g*Gly-*m*Aib-OBn (**4**). More specifically, Boc-*g*Ala-*m*Aib-OBn (**15**) was prepared in 43% yield by treatment of BnO-*m*Aib-L-Ala-OH (**14**) with DPPA in *tert*-butyl alcohol at reflux. Again, a large excess of *tert*-butyl alcohol is required for the formation of **15** in good yield. The synthesis of the sequential oligomers Boc-(*g*Ala-*m*Aib)$_n$-OBn by selective deprotection was attempted. The benzyl group at the *m*Aib end was successfully removed by hydrogenolysis with H_2 in the presence of Pd (10%) on charcoal in a quantitative yield. Unfortunately, removal of the Boc group at the *g*Ala end did not afford the desired product, but instead gave the benzyl dimethylmalonate amide (H-*m*Aib-OBn). This unwanted result was probably due to the spontaneous decomposition of $^+H_2$-*g*Ala-*m*Aib-OBn, possibly favoured by traces of water,

with formation of acetaldehyde and ammonia as by-products.

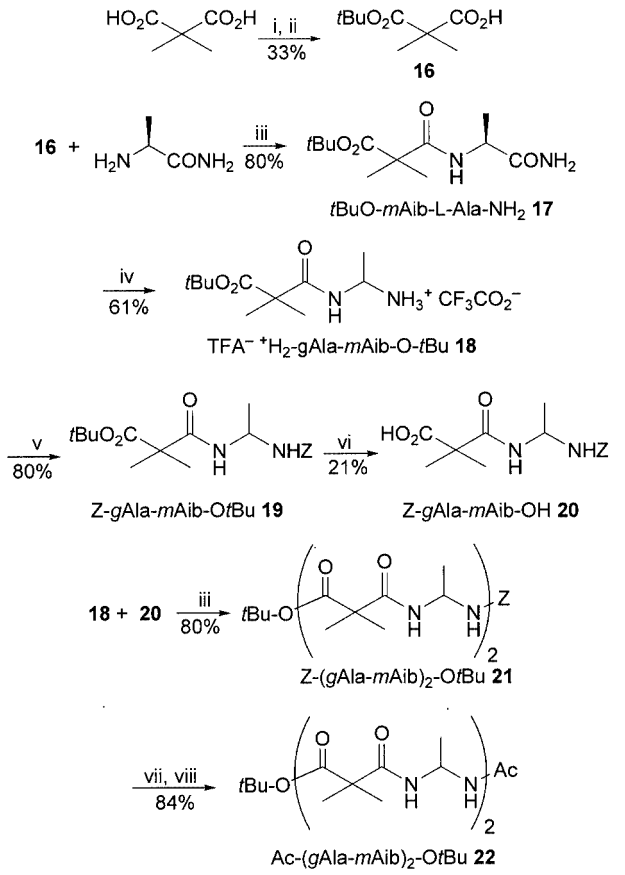


Scheme 3. Synthetic routes for Boc-*g*Ala-*m*Aib-OBn **(15)**; (i) HCl•H-L-Ala-O*t*Bu (1 equiv.), Et_3N (3 equiv.), HCTU (1 equiv.), acetonitrile, 20 min, r.t.; (ii) TFA (9 equiv.), CH_2Cl_2, 4 h, r.t.; (iii) DPPA (1 equiv.), Et_3N (1.1 equiv.), *t*BuOH, 5 h, reflux

We were thus forced to try an alternative approach to circumvent the acidic deprotection step of the sensitive *g*Ala unit. Dimethylmalonic acid was transformed into the corresponding di-*tert*-butyl ester by treatment with a large excess of isobutene under acidic conditions, and this was partially hydrolysed with TFA to afford the mono *tert*-butyl ester **16** (Scheme 4). The L-Ala residue was introduced by treatment of **16** with H-L-Ala-NH_2 in the presence of 1-hydroxy-7-aza-1,2,3-benzotriazole (HOAt), 1-(3-dimethylamino)propyl-3-ethylcarbodiimide hydrochloride (EDC·HCl) and *N*-methylmorpholine (NMM) to afford **17** in very good yield. Formation of the *g*Ala unit was achieved in satisfactory yield by treatment of **17** with TIB.[24−27] As this reagent promotes the Curtius rearrangement of a primary amide under very mild conditions, we judged that it might be appropriate for this sensitive compound. Formation of **18** by this reaction was confirmed by X-ray diffraction analysis (Figure 6), which clearly showed the presence of an aminal function.

Compound **18** contains a free amino group, ready for the formation of the oligomer Z-(*g*Ala-*m*Aib)$_2$-O*t*Bu (**21**). To prepare the carboxylic unit for the coupling reaction, **18** was transformed into the corresponding carbobenzoxy (Z) derivative Z-*g*Ala-*m*Aib-O*t*Bu (**19**) in high yield, by treatment with Z−OSu (OSu, oxysuccinimide). Subsequently, the *tert*-butyl ester of **19** was cleaved under acidic conditions to afford Z-*g*Ala-*m*Aib-OH (**20**).

The synthesis of the oligomer Z-(*g*Ala-*m*Aib)$_2$-O*t*Bu (**21**) was achieved in high yield by coupling of **18** with **20** in the presence of HOAt, EDC·HCl and NMM under standard conditions. However, HPLC and ^{1}H NMR analyses of **21**, containing two *g*Ala units, suggested that a mixture of diastereomers might be present. It is reasonable to assume that the formation of a set of diastereomeric products could have originated from (partial) racemization either during the rearrangement of the L-Ala residue to the *g*Ala unit or during the subsequent deprotonation step required for coupling. The result of a X-ray diffraction study of compound **18** does not clash with this hypothesis, because a single crystalline enantiomer, as found in our case, can be



Scheme 4. Synthetic routes for Ac-(*g*Ala-*m*Aib)$_2$-O*t*Bu **(22)**; reagents and conditions: (i) isobutene (35 equiv.), H_2SO_4 (0.24 equiv.), CH_2Cl_2, 240 h, r.t.; (ii) TFA (8 equiv.), CH_2Cl_2, 20 min, r.t;. (iii) EDC·HCl (1.1 equiv.), HOAt (1 equiv.), NMM (2 equiv.), CH_2Cl_2, 48 h, r.t.; (iv) TIB (1.4 equiv.), CH_3CN/H_2O (6:4), 48 h, r.t.; (v) Z-OSu (1.3 equiv.), NMM (2 equiv.), CH_2Cl_2, 48 h, r.t.; (vi) TFA (18 equiv.), CH_2Cl_2, 35 min, 0 °C; (vii) H_2 (3 atm), C/Pd (10%), MeOH, 12 h, r.t.; (viii) Ac_2O (10 equiv.), CH_2Cl_2, 24 h, r.t.

