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New 21- and 24-atom Aib-containing cyclopeptides

TATJANA JEREMIC, ANTHONY LINDEN and HEINZ HEIMGARTNER*

Institute of Organic Chemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

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Abstract: The synthesis of two Aib-containing cyclic octapeptides, cyclo(Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib) (**1**) and cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**), and a cyclic heptapeptide cyclo(Gly-Aib-Val-Aib-Leu-Aib-Phe) (**3**), is described. The linear precursors of **1**–**3** were prepared using solution-phase techniques, and the cyclisation was also accomplished in solution. Among the coupling reagents examined in the final macrolactamisation step, PyAOP, HATU and DEPC/DEPBT efficiently yielded cyclised products. However, the success of the cyclisation was found to be dependent on the coupling reagent used. The two octapeptides **1** and **2** were obtained in much better yields (up to 63%) than the cycloheptapeptide **3** (30–37%). In addition, crystal-state conformational analysis of **2** was performed by X-ray crystallography. Six intramolecular hydrogen bonds stabilise the structure characterised by two consecutive type II′/I β-turns, two consecutive type II/III′ β-turns, one γ-turn, and one inverse γ-turn. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: cyclopeptides; peptide synthesis; Aib-peptides; crystal structure

INTRODUCTION

The development of efficient and convenient methods for the synthesis of cyclic peptides has gained considerable attention in recent years [1–8]. Cyclic peptides often exhibit improved metabolic stabilities, and increased potencies and bioavailabilities than their linear counterparts. In addition, cyclisation may also be employed to constrain a bioactive peptide in its active conformation, thereby increasing its specificity [9]. Cyclic peptides are, therefore, of great interest as initial synthetic targets from a drug-lead discovery perspective [10]. Although some rules have been derived for specific cases, it is difficult to predict the correct cyclisation strategy for maximum yields. The yields of the cyclisation depend upon many factors such as the ring size, the site of cyclisation, the number and position of turn-inducing amino acid residues (if any), the sequence of the linear precursor, and finally the reaction conditions (coupling reagents, solvent, concentration, etc.) [11].

We are interested in the design and synthesis of cyclic peptides containing C(2)-tetrasubstituted α-amino acids in order to achieve a greater degree of conformational rigidity in a cyclopeptide structure. Our previous successful synthesis of cyclic hexapeptides containing several Aib residues [12–14] prompted us to investigate the cyclisation tendencies of longer Aib-containing peptides. The cyclisation of peptides consisting of more than seven amino acid residues is generally considered easier to accomplish in comparison with the cyclisation of small linear peptides (three to six amino acids) [15]. The information on cyclisation of Aib-containing hepta- and octapeptides is scarce. The preparation of only two cyclic heptapeptides containing an Aib residue, i.e. cyclo(Ala-Ile-Val-Ser(Bzl)-Aib-Phe-Gly) and cyclo(Leu-Pro-MeLeu-Aib-Phe-MePhe-Phe) (scytalidamide A), has been reported in the literature so far [16,17]. We have started our investigation with the synthesis and cyclisation of octapeptides, since there are, to the best of our knowledge, no literature reports about the synthesis, conformational analysis or isolation of cyclooctapeptides containing Aib residues. Having in mind that the cyclisation of hexapeptides bearing Leu-Aib-Phe or Gly-Aib-Aib tripeptide sequences, or alternating Aib and proteinogenic amino acid residues, resulted in conformations that were highly favourable for the cyclisation [12–14], we decided to incorporate such sequences also in our longer chains. The present work reports on the synthesis of two cyclic octapeptides cyclo(Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib) (**1**) and cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**), and a cycloheptapeptide cyclo(Gly-Aib-Val-Aib-Leu-Aib-Phe) (**3**) (Figure 1).

Abbreviations: Amino acid and peptide nomenclature conforms to IUPAC-IUB rules (*J. Peptide Sci.* 2003; **9**: 1–8).
Other abbreviations: Aib, α-aminoisobutyric acid; CC, column chromatography; DEPBT, 3-[(diethoxyphosphoryl)oxy]-1,2,3-benzotriazin-4-(3*H*)-one; DEPC, diethyl phosphorocyanidate; DIEA, *N*-ethyl-*N*,*N*-diisopropylamine; ESI-MS, electrospray-ionisation mass spectrometry; HATU, *N*-{[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridino-1-yl]methylene}-*N*-methylmethanaminium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzo-1,2,3-triazole; PLC, preparative layer chromatography; PyAOP, (7-azabenzo-1,2,3-triazol-1-yloxy)tris(pyrrolidino) phosphonium hexafluorophosphate; PyBOP, (1*H*-benzo-1,2,3-triazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate; PyBrOP, bromo-tris(pyrrolidino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; TLC, thin layer chromatography.

*Correspondence to: Heinz Heimgartner, Institute of Organic Chemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland; e-mail: heimgart@oci.uzh.ch

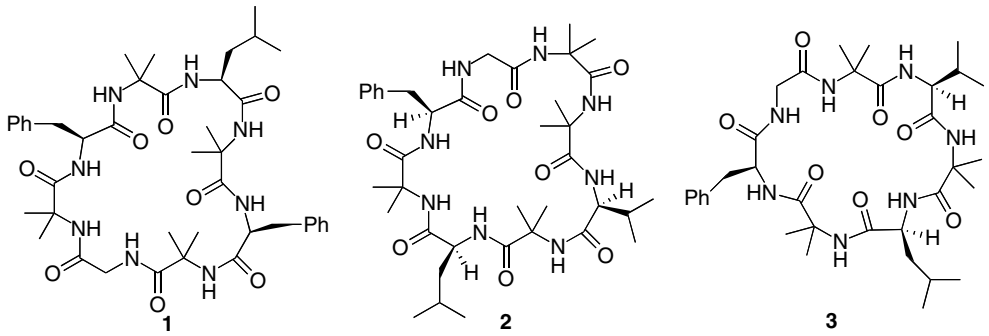

Figure 1 Structure of compounds cyclo(Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib) (**1**) and cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**), and a cycloheptapeptide cyclo(Gly-Aib-Val-Aib-Leu-Aib-Phe) (**3**).

MATERIALS AND METHODS

General

Solvents were purified by standard procedures. ^{1}H-NMR and ^{13}C-NMR spectra of linear peptides were recorded on a Bruker ARX 300 spectrometer at 300 and 75.5 MHz respectively, employing DMSO-d_6 or $CDCl_3$ as solvent at 300 K, and using TMS as an internal standard (δ in ppm, coupling constant J in Hz). ^{1}H,^{13}C, heteronuclear single quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC) and ROESY (mixing time, 300 ms) spectra of the cyclic peptide **1** were recorded on a Bruker DRX 500 spectrometer. TOCSY (mixing time, 100 ms) spectra of **1** were recorded on a Bruker DRX 600 spectrometer. ^{1}H,^{13}C, HSQC, HMBC, TOCSY (mixing time, 100 ms) and ROESY (mixing time, 300 ms) spectra of the cyclic peptide **2** were recorded on a Bruker AV 300 spectrometer at 340 K. ^{1}H,^{13}C, HSQC, HMBC, TOCSY (mixing time, 100 ms) and ROESY (mixing time, 300 ms) spectra of the cyclic peptide **3** were recorded on a Bruker AV 600 spectrometer. Mass spectra were measured on a Finnigan TSQ-700 triple-stage quadrupole instrument for ESI-MS. Melting points (Mp) were measured on Büchi B-540 apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer Spectrum One spectrometer, absorption in cm^{-1}, using KBr. For the TLC, Merck TLC aluminium sheets and silica gel 60 F254 were used. The products were isolated by means of CC, using Uetikon–Chemie silica gel (Chromatographiegel C-560), or by means of preparative TLC (PLC), using Merck PLC plates (glass), and silica gel F254 (40–63 μm = grain size; 2 mm = thickness of layer).

Peptide Synthesis and Characterisation

General procedure A (GP A): hydrogenolytic deprotection. To a solution of a Z-protected peptide in MeOH was added Pd/C (10% on activated charcoal) and the mixture was hydrogenated overnight under atmospheric pressure using an H_2-filled balloon. The catalyst was removed by filtration through a pad of celite and the solvent evaporated under reduced pressure. The crude product was dried under vacuum and used directly in the next reaction step without further purification.

General procedure B (GP B): peptide coupling. To a solution of an *N*-protected peptide acid (or *N*-protected amino acid) in absolute CH_2Cl_2 (or CH_2Cl_2/THF, or CH_2Cl_2/DMF mixture) were added the amino component (1.0 or 1.1 equiv.), coupling reagent (PyAOP, PyBOP or HATU, 1.1 equiv.), HOAt (1.1 equiv., 0.5 M solution in DMF), and DIEA (2 equiv.). The mixture was stirred at room temperature (rt) under N_2 until the starting material was consumed (TLC). The solvent was then evaporated, the residue was dissolved in EtOAc and washed with 5% aq. $KHSO_4$ solution, 5% aq. $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), concentrated, purified by CC and dried under high vacuum.

Z-Gly-Aib-Phe-Aib-N(Me)Ph (4)

Z-Phe-Aib-N(Me)Ph [18] (0.79 g, 1.67 mmol) was *N*-deprotected by following GP A (H_2, 80 mg Pd/C, 15 ml MeOH, overnight) to give 0.566 g (quantitative yield) H-Phe-Aib-N(Me)Ph as a foam, which was used directly in the next step.

Z-Gly-Aib-OH [12] (0.447 g, 1.52 mmol) was coupled with H-Phe-Aib-N(Me)Ph (0.566 g, 1.67 mmol) using PyAOP (0.87 g, 1.67 mmol) and DIEA (0.43 g, 3.34 mmol) in abs. CH_2Cl_2/THF (1 : 3, 16 ml) according to GP B. Reaction time: 24 h at rt. Purification by CC (SiO_2, EtOAc) and crystallisation from EtOAc/Et_2O yielded 0.79 g (84%) tetrapeptide **4** as a powder.

