Journal of Peptide Science
J. Peptide Sci. **9**: 187–199 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.445

JOURNAL OF PEPTIDE SCIENCE

Synthesis and Conformational Investigation of Cyclic Dipeptides: 7-Membered Rings Containing α- and β-Amino Acids

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Received 10 July 2002
Accepted 27 August 2002

Abstract: The synthesis of heterocyclic compounds containing the 7-membered ring system [1,4]diazepane-2,5-dione is described. The aim of this study was to elaborate the solid phase and solution synthesis of eight representatives of the cyclic scaffold and to investigate their chemical stability and their conformational properties. The solid phase synthesis was performed on aminomethyl polystyrene resin using 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid as a backbone linker system (BAL-linker). After attachment of the α- and β-amino acid and deprotection of the amino function, the dipeptide ester was obtained. The molecule was cyclized on the solid support by treatment with NaOMe in MeOH/NMP. The product was cleaved from the resin by TFA. For the solution pathway the linear dipeptides were synthesized by coupling of the BOC-protected L-α-amino acid with the β^2-amino acid ester (EDC/HOBT). After *N*- and *C*-terminal deprotection of the dipeptide, the linear species was cyclized with EDC/HOBT at a concentration of 3 mM in DMF. The products showed high chemical stability after storage in DMSO at room temperature for weeks. The x-ray and two dimensional NMR investigations were performed to investigate the conformation of the molecules. Three types of configuration could be distinguished by NMR, depending on the substitution pattern of the cyclic compounds. The x-ray results confirmed the NMR observations. In general the 7-membered rings showed rigidity, thus they could represent optimal scaffolds for new receptor ligands. Copyright © 2003 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: cyclic dipeptides; β-amino acids; 7-membered rings: conformation; NMR structure; x-ray structure; combinatorial chemistry

INTRODUCTION

For pharmaceutical purposes, new drug-like scaffolds are a matter of particular interest. Such scaffolds should be orally bioavailable, should have good properties as receptor ligands and show high stability towards enzymatic degradation. Further, their structures should fulfil the rule of five [1]. Peptides often show high biological activities, but just partly fulfil the criteria mentioned above. For example they

Abbreviations: 2D-NMR, 2-dimensional nuclear magnetic resonance spectroscopy; BAL, backbone amide linker; BOC, *tert*-butoxycarbonyl; COSY, correlated spectroscopy; DCM, dichloromethane; DIEA, N,N′-diisopropylethylamine; DIPCDI, N,N′-diisopropylcarbodiimide; DMF, N,N′-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ESI-MS, electrospray ionization mass spectrometry; Fmoc, fluorenylmethoxycarbonyl-; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium- hexafluorophosphate; HOAc, acetic acid; HOBT, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; HSQC, heteronuclear single quantum coherence; IR spectroscopy, infrared spectroscopy; MeOH, methanol; NMM, N-methyl-morpholine; NMP, 1-methyl-2-pyrrolidinone; ROESY, rotating frame Overhauser effect spectroscopy; TBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TES, triethylsilane; TFA, trifluoroacetic acid; TFFH, tetramethylfluoroformamidinium hexafluorophosphate; THF, tetrahydrofuran.

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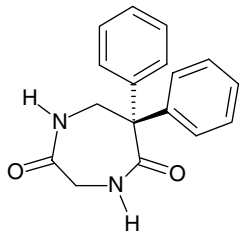
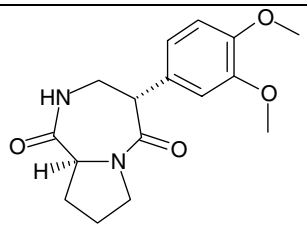
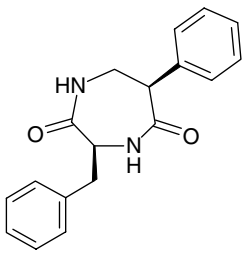
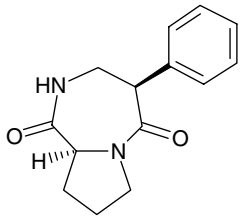
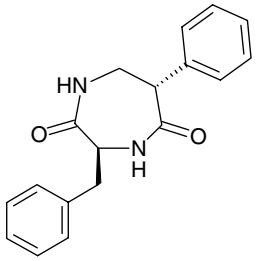
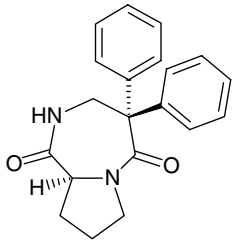
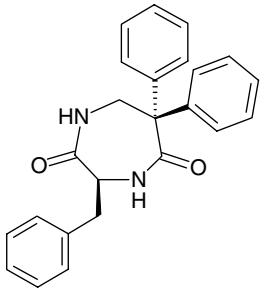
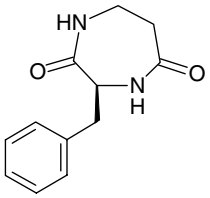
can be cleaved by proteases and in part they show high flexibility.

Two concepts were applied here to improve the stability of peptides. Cyclization [2] and the use of β-amino acids [3]: both lead to enzymatically stable structures [4], which could represent interesting receptor ligands. The combination of those concepts led us to the presented 7-membered ring system, a cyclic scaffold containing one α- and one β-amino acid subunit (Table 1).

There are numerous literature reports concerning the synthesis of the [1,4]benzodiazepane-2,5-dione ring system [5–9] but only very few describing the synthesis of the [1,4]diazepane-2,5-dione system [10,11] and even fewer synthesizing combinatorial libraries [12].

In particular, 7-membered ring cyclic dipeptides from functionalized β-amino acids are described by El Mahdi *et al.* [10], whereby *N*-substituted amino acids and β^2/β^3 disubstituted β-amino acids are

Table 1 Cyclic Dipeptides Containing α- and β-Amino Acids

No.	Structure	No.	Structure
1		5	
2		6	
3		7	
4		8	

used. In contrast to this work, in the current study *N*-unsubstituted compounds were synthesized and conformationally investigated. All substances will be extensively screened against various targets of pharmacological interest. Further work should show whether the reaction methodology has a general applicability (use of natural amino acid residues, of β^2 and β^3 substituted β-amino acids or of more complex unnatural amino acids).