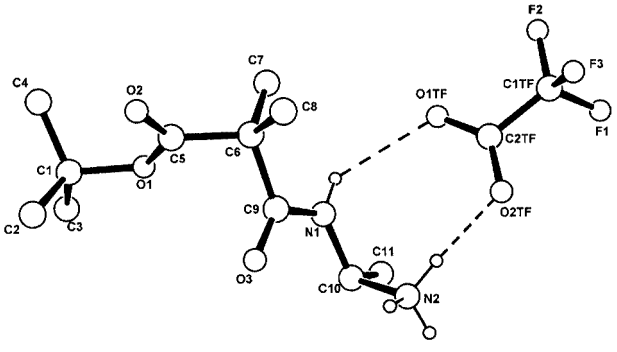


Figure 6. X-ray diffraction structure of TFA·$^+$H$_2$-*g*Gly-*m*Aib-O*t*Bu (**18**) with numbering of the atoms; the N−H···O=C H-bonds are represented by dashed lines

obtained even from a solution of a racemate. In addition, we have been unable to assign the absolute configuration of the single *g*Ala stereogenic centre of **18** from our X-ray diffraction analysis, as this compound lacks atoms heavier

than oxygen, thus preventing determination by the anomalous dispersion method.

Analytical HPLC of the more polar oligomer Ac-(*g*Ala-*m*Aib)$_2$-O*t*Bu (**22**) obtained by hydrogenolysis of the carbobenzoxy *N*-protecting group of **21**, followed by acetylation of the resulting free amino function, indicated more clearly that two peaks (most probably each corresponding to a diastereomer formed by a pair of unseparated enantiomers) were present in the product mixture. However, even with the more promising **22**, HPLC separation of the diastereomeric compounds at the preparative level proved impossible. Nevertheless, the ^{13}C NMR spectrum of the product mixture confirmed the presence of two diastereomers, as each carbon signal was doubled (Figure 7).

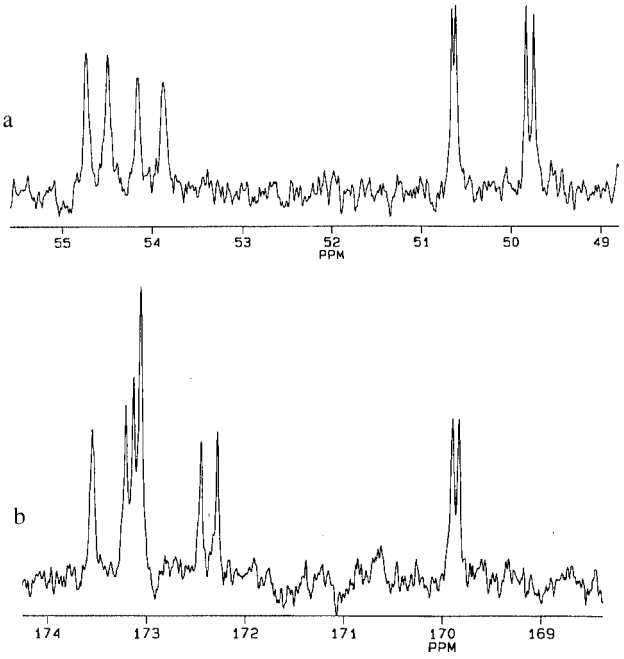


Figure 7. Partial ^{13}C NMR spectrum of Ac-(*g*Ala-*m*Aib)$_2$-O*t*Bu (**22**) in $CDCl_3$ solution; only the spectral regions of the α-carbons (N−*C*−N and CO−*C*−CO) (**a**) and carbonyl carbons (**b**) are shown

Conclusions

The sequential oligomers Boc-(*g*Gly-*m*Aib)$_n$-OBn (n = 1−4) have been synthesized in good yields. The key step was the formation of the >N−CH_2−N< *g*Gly unit, which was easily obtained in a one-pot/three-step reaction starting from BnO-*m*Aib-Gly-OH and DPPA. The reaction mechanism involves formation of an acylazide, which spontaneously decomposes to generate N_2 and a nitrene, which in turn rearranges to provide the corresponding isocyanate. This latter intermediate reacts with *tert*-butyl alcohol to afford a carbamate moiety. The oligomers were easily prepared, but a conformational analysis, carried out by IR absorption and ^{1}H NMR techniques in a structure-supporting solvent, did not afford clear-cut results. The synthesis of the more conformationally restricted, sequential oligomers (*g*Ala-*m*Aib)$_n$ was therefore attempted. Unfortunately, although two different approaches were used, neither was successful, owing to the presence of the very sensitive *g*Ala unit, which has a high tendency to decompose and racemize. Further studies directed towards the synthesis of this series of sequential retro-modified peptides are currently in progress in the Bologna laboratory.

Experimental Section

Synthesis and Chemical Characterization: Materials and reagents were of the highest commercially available grade and were used without further purification. Reactions were monitored by thin-layer chromatography on Merck 60 F254 silica gel covered plastic plates. Compounds were viewed under UV light and by use of ceric ammonium molybdate. Flash chromatography was performed on a Merck 60 silica gel stationary phase. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 300 or Varian Mercury 400 spectrometers. Chemical shifts are reported in δ values relative to the solvent peak of $CHCl_3$, set at δ = 7.27 ppm. Infrared absorption spectra were recorded with a Nicolet 210 FT-IR absorption spectrometer. Melting points were determined in open capillaries and are uncorrected.