ESI-MS (MeOH + NaI): m/z 638.4 ([M + Na]$^+$); calcd. 638.3. Mp 182–183 °C. R_f (EtOAc/MeOH 15 : 1) = 0.4. ^{1}H-NMR ($CDCl_3$): 7.34–7.12 (m, 15 arom. H, 1 NH); 6.94, 6.80, 6.22 (3 br. s, 3 NH); 5.10 (br. s, $PhCH_2O$); 4.48 (m, H_α of Phe); 3.63–3.61 (m, 2 H_α of Gly); 3.38–3.33 (m, 1 H_β of Phe); 3.25 (s, NMe); 2.95–2.87 (m, 1 H_β of Phe); 1.47, 1.46, 1.38. 1.10 (4s, 4 Me of 2 Aib). ^{13}C-NMR ($CDCl_3$): 173.6, 173.4, 170.4, 170.0 (4s, 4 CO(amide)); 157.0 (s, CO(urethane)); 145.0, 137.6, 135.9 (3s, 3 arom. C); 129.2, 129.0, 128.5, 128.3, 128.2, 127.9, 127.3, 126.5 (8d, 15 arom. CH); 67.1 (t, $PhCH_2O$); 57.4, 56.8 (2s, 2 C_α of 2 Aib); 54.1 (d, C_α of Phe); 45.5, 36.8 (2t, C_α of Gly and C_β of Phe); 40.7 (q, NMe); 26.3, 25.8, 23.7 (3q, 4 Me of 2 Aib). IR (KBr): 3398m, 3363s, 3337s, 3305s, 3065w, 3028w, 2995m, 2927m, 1699s, 1686s, 1669s, 1648s, 1593m, 1536s, 1512s, 1494s, 1461m, 1444m, 1395m, 1373m, 1328m, 1267s, 1247m, 1209m, 1198m, 1156m, 1087m, 1045m, 1004w, 993w, 924w, 786m, 777w, 751m, 732m, 710m, 700m, 661w, 642w, 617w, 524m.

Z-Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib-N(Me)Ph (5)

Z-Gly-Aib-Phe-Aib-N(Me)Ph (**4**) (0.68 g, 1.1 mmol) was *N*-deprotected according to GP A (H_2, 70 mg Pd/C, 30 ml MeOH, 16 h) to afford 0.53 g (quantitative yield) of H-Gly-Aib-Phe-Aib-N(Me)Ph as a foam. This material was coupled with Z-Phe-Aib-OH [18] (0.384 g, 1.0 mmol) by following GP B, using

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PyBOP (0.573 g, 1.1 mmol) and DIEA (0.284 g, 2.2 mmol) in absolute CH_2Cl_2 (15 ml). Reaction time: 20 h at rt. Purification by CC (SiO_2, CH_2Cl_2/MeOH 20 : 1, EtOAc/MeOH 15 : 1, EtOAc/hexane/MeOH 10 : 2 : 1) gave 0.815 g (96%) hexapeptide Z-Phe-Aib-Gly-Aib-Phe-Aib-N(Me)Ph as a foam. This material was *N*-deprotected by following GP A (H_2, 82 mg Pd/C, 20 ml MeOH, 20 h) to give 0.69 g (quantitative yield) of H-Phe-Aib-Gly-Aib-Phe-Aib-N(Me)Ph as a foam, which was used directly in the next step without further purification. This hexapeptide was coupled with Z-Leu-Aib-OH [19] (0.305 g, 0.87 mmol) using HATU (0.403 g, 1.06 mmol), HOAt (1.06 mmol, 2.12 ml 0.5 M DMF solution) and DIEA (0.247 g, 2.12 mmol) in absolute CH_2Cl_2/THF (1 : 1, 20 ml) according to GP B. Reaction time: 24 h at rt. Purification by CC (SiO_2, EtOAc/hexane/MeOH 10 : 3 : 1) yielded 0.83 g (91%) octapeptide **5** as a foam.

ESI-MS (MeOH + NaI): *m/z* 1068.6 ($[M + Na]^+$); calcd. 1068.6. Mp 140–142 °C. R_f (EtOAc/hexane/MeOH 10 : 3 : 1) = 0.25. ^{1}H-NMR ($CDCl_3$): 7.83–7.78 (m, 2 NH); 7.60, 7.43 (2s, 2 NH); 7.37–7.10 (m, 20 arom. H, 3 NH); 6.67 (s, NH); 5.12–4.91 (m, $PhCH_2O$); 4.53–4.47, 4.24–4.19, 3.93–3.87 (3m, H_α of Leu and 2 H_α of 2 Phe); 3.76–3.54, 3.32–3.22, 3.05–2.86 (3m, 2 H_α of Gly, 2 × 2 H_β of 2 Phe); 1.77–1.15 (m, 2 H_β and H_γ of Leu, 8 Me of 4 Aib); 0.95–0.90 (m, 2 Me of Leu). ^{13}C-NMR ($CDCl_3$): 176.8, 176.4, 174.7, 174.1, 173.6, 172.4, 171.1, 170.2 (8s, 8 CO(amide)); 157.6 (s, CO(urethane)); 138.3, 137.1, 135.8 (3s, 4 arom. C); 129.0, 128.5, 128.4, 128.2, 128.1, 127.6, 127.2, 126.7, 126.1 (9d, 20 arom. CH); 67.0 (t, $PhCH_2O$); 57.0, 56.8 (2s, 4 C_α of 4 Aib); 56.5, 55.8, 54.8 (3d, C_α of Leu and 2 C_α of 2 Phe); 40.5 (q, NMe); 45.1, 39.4. 36.8, 36.1 (4t, C_α of Gly, C_β of Leu and 2 C_β of 2 Phe); 26.2, 24.7, 23.7, 23.4, 23.0, 22.6, 21.6 (7q, 8 Me of 4 Aib, 2 Me of Leu). IR (KBr): 3309s, 3062m, 3031m, 2985m, 2936m, 2871w, 1660s, 1594m, 1533s, 1497s, 1468m, 1454s, 1385m, 1363m, 1330m, 1267s, 1218s, 1196m, 1172m, 1117w, 1090m, 1046w, 1028w, 936w, 743m, 699m, 618m, 585m, 550m, 516m.

Cyclo(Leu1-Aib2-Phe3-Aib4-Gly5-Aib6-Phe7-Aib8) (1)

Octapeptide **5** (0.69 g, 0.66 mmol) was dissolved in MeCN (4 ml) and then 4 ml of 6 N HCl were added dropwise. The mixture was stirred at rt overnight. The MeCN was evaporated under reduced pressure and H_2O was added (4 ml). The product was extracted with CH_2Cl_2, the organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. After drying under vacuum, 0.64 g (quantitative yield) of Z-Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib-OH were obtained as a foam, and were subjected to *N*-deprotection by following GP A (H_2, 70 mg Pd/C, 15 ml MeOH, overnight). After drying under vacuum, 0.457 g (84%) of the free linear octapeptide were obtained as a powder, which was used in the cyclisation step without further purification.

Cyclisation with HATU/HOAt. The free linear octapeptide (0.08 g, 0.1 mmol) was dissolved in absolute DMF (100 ml, 1 mM solution) and cooled to 0 °C in an ice bath. To the solution were added HATU (0.046 g, 0.12 mmol), HOAt (0.24 mmol, 0.5 ml of 0.5 M solution in DMF) and DIEA (0.155 g, 1.2 mmol) under stirring. The solution was kept at 0 °C for 2 h and at rt for 22 h. The solvent was removed under reduced pressure, the residue dissolved in EtOAc and washed with 1 M HCl solution, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude cyclopeptide was purified by CC (SiO_2, performed twice; EtOAc/MeOH/hexane 15 : 1 : 3; EtOAc/CH_2Cl_2/MeOH 5 : 1 : 0.1) to afford 45 mg (58%) of pure **1** as a powder.

Cyclisation with PyAOP/HOAt. The free linear octapeptide (0.08 g, 0.1 mmol) was dissolved in absolute DMF (150 ml, 0.67 mM solution) under stirring. PyAOP (0.156 g, 0.3 mmol), HOAt (0.3 mmol, 0.6 ml of 0.5 M solution in DMF) and DIEA (0.129 g, 1 mmol) were added at rt and the solution was stirred for an additional 3 days. The solvent was then removed under reduced pressure, the residue dissolved in EtOAc and washed with 10% citric acid solution, 5% $NaHCO_3$ solution, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated to afford a yellow oil which was purified by CC (SiO_2, performed twice; EtOAc/MeOH/hexane 15 : 1 : 3; EtOAc/CH_2Cl_2/MeOH 5 : 1 : 0.1) to give 45 mg (58%) of pure cyclic octapeptide **1** as a powder.

Cyclisation with PyBOP/HOAt. As described for the cyclisation with PyAOP, with linear octapeptide (0.118 g, 0.143 mmol) dissolved in absolute CH_2Cl_2/DMF 2 : 1 (300 ml, 0.5 mM solution), PyBOP (0.224 g, 0.43 mmol), HOAt (0.43 mmol, 0.9 ml of 0.5 M solution in DMF), and DIEA (3 ml, 1% v/v). Reaction time: 1 day. Purification by CC (SiO_2, performed thrice; EtOAc/hexane/MeOH 15 : 3 : 1; CH_2Cl_2/MeOH/hexane 30 : 1 : 3, EtOAc/CH_2Cl_2/MeOH 5 : 1 : 0.1) yielded 40 mg (35%) of pure **1** as a solid.

Cyclisation with PyBrOP/HOAt. As described for the cyclisation with PyAOP, with linear octapeptide (0.08 g, 0.1 mmol) dissolved in absolute CH_2Cl_2/DMF 10 : 1 (165 ml, 0.6 mM solution), PyBrOP (0.227 g, 0.5 mmol), HOAt (0.5 mmol, 1 ml of 0.5 M solution in DMF), and DIEA (0.258 g, 2 mmol). Reaction time: 3 days. Purification by CC as described for the cyclisation with PyAOP yielded 34 mg (44%) of pure **1** as a solid.