MATERIALS AND METHODS

α-Amino acid derivatives were commercially available, β-amino acid derivatives were synthesized by in-house preparative laboratories and were used as racemates.

The solvents used were reagent grade; commercially available organic reagents were used without further purification.

The resin used was PS-resin, size distribution 125–160 μm (Rapp Polymere GmbH, Tübingen, PS-AM NH_2/H125 160.02, 1.18 mmol/g loading).

Silica gel plates Alugram Sil G/UV_{254} Macherey-Nagel were visualized by ultraviolet (UV) lamp and/or $KMnO_4$ reaction.

Column chromatography was performed on silica gel Merck 60.

HPLC analyses were performed on a Merck Hitachi system equipped with an L-6200 pump, L-4000 UV detector and D-2500 chromato-Integrator. A C_{18} column, 5 cm (Macherey — Nagel) was used. ACN/H_2O was used as the solvent system.

HPLC purifications were carried out on a Waters Delta Prep 300 system with a Waters 600E system controller, a Waters 484 tunable absorbance detector and a Kontron w + w 340 recorder. A XTera RP C_{18} column, 7 μM 19 × 150 mm (Waters) was used. ACN/H_2O was used as the solvent system.

HPLC-chromatography on chiral columns was performed on Chirobiotic T 11 32 columns, 125 × 2 mm, H_2O/EtOH was used as the solvent system.

The IR spectra of resin samples were measured on a Bruker IFS66 FT-IR spectrometer, spectral resolution 2 cm^{-1} (3 mg resin/400 mg KBr).

Absorption values were measured in 1 cm cuvettes on a Shimadzu UV-1602 spectrometer.

The ESI-MS spectra were measured on a Waters ZMD (Micromass) equipped with a CTC PAL autosampler and HP1100 Quat. LC-pump (Agilent).

The NMR spectra were recorded on a Bruker DPX 400 (Faellanden, Switzerland) spectrometer using a triple inverse probe. 1H shifts were referenced to DMSO-d6 at 2.49 ppm and CDCl3 at 7.25 ppm. The following NMR experiments were carried out: 1D-1H, 1H-1H-COSY, 1H-1H-ROESY, 1H-13C-COSY (HSQC).

The x-ray spectra were measured on a Nonius Cad 4 spectrometer. For structure refinement the program SHELXL-97 was used (G.M. Sheldrick, SHELXL-97, University of Göttingen).

Optical rotation was measured on a Perkin Elmer 241 polarimeter at 589 nm.

Synthesis: General Procedures (Solid Phase)

Linker attachment. Aminomethylpolystyrol resin (1 mmol) was swelled in NMP. A solution of 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid (BAL-linker, 1.5 mmol), DIPCDI (1.5 mmol), HOBT (1.6 mmol) in NMP (5 ml) was reacted with the resin for 16 h at room temperature. The resin was washed thoroughly three times for 5 min with each solvent (DMF, DCM, THF, isopropanol). The resin was dried under high vacuum at 40 °C for 16 h. Spec. loading: 0.9 mmol/g. The IR of the resin showed the aromatic aldehyde function with a strong band at 1670 cm^{-1}.

Reductive amination. BAL-linker loaded resin (2.7 mmol, spec. loading 0.9 mmol/g) was swelled in pyridine. A solution of β-homo amino acid ester hydrochloride (16.2 mmol) and $NaBH_3CN$ (510 mg, 8.1 mmol) in pyridine (50 ml) was added and finally $Ti(OEt)_4$ (3.6 ml, 16.2 mmol) was added. The reaction solution was kept at 50 °C for 16 h. The resin was washed thoroughly three times for 5 min with each solvent (DMF, 20% HOAc/DMF, DMF, DCM, THF, isopropanol) and dried under high vacuum at 40 °C for 16 h. The IR of the resin showed a strong band at 1720 cm^{-1}, due to the ester function. The characteristic pattern for the aldehyde group at 2780 cm^{-1} had disappeared.

Coupling to the secondary amine. The resin (0.9 mmol) was swelled in NMP. The Fmoc-L-α amino acid (2.7 mmol) and HATU (1030 mg, 2.7 mmol) was dissolved in NMP (6 ml), DIEA (925 μl, 5.4 mmol) was added. After 20 min of activation, the solution was added to the resin. The reaction remained at room temperature for 40 h.

The resin was washed thoroughly three times for 5 min with each solvent (DMF, DCM, THF, isopropanol) and dried under high vacuum at 40 °C for 16 h. Completeness of the coupling step was confirmed by a negative chloranil test [13] for secondary amines. The IR spectra showed

significant bands at 740/756 cm^{-1} due to Fmoc-vibrations.

To determine the yield of a Fmoc amino acid coupling reaction the Fmoc-concentration on the resin was determined: the resin (3 mg) was treated with 20% piperidine in DMF (1 ml) for 1 h. Methanol (9 ml) was added and the absorption of the solution was determined at 301 nm.

$$\text{Loading} = \frac{A(301)}{7800\ M^{-1}\ cm^{-1}\ 1\ cm}\frac{V}{m}\ \text{loading in mmol/g}$$

where A is the absorption at 301 nm, V is the volume of the solution in ml and m is the mass of resin.

Fmoc-cleavage. The resin (0.9 mmol) was treated with 20% piperidine in DMF for 2, 5 and 20 min. Between the cleavage steps, the piperidine solution was filtered. The resin was washed thoroughly three times for 5 min with each solvent (DMF, DCM, THF, isopropanol) and dried under high vacuum at 40 °C for 16 h. In the IR spectra the Fmoc-characteristic bands at 740/756 cm^{-1} had disappeared.

Cyclization. The resin (0.9 mmol) was treated with a 0.3 M solution of NaOMe in DMF/MeOH 4 : 1 for 68 h at room temperature. The resin was thoroughly washed three times for 5 min with each solvent (dry MeOH, DMF, 20% acetic acid/DMF, DMF, DCM, isopropanol) and dried under high vacuum at 40 °C for 16 h.

Cleavage from the resin. The resin was treated with a mixture of TFA/TES/H_2O (95 : 3 : 2) at room temperature for 16 h. The solution was filtered, the resin was washed once with TFA and the solution was concentrated *in vacuo* to dryness. The residue was dried under high vacuum and purified by preparative HPLC (ACN/H_2O/TFA).