Phenylmethyl Dimethylmalonate (1): DIEA (266 mmol, 44 mL) and DMAP (10 mmol, 1.22 g) were added to a stirred solution of 2,2-dimethylmalonic acid (76 mmol, 10 g) in dichloromethane (60 mL) under inert atmosphere. Benzyl bromide (190 mmol, 22 mL) was then added and the mixture was stirred at room temp. for 24 h. Water (100 mL) and dichloromethane (40 mL) were added, and the organic layer was separated, washed with brine, aqueous HCl (1 N, 3 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over sodium sulfate and concentrated in vacuo. Dibenzyl dimethylmalonate was obtained pure in 84% yield (19.9 g) as a liquid, after silica gel chromatography (cyclohexane/ethyl acetate, 9:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.48 (s, 6 H, 2 × CH_3), 5.12 (s, 4 H, 2 × OCH_2Ph), 7.20−7.39 (m, 10 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.7, 49.8, 66.7, 127.6, 127.9, 128.2, 135.4, 172.2. IR (film): $\tilde{\nu}$ = 1729 (C=O) cm^{-1}. A solution of KOH (70 mmol, 4 g) in benzyl alcohol (96 mL) was added to a stirred of dibenzyl dimethylmalonate (64 mmol, 20 g) and the mixture was stirred for 72 h. The mixture was diluted with diethyl ether (1.5 L) and washed with water (2 × 100 mL). HCl (6 N) was added to the aqueous layer to pH 2, and the product **1** was extracted with ethyl acetate (2 × 150 mL). The ethyl acetate solution was dried over sodium sulfate and concentrated in vacuo. Benzyl dimethylmalonate was obtained pure in 72% yield (10.2 g) as a solid after recrystallization from cyclohexane/ethyl acetate; m.p. 48−50 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.51 (s, 6 H, 2 × CH_3), 5.20 (s, 2 H, OCH_2Ph), 7.27−7.38 (m, 5 H, Ph), ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.6, 49.9, 67.1, 127.7, 128.1, 128.4, 135.3, 172.1, 178.8. IR (nujol): $\tilde{\nu}$ = 1747, 1703 cm^{-1} (C=O).

Phenylmethyl 2-Methyl-2-(2-propenyl)amidopropanoate (2): HCTU (4.5 mmol, 1.86 g), allylamine (4.5 mmol, 0.34 mL) and triethylamine (9 mmol, 1.25 mL) were added at room temp. to a stirred solution of benzyl dimethylmalonate (**1**, 4.5 mmol, 1 g) in acetonitrile (20 mL). After 20 min the mixture was washed with brine, aqueous HCl (1 N, 3 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over sodium sulfate and concentrated in vacuo. Compound **2** was obtained pure as a liquid in 92% yield (1.08 g) after silica gel chromatography (cyclohexane/ethyl acetate, 8:2). ^{1}H

NMR (300 MHz, $CDCl_3$): δ = 1.49 (s, 6 H, 2 × CH_3), 3.83 [tt, $^3J_{H,H}$ = 1.5, 5.5 Hz; 2 H, CH_2−CH=CH_2], 5.04−5.16 (m, 2 H, CH_2−CH=CH_2), 5.18 (s, 2 H, OCH_2Ph), 5.68−5.82 (m, 1 H, CH_2−CH=CH_2), 6.37 (br. s, 1 H, NH), 7.25−7.40 (m; 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.4, 41.9, 49.9, 66.9, 115.9, 127.8, 128.2, 128.4, 133.7, 135. 3, 171.4, 174.3. IR (film): $\tilde{\nu}$ = 3430, 3337 (N−H), 1733, 1653 (C=O) cm^{-1}.

BnO-*m*Aib-Gly-OH (3): $KMnO_4$ (105 mmol, 16.6 g) was added portionwise at 0 °C to a stirred solution of **2** (30 mmol, 8 g) in acetone (175 mL), water (25 mL) and acetic acid (14 mL). The mixture was stirred for 1 h, and Na_2SO_3 (3 g) was then added until the purple colour disappeared. Acetone was removed under reduced pressure, and HCl (1 N) was added to pH ≈ 2. Ethyl acetate (200 mL) was added, and the organic layer was separated, washed with brine (100 mL) and water (100 mL), dried over sodium sulfate and concentrated under reduced pressure. Compound **3** was obtained pure as a solid in 70% yield (5.86 g); m.p. 69−71 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.50 (s, 6 H, 2 × CH_3), 4.03 [d, $^3J_{H,H}$ = 5.4 Hz; 2 H, NCH_2CO), 5.18 (s, 2 H, OCH_2Ph), 7.07 [br. t, $^3J_{H,H}$ = 5.4 Hz; 1 H, NH], 7.31−7.38 (m, 5 H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.8, 41.9, 50.2, 67.6, 128.3, 128.7, 128.9, 135.6, 173.0, 173.5, 174.5 ppm. IR (nujol): $\tilde{\nu}$ = 3380 (N−H), 1733, 1653 (C=O) cm^{-1}.