ESI-MS (MeOH + NaI): *m/z* 827.7 ($[M + Na]^+$), 805.7 ($[M + H]^+$); calcd. 805.5. Mp 214.5–216 °C. R_f (EtOAc/CH_2Cl_2/MeOH 5 : 1 : 0.1) = 0.15. ^{1}H-NMR (500 MHz, DMSO-d_6): 8.42 (s, NH of Aib6); 8.11 (s, NH of Aib4); 8.02 (s, NH of Aib2); 7.97 (br.d, $J = 5.95$, NH of Phe3); 7.67 (d, $J = 8.57$, NH of Phe7); 7.57 (s, NH of Aib8); 7.43 (br.t, $J = 5.24$, NH of Gly5); 7.27–7.13 (m, 10 arom. H of Phe3 and Phe7); 7.02 (br.d, $J = 6.8$, NH of Leu1); 4.50–4.45 (m, H_α of Phe^7); 4.36–4.28 (m, H_α of Phe3 and H_α of Leu1); 3.83 (dd, $J = 5.3$, 15.85, 1 H_α of Gly5); 3.51–3.44 (m, 1 H_α of Gly5); 3.36–3.30 (m, overlap with the H_2O peak, 1 H_β of Phe7); 3.00–2.90 (m, 2 H_β of Phe3); 2.75–2.70 (m, 1 H_β of Phe7); 1.74–1.62 (m, H_γ of Leu1); 1.60–1.57, 1.52–1.46 (2m, 2 H_β of Leu1); 1.44, 1.40 (2s, 2 Me of Aib2); 1.39, 1.34 (2s, 2 Me of Aib4);1.26 (s, Me of Aib6); 1.24, 1.23 (2s, 2 Me of Aib8); 0.99 (s, Me of Aib6); 0.87, 0.83 (2d, $J = 6.6$, 6.4, 2 Me of Leu1). ^{13}C-NMR (125.8 MHz, DMSO-d_6): 174.9 (s, CO of Aib2); 174.31 (s, CO of Aib4); 174.29 (s, CO of Aib8); 173.7 (s, CO of Aib6); 171.4 (s, CO of Leu1); 170.6 (s, CO of Phe3); 170.4 (s, CO of Phe7); 170.1 (s, CO of Gly5); 138.2 (s, 1 arom. C of Phe7); 137.4 (s, 1 arom. C of Phe3); 129.1, 128.6, 128.0, 127.9, 126.2, 126.0 (6d, 10 arom. CH of Phe3 and Phe7); 56.2 (s, C_α of Aib2); 55.73 (s, C_α of Aib4); 55.70 (s, C_α of Aib8); 55.65 (s, C_α of Aib6); 55.0 (d, C_α of Phe3); 53.2 (d, C_α of Phe7); 51.0 (d, C_α of Leu1); 42.8 (t, C_α of Gly5); 40.1 (t, C_β of Leu1); 35.8 (t, C_β of Phe7); 35.6

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(t, C_β of Phe3); 27.5 (q, Me of Aib4); 26.8 (q, Me of Aib8); 25.9 (q, Me of Aib6); 24.1, 23.75 (2q, 2 Me of Aib2); 23.7 (q, Me of Aib6); 23.4 (q, Me of Leu1); 23.1 (d, C(4) of Leu1); 22.78 (q, Me of Aib8); 22.77 (q, Me of Aib4); 21.0 (q, Me of Leu1). IR (KBr): 3349s, 3062w, 3030w, 2932m, 2871w, 1672s, 1526s, 1468m, 1455m, 1385m, 1364m, 1335w, 1281m, 1221m, 1182m, 1112w, 1081w, 1030w, 869w, 851w, 746w, 700m, 620w.

Z-Val-Aib-Leu-Aib-Phe-O*t*Bu (6)

Z-Leu-Aib-Phe-O*t*Bu [14] (0.9 g, 1.62 mmol) was *N*-deprotected according to GP A (H_2, 90 mg Pd/C, 15 ml MeOH, 20 h). The crude material was dried under vacuum to give 0.643 g (quantitative yield) of H-Leu-Aib-Phe-O*t*Bu as a foam which was used directly in the next step without further purification. This material was then coupled with Z-Val-Aib-OH [20] (0.5 g, 1.49 mmol) by following GP B, using PyBOP (0.833 g, 1.6 mmol), HOAt (1.6 mmol, 3.2 ml 0.5 M solution in DMF) and DIEA (0.414 g, 3.2 mmol) in absolute CH_2Cl_2 (10 ml). Reaction time: 24 h at rt. Purification by CC (SiO_2, performed twice; once with CH_2Cl_2/MeOH/hexane 30 : 1 : 4, once with gradient CH_2Cl_2/EtOAc 5 : 1 → EtOAc) yielded 1.04 g (95%) pentapeptide **6** as a powder.

ESI-MS (MeOH + NaI): *m/z* 760.5 ($[M + Na]^+$); calcd. 760.5. Mp 180–182 °C. R_f (CH_2Cl_2/MeOH/hexane 30 : 1 : 4) = 0.25. ^{1}H-NMR ($CDCl_3$): 8.28, 7.71 (2s, 2 NH); 7.46–7.18 (m, 10 arom. H, 3 NH); 5.05 (br. s, $PhCH_2O$); 4.32–4.30, 4.05–3.92, 3.79–3.31 (3m, 3 H_α of Leu, Val and Phe); 2.97–2.94 (m, H_β of Phe); 1.99–1.97 (m, H_β of Val); 1.62–1.48 (m, 2 H_β and H_γ of Leu); 1.38, 1.35, 1.34, 1.30 (4s, 4 Me of 2 Aib and *t*Bu); 0.90–0.79 (m, 2 Me of Leu, 2 Me of Val). ^{13}C-NMR ($CDCl_3$): 174.3, 173.8, 171.6, 171.1, 170.2 (5s, 4 CO (amide) and CO (ester)); 156.7 (s, CO(urethane)); 137.3, 136.7 (2s, 2 arom. C); 129.2, 128.3, 128.0, 127.8, 127.4, 126.3 (6d, 10 arom. CH); 80.4 (s, *t*Bu); 65.5 (t, $PhCH_2O$); 60.9 (d, C_α of Val); 56.01, 55.94 (2s, 2 C_α of 2 Aib); 54.4, 52.0 (2d, C_α of Leu and C_α of Phe); 39.0, 36.9 (2t, C_β of Leu and C_β of Phe); 27.3 (q, *t*Bu); 29.5, 25.6, 25.3, 24.0, 22.9, 21.2 (6q, 4 Me of 2 Aib, 2 Me of Leu); 18.9, 18.5 (2q, 2 Me of Val). IR (KBr): 3352s, 3316s, 3066w, 3030w, 2964m, 2938m, 2872w, 1721s, 1704s, 1685s, 1672s, 1651s, 1531s, 1467m, 1453m, 1442m, 1382m, 1368m, 1287s, 1273s, 1255s, 1241s, 1168s, 1111m, 1029w, 1020w, 1011w, 981w, 935w, 849w, 787w, 736m, 702m, 650m, 557w, 519w.

H-Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu (7)

Z-Val-Aib-Leu-Aib-Phe-O*t*Bu (**6**) (0.92 g, 1.25 mmol) was *N*-deprotected according to GP A (H_2, 95 mg Pd/C, 20 ml MeOH, 20 h). The crude product was dried under vacuum to give 0.753 g (quantitative yield) of H-Val-Aib-Leu-Aib-Phe-O*t*Bu as a foam. A portion of this material (0.36 g, 0.6 mmol) was then coupled with Z-Gly-Aib-Aib-OH [12] (0.206 g, 0.54 mmol) by following GP B, using PyBOP (0.312 g, 0.6 mmol), HOAt (0.6 mmol, 1.2 ml 0.5 M solution in DMF) and DIEA (0.155 g, 1.2 mmol) in absolute CH_2Cl_2 (14 ml). Reaction time: 24 h. The product was purified by CC (SiO_2, EtOAc/MeOH/hexane 20 : 1 : 2 and CH_2Cl_2/MeOH 16 : 1 and used directly in the next step. The octapeptide (0.521 g, 0.54 mmol) was *N*-deprotected according to GP A (H_2, 55 mg Pd/C, 12 ml MeOH, 20 h). The crude product was purified by CC (SiO_2, with gradient CH_2Cl_2/MeOH 12 : 1 → CH_2Cl_2/MeOH/NH_3 12: 1 : 0.1) to afford 0.42 g (93%) of pure **7** as a foam.

ESI-MS (MeOH + NaI): *m/z* 829.6 ($[M - H]^+$); calcd. 829.5. Mp 120–122 °C. R_f (CH_2Cl_2/MeOH 12 : 1) = 0.2. ^{1}H-NMR ($CDCl_3$): 8.26 (s, NH); 7.86 (d, *J* = 5.9, NH); 7.64, 7.50 (2s, 2 NH); 7.24–7.10 (m, 5 arom. H, 5 NH); 4.38–4.35, 4.10–4.00, 3.69–3.65 (3m, 3 H_α of Leu, Val and Phe); 3.26–3.12, 2.95–2.86 (2m, 2 H_α of Gly and 2 H_β of Phe); 2.22–2.19 (m, H_β of Val); 1.64–1.56 (m, 2 H_β and H_γ of Leu); 1.45, 1.42, 1.40, 1.38, 1.36, 1.35, 1.34, 1.32 (8s, 8 Me of 4 Aib); 1.31 (s, *t*Bu); 1.00–0.93, 0.85–0.79 (2m, 2 Me of Leu and 2 Me of Val). ^{13}C-NMR ($CDCl_3$): 176.8, 174.7, 174.2, 174.0, 173.8, 172.6, 170.8, 170.1 (8s, 7 CO(amide) and CO(ester)); 137.2 (s, 1 arom. C); 128.9, 127.9, 126.2 (3d, 5 arom. CH); 80.3 (s, *t*Bu); 61.8 (d, C_α of Val); 56.1, 55.9, 55.4 (3s, 4 C_α of 4 Aib); 53.9, 51.1 (2d, C_α of Leu and C_α of Phe); 44.8, 39.1, 37.0 (3t, C_α of Gly, C_β of Leu and C_β of Phe); 27.3 (q, *t*Bu); 28.4, 26.8, 26.3, 25.7, 24.0, 23.5, 23.1, 22.9, 22.4, 20.3, 19.3, 18.7 (12s, 8 Me of 4 Aib, 2 Me of Leu and 2 Me of Val). IR (KBr): 3318s, 2979m, 2037m, 2873w, 1730m, 1659s, 1530s, 1468m, 1454m, 1385m, 1365m, 1282m, 1225m, 1157m, 1080w, 1031w, 932w, 846w, 741w, 701w, 660w, 590w.