The raw products were not stable at room temperature, hydrolysis to the linear dipeptides could be observed.

Synthesis: General Procedures (Solution)

Coupling reaction (procedure A). The BOC protected L-α-amino acid (1 mmol), the homo-β-amino acid ethyl ester (1 mmol), HOBT (1 mmol) and EDC (1 mmol) were dissolved in DMF (10 ml), the pH was adjusted to 8 by addition of NMM (700 µl). The solution was left at room temperature for 16 h. The solution was concentrated *in vacuo* and DCM (50 ml) was added. The solution was washed with 5% $NaHSO_4$, 5% $NaHCO_3$ and saturated brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained residue was dried under high vacuum and used for further reactions.

BOC-cleavage (procedure B). The BOC-protected compound (1 mmol) was dissolved in 20% TFA in DCM (10 ml), the solution was left at room temperature for 4 h. The reaction solution was concentrated *in vacuo* at 40 °C. The residue was dried under high vacuum and used for further reactions.

Ester saponification (procedure C). The ester (1 mmol) was dissolved in THF/H_2O (9 : 1, 4 ml) and 3 M LiOH in water (1.3 ml) was added. The solution was kept at 50 °C for 16 h. The reaction solution was concentrated *in vacuo* and coevaporated with toluene and diisopropylether. The residue was dried under high vacuum and used for cyclization.

Cyclization (procedure D). The linear dipeptide (1 mmol) was dissolved in DMF (100 ml). This solution was combined with a solution of EDC (1.5 mmol) and HOBT (1.5 mmol) in DMF (234 ml). DIEA (3 mmol) was added to adjust the pH to 8. The reaction solution was kept at 40 °C for 16 h.

The solvent was concentrated *in vacuo* and DCM was added (50 ml). The solution was washed with 5% $NaHSO_4$, 5% $NaHCO_3$ and saturated brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was then chromatographed on silica gel (DCM/MeOH 40 : 1).

6,6-Diphenyl-[1,4] diazepane-2,5-dione (1). Compound **1** was synthesized according to the general procedures for the solid phase strategy.

Linker attachment: according to general procedures.

Reductive amination: 3-amino-2,2-diphenyl-propionic acid ethyl ester hydrochloride (4.95 g, 16.2 mmol) was used. IR showed a strong band at 1720 cm^{-1}.

Coupling: Fmoc-Gly-OH (803 mg, 2.7 mmol) was used. Fmoc-determination resulted in 31% yield over 3 steps (linker attachment, reductive amination, coupling).

Fmoc cleavage: according to general procedures.

Cyclization and cleavage: HPLC of the raw oil showed 35% product, after standing at room temperature a new peak (M + 18) appeared, the product partly decomposed. By HPLC purification besides the desired product, 38 mg (0.13 mmol) of the open ring could be isolated.

Yield after HPLC purification: 12 mg, 0.043 mmol, 5% over all steps.

ESI-MS: $[M + H]^+ = 281$.

^{1}H NMR (d^6-DMSO): δ(ppm) = 3.85 (d, 2H, Gly-CHα); 4.07 (d, 2H, β^3-CH_2); 7.18 (d, 4H, phenyl-*o*); 7.27 (m, 2H, phenyl-*p*); 7.34 (m, 4H, phenyl-*m*); 7.86 (t, 1H, NH); 8.12 (t, 1H, NH).

±(3S,6R)/(3R,6S)-3-Benzyl-6-phenyl-[1,4]diazepane-2,5-dione (2)

±(3S,6S)/(3R,6R)-3-Benzyl-6-phenyl-[1,4]diazepane-2,5-dione (3)**.** Compounds **2** and **3** were synthesized according to the general procedures for the solution strategy.

*3-(2-tert-Butoxycarbonylamino-3-phenyl-propionylamino)-2-phenyl-propionic acid ethyl ester (**2a**, **3a** — procedure A).* BOC-L-Phe-OH (531 mg, 2 mmol), *rac* 3-amino-2-phenyl-propionic acid ethyl ester hydrochloride (460 mg, 2 mmol), EDC (383 mg, 2 mmol), HOBT (270 mg, 2 mmol), THF (20 ml), DMF (10 ml), NMM to pH 8.

Yield: 850 mg, 1.93 mmol, 96%.

ESI-MS: $[M + H]^+ = 441$.

*3-(2-Amino-3-phenyl-propionylamino)-2-phenyl-propionic acid ethyl ester (**2b**, **3b** — procedure B).* 1.93 mmol (850 mg) of the BOC-protected compound was reacted.

Yield: 610 mg, 1.79 mmol, 93% (TFA-salt).

ESI-MS: $[M + H]^+ = 341$.

*3-(2-Amino-3-phenyl-propionylamino)-2-phenyl-propionic acid*LiTFA (**2c**, **3c** — procedure C).* 1.79 mmol (610 mg) of the ester was reacted.

Yield: 660 mg, 1.47 mmol 82%.

ESI-MS: $[M + H]^+ = 313$.

*±(3S,6R)/(3R,6S)-3-Benzyl-6-phenyl-[1,4]diazepane-2,5-dione (**2** — procedure D)*

*±(3S,6S)/(3R,6R)-3-Benzyl-6-phenyl-[1,4]diazepane-2,5-di-one (**3** — procedure D).* 0.6 mmol (200 mg) of dipeptide was reacted.

Raw product: 95 mg, 0.32 mmol, 54%.

The raw product was purified by chromatography on silica gel (DCM/MeOH 40 : 1), whereupon the two diastereomers **2** and **3** could be separated.

*±(3S,6R)/(3R,6S)-3-Benzyl-6-phenyl-[1,4]diazepane-2,5-dione (**2**).* Yield: 32 mg (0.11 mmol), 18%.

ESI-MS: $[M + H]^+ = 295$.

^{1}H NMR ($CDCl_3$): δ(ppm) = 3.00/3.50 (2xm, 2H, Bn-CH_2); 3.38/4.10 (2xm, 2H, β^3-CH_2); 4.00 (m, 1H, β^2-CH); 4.61 (m, 1H, α-CH); 5.82 (m, 2H, 2xNH); 7.19 (d, 2H, phenyl-o); 7.35–7.42 (m, 8H, phenyl-m,p, Bn-o,m,p).