Boc-*g*Gly-*m*Aib-OBn (4): A solution of **3** (3.6 mmol, 1.0 g), DPPA (0.36 mmol, 0.78 mL) and triethylamine (3.6 mmol, 0.5 mL) in *tert*-butyl alcohol (20 mL) was stirred at reflux for 4 h. The solvent was then removed and replaced with ethyl acetate (20 mL). The resulting solution was washed with HCl (1 N, 15 mL), water (15 mL), 5% aqueous $NaHCO_3$ (15 mL) and brine (15 mL), dried over sodium sulfate and concentrated in vacuo. Compound **4** was obtained pure as a liquid in 50% yield (0.63 g) after silica gel chromatography (cyclohexane/ethyl acetate, 8:2); m.p. 79−81 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*Bu), 1.45 (s, 6 H, 2 × CH_3), 4.48 [t, $^3J_{H,H}$ = 6.3 Hz; 2 H, NCH_2N], 5.17 (s, 2 H, OCH_2Ph), 5.50 (br. s, 1 H, NH), 7.08 (br. s, 1 H, NH), 7.26−7.39 (m, 5 H, Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 23.2, 28.2, 46.3, 49.9, 67.0, 79.8, 127.7, 128.1, 128.4, 135.3, 155.8, 172.7, 173.5. IR (nujol): $\tilde{\nu}$ = 3317 (N−H), 1739, 1699, 1646 (C=O) cm^{-1}.

TFA·$^+$H$_2$-*g*Gly-*m*Aib-OBn (5): Boc-*g*Gly-*m*Aib-OBn (**4**, 1.6 mmol, 0.55 mg) in dry dichloromethane (10 mL) and TFA (28.8 mmol, 2.2 mL) was stirred for 4 h at room temp., and the volatiles were removed in vacuo. Compound **5** was obtained in quantitative yield and was used without any further purification; m.p. 88−90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 6 H, 2 × CH_3), 4.52 (d, $^3J_{H,H}$ = 5.4 Hz; 2 H,), 5.14 (s, 2 H, OCH_2Ph), 7.23−7.39 (m, 5 H, Ph), 8.19 (br. s, 3 H, NH_3), 8.46 [br. t, $^3J_{H,H}$ = 5.4 Hz; 1 H, NH]. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.8, 46.7, 50.2, 67.7, 127.9, 128.1, 128.6, 135.0, 161.3, 173.7, 175.4. IR (nujol): $\tilde{\nu}$ = 3357 (N−H), 1719, 1672 (C=O) cm^{-1}.

General Method for the Hydrogenolysis of the Benzyl Esters: Palladium on charcoal (10%, 20 mg) was added to a solution of Boc(-*g*Gly-*m*Aib)$_n$-OBn (1 mmol) in methanol (20 mL), and the mixture was stirred under H_2 (≈3 atm) for 12 h. The catalyst was then filtered through a celite pad and the mixture was concentrated. The corresponding carboxylic acid was obtained pure as a solid in quantitative yield without any further purification.

General Method for Coupling: A solution of TFA·$^+$H$_2$-*g*Gly-*m*Aib-OBn (**5**, 2.6 mmol, 0.95 g) and triethylamine (9.1 mmol, 1.3 mL) in acetonitrile (20 mL) was stirred for 30 min at room temp. Separately, Boc(-*g*Gly-*m*Aib)$_n$-OH (2.6 mmol) and HCTU (2.6 mmol) in dry acetonitrile (20 mL) were stirred for 10 min at room temp. The two solutions were mixed dropwise and the mixture was stirred for an additional 20 min. The acetonitrile was then removed under reduced pressure and replaced with ethyl acetate (50 mL). The resulting solution was washed with aqueous HCl (1 N, 3 × 30 mL) and aqueous $NaHCO_3$ (5%, 1 × 30 mL), dried over sodium sulfate and concentrated in vacuo.

Boc-*g*Gly-*m*Aib-OH (6): Quantitative yield; m.p. 138−139 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, *t*Bu), 1.47 (s, 6 H, 2 × CH_3), 4.53 [t, $^3J_{H,H}$ = 6.3 Hz; 2 H, NCH_2CO], 5.99 [br. t, $^3J_{H,H}$ = 6.3 Hz; 1 H, NH], 8.00 (br. s, 1 H). ^{13}C NMR (100 MHz, CD_3OD): δ = 22.6, 27.5, 45.9, 49.8, 79.5, 157.0, 174.8, 176.0. IR (nujol): $\tilde{\nu}$ = 3336 (N−H), 1699, 1652 (C=O) cm^{-1}.

Boc(-*g*Gly-*m*Aib)$_2$-OBn (7): 65% yield; m.p. 155−156 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.37 (s, 6 H, 2 × CH_3), 1.43 (s, 6 H, 2 × CH_3), 1.44 (s, 9 H, *t*Bu), 4.48 (t, $^3J_{H,H}$ = 6.4 Hz; 2 H, NCH_2N), 4.59 (t, $^3J_{H,H}$ = 6.3 Hz; 2 H, NCH_2N), 5.15 (s, 2 H, OCH_2Ph), 5.61 [br. t, $^3J_{H,H}$ = 6.4 Hz; 1 H, NH], 7.24 [br. t, ^3J (H, H) = 6.3 Hz; 1 H, NH], 7.30−7.41 (m, 6 H, Ph + NH), 7.46 [br. t, $^3J_{H,H}$ = 6.4 Hz; 1 H, NH]. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.5, 23.7, 28.5, 45.5, 46.6, 49.6, 50.3, 67.5, 80.4, 128.2, 128.6, 128.9, 135.6, 156.2, 173.4, 174.1, 174.5, 174.7. IR (nujol): $\tilde{\nu}$ = 3340, 3318 (N−H), 1746, 1695, 1645 (C=O) cm^{-1}.

Boc(-*g*Gly-*m*Aib)$_2$-OH (8): Quantitative yield; m.p. 205−207 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 6 H, 2 × CH_3), 1.44 (s, 6 H, 2 × CH_3), 1.47 (s, 9 H, *t*Bu), 4.50 [t, $^3J_{H,H}$ = 5.7 Hz; 2 H, NCH_2N], 4.64 [t, $^3J_{H,H}$ = 5.7 Hz; 2 H, NCH_2N], 5.87 (br. s, 1 H, NH), 7.30 (br. s, 1 H, NH), 7.53 (br. s, 1 H, NH), 7.69 (br. s, 1 H, NH). ^{13}C NMR (75 MHz, CD_3OD): δ = 23.7, 23.8, 28.7, 46.2, 47.2, 51.1, 51.2, 80.7, 175.9, 176.0, 176.3, 176.9. IR (nujol): $\tilde{\nu}$ = 3326 (N−H), 1737, 1635 (C=O) cm^{-1}.