Cyclo(Gly1-Aib2-Aib3-Val4-Aib5-Leu6-Aib7-Phe8) (2)

The *N*-deprotected octapeptide (**7**) (0.315 g, 0.38 mmol) was dissolved in CH_2Cl_2 (10 ml), and TFA (10 ml) was then added. The mixture was stirred for 2 h at rt. Excess TFA was evaporated, and the remaining TFA was removed by co-evaporation with Et_2O. The residue was triturated with Et_2O, the solution decanted, and the operation was repeated twice to afford, after drying under vacuum, 0.34 g (0.38 mmol, 100%) of the deprotected octapeptide as its TFA salt.

Cyclisation with HATU/HOAt. The free linear octapeptide TFA salt (0.112 g, 0.126 mmol) was dissolved in absolute DMF (200 ml, 0.63 mM solution) and cooled to 0 °C in an ice bath. To the solution were added HATU (0.06 g, 0.16 mmol), HOAt (0.31 mmol, 0.63 ml of 0.5 M solution in DMF) and collidine (0.3 g, 2.52 mmol) under stirring. The solution was kept at 0 °C for 2 h and at rt for 2 days. The solvent was removed under reduced pressure, the residue dissolved in EtOAc and washed with 1 M HCl solution, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude cyclopeptide was purified by CC (SiO_2, performed thrice; EtOAc/MeOH 15 : 1; CH_2Cl_2/MeOH 22 : 1; CH_2Cl_2MeOH 13 : 1) to afford 30 mg (31%) of pure **2** as a powder.

Cyclisation with PyAOP/HOAt. The free linear octapeptide TFA salt (0.112 g, 0.126 mmol) was dissolved in absolute DMF (200 ml, 0.63 mM solution) under stirring. PyAOP (0.2 g, 0.38 mmol), HOAt (0.38 mmol, 0.8 ml of 0.5 M solution in DMF) and DIEA (0.244 g, 1.89 mmol) were added at rt and the solution was stirred for an additional 3 days. The solvent was then removed under reduced pressure, the residue dissolved in EtOAc and washed with 10% citric acid solution, 5% $NaHCO_3$ solution, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated to afford a yellow oil which was purified by CC (SiO_2, performed twice; EtOAc/MeOH 15 : 1; CH_2Cl_2/EtOAc/MeOH 10 : 5 : 1), and PLC (CH_2Cl_2/MeOH

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16 : 1) to give 60 mg (63%) of pure cyclic octapeptide **2** as a powder.

Cyclisation with DEPC/DEPBT. The free linear octapeptide TFA salt (0.103 g, 0.116 mmol) was dissolved in absolute DMF (150 ml, 0.8 mM solution) and the solution was cooled to 0 °C in an ice bath. Then, DEPC (0.114 g, 0.7 mmol), DEPBT (0.209 g, 0.7 mmol) and DIEA (0.6 g, 4.64 mmol) were added under stirring. The solution was warmed to rt and stirred for an additional 5 days. The solvent was then evaporated under reduced pressure, the residue taken up in EtOAc and washed with 5% $KHSO_4$ solution, 5% $NaHCO_3$ solution, and brine. The organic phase was then dried ($MgSO_4$) and concentrated to give the crude cyclic octapeptide, which was purified by CC and PSC. CC (SiO_2, CH_2Cl_2/MeOH 17 : 1). PLC (SiO_2, performed thrice; CH_2Cl_2/MeOH 10 : 1, EtOAc/CH_2Cl_2/MeOH 6 : 6 : 1; CH_2Cl_2/MeOH 16 : 1). 48 mg (54%) of pure **2** were obtained as a powder.

ESI-MS (MeOH + NaI): *m/z* 779.4 ($[M + Na]^+$); calcd. 779.4. Mp 232–234 °C. R_f (CH_2Cl_2/MeOH 15 : 1) = 0.2. ^{1}H-NMR(300 MHz, DMSO-d_6, 340K): 8.41 (s, NH of Aib2); 8.36 (s, NH of Aib7); 7.74 (br. t, NH of Gly1); 7.67 (s, NH of Aib3); 7.50 (s, NH of Aib5); 7.31 (d, $J = 8.7$, NH of Val4); 7.26–7.16 (m, 5 arom. H); 7.07–7.04 (br. d, $J = 8.5$, NH of Leu6 and NH of Phe8, signals overlapped); 4.48–4.32 (m, H_α of Phe8 and H_α of Leu6); 4.11–4.06 (m, H_α of Val4); 4.02–3.95, 3.36–3.28 (2m, 2 H_α of Gly1); 3.25–3.17, 2.95–2.85 (2m, 2 H_β of Phe8); 2.38–2.23 (m, H_β of Val4); 1.77–1.25 (m, 2 H_β and H_γ of Leu6); 1.42, (s, Me of Aib3); 1.38, (s, Me of Aib5); 1.37 (s, Me of Aib3); 1.36 (s, Me of Aib2); 1.33 (s, Me of Aib2, Me of Aib5); 1.20, 1.18 (2s, 2 Me of Aib7); 0.93–0.77 (m, 2 Me of Leu6, 2 Me of Val4). ^{13}C-NMR (75.5 MHz, DMSO-d_6, 340 K): 174.5 (s, CO of Aib3); 173.6 (s, CO of Aib2); 173.5 (s, CO of Aib5); 173.0 (s, CO of Aib7); 172.6 (s, CO of Leu6); 171.0 (s, CO of Phe8); 169.8 (s, CO of Val4); 168.7 (s, CO of Gly1); 138.1 (s, 1 arom. C of Phe8); 128.5, 127.7, 125.8 (3d, 5 arom. CH of Phe8); 57.8 (d, C_α of Val4); 55.9 (s, C_α of Aib2, Aib3); 55.8 (s, C_α of Aib5); 55.7 (s, C_α of Aib7); 53.4 (d, C_α of Phe8); 50.6 (d, C_α of Leu6); 44.1 (t, C_α of Gly1); 41.5 (t, C_β of Leu6); 36.1 (t, C_β of Phe8); 28.3 (d, C_β of Val4); 26.3 (q, Me of Aib2 or Aib5); 26.0 (q, Me of Aib3); 25.1 (q, Me of Aib2); 24.53, 24.48 (2q, 2 Me of Aib7); 24.2 (q, Me of Aib2 or Aib5); 23.8 (q, Me of Aib3); 23.2 (d, C_γ of Leu6); 22.9 (q, Me of Leu6); 22.6 (q, Me of Aib5); 21.1 (q, Me of Leu6); 19.1, 17.3 (2q, 2 Me of Val4). IR (KBr): 3337s, 3062w, 3031w, 2961m, 2936m, 2872m, 1663s, 1527s, 1467m, 1386m, 1364m, 1278m, 1224m, 1184m, 1123w, 1031w, 944w, 748m, 701m, 573m, 506m.

Z-Gly-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu (8)

The coupling of Z-Gly-Aib-OH (0.171 g, 0.58 mmol) with H-Val-Aib-Leu-Aib-Phe-O*t*Bu (0.385 g, 0.64 mmol) in absolute CH_2Cl_2/DMF (3 : 1, 12 ml) was achieved according to GP B, using PyBOP (0.34 g, 0.65 mmol), HOAt (0.65 mmol, 1.3 ml 0.5 M solution in DMF) and DIEA (0.168 g, 1.3 mmol). Reaction time: 24 h. Purification by CC (SiO_2, performed twice; once with pure EtOAc and once with CH_2Cl_2/MeOH 13 : 1) afforded 0.3 g (59%) of heptapeptide **8** as a foam.

ESI-MS (MeOH + NaI): *m/z* 902.6 ($[M + Na]^+$); calcd. 902.5. Mp 122–123 °C. R_f (EtOAc) = 0.3. ^{1}H-NMR ($CDCl_3$): 8.49 (s, NH); 7.78 (br.d, $J = 4.98$, NH); 7.62 (s, NH); 7.56 (br.t, NH of Gly); 7.52 (s, NH); 7.36–7.16 (m, 10 arom. H, 2 NH); 5.06–5.03 (m, $PhCH_2O$); 4.36–4.34, 4.15–4.02, 3.77–3.64 (3m, 3 H_α of Leu, Phe, Val and 2 H_α of Gly); 2.94–2.86 (m, 2 H_β of Phe); 2.15–2.03 (m, 2 H_β of Val); 1.70–1.50 (m, 2 H_β and H_γ of Leu); 1.40, 1.39, 1.37, 1.34, 1.30 (5s, 6 Me of 3 Aib, *t*Bu); 0.94 (dd, $J = 16.0$, 6.7, 2 Me of Leu); 0.79 (dd, $J = 13.8$, 5.8, 2 Me of Val). ^{13}C-NMR ($CDCl_3$): 176.4, 174.2, 173.8, 172.1, 170.9, 170.1 (6s, 6 CO(amide) and CO(ester)); 156.6 (s, CO(urethane)); 137.2, 136.7 (2s, 2 arom. C); 128.9, 128.2, 127.9, 127.7, 127.4, 126.2 (6d, 10 arom. CH); 80.3 (s, *t*Bu); 65.4 (t, $PhCH_2O$); 61.6 (d, C_α of Val); 56.1, 55.9, 55.7 (3s, 3 C_α of 3 Aib); 54.0, 51.2 (2d, C_α of Phe and C_α of Leu); 43.6, 39.1, 37.0 (3t, C_α of Gly, C_β of Leu and C_β of Phe); 28.4 (d, C_β of Val); 27.3 (q, *t*Bu); 24.1 (d, C_γ of Leu); 26.2, 25.9, 23.6, 23.3, 23.0, 20.3, 19.5, 18.6 (8q, 6 Me of 3 Aib, 2 Me of Leu and 2 Me of Val). IR (KBr): 3335s, 3063w, 3032w, 2964m, 2936m, 2873w, 1664s, 1529s, 1467m, 1454m, 1385m, 1368m, 1245m, 1156s, 1049w, 851s, 739w, 699m, 558m.