*±(3S,6S)/(3R,6R)-3-Benzyl-6-phenyl-[1,4]diazepane-2,5-dione (**3**).* Yield: 36 mg (0.12 mmol), 20%.

ESI-MS: $[M + H]^+ = 295$.

^{1}H NMR (d^6-DMSO): δ(ppm) = 2.85/3.08 (2xm, 2H, Bn-CH_2); 3.16/3.87 (2xm, 2H, β^3-CH_2); 3.70 (m, 1H, β^2-CH); 4.90 (m, 1H, α-CH); 7.18–7.32 (m, 8H, phenyl-o,m,p, Bn-m,p); 7.40 (d, 2H, Bn-o); 7.72 (d, 1H, NH α-aa); 8.05 (t, 1H, NH β-aa).

(S)-3-Benzyl-6,6-diphenyl-[1,4]diazepane-2,5-dione† (4)**.** Compound **4** was synthesized according to the general procedures for the solution strategy.

3-((S)-2-tert-Butoxycarbonylamino-3-phenyl-propionylamino)-2,2-diphenyl-propionic acid ethyl ester† *(**4a** — procedure A).* BOC-L-Phe-OH (265 mg, 1 mmol), 3-amino-2,2-diphenyl-propionic acid ethyl ester hydrochloride (306 mg, 1 mmol), EDC (192 mg, 1 mmol), HOBT (135 mg, 1 mmol), DMF (10 ml), NMM to pH 8.

Yield: 490 mg, 0.95 mmol, 95%.

ESI-MS: $[M + H]^+ = 517$.

3-((S)-2-Amino-3-phenyl-propionylamino)-2,2-diphenyl-propionic acid ethyl ester† *(**4b** — procedure B).* 0.87 mmol (450 mg) of the BOC-protected compound was reacted.

Yield: 362 mg, 0.87 mmol, 100% (TFA-salt).

ESI-MS: $[M + H]^+ = 417$.

3-((S)-2-Amino-3-phenyl-propionylamino)-2,2-diphenyl-propionic acid† *(**4c** — procedure C).* 0.87 mmol (362 mg) of the ester was reacted.

Yield: 370 mg > 100% (contains salts).

ESI-MS: $[M + H]^+ = 389$.

(S)-3-Benzyl-6,6-diphenyl-[1,4]diazepane-2,5-dione† *(**4** — procedure D).* 0.47 mmol (200 mg) of dipeptide was reacted.

The product was used without further purification.

Yield: 152 mg, 0.41 mmol, 87%.

ESI-MS: $[M + H]^+ = 371$.

^{1}H NMR ($CDCl_3$): δ(ppm) = 2.86/3.35 (2xm, 2H, Bn-CH_2); 3.70/4.52 (2xm, 2H, β^3-CH_2); 4.55 (m, 1H, α-CH); 5.70 (t, 1H, NH β-aa); 5.77 (m, 1H, NH α-aa); 7.03–7.30 (m, 15H, 2xphenyl, Bn).

(4S,9aS)-4-(3,4-Dimethoxy-phenyl)-hexahydro-pyrrolo[1,2-a][1,4]diazepine-1,5-dione† (5)**.**

Compound **5** was synthesized according to the general procedures for the solid phase strategy.

Linker attachment: according to general procedures.

Reductive amination: 3-Amino-2-(3,4-dimethoxy-phenyl)-propionic acid ethyl ester hydrochloride

† Expected stereochemistry, not investigated.

(4.69 g, 16.2 mmol) was used. IR showed a strong band at 1720 cm^{-1}.

Coupling: Fmoc-L-Pro-OH (911 mg, 2.7 mmol) was used. Fmoc-determination resulted in 75% yield over three steps (linker attachment, reductive amination, coupling).

Fmoc cleavage: according to general procedures.

Cyclization and cleavage: HPLC of the raw oil showed 47% product, after standing at room temperature the product partly decomposed, three purification steps were necessary to isolate pure product. By HPLC purification just one isomer was isolated.

Yield after HPLC purification: 5 mg, 2% over all steps.

ESI-MS: $[M + H]^+ = 305$.

^1H NMR (d^6-DMSO): δ(ppm) = 1.78 (m, 2H, γ-CH_2-Pro); 2.00/2.44 (2xm, 2H, β-CH_2-Pro); 3.12/3.88 (2xm, 2H, β^3-CH_2); 3.38–3.53 (m, 2H, δ-CH_2-Pro); 3.68 (m, 1H, β^2-CH); 3.73/3.77 (2xs, 6H, O-CH_3); 5.02 (m, 1H, α-CH); 6.77–6.90 (m, 3H, aryl); 8.13 (t, 1H, NH).

(4R,9aS)-4-Phenyl-hexahydro-pyrrolo[1,2-a][1,4]diazepine-1,5-dione† (6). Compound **6** was synthesized according to the general procedures for the solid phase strategy.

Linker attachment: according to general procedures.

Reductive amination: 3-amino-2-phenyl-propionic acid ethyl ester hydrochloride (3.72 g, 16.2 mmol) was used. IR showed a strong band at 1720 cm^{-1}.

Coupling: Fmoc-L-Pro-OH (911 mg, 2.7 mmol) was used. Fmoc-determination showed almost quantitative reactions over three steps (linker attachment, reductive amination, coupling).

Cyclization: HPLC of the raw oil showed 37% product. By HPLC purification just one isomer was isolated.

Yield after HPLC purification: 11 mg, 0.045 mmol, 5% over all steps

ESI-MS: $[M + H]^+ = 245$.

^1H NMR (d^6-DMSO): δ(ppm) = 1.76 (m, 2H, γ-CH_2-Pro); 2.00/2.45 (2xm, 2H, β-CH_2-Pro); 3.13/3.87 (2xm, 2H, β^3-CH_2); 3.30–3.50 (m, 2H, δ-CH_2-Pro); 3.72 (m, 1H, β^2-CH); 5.07 (m, 1H, α-CH); 7.25–7.37 (m, 5H, phenyl); 8.17 (t, 1H, NH).