Boc(-*g*Gly-*m*Aib)$_3$-OBn (9): 62% yield; m.p. 214−215 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (s, 6 H, 2 × CH_3), 1.41 (s, 6 H, 2 × CH_3), 1.44 (s, 6 H, 2 × CH_3), 1.46 (s, 9 H, *t*Bu), 4.51 [t, $^3J_{H,H}$ = 6.3 Hz; 2 H, NCH_2N], 4.59 [t, $^3J_{H,H}$ = 6 Hz; 2 H, NCH_2N], 4.60 [t, $^3J_{H,H}$ = 6 Hz; 2 H, NCH_2N], 5.17 (s, 2 H, OCH_2Ph), 5.90 (br. s, 1 H, NH), 7.21−7.48 (m, 10 H, Ph +5 × NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.2, 23.4, 28.3, 45.4, 46.5, 49.7, 50.1, 67.1, 79.9, 127.8, 128.3, 128.6, 135.5, 155.0, 173.2, 173.8, 174.4. IR (nujol): $\tilde{\nu}$ = 3410, 3337 (N−H), 1711, 1649 (C=O) cm^{-1}.

Boc(-*g*Gly-*m*Aib)$_3$-OH (10): Quantitative yield; m.p. 220−223 °C (dec.). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 6 H, 2 × CH_3), 1.43 (s, 6 H, 2 × CH_3), 1.45 (s, 6 H, 2 × CH_3), 1.48 (s, 9 H, *t*Bu), 4.55 [t, $^3J_{H,H}$ = 6.3 Hz; 2 H, NCH_2N], 4.60−4.66 [m, 4 H, 2 × NCH_2N], 5.94 (br. s, 1 H, NH), 7.27−7.32 (br. s, 2 H, 2 × NH), 7.57 (br. s, 3 H, 3 × NH). ^{13}C NMR (75 MHz, CD_3OD): δ = 23.7, 23.8, 28.7, 46.2, 46.4, 47.3, 51.1, 51.2, 51.3, 66.5, 80.7, 158.1, 175.9, 176.3, 177.0. IR (nujol): $\tilde{\nu}$ = 3324 (N−H), 1733, 1714, 1704, 1652 (C=O) cm^{-1}.

Boc(-*g*Gly-*m*Aib)$_4$-OBn (11): 40% yield; m.p. 235−237 °C. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.21 (s, 6 H, 2 × CH_3), 1.23 (s, 12 H, 4 × CH_3), 1.31 (s, 6 H, 2 × CH_3), 1.36 (s, 9 H, *t*Bu), 4.29 [t, $^3J_{H,H}$ = 4.0 Hz; 2 H, NCH_2N], 4.39−4.44 (m, 6 H, 3 × NCH_2N), 5.08 (s, 2 H, OCH_2Ph), 7.09 (br. s, 1 H, NH), 7.29−7.37 (m, 5 H, Ph), 7.95 (br. s, 6 H, NH), 8.14 (br. s, 1 H, NH). ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 22.7, 23.2, 28.0, 30.5, 44.8, 49.1, 49.7, 65.8, 127.2, 127.7, 128.2, 136.0, 159.2, 171.9, 172.8, 172.9, 173.0. IR (nujol): $\tilde{\nu}$ = 3403, 3337 (N−H), 1712, 1649 (C=O) cm^{-1}.

Boc(-*g*Gly-*m*Aib)$_4$-OH (12): Quantitative yield; m.p. 255−260 °C (dec.). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.20 (s, 6 H, 2 ×

CH_3), 1.22 (s, 18 H, 6 × CH_3), 1.34 (s, 9 H, *t*Bu), 4.28 [t, $^3J_{H,H}$ = 5.8 Hz; 2 H, NCH_2N], 4.38−4.43 (m, 6 H, 3 × NCH_2N), 7.08 (br. s, 1 H, NH), 7.91 [t, 1 H, $^3J_{H,H}$ = 5.4 Hz; NH], 7.92−8.20 (m, 5 H, 5 × NH), 8.10 (br. s, 1 H, NH). ^{13}C NMR (150 MHz, [D_6]DMSO): δ = 23.8, 23.9, 24.0, 28.8, 31.4, 45.4, 45.6, 49.9, 50.0, 173.8, 173.9. IR (nujol): ṽ = 3330 (N−H), 1720, 1708, 1688, 1653 (C=O) cm^{-1}.

BnO-*m*Aib-L-Ala-O*t*Bu (13): A solution of HCl·H-L-Ala-O*t*Bu (24 mmol, 4.36 g) and triethylamine (72 mmol, 10 mL) in acetonitrile (50 mL) was stirred for 30 min at room temp. Separately, a solution of benzyl dimethylmalonate (**1**, 24 mmol, 5.29 g) and HCTU (24 mmol, 9.93 g) in acetonitrile (50 mL) was stirred at room temp. for 5 min. The first solution was then added dropwise at room temp., and the resulting mixture was stirred for an additional 20 min. The acetonitrile was then removed under reduced pressure and replaced with dichloromethane (20 mL). The mixture was washed with aqueous HCl (1 N, 3 × 30 mL) and aqueous $NaHCO_3$ (5%, 1 × 30 mL), dried with sodium sulfate and concentrated in vacuo. The product was obtained pure as a liquid after silica gel chromatography (cyclohexane/ethyl acetate, 8:2) in 83% yield (6.95 g). $[\alpha]_D^{20}$ = −1.1 (*c* = 1.0, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.28 [d, $^3J_{H,H}$ = 7.2 Hz; 3 H, Ala−CH_3], 1.45 (s, 9 H, *t*Bu), 1.47 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 4.38 [q, $^3J_{H,H}$ = 7.2 Hz; 1 H, Ala-CH), 5.18 (s, 2 H, OC*H*$_2$Ph), 6.83 [d, $^3J_{H,H}$ = 6.8 Hz; 1 H, NH], 7.34 (m, 5 H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.0, 23.2, 23.3, 27.8, 48.7, 49.8, 66.9, 81.7, 127.9, 128.1, 128.4, 135.4, 165.6, 170.9, 171.8, 174.0. IR (film): ṽ = 3350 (N−H), 1739, 1666 (C=O) cm^{-1}.