Cyclo(Gly1-Aib2-Val3-Aib4-Leu5-Aib6-Phe7) (3)

Z-Gly-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu (**8**) (0.22 g, 0.25 mmol) was *N*-deprotected according to GP A (H_2, 23 mg Pd/C, 6 ml MeOH, 20 h). Thus, 0.19 g (quant. yield) of H-Gly-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu were obtained as a foam, which was dissolved in CH_2Cl_2 (6 ml), and TFA (4 ml) was added at rt. The mixture was stirred for 2 h at rt. Excess TFA was evaporated, and the remaining TFA was removed by co-evaporation with Et_2O. The residue was triturated with Et_2O, the solution decanted, and the operation was repeated twice to afford, after drying under vacuum, 0.2 g (0.25 mmol, 100%) of the deprotected octapeptide as its TFA salt.

Cyclisation with PyAOP/HOAt. The free linear heptapeptide TFA salt (0.1 g, 0.125 mmol) was dissolved in absolute DMF (250 ml, 0.5 mM solution) under stirring. PyAOP (0.324 g, 0.62 mmol), HOAt (0.62 mmol, 1.25 ml of 0.5 M solution in DMF) and DIEA (0.48 g, 3.73 mmol) were added at rt and the solution was stirred for an additional 3 days. The solvent was then removed under reduced pressure, the residue dissolved in EtOAc and washed with 10% citric acid solution, 5% $NaHCO_3$ solution, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated to afford a yellow oil which was purified by CC (SiO_2, performed twice; once with EtOAc and once with EtOAc/MeOH 20 : 1), and PLC (CH_2Cl_2/MeOH 20 : 1) to give 25 mg (30%) of pure cyclic heptapeptide **3** as a powder.

Cyclisation with DEPC/DEPBT. The free linear heptapeptide TFA salt (0.1 g, 0.125 mmol) was dissolved in absolute DMF (125 ml, 1 mM solution) and the solution was cooled to 0 °C in an ice bath. Then, DEPC (0.209 g, 1.25 mmol), DEPBT (0.372 g, 1.25 mmol) and DIEA (1.25 ml, 1% v/v) were added under stirring. The solution was warmed to rt and stirred for an additional 5 days. The solvent was then evaporated under reduced pressure, the residue taken up in EtOAc and washed with 5% $KHSO_4$ solution, 5% $NaHCO_3$ solution, and brine. The organic phase was then dried ($MgSO_4$) and concentrated to give the crude cyclic heptapeptide, which was purified by CC and PLC. CC (SiO_2, performed twice, once with EtOAc, once with EtOAc/MeOH 20 : 1). PLC (SiO_2, performed twice, CH_2Cl_2/MeOH 15 : 1, CH_2Cl_2/MeOH/hexane 15 : 1 : 3). 31 mg (37%) of pure **3** were obtained as a powder.

ESI-MS (MeOH + NaI): *m/z* 694.4 ($[M + Na]^+$); calcd. 694.4. R_f (EtOAc/MeOH 15 : 1) = 0.35. ^{1}H-NMR(600 MHz, DMSO-d_6): 8.53 (s, NH of Aib6); 8.16 (s, NH of Aib2); 7.89 (br. t,

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$J = 5.7$, NH of Gly1); 7.79–7.72 (m, NH of Phe7 and NH of Aib4); 7.27–7.21 (m, 2 arom. H and NH of Val3); 7.19–7.12 (m, 3 arom. H); 6.9 (d, $J = 8.3$, NH of Leu5); 4.34–4.28 (m, H_α Leu5); 4.17–4.08 (m, H_α of Phe7, H_α of Val3); 4.00–3.93 (m, 1 H_α of Gly1); 3.70 (dd, $J = 16.8$, 5.1, 1 H_α of Gly1); 3.25 (dd, $J = 13.8$, 4.2, 1 H_β of Phe7); 3.10–2.95 (m, 1 H_β of Phe7); 2.28–2.20 (m, H_β of Val3); 1.60–1.52 (m, H_γ of Leu5); 1.40 (s, Me of Aib4); 1.39, 1.38 (2s, 2 Me of Aib2); 1.37–1.29 (m, 2 H_β of Leu5); 1.28 (s, Me of Aib4); 1.21, 1.12 (2s, 2 Me of Aib6); 0.89–0.83 (m, 2 Me of Leu5, 2 Me of Val3). ^{13}C–NMR (150.9 MHz, DMSO-d_6): 174.1 (s, CO of Aib2); 173.25 (s, CO of Aib6); 173.20 (s, CO of Aib4); 172.7 (s, CO of Leu5); 171.1 (s, CO of Phe7); 170.3 (s, CO of Val3); 168.9 (s, CO of Gly1); 138.7 (*s*, 1 arom. C); 129.0, 127.8, 125.9 (3d, 5 arom. CH); 57.6 (d, C_α of Val3); 56.3 (s, C_α of Aib4); 56.0 (s, C_α of Aib2); 55.9 (s, C_α of Aib6); 54.6 (d, C_α of Phe7); 50.8 (d, C_α of Leu5); 42.0 (t, C_α of Gly1); 41.2 (t, C_β of Leu5); 35.0 (t, C_β of Phe7); 28.9 (d, C_β of Val3); 27.2 (q, Me of Aib4); 26.0 (q, Me of Aib2); 25.2 (q, Me of Aib6); 24.0 (q, Me of Aib2, Me of Aib6); 23.8 (d, C_γ of Leu5); 23.1 (q, Me of Leu5); 22.7 (q, Me of Aib4); 21.7 (q, Me of Leu6); 19.2, 17.5 (2q, 2 Me of Val3). IR (KBr): 3331s, 3062w, 3031w, 2962m, 2935m, 2872m, 1663s, 1532s, 1468m, 1386m, 1363m, 1335w, 1284m, 1229m, 1177m, 1124w, 1031w, 945w, 847w, 748w, 700m, 604w, 575w.

X-ray Crystal Structure Determination of 2[1]

Colourless single crystals of cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**) were obtained by slow evaporation at room temperature from a $CHCl_3/Et_2O$/EtOH solution. All measurements were made on a *Nonius Kappa CCD* area-detector diffractometer [21] using graphite-monochromated MoK_α radiation (λ 0.71073 Å) and an *Oxford Cryosystems Cryostream 700* cooler. The intensities were corrected for Lorentz and polarisation effects, and an absorption correction based on the multi-scan method was applied [22]. Equivalent reflections, other than Friedel pairs, were merged. Data collection and refinement parameters are given in Table 1. The structure was solved by direct methods using *SHELXS97* [23], which revealed the positions of all non-hydrogen atoms. The asymmetric unit contains two molecules of the peptide and five molecules of $CHCl_3$. The atomic coordinates were tested carefully for a relationship from a higher symmetry space group using the program *PLATON* [24], but none could be found. The $CHCl_3$ molecules are probably disordered, but the disorder could not be modelled successfully, nor could any improvement be achieved by omitting the solvent molecules and applying the *SQUEEZE* [25] routine of the *PLATON* program. In peptide molecule A, the phenyl ring is disordered over two conformations. Two sets of overlapping positions were defined for the atoms of the disordered ring and the site occupation factor of the major conformation of the ring refined to 0.53(2). The two orientations of the disordered phenyl ring were refined as idealised rigid groups. Similarity restraints were applied to the C–C bond lengths between the disordered phenyl ring and the benzyl methylene group. Neighbouring atoms within and between each conformation of the disordered phenyl ring were restrained to have similar atomic displacement parameters. The non-hydrogen atoms were refined anisotropically. All of the H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 U_{eq} of its parent atom (1.5 U_{eq} for the methyl groups). Refinement of the structure was carried out on F^2 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 not applied. Five reflections, whose intensities were considered to be extreme outliers, were omitted from the

Table 1 Crystallographic data of **2**

Crystallised from	$CHCl_3/Et_2O$/EtOH
Empirical formula	2 ($C_{38}H_{60}N_8O_8$).5 ($CHCl_3$)
Formula weight (g mol^{-1})	2110.72
Crystal colour, habit	Colourless, plate
Crystal dimensions (mm)	0.15 × 0.35 × 0.35
Temperature (K)	160 (1)
Crystal system	Monoclinic
Space group	$P2_1$
Z	2
Reflections for cell determination	167 817
2θ range for cell determination (°)	4–50
Unit cell parameters	
a (Å)	14.9157 (2)
b (Å)	10.3783 (1)
c (Å)	36.2719 (5)
β (°)	101.5641(6)
V (Å^3)	5500.9 (1)
F(000)	2212
D_x (g cm^{-3})	1.274
μ(Mo $K\alpha$) (mm^{-1})	0.436
Scan type	ω
$2\theta_{(max)}$ (°)	50
Transmission factors (min; max)	0.736; 0.942
Total reflections measured	76 088
Symmetry-independent reflections	19 399
R_{int}	0.076
Reflections with $I > 2\sigma(I)$	13 570
Reflections used in refinement	19 394
Parameters refined; restraints	1208; 170
Final $R(F)$ [$I > 2\sigma(I)$ reflections]	0.0794
$wR(F^2)$ (all data)	0.2349
Weights:	$w = [\sigma^2(F_o^2) + (0.1348\ P)^2 + 5.7910\ P]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$
Goodness of fit	1.028
Final Δ_{max}/σ	0.002
$\Delta\rho$ (max; min) (e Å^{-3})	0.92; −0.73
$\sigma(d_{(C—C)})$ (Å)	0.006–0.01

[1] CCDC-675386 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from the *Cambridge Crystallographic Data Center via* http://www.ccdc.cam.ac.uk/data_request/cif.