(S)-4,4-Diphenyl-hexahydro-pyrrolo[1,2-a][1,4]diazepine-1,5-dione† (7). Compound **7** was synthesized according to the general procedures for the solution strategy.

*(S)-2-(2-Ethoxycarbonyl-2,2-diphenyl-ethylcarbamoyl)-pyrrolidine-1-carboxylic acid tert-butyl ester† (**7a** — procedure A).* BOC-L-Pro-OH (215 mg, 1 mmol), 3-amino-2,2-diphenyl-propionic acid ethyl ester hydrochloride (306 mg, 1 mmol), EDC (192 mg, 1 mmol), HOBT (135 mg, 1 mmol), DMF (10 ml), NMM to pH 8.

Yield: 410 mg, 0.88 mmol, 88%.

ESI-MS: $[M + H]^+ = 467$.

*2,2-Diphenyl-3-[((S)-pyrrolidine-2-carbonyl)-amino]-propionic acid ethyl ester† (**7b** — procedure B).* 0.88 mmol (410 mg) of the BOC-protected compound was reacted.

Yield: 420 mg, 0.88 mmol, 100% (TFA-salt).

ESI-MS: $[M + H]^+ = 367$.

*2,2-Diphenyl-3-[((S)-pyrrolidine-2-carbonyl)-amino]-propionic acid† (**7c** — procedure C).* 0.88 mmol (420 mg) of the ester was reacted.

Yield: >100% (contains salts).

ESI-MS: $[M + H]^+ = 339$.

*(S)-4,4-Diphenyl-hexahydro-pyrrolo[1,2-a][1,4]diazepine-1,5-dione† (**7** — procedure D).* 0.88 mmol (305 mg) of dipeptide was reacted. The reaction was kept at 45 °C for 60 h and additional 2 equivalents of DIPEA were added to keep the pH high enough.

Raw product: 80 mg, 0.25 mmol, 28%. The product was purified by chromatography on silica gel (DCM/MeOH 40 : 1).

Yield: 48 mg, 0.15 mmol, 17%.

ESI-MS: $[M + H]^+ = 321$.

^1H NMR (d^6-DMSO): δ(ppm) = 1.79 (m, 2H, γ-CH_2-Pro); 2.00/2.38 (2xm, 2H, β-CH_2-Pro); 3.57 (m, 2H, δ-CH_2-Pro); 3.59/4.66 (2xm, 2H, β^3-CH_2); 4.94 (m, 1H, α-CH); 7.14–7.34 (m, 10H, 2xphenyl); 7.96 (t, 1H, NH).

(S)-3-Benzyl-[1,4]diazepane-2,5-dione† (8). Compound **8** was synthesized according to the general procedures for the solid phase strategy.

Linker attachment: according to general procedures.

Reductive amination: 3-amino-propionic acid ethyl ester hydrochloride (2.49 g, 16.2 mmol) was used. IR showed a strong band at 1720 cm^{-1}.

Coupling: Fmoc-L-Phe-OH (1.05 g, 2.7 mmol) was used. Fmoc-determination resulted in 55% yield over three steps (linker attachment, reductive amination, coupling).

Fmoc cleavage: according to general procedures.

Cyclization and cleavage: HPLC of the raw oil showed 52% product.

† Expected stereochemistry, not investigated.

† Expected stereochemistry, not investigated.

ESI-MS: $[M + H]^+ = 219$.

The product was not isolated and no NMR was measured.

X-Ray Investigation

Colourless single crystals of **2** and **7** were grown by slow evaporation at room temperature from acetone respectively methanol (Table 2).

Optical Rotation of 2

The optical rotation of compound **2** was determined and turned out to be 0 (methanol, c = 0.3).

Test of Epimerization for Compounds 2 and 3

1 mg of the sample was hydrolysed in 1 ml of 6 N HCl at 110 °C for 24 h in a closed vessel. The solvent was evaporated and the residue was analysed on a chiral column. By comparison with pure L- and D-phenylalanine and both isomers of the homo-β-amino acid, the ratio of the four components in the hydrolysed sample was determined. The chromatogram clearly indicated that the product was not enantiomerically pure. D and L-Phe could be found, as well as both isomers of the homo-β-amino acid moiety.

RESULTS AND DISCUSSION

Solid Phase Synthesis (Scheme 1)

In a first attempt the synthesis of the 7-membered cyclic dipeptides was performed in the same manner as the solid phase synthesis of diketopiperazines [14], where 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid was used as a backbone linker system suitable for head-to-tail cyclizations [15–18]. The choice of linkers is an important factor in achieving good success in solid phase synthesis. The linker has to be stable under all conditions of synthesis, and afterwards a quantitative cleavage without side reactions is required to release the product.

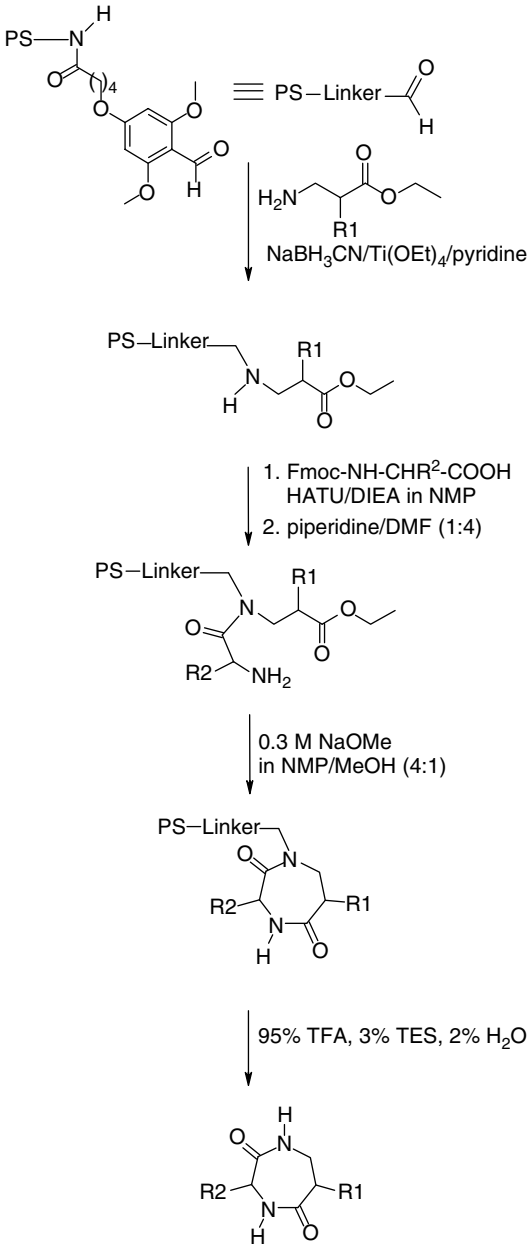
A one step reductive amination reaction was used for the attachment of the first amino acid ester to the solid support. $Ti(OEt)_4$ acts as a water withdrawing agent and sodium cyanoborohydride as a reducing agent [19–21].