BnO-*m*Aib-L-Ala-OH (14): BnO-*m*Aib-L-Ala-O*t*Bu (**13**, 20 mmol, 6.9 g) in dry dichloromethane (50 mL) and TFA (180 mmol, 14 mL) was stirred for 4 h at room temp. The volatiles were then removed in vacuo, and the product **14** was obtained as a thick liquid in quantitative yield and was used without any further purification. $[\alpha]_D^{20}$ = −18.2 (*c* = 1.1, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.38 [d, $^3J_{H,H}$ = 7.0 Hz; 3 H, Ala−CH_3], 1.49 (s, 6 H, *t*Bu), 4.52 [dq, $^3J_{H,H}$ = 7.0 Hz; 1 H, Ala−CH], 5.19 (s, 2 H, OC*H*$_2$Ph), 6.97 [d, $^3J_{H,H}$ = 6.6 Hz; 1 H, NH], 7.32−7.39 (m, 5 H, Ph), 7.80 (s, 1 H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.5, 23.2, 23.4, 48.4, 49.8, 67.3, 128.0, 128.3, 128.5, 135.2, 172.3, 174.0, 176.5. IR (film): ṽ = 3364 (N−H), 1733, 1716, 1652 (C=O) cm^{-1}.

Boc-*g*Ala-*m*Aib-OBn (15): A solution of **14** (4.7 mmol, 1.38 g), DPPA (4.7 mmol, 1.02 mL) and triethylamine (5.2 mmol, 0.72 mL) in *tert*-butyl alcohol (25 mL) was stirred at reflux for 5 h. The solvent was then removed and replaced with ethyl acetate (20 mL). The resulting solution was washed with HCl (1 N, 15 mL), water (15 mL), aqueous $NaHCO_3$ (5%, 15 mL) and brine (15 mL), dried over sodium sulfate and concentrated in vacuo. Compound **15** was obtained pure as a liquid in 43% yield (0.74 g), after silica gel chromatography (cyclohexane/ethyl acetate, 8:2); m.p. 150−153 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 [d, $^3J_{H,H}$ = 6.6 Hz; 3 H, Ala−CH_3], 1.46 (s, 6 H, 2 × CH_3), 1.49 (s, 9 H, *t*Bu), 5.18 [AB, $^2J_{H,H}$ = 12.4 Hz; 2 H, OC*H*$_2$Ph], 5.49 (m, 1 H, Ala-CH), 6.98 [d, $^3J_{H,H}$ = 6.9 Hz; 1 H, NH], 7.30−7.40 (m, 5 H, Ph), 8.20 (d, $^3J_{H,H}$ = 7.8 Hz; 1 H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.6, 23.2, 23.3, 28.0, 50.0, 54.5, 67.0, 82.8, 127.9, 128.2, 128.5, 135.5, 152.6, 152.8, 170.8, 174.2. IR (film): ṽ = 3344, 3304 (N−H), 1759, 1738, 1690, 1639 (C=O) cm^{-1}.

***tert*-Butyl Dimethylmalonate (16):** A solution of dimethylmalonic acid (227 mmol, 30 g) in dichloromethane (750 mL) was stirred at −60 °C, and gaseous isobutene (8 mol, 450 g) was bubbled through. Sulfuric acid (56 mmol, 3 mL) was then added, and the mixture was stirred at room temp. in a closed flask for 240 h. The mixture was washed with aqueous $NaHCO_3$ (5%, 2 × 250 mL) and water (150 mL), dried over sodium sulfate and concentrated in vacuo. Di-*tert*-butyl dimethylmalonate was obtained pure as a liquid in 95% yield (52.6 g). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.34 (s, 6 H, 2 × CH_3), 1.45 (s, 18 H, 2 × *t*Bu). IR (KBr): ṽ = 1726 (C=O) cm^{-1}.

Di-*tert*-butyl dimethylmalonate (24.5 mmol, 6 g) in dry dichloromethane (40 mL) and TFA (196 mmol, 15 mL) was stirred for 20 min at 0 °C. The volatiles were then removed in vacuo, and dichloromethane (50 mL) and water (50 mL) were added. The aqueous layer was separated and solid $KHSO_4$ was added to pH 2. CH_2Cl_2 (4 × 200 mL) was then added and the layers were separated. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. Compound **16** was obtained pure as a white solid in 35% yield (1.61 g); m.p. 70−72 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 1.43 (s, 6 H, 2 × CH_3), 1.46 (s, 9 H, *t*Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5, 27.6, 50.5, 81.7, 171.6, 179.3. IR (KBr): ṽ = 1745, 1698 (C=O) cm^{-1}.