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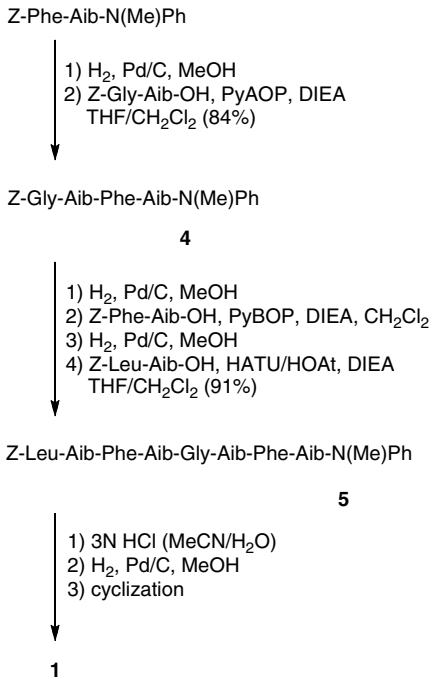
final refinement. Refinement of the absolute structure parameter [26,27] yielded a value of −0.03 (7), which confidently confirms that the refined coordinates represent the true enantiomorph. Neutral atom scattering factors for non-hydrogen atoms were taken from Ref. 28, and the scattering factors for H-atoms were taken from Ref. 29. Anomalous dispersion effects were included in F_c [30]; the values for f' and f'' were those of Ref. 31. The values of the mass attenuation coefficients are those of Ref. 32. All calculations were performed using the *SHELXL97* program [33].

RESULTS AND DISCUSSION

The linear octa and heptapeptides Z-Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib-N(Me)Ph (**5**), H-Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu (**7**), and Z-Gly-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu (**8**) were synthesised by solution-phase methods as shown in Schemes 1 and 2. For the synthesis of the octapeptide **5**, the disconnection approach was used. Thus, **5** was disconnected into four dipeptide units, Z-Gly-Aib-OH, Z-Phe-Aib-N(Me)Ph, Z-Phe-Aib-OH and Z-Leu-Aib-OH. These required dipeptide units were prepared according to the previously described procedures [12,18,19]. The dipeptide amide Z-Phe-Aib-N(Me)Ph [18] was first *N*-deprotected under hydrogenation conditions, and then coupled with Z-Gly-Aib-OH [12] by using PyAOP, which led to the tetrapeptide Z-Gly-Aib-Phe-Aib-N(Me)Ph (**4**) in high yield. After deprotection of the *N*-terminus of the latter, the reaction with Z-Phe-Aib-OH [18] using PyBOP as activating agent afforded hexapeptide Z-Phe-Aib-Gly-Aib-Phe-Aib-N(Me)Ph in nearly quantitative yield. However, traces of the coupling reagent could not be separated from the hexapeptide. Removal of the Z protecting group, followed by the coupling of the resulting H-Phe-Aib-Gly-Aib-Phe-Aib-N(Me)Ph with Z-Leu-Aib-OH [19] in the presence of HATU/HOAt afforded the linear octapeptide **5** in high yield.

For the synthesis of the linear octapeptide **7**, a convergent (3 + 2 + 3) strategy was employed. After removal of the Z group in Z-Leu-Aib-Phe-O*t*Bu [14], condensation with Z-Val-Aib-OH [20] by using PyBOP/HOAt provided the pentapeptide Z-Val-Aib-Leu-Aib-Phe-O*t*Bu (**6**) in high yield. The latter was *N*-deprotected by means of catalytic hydrogenation and the obtained H-Val-Aib-Leu-Aib-Phe-O*t*Bu was devided in two portions. One portion of this peptide was coupled with Z-Gly-Aib-Aib-OH [12] using PyBOP, to give, after the Z protecting group has been removed, pure octapeptide **7**. The condensation of the second portion of H-Val-Aib-Leu-Aib-Phe-O*t*Bu with Z-Gly-Aib-OH [12] afforded heptapeptide Z-Gly-Aib-Val-Aib-Leu-Aib-Phe-O*t*Bu (**8**) in good yield when PyBOP was used as the coupling reagent.

The obtained linear peptides were then deprotected and subjected to macrolactamisation in order to estimate the cyclisation tendency of each peptide. Since



Scheme 1 Synthesis of octapeptide (**5**).

no activating reagent has been found to be generally applicable in the cyclisation step, several coupling reagents and reaction conditions were tested. All of the cyclisation reactions were performed in diluted DMF or DMF/CH_2Cl_2 solutions (0.5–1.0 mM) using an excess of coupling reagents and base. The results of the cyclisations are summarised in Table 2. In the case of cyclic octapeptide cyclo(Leu-Aib-Phe-Aib-Gly-Aib-Phe-Aib) (**1**), both HATU and PyAOP were highly efficient and interestingly afforded **1** in exactly the same cyclisation yield. We used only a slight excess of HATU over the linear peptide since HATU is known to participate in a side reaction at the amino terminus to give a guanidino derivative [34]. This side reaction usually occurs when an excess of the aminium salt–based coupling reagents and prolonged reaction times are used. Remarkably, the previously synthesised Aib-containing cyclic hexapeptides were obtained only in relatively low yields (<25%) when HATU was used in the cyclisation step [14]. In the case of cyclic octapeptide cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**), the phosphonium reagent PyAOP was superior to HATU during cyclisation, which is in accordance with our previously observed results in the case of cyclic hexapeptides. Next, the macrolactamisation ability of the organophosphorus reagent DEPC [35] was tested. This reagent showed high efficiency during the cyclisation of Aib-containing penta- and hexapeptides [12–14,18]. However, the cyclisation step leading to cyclic octapeptide **1** resulted only in a complex mixture of products. Therefore, we decided to use DEPC in combination with the coupling reagent DEPBT. This reagent has proven to be very efficient in the synthesis of cyclic peptides with

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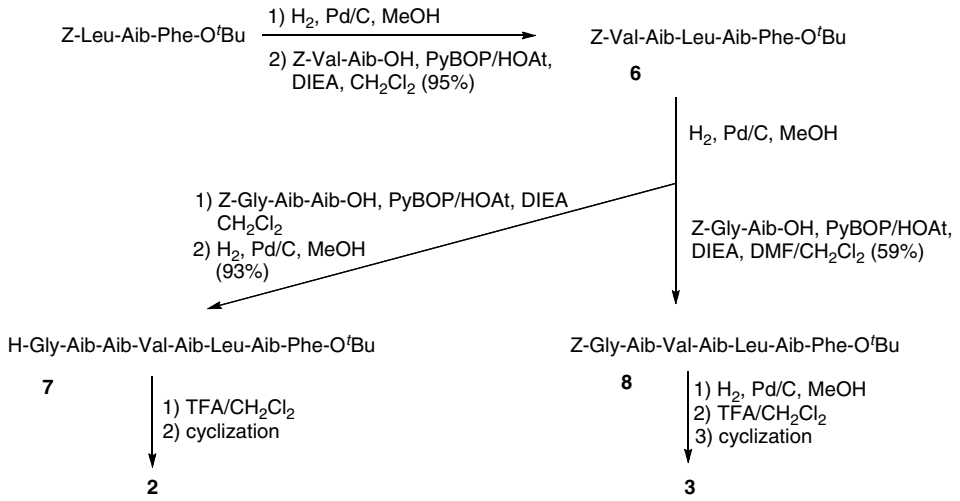

Scheme 2 Synthesis of octapeptide (**7**) and heptapeptide (**8**).

Table 2 Conditions used for the cyclisation of Aib-containing peptides

Cyclopeptide	Cyclisation reagents (equivalents)	Reaction time (*d*)	Yield (%)
1	HATU (1.25)/HOAt (2.5)/DIEA (10)	1	58
	PyAOP (3)/HOAt (3)/DIEA (10)	3	58
	PyBOP (3)/HOAt (3)/DIEA (1% v/v)	1	35
	PyBrOP (5)/HOAt (5)/DIEA (20)	3	44
2	HATU (1.25)/HOAt (2.5)/collidine (20)	2	31
	PyAOP (3)/HOAt (3)/DIEA (15)	3	63
	DEPC (6)/DEPBT (6)/DIEA (40)	5	54
3	PyAOP (5) /HOAt (5)/DIEA (30)	3	30
	DEPC (10)/DEPBT (10)/DIEA (1% v/v)	5	37

remarkable resistance to racemisation [36]. Thus, the cyclic peptides **2** and **3** were also obtained in good yields when DEPC/DEPBT cyclisation conditions were employed. A similar application of two or more coupling reagents at the same time (one-pot) has previously been reported to facilitate efficient ring-closing reactions by providing a choice of reagents for the specific substrate, which is in lieu of optimising each individual reaction for each individual coupling agent [10, 37]. So far, no bioassays have been performed with the prepared cyclopeptides.

Solid State Conformation and Hydrogen-Bonding Pattern

We carried out an X-ray crystallographic investigation on the molecular structure of **2** to study the effect that a high content of Aib residues might have on the conformation of a cyclic octapeptide. The asymmetric unit contains two molecules of the cyclic peptide and five molecules of $CHCl_3$. The symmetry-independent peptide molecules have almost identical conformations. The phenyl ring in peptide molecule A is disordered over two slightly different, almost equally occupied orientations, which result from a small rotation about the C(23)–C(40) bond. The ORTEP plots [38] of the molecular structure with the atom numbering schemes are presented in the Figures 2 and 3. The relevant torsion angles for **2** are summarised in Table 3, and the hydrogen-bond parameters are shown in Table 4.