On bead FT-IR-spectroscopy [22] indicated a strong absorption at 1720 cm^{-1}due to the C=O stretching vibration. The characteristic band for the aldehyde group at 2780 cm^{-1} had disappeared.

The optimization of the acylation step of the resulting secondary amine required several experiments. As also mentioned by Jensen *et al.* [18]

Table 2 Crystallographic Data for **2** and **7**

Empirical formula	C_{18} H_{18} N_2 O_2	C_{20} H_{21} N_2 $O_{2.5}$
Formula weight	294.34	329.39
Crystallization solvent	Acetone	Methanol
Crystal system	Monoclinic	Monoclinic
Space group	P21/n	C2
Unit cell dimensions	a = 15.652 (2) Å α = 90.000 (10)° b = 5.4390 (10) Å β = 106.800 (10)° c = 19.3600 (10) Å γ = 90.000 (10)°	a = 22.5670 (10) Å α = 90.000 (10)° b = 6.2730 (10) Å β = 103.150 (10)° c = 12.5080 (10) Å γ = 90.000 (10)°
Volume	1577.8 (4) $Å^3$	1724.2 (3) $Å^3$
Z (molecules/unit cell)	4	4
Density (calc.)	1.239 mg/mm^3	1.269 mg/mm^3
Independent reflections	3219 [R(int) = 0.0247]	1920 [R(int) = 0.0203]
Reflections collected	3314	2009
Final R indexes [$I > 2\sigma(I)$]	R1 = 0.0559, ωR2 = 0.1711	R1 = 0.0643, ωR2 = 0.1747
Temperature	293 (2) K	293 (2) K
Radiation (λ, Å)	CuK α (1.54178)	CuK α (1.54178)



Scheme 1 Synthesis on solid phase.

the sterically hindered *N*-substituted handle resin is much more difficult to acylate than a comparable unsubstituted primary amine. Different coupling reagents like DIPCDI, TBTU or EDC were used. The yields were between 20% and 40% over three steps (coupling of the linker, reductive amination and coupling of the amino acid). Better results could be obtained with TFFH or by using the method of the symmetrical anhydride (50%–70%). Finally HATU [23] gave the best results, contrary to findings of Boojamra *et al.* [5], who described EDC as the best reagent for this acylation. The advantage of the HATU method is that only 2 equivalents of Fmoc-amino acid, 2 equiv. of HATU and 4 equiv. of DIEA are needed. Yields of up to 95% could be obtained, depending on the amino acid and the resin. Those yields were determined by the 9-fluorenylmethoxycarbonyl-test [24]. IR analysis of the resin showed significant bands at 740/756 cm^{-1} due to Fmoc-vibrations.

Cleavage of the Fmoc-protecting group was performed in 20% piperidine in DMF, using standard protocols. To a very small extent, cyclic 7-membered product could be detected after the deprotection of the amino group. In contrast, the 6-membered analogues, the diketopiperazines cyclize spontaneously [14].

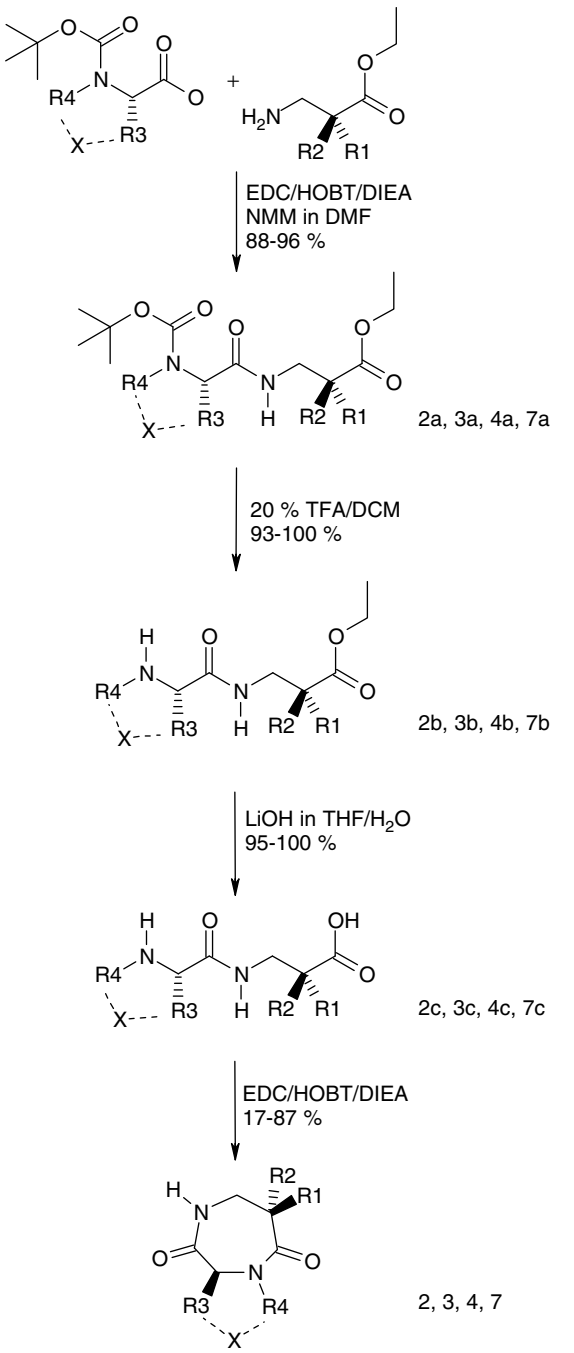
After Fmoc-deprotection the resulting ester had to be cyclized. Although this cyclization step is described as difficult, we succeeded in running the reaction on solid phase using the backbone linker system.