***t*BuO-*m*Aib-L-Ala-NH_2 (17):** EDC·HCl (58.3 mmol, 7.2 g) was added at 0 °C to a stirred solution of mono *tert*-butyl dimethylmalonate (**16**, 53 mmol, 10 g) and HOAt (53 mmol, 7.2 g) in dichloromethane (100 mL). The mixture was stirred at room temp. until the solution became clear. H-L-Ala-NH_2 (63.6 mmol, 14.1 g) and NMM (63.6 mmol, 14.1 g) were then added, and the mixture was stirred for 48 h at room temp. Dichloromethane was removed in vacuo and replaced with ethyl acetate (100 mL). The organic solvent was washed with aqueous $KHSO_4$ (10%, 50 mL), water (50 mL), aqueous $NaHCO_3$ (5%, 50 mL) and water (50 mL), dried over sodium sulfate and concentrated in vacuo. Compound **17** was obtained pure as a solid in 80% yield (10.9 g) after recrystallization from ethyl acetate/petroleum ether; m.p. 119−120 °C. $[\alpha]_D^{20}$ = −9.2 (*c* = 0.5, MeOH). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.37 [d, $^3J_{H,H}$ = 6.0 Hz; 3 H, Ala−CH_3], 1.41 (s, 6 H, 2 × CH_3), 1.46 (s, 9 H, *t*Bu), 4.54 (m, 1 H, Ala CH), 5.86 (s, 1 H, N*H*H), 6.94 (s, 1 H, NH*H*), 6.97 [d, $^3J_{H,H}$ = 7.8 Hz; 1 H, Ala NH]. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.2, 23.2, 23.3, 27.7, 48.6, 50.6, 82.1, 172.4, 173.3, 174.6. IR (KBr): ṽ = 3389, 3277 (N−H), 1731, 1641, 1620 (C=O) cm^{-1}.

TFA·$^+$$H_2$-*g*Ala-*m*Aib-O*t*Bu (18): A solution of *t*BuO-*m*Aib-L-Ala-NH_2 (**17**, 5.8 mmol, 1.5 g) and TIB (8.1 mmol, 3.4 mmol) in acetonitrile (60 mL) and water (40 mL) was stirred for 6 h at room temp. under an inert atmosphere. The solvents were then removed under an inert atmosphere. Compound **18** was obtained pure as a solid in 61% yield (1.22 g) after repeated crystallization from diethyl ether; m.p. 108−109 °C. ^{1}H NMR (200 MHz, DMSO): δ = 1.26 (s, 3 H, CH_3), 1.28 (s, 3 H, CH_3), 1.36 (br. s, 12 H, O*t*Bu and Ala−CH_3), 5.05 (m, 1 H, CHN), 8.42 (s, 3 H, $^+NH_3$), 8.61 [d, $^3J_{H,H}$ = 7.2 Hz, 1 H, NH]. ^{13}C NMR (50 MHz, DMSO): δ = 18.4, 22.5, 22.7, 27.5, 50.3, 54.3, 80.6, 117.0 [q, $^2J_{C,F}$ = 296 Hz, CF_3], 159.4 [q, $^3J_{C,F}$ = 36.8 Hz, CF_3CO], 171.9, 172.4. IR (KBr): ṽ = 3394 (N−H), 1717, 1677 (C=O) cm^{-1}.

***Z*-*g*Ala-*m*Aib-O*t*Bu (19):** NMM (11.6 mmol, 1.28 mL) was added at 0 °C to a stirred solution of TFA·$^+$$H_2$-*g*Ala-*m*Aib-O*t*Bu (**18**, 5.8 mmol, 2.0 g) and Z−OSu (7.5 mmol, 1.9 g) in dichloromethane (25 mL). The mixture was stirred for 48 at room temp., and the dichloromethane was removed in vacuo and replaced with ethyl acetate (40 mL). The organic solution was washed with aqueous $KHSO_4$ (10%, 20 mL), water (20 mL), aqueous $NaHCO_3$ (5%, 20 mL) and water (20 mL), dried over sodium sulfate and concentrated in vacuo. Compound **19** was obtained pure as a solid in 80% yield (0.99 g) after silica gel chromatography (dichloromethane/

ethanol, 98:2); m.p. 78−81 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35 (s, 6 H, 2 × CH_3), 1.43 (s, 9 H, *t*Bu), 1.51 [d, $^3J_{H,H}$ = 5.6 Hz; 3 H, CH_3], 5.08 (s, 2 H, OCH_2Ph), 5.30 (m, 1 H, CHN), 5.76 (br. s, 1 H, NH), 7.04 (br. s, NH), 7.33 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5, 23.2, 23.4, 27.7, 50.5, 55.6, 66.7, 81.8, 128.0, 128.1, 128.5, 136.2, 155.3, 172.0, 173.5. IR (KBr): $\tilde{\nu}$ = 3340 (N−H), 1728, 1681, 1662 (C=O) cm^{-1}.

Z-*g*Ala-*m*Aib-OH (20): Z-*g*Ala-*m*Aib-O*t*Bu (4.1 mmol, 1.50 g) in dry dichloromethane (20 mL) and TFA (73.8 mmol, 5.6 mL) was stirred for 35 min at 0 °C. The volatiles were then removed in vacuo, and dichloromethane (50 mL) and water (50 mL) were added. The aqueous layer was separated and solid $KHSO_4$ was added to pH 2. Dichloromethane (4 × 200 mL) was added and the layers were separated. The organic layer was dried over sodium sulfate and concentrated in vacuo. Compound **20** was obtained pure as a solid in 21% yield (0.14 g); m.p. 148−150 °C. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.25 (s, 9 H, 3 × CH_3), 5.00 (br. s, 2 H, OCH_2Ph), 5.31 (m, 1 H, CHN), 6.45 (br. s, 1 H, NH), 7.32 (m, 5 H, Ph), 7.68 [d, $^3J_{H,H}$ = 6.8 Hz; 1 H, NH], 10.09 (br. s, 1 H, OH). ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 20.9, 22.9, 23.0, 49.3, 54.6, 65.3, 127.7, 127.8, 128.3, 136.9 154.8, 171.1, 175.0. IR (KBr): $\tilde{\nu}$ = 3419, 3328 (N−H), 1726, 1693, 1645 (C=O) cm^{-1}.