Peptide molecules A and B display identical hydrogen-bonding patterns. The pattern for molecule A is described below. Six of the amide groups in

Table 3 Backbone torsion angles (°) for cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**)

Residue	ϕ (Molecule A)	ψ (Molecule A)	ϕ (Molecule B)	ψ (Molecule B)
Gly1	−80.7(6)	+51.7(7)	−80.1(6)	+49.5(7)
Aib2	+53.4(7)	−124.0(5)	+54.3(7)	−125.1(5)
Aib3	−53.0(6)	−32.0(7)	−54.3(6)	−30.8(6)
Val4	−83.3(5)	−9.2(6)	−81.3(5)	−7.8(6)
Aib5	+71.0(6)	−54.6(6)	+72.5(6)	−56.5(6)
Leu6	−49.4(6)	+135.3(4)	−49.4(6)	+133.4(4)
Aib7	+57.8(6)	+25.2(6)	+58.4(6)	+25.9(6)
Phe8	+62.5(6)	+24.5(6)	+64.7(6)	+24.2(6)

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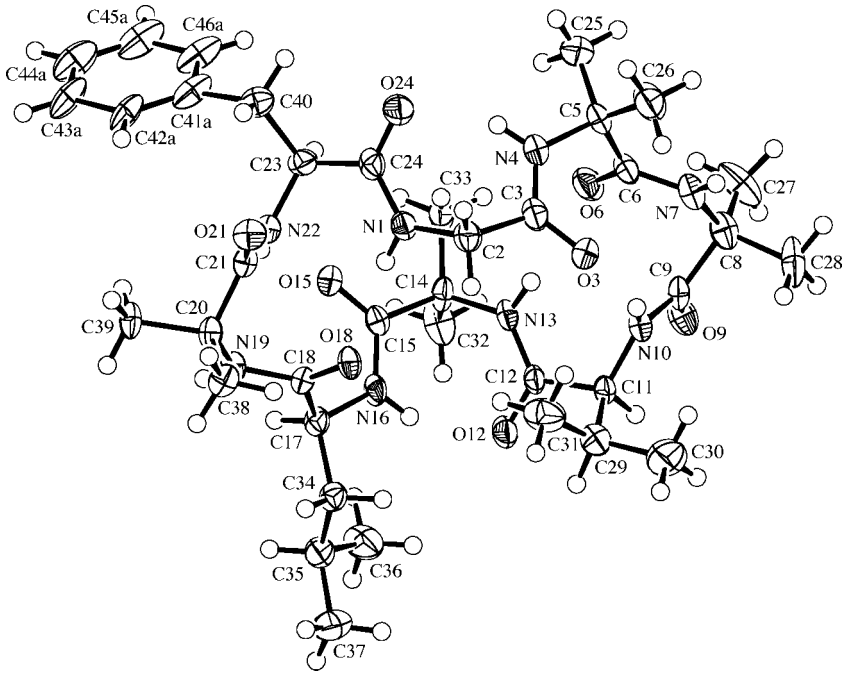

Figure 2 ORTEP plot [38] of the molecular structure of molecule A of **2** (50% probability ellipsoids; arbitrary numbering of atoms; only one conformation of the disordered phenyl ring is shown).

Table 4 Hydrogen-bonding geometry for cyclo(Gly-Aib-Aib-Val-Aib-Leu-Aib-Phe) (**2**)

Donor (D–H)	Acceptor (A)	D–H (Å)	H···A (Å)	D···A (Å)	D-H···A (°)
N(1)–H(1)	O(18)	0.88	2.08	2.904(5)	156
N(4)–H(4)	O(24)	0.88	2.06	2.853(6)	150
N(7)–H(7)	O(9[a])	0.88	2.05	2.901(6)	161
N(10)–H(10)	O(3)	0.88	2.32	3.151(6)	159
N(13)–H(13)	O(6)	0.88	2.19	3.032(6)	160
N(16)–H(16)	O(12)	0.88	1.90	2.706(5)	152
N(19)–H(19)	O(71)	0.88	2.01	2.883(5)	171
N(22)–H(22)	O(15)	0.88	2.21	2.893(5)	134
N(51)–H(51)	O(68)	0.88	2.10	2.936(5)	159
N(54)–H(54)	O(74)	0.88	2.09	2.875(5)	148
N(57)–H(57)	O(59[b])	0.88	2.06	2.912(6)	164
N(60)–H(60)	O(53)	0.88	2.44	3.252(6)	155
N(63)–H(63)	O(56)	0.88	2.09	2.939(5)	162
N(66)–H(66)	O(62)	0.88	1.91	2.724(5)	153
N(69)–H(69)	O(21[c])	0.88	2.02	2.890(5)	169
N(72)–H(72)	O(65)	0.88	2.23	2.929(5)	136

Atom labels with superscripts refer to a molecule in the following symmetry related positions.
[a] $1-x, -1/2+y, 1-z$.
[b] $2-x, -1/2+y, -z$.
[c] $x, 1+y, z$.

molecule A form intramolecular hydrogen bonds with amide O-atoms, while the remaining two amide groups [N(7)–H and N(19)–H] are involved in intermolecular interactions. Two types of intramolecular interactions are present. N(1)–H, N(10)–H, N(13)–H and N(22)–H form intramolecular hydrogen bonds with the amide O-atom that is two peptide units back along the peptide backbone. Each of these interactions creates a ten-membered loop and can be described by a graph-set motif of S(10) [39]. N(4)–H and N(16)–H form intramolecular hydrogen bonds with the amide O-atom that is in the first peptide unit back along the peptide backbone. Each of these interactions creates a seven-membered loop and can be described by a graph-set motif of S(7). N(7)–H forms an intermolecular hydrogen bond with the amide O-atom, O(9), from an adjacent molecule A and thereby links the A molecules into extended zig-zag chains which run parallel to the [0 1 0] direction and have a graph-set motif of C(5). The B molecules are linked in the same way into extended zig-zag chains which also run parallel to the [0 1 0] direction. N(19)–H of molecule A forms an intermolecular hydrogen bond with the amide O-atom, O(71), from an adjacent molecule B. Molecule B then interacts in the same way with O(21) of a different molecule A. These interactions link the A and B molecules into extended ···A···B···A···B··· chains that run parallel to the [0 1 0] direction and have a graph-set motif of $C_2^2(10)$. Together, the intermolecular interactions link the peptide molecules into two-dimensional networks that lie parallel to the (1 0 1) plane.

The overall conformation of cyclic peptide **2** includes four β-turns, a γ-turn, and an inverse γ-turn [40,41], all being stabilised by hydrogen bonds (Figure 4). The Gly1-Aib2-Aib3-Val4-Aib5 segment is structured with

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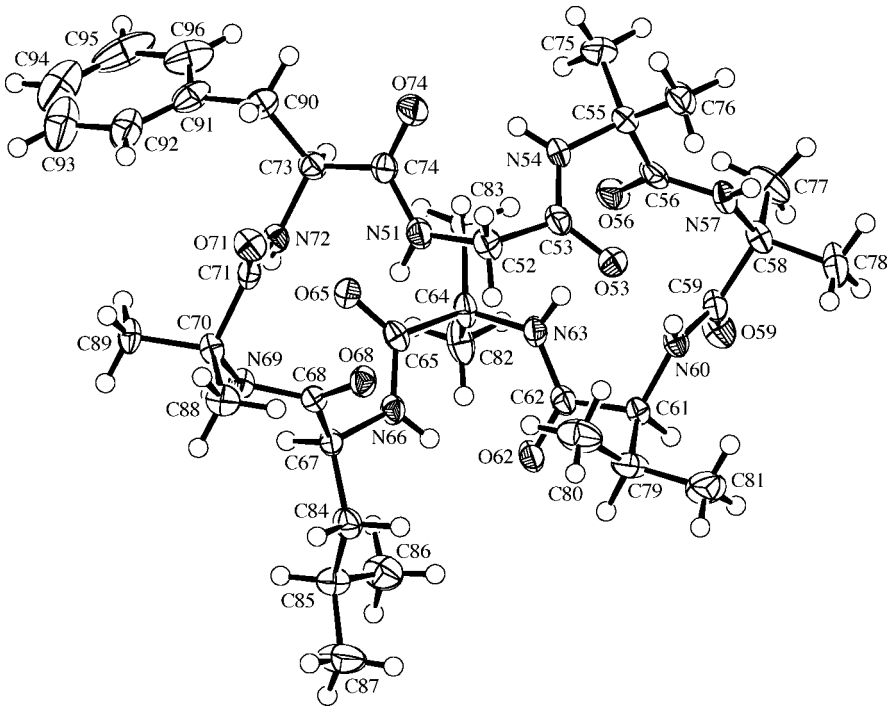

Figure 3 ORTEP plot [38] of the molecular structure of molecule B of **2** (50% probability ellipsoids; arbitrary numbering of atoms).

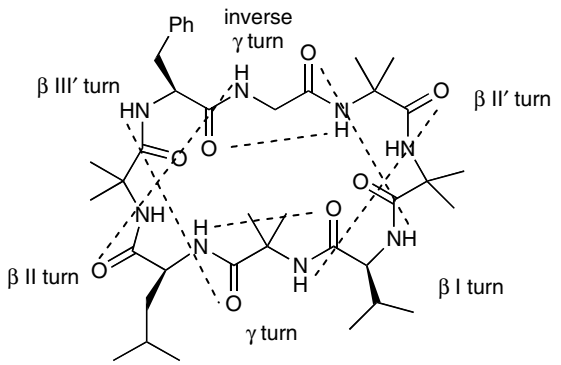

Figure 4 Schematic diagram of the turn types and the hydrogen bonds (dashed lines) for **2**.

two consecutive β-turns of type II′ along Gly1-Aib2-Aib3-Val4, and type I over the sequence Aib2-Aib3-Val4-Aib5. In the segment Aib5-Leu6-Aib7-Phe8-Gly1, two consecutive β-turns of type II/III′ are also found with intramolecular hydrogen bonds between the NH of Phe8 and the CO of Aib5, and between the NH of Gly1 and the CO of Leu6. The two observed β-turns of type II′/II are slightly distorted because of the ϕ, ψ values of the $(i+2)$ residues Aib3 and Aib7, respectively (Table 3). Three Aib residues show torsion angles which are in good agreement with the expected values in the helical region of the conformational space. The residue Aib2 is obviously forced to assume the conformation of a D-amino acid [42] as it prefers the $(i+1)$ position of a β-turn of type II′, which has rarely been observed for this residue. A rather similar conformation was previously found for the residue Aib2 of the cyclic hexapeptide cyclo(Gly1-Aib2-Leu3-Aib4-Phe5-Aib6) [14]. The structure is further stabilised by intramolecular hydrogen bonds between the NH of Aib2 and the CO of Phe8, and between the NH of Leu6 and the CO of Val4, which form γ-turns. The torsion angles of Gly1 and Aib5 are consistent with the ideal values for an inverse γ- and a γ-turn, respectively. The only known case of Aib being within a γ-turn is that of the cyclotetrapeptide dihydrochlamydocin [43].