Several reaction conditions for the cyclization were investigated. Reagents such as $SiCl_4$, Me_3SiCl or $AlMe_3$ as well as triethylamine were tested to convert the dipeptide ester into the cyclic product. By treatment of the resin with 0.3 M NaOMe in MeOH/NMP (1 : 4), the 7-membered ring product was obtained. Depending on the amino acids and their substituents, the free dipeptide (as a free acid) could be detected as a side product. This side reaction is also described by Malenfant *et al.* [25].

Finally the product was cleaved from the solid support by treatment of the resin with 95% TFA (Scheme 1) [16,18,26]. The reaction pathway shown in Scheme 1 seemed to work very well for model compound **8**. When it was applied to other derivatives, compounds **1–7**, several side reactions were detected and low yields of the desired products were obtained. In some cases β-elimination was observed to give the α-amino acid amide as a side product. Most likely this happens during TFA cleavage from the solid support. Not only the cyclic product undergoes this reaction, but it was also found when linear intermediates were cleaved from the resin. However, for some of the characterized products the solid phase pathway had been used and the isolated material was sufficient to perform NMR conformational analysis (see experimental part).

Synthesis in Solution (Scheme 2)

In a second approach, solution methods were applied to obtain the cyclic dipeptides in larger



	R1	R2	R3	R4	X
2, 2a, 2b, 2c		-H		-H	--
3, 3a, 3b, 3c	-H			-H	--
4, 4a, 4b, 4c,				-H	--
7, 7a, 7b, 7c			$-CH_2-$	$-CH_2-$	$-CH_2-$

Scheme 2 Synthesis in solution.

amounts. According to standard peptide coupling procedures (EDC/HOBT), the BOC-protected L-α-amino acid was coupled to racemic β^2-amino acid ester, with EDC/HOBT. After cleavage of the BOC-group and saponification of the ester by LiOH in THF, the dipeptide had to be cyclized. Due to the preferred transoid conformation of the peptide bond in the linear precursor, the molecule does not easily undergo intramolecular coupling. For this reason El Mahdi *et al.* [10] used *N*-substituted amino acids in order to constrain the amide to the cisoid conformation. They worked with BOP as the coupling reagent at $-30\,^{\circ}C$. Seebach *et al.* performed similar cyclizations of tetrapeptides by the help of pentafluorophenyl active esters [27].

Finally we succeeded in running the reaction with EDC/HOBT in high dilution (Scheme 2). Depending on the concentration during the cyclization reaction, besides the cyclic product, higher membered rings could be detected. The best results with yields of up to 87% could be obtained, using concentrations of 3 mM for the reaction. For this reaction pathway, no β-elimination could be detected.

Configurational and Conformational Analysis

Compounds **1–7** were analysed by ^{1}H-NMR measurements. Except for compound **1**, which did not show restricted mobility, all substances were further examined by 1H-1H-COSY, 1H-13C-COSY (HSQC) and 1H-1H-ROESY experiments.

Compound **1** shows very high ring flexibility, although the molecule contains two amide bonds and a disubstituted β^2-position. Protons belonging to the CH_2-group like the Gly-CHα or the β^3-CH_2 can not be distinguished from each other in the ^{1}H-spectrum.

Higher substituted compounds such as **2**, **3** or **4** on the other hand, show more complex ^{1}H-spectra due to their restricted flexibility. For those compounds and for the proline containing bicyclic structures **5**, **6** and **7** 2D-NMR spectra were performed.

In principle three types of configurations can be distinguished (Figure 1a,b,c). In case (a) the residues at the C-α atom of the α-amino acid and at the C-β^2 atom of the β-amino acid are oriented towards the same side of the 7-membered ring, which leads to either R/S or S/R configuration. By evaluation of NOE signals (arrows) the relative configuration of the two stereocentres can be defined clearly. There is a strong NOE signal between H-C5

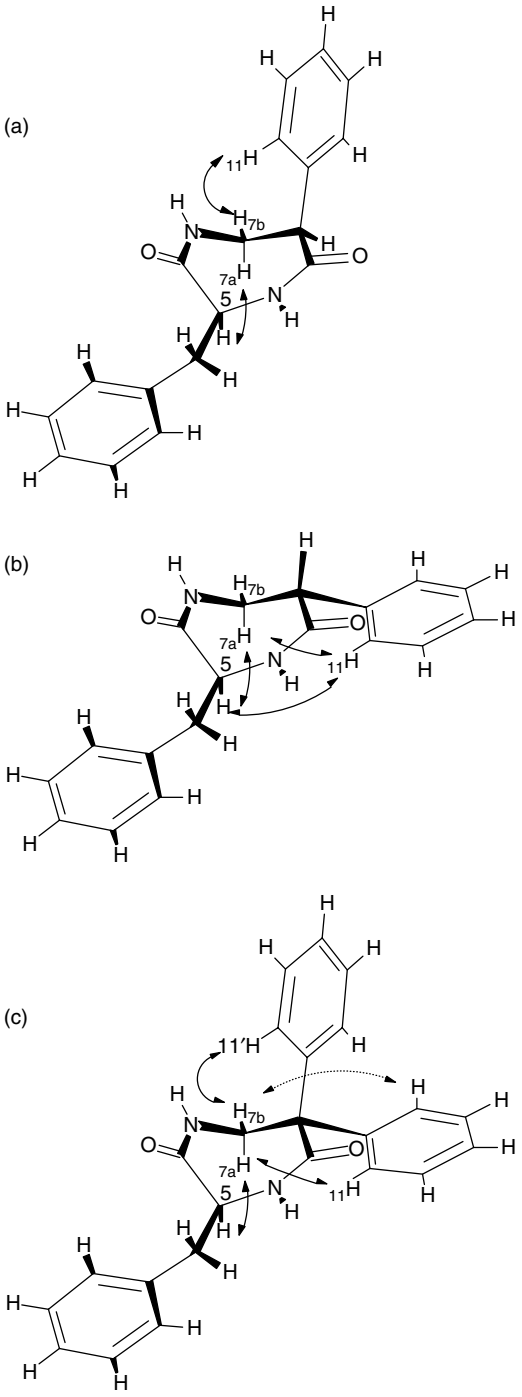


Figure 1 Configurations determined by NOE measurements.

and H-C7a, another strong signal can be observed between H-C7b and H-C11. This configuration is found for compounds **2** and **6**.