Z-(*g*Ala-*m*Aib)$_2$-O*t*Bu (21): EDC·HCl (1.53 mmol, 0.29 g) was added at 0 °C to a stirred solution of Z-*g*Ala-*m*Aib-OH (1.39 mmol, 0.43 mg) and HOAt (1.39 mmol, 0.19 g) in dichloromethane (30 mL). The mixture was stirred at room temp. until the solution became clear. Separately, *t*BuO-*m*Aib-*g*Ala-H·TFA (1.53 mmol, 0.53 g) and NMM (2.78 mmol, 0.31 mL) were stirred in dry dichloromethane (15 mL) for 5 min at room temp. The two solutions were then mixed, and the resulting mixture was stirred for 48 h. The volatiles were removed in vacuo and the product **21** was obtained pure as a solid in 80% yield (0.58 g) after silica gel chromatography (dichloromethane/ethanol, 98:2); m.p. 115−117 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35−1.60 (m, 27 H, 9 × CH_3). 5.09 (m, 2 H, OCH_2Ph), 5.35−5.38 (m, 2 H, 2 × NCHN), 5.60 [br. d, $^3J_{H,H}$ = 13.1 Hz; 1 H, NH], 7.15−6.95 (m, 3 H, 3 × NH), 7.32−7.35 (s, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$, mixture of diastereoisomers): δ = 20.1, 20.7, 23.2, 23.3, 23.5, 27.7, 27.8, 49.5, 50.5, 54.5, 54.6, 55.5, 66.6, 81.9, 128.0, 128.1, 128.4, 136.2, 155.2, 172.0, 172.1, 172.2, 172.6, 172.7, 173.2, 173.3, 173.6. IR (KBr): $\tilde{\nu}$ = 3322 (N−H), 1718, 1665 (C=O) cm^{-1}.

Ac-(*g*Ala-*m*Aib)$_2$-O*t*Bu (22): Palladium on charcoal (10%, 5 mg) was added to a solution of Z-(*g*Ala-*m*Aib)$_2$-O*t*Bu (**21**, 0.13 mmol, 68 mg) in methanol (10 mL) and the mixture was stirred under H_2 (ca. 3 atm) for 12 h. The catalyst was then filtered through a celite pad and the mixture was concentrated. The corresponding amine *t*BuO-(*m*Aib-*g*Ala)$_2$-H, obtained pure as a solid in quantitative yield, was dissolved in dichloromethane (10 mL). Acetic anhydride (10.6 mmol, 1 mL) was added, and the mixture was stirred for 24 h at room temp. The volatiles were removed in vacuo. Compound **22** was obtained as a solid in 84% overall yield (47 mg) after recrystallization from diethyl ether; m.p. 162−164 °C. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of diastereoisomers): δ = 1.30−1.10 (m, 18 H, 6 × CH_3); 1.52−1.44 (m, 15 H, 5 × CH_3), 1.97 (s, 3 H, CH_3), 5.35 (m, 1 H, NCHN), 5.49 (m, 1 H, NCHN), 6.52 and 6.60 (2d, *J* = 6.9 Hz and 5.6 Hz; 1 H, NH) 7.11 (m, 3 H, 3 × NH). ^{13}C NMR (50 MHz, $CDCl_3$, mixture of diastereoisomers): δ = 20.0 and 20.1 ppm, 20.4 and 20.5, 23.1, 23.2, 23.3, 23.4, 23.5, 27.8, 49.7 and 49.8, 50.6 and 50.7, 53.9 and 54.2, 54.5 and 54.7, 169.8 and 169.9, 172.3 and 172.4, 173.0, 173.1 and 173.2, 173.5. IR (KBr): $\tilde{\nu}$ = 3320 (N−H), 1738, 1666 (C=O) cm^{-1}.

FT-IR Absorption: The FT-IR absorption spectra were recorded with a Perkin−Elmer 1720X spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (base-line) spectra were recorded under the same conditions. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde $CDCl_3$ (99.8% D) was purchased from Fluka.

^{1}H NMR: The ^{1}H NMR spectra were recorded with a Bruker AM 400 spectrometer. Measurements were carried in deuteriochloroform (99.96% D; Aldrich) with tetramethylsilane as the internal standard.

X-ray Crystallography: Formula $C_{13}H_{23}F_3N_2O_5$. Crystal (by slow evaporation of a diethyl ether solution) dimensions 0.4 × 0.3 × 0.2 mm. Monoclinic, $P2_1$; a = 9.160(2) Å, b = 10.946(3) Å, c = 9.282(2) Å, β = 98.93(2)°; V = 919.4(4) Å^3; $\rho_{calcd.}$ = 1.244 Mg·m^{-3}; $2\theta_{max.}$ = 120°; Cu-$K\alpha$ radiation (λ = 1.54178 Å), $\theta-2\theta$ scan mode, T = 293 K; 1521 collected reflections, 1435 of which independent; *hkl* limits: $-10 \le h \le 10$, $0 \le k \le 12$, $0 \le l \le 10$. Data collection was performed on a Philips PW1100 four-circle diffractometer. Intensities were corrected for Lorentz and polarization effects, but not for absorption (μ = 0.988 mm^{-1}). The structure was solved by direct methods (SHELXS 97 program[33]) and refined by full-matrix block least-squares on F^2, using all data, by application of the SHELXL 97 program,[34] with all non-H atoms anisotropic, and allowing the positional parameters and anisotropic displacement parameters of the non-H atoms to refine at alternate cycles. The $-CF_3$ moiety of the trifluoroacetate anion shows rotational disorder. It was refined with the three fluorine atoms in two sets of positions with population parameters of 0.60 and 0.40, respectively. Restraints were applied to the C−F bond lengths and to the anisotropic displacement parameters of the fluorine atoms, the latter to approach isotropic behaviour. Data/restraints/parameters: 1435/43/242. The positions of the H-atoms of the terminal $-NH_3^+$ group were recovered from a difference Fourier map, while the remaining H-atoms were calculated at the idealized positions. All H-atoms were refined as riding. The refinement converged to R_1 = 0.067 [on $F = 4\sigma(F)$]; wR_2 = 0.180 (on F^2, all data). Goodness of fit on F^2 = 1.071; max. and min. residual electron density peaks +0.238 and −0.292 e·Å^3.

CCDC-234290 contains the supplementary crystallographic data for this paper. These data can be obtained online free of charge [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax (+44)-1223-336-033; or deposit@ccdc.cam.ac.uk].

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