CONCLUSIONS

In the synthesis of Aib-containing cyclic octapeptides **1** and **2** reported here we have obtained high yields of cyclisation using PyAOP and/or HATU coupling reagents. These high yields probably indicate the presence of favourable conformations in the linear precursor peptides. The four conformationally constrained Aib residues obviously contribute to the formation of stable-folded structures, which bring the *N*- and *C*-terminal residues closer together and thereby facilitating the cyclisation reaction. The proximity of the latter is seemingly not ideal for the cyclisation of a heptapeptide containing alternating Aib and proteinogenic amino acid residues. This results in only moderate yields obtained in the case of cyclic peptide **3**.

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REFERENCES

1. Lambert JN, Mitchell JP, Roberts KD. The synthesis of cyclic peptides. *J. Chem. Soc., Perkin Trans. 1* 2001; 471–484.
2. Li P, Roller PP, Xu J. Current synthetic approaches to peptide and peptidomimetic cyclization. *Curr. Org. Chem.* 2002; **6**: 411–440.
3. Davies JS. The cyclization of peptides and depsipeptides. *J. Pept. Sci.* 2003; **9**: 471–501.
4. Kazmeier U, Hebach C, Watzke A, Maier S, Mues H, Huch V. A straightforward approach towards cyclic peptides via ring-closing metathesis – scope and limitation. *Org. Biomol. Chem.* 2005; **3**: 136–145.
5. Ravn J, Bourne GT, Smythe ML. A safety catch linker for Fmoc-based assembly of constrained cyclic peptides. *J. Pept. Sci.* 2005; **11**: 572–578.
6. Balraju V, Iqbal J. Synthesis of cyclic peptides constrained with biarylamine linkers using Buchwald-Hartwig C-N coupling. *J. Org. Chem.* 2006; **71**: 8954–8956.
7. Tegge W, Bautsch W, Frank R. Synthesis of cyclic peptides and peptide libraries on an new disulfide linker. *J. Pept. Sci.* 2007; **13**: 693–699.
8. Oike H. Supramolecular approach for synthesis and functionalization of cyclic macromolecules. *React. Funct. Polym.* 2007; **67**: 1157–1167.
9. Teixido M, Altamura M, Quartara L, Giolitti A, Maggi CA, Giralt E, Albericio F. Bicyclic homodetic peptide libraries: comparison of synthetic strategies for their solid-phase synthesis. *J. Comb. Chem.* 2003; **5**: 760–768.
10. Bolla ML, Azevedo EV, Smith JM, Taylor RE, Ranjit DK, Segall AM, McAlpine SR. Novel antibiotics: macrocyclic peptides designed to trap holliday junctions. *Org. Lett.* 2003; **5**: 109–112.
11. Gilon C, Mang C, Lohof E, Friedler A, Kessler H. Cyclization of peptides. In *Houben-Weyl Methods of Organic Chemistry*, Vol. E22b, Goodman M, Felix A, Moroder L, Toniolo C (eds). Thieme Publisher: Stuttgart, 2003; 461–542.
12. Jeremic T, Linden A, Heimgartner H. Solution-phase synthesis of Aib-containing cyclic hexapeptides. *Chem. Biodivers.* 2004; **1**: 1730–1761.
13. Jeremic T, Linden A, Heimgartner H. Synthesis of cyclohexapeptides containing Pro and Aib residues. *Helv. Chim. Acta* 2004; **87**: 3056–3079.
14. Jeremic T, Linden A, Moehle K, Heimgartner H. Synthesis and conformational analysis of 18-membered Aib-containing cyclohexapeptides. *Tetrahedron* 2005; **61**: 1871–1883.
15. Ehrlich A, Heyne HU, Winter R, Beyermann M, Haber H, Carpino LA, Bienert M. Cyclization of *all*-L-pentapeptides by means of 1-hydroxy-7-azabenzotriazole-derived uronium and phosphonium reagents. *J. Org. Chem.* 1996; **61**: 8831–8838.
16. Kessler H, Bernd M. Konformationsuntersuchungen an vier cyclischen Heptapeptiden durch NMR-Spektroskopie. *Liebigs Ann. Chem.* 1985; 1145–1167.
17. Gu W, Silverman RB. Solid-phase total synthesis of scytalidamide A. *J. Org. Chem.* 2003; **68**: 8774–8779.
18. Arnhold FA. Synthese von Aib- und Phe(2Me)-haltigen Pentapeptiden und deren Cyclisierung. PhD thesis, Universität Zürich, 1997.
19. Brun KA, Linden A, Heimgartner H. New optically active 2*H*-azirin-3-amines as synthons for enantiomerically pure 2,2-disubstituted glycines. *Helv. Chim. Acta* 2001; **84**: 1756–1777.
20. Lehmann J, Heimgartner H. Synthesis of endothiopeptides and their cyclization to 1,3-thiazol-5(4*H*)-imines. *Helv. Chim. Acta* 1999; **82**: 1899–1915.
21. Hooft R. *KappaCCD Collect Software*, Nonius BV: Delft, 1999.
22. Blessing RH. An empirical correction for absorption anisotropy. *Acta Crystallogr., Sect. A* 1995; **51**: 33–38.
23. Sheldrick GM. *SHELXS97, Program for the Solution of Crystal Structures*. University of Göttingen: Göttingen, 1997.
24. Spek AL. *PLATON, Program for the Analysis of Molecular Geometry*. University of Utrecht: Utrecht, 2005.
25. van der Sluis P, Spek AL. BYPASS: an effective method for the refinement of crystal structures containing disordered solvent regions. *Acta Crystallogr.* 1990; **A46**: 194–201.
26. Flack HD, Bernardinelli G. Absolute structure and absolute configuration. *Acta Crystallogr., Sect. A* 1999; **55**: 908–915.
27. Flack HD, Bernardinelli G. Reporting and evaluating absolute-structure and absolute-configuration determinations. *J. Appl. Crystallogr.* 2000; **33**: 1143–1148.
28. Maslen EN, Fox AG, O'Keefe MA. In *International Tables for Crystallography*, Vol. C, Table 6.1.1.1, Wilson AJC (ed.). Kluwer Academic Publishers: Dordrecht, 1992; 477–486.
29. Stewart RF, Davidson ER, Simpson WT. Coherent X-ray scattering for hydrogen atom in hydrogen molecule. *J. Chem. Phys.* 1965; **42**: 3175–3187.
30. Ibers JA, Hamilton WC. Dispersion corrections + crystal structure refinements. *Acta Crystallogr.* 1964; **17**: 781–782.
31. Creagh DC, McAuley WJ. In *International Tables for Crystallography*, Vol. C, Table 4.2.6.8, Wilson AJC (ed.). Kluwer Academic Publishers: Dordrecht, 1992; 219–222.
32. Creagh DC, Hubbell JH. In *International Tables for Crystallography*, Vol. C, Table 4.2.4.3, Wilson AJC (ed.). Kluwer Academic Publishers: Dordrecht, 1992; 200–206.
33. Sheldrick GM. *SHELXL97, Program for the Refinement of Crystal Structures*. University of Göttingen: Göttingen, 1997.
34. Albericio F, Bofill JM, El-Faham A, Kates SA. Use of onium salt-based coupling reagents in pepide synthesis. *J. Org. Chem.* 1998; **63**: 9678–9683.
35. Yokokawa F, Sameshima H, Katagiri D, Aoyama T, Shioiri T. Total syntheses of lyngbyabellins A and B, potent cytotoxic lipopeptides from the marine cyanobacterium *Lyngbya majuscula*. *Tetrahedron* 2002; **58**: 9445–9458.
36. Goodman M, Zapf C, Rew Y. New reagents, reactions, and peptidomimetics for drug design. *Biopolymers* 2001; **60**: 229–245.
37. Robinson JL, Taylor RE, Liotta LA, Bolla ML, Azevedo EV, Medina I, McAlpine SR. A progressive synthetic strategy for class B synergimycins. *Tetrahedron Lett.* 2004; **45**: 2147–2150.
38. Johnson CK. *ORTEPII, Report ORNL-5138*. Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
39. Bernstein J, Davis RE, Shimoni L, Chang N-L. Patterns in hydrogen bonding-functionality and graph set analysis in crystals. *Angew. Chem., Int. Ed. Engl.* 1995; **34**: 1555–1573.
40. Rose GD, Gierasch LM, Smith JA. Turns in peptides and proteins. *Adv. Protein Chem.* 1985; **37**: 1–109.
41. Müller G, Gurrath M, Kurz M, Kessler H. βVI turns in peptides and proteins: a model peptide mimicry. *Proteins: Struct., Funct., Genet.* 1993; **15**: 235–251.
42. Kessler H, Gratias R, Hessler G, Gurrath M, Mueller G. Conformation of cyclic peptides. Principle concepts and design of selectivity and superactivity in bioactive sequences by spatial screening. *Pure Appl. Chem.* 1996; **68**: 1201–1205.
43. Flippen JL, Karle IL. Conformation of the cyclic tetrapeptide dihydrochlamydocin. Iabu-L-Phe-D-Pro-LX, and experimental values for $3 \rightarrow 1$ intramolecular hydrogen bonds by X-ray diffraction. *Biopolymers* 1976; **15**: 1081–1092.