In case (b) the residues at the C-α atom of the α-amino acid and at the C-β^2 atom of the β-amino acid are oriented towards different sides of the 7-membered ring, which leads to either S/S or a R/R configuration. By evaluation of NOE signals the relative configuration of the two stereocentres can be defined clearly. There is a strong NOE signal between H-C5 and H-C7a, another strong signal can be observed between H-C7a and H-C11 and there is a third signal between H-C5 and H-C11. This configuration is found for compounds **3** and **5**.

Compounds **4** and **7** with the disubstituted C-β^2 atom of the β-amino acid are represented in Figure 1c. There are strong NOE signals between H-C5 and H-C7a and between H-C7a and H-C11, and further between H-C7b and H-C11. A weaker NOE can be observed between H-C7b and H-C11.

In summary all 7-membered rings show similar behaviour, all compounds perform strong NOEs between H-C5 and H-C7, which leads to the conformations illustrated in Figure 1. Relative configurations of the stereocentres could clearly be determined.

Compound **2** was crystallized from acetone, Compound **7** was crystallized from methanol to give needles suitable for x-ray diffraction analysis. The observed structures and solid-state conformations are presented in Figure 2.

In both structures the atoms of the 7-membered ring are members of two planes, one formed by C5, C6, N3, C7, the other by C7, C8, C9, N4, C5. Both *cis*-amide bonds are planar. The two hydrogen atoms H-C7a and H-C7b and the phenyl residues at C8 are staggered to each other.

These observations confirmed the results we had previously obtained by NMR investigations of **2**. Small coupling constants in the ^{1}H-spectrum between H-C8 and H-C7a/b indicate dihedral angles of about 60° each.

A small coupling constant in the 1H-1H-COSY spectrum indicates a torsion angle of about 90° between H-N4 and H-C5. Both facts can be confirmed by the x-ray analysis.

Epimerization

The fact that space group P21/n can be found for compound **2**, points to the substance being racemic. This observation led to the measurement of the optical rotation, and indeed, it was 0. This means an epimerization of the stereo centre of the L-α moiety must have taken place. Probably this did not happen during a coupling step, more likely it happened during the saponification step, when the solution was heated under basic conditions.

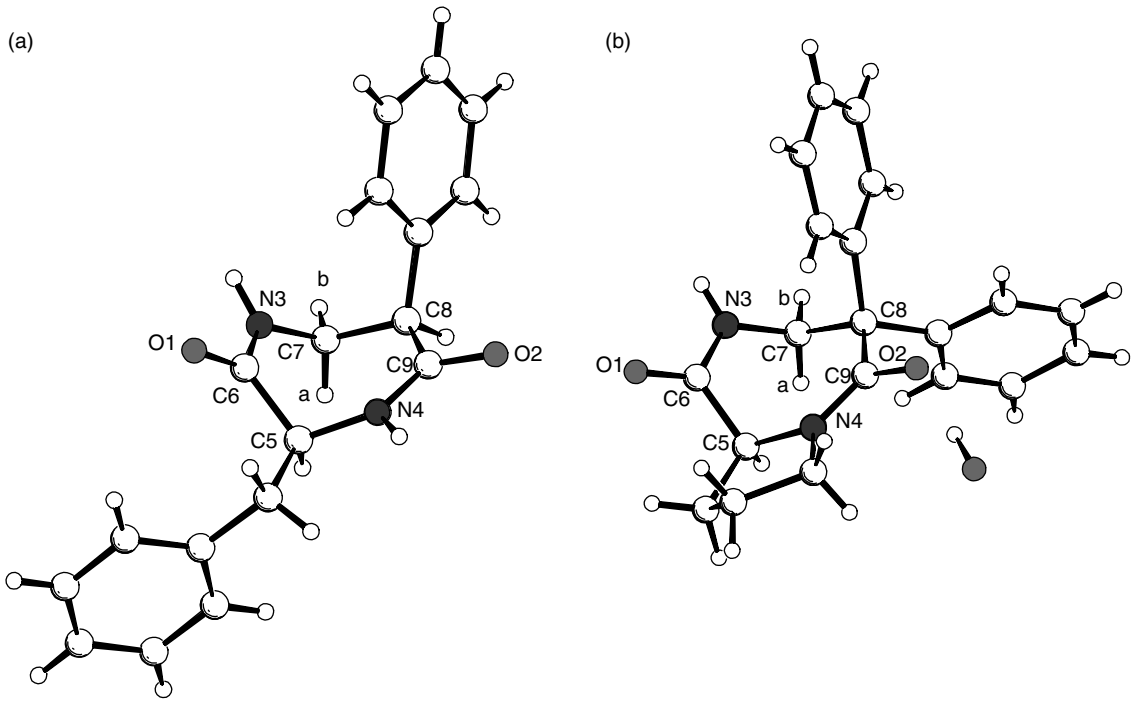


Figure 2 X-ray diffraction structure of (a) compound **2** and (b) compound **7**.

Hydrolysis of compounds **2** and **3** followed by HPLC-analysis on chiral columns clearly indicated that indeed both enantiomers in a racemic mixture were present in the solution.

Stability

NMR-samples in d^6-DMSO were kept at room temperature for 5 weeks. Except for an increase of the water content, the spectra measured afterwards did not show any change compared with those measured with fresh solutions.

CONCLUSIONS

β-Amino acids can be found in naturally occurring compounds with important pharmacological properties [28–30]. Their use as building blocks in pharmaceutical active substances is of interest. The aim of the current study was to synthesize cyclic dipeptides, whereby one of the amino acids was replaced by a β^2-amino acid, thus forming 7-membered ring structures. Several compounds carrying aromatic residues were synthesized and characterized. When proline was used as the α-amino acid unit, the synthesis resulted in bicyclic rigid products.

In a first attempt a solid phase pathway was considered the optimal strategy to synthesize the desired 7-membered ring molecules. It worked quite well for model compound **8**. When it was applied to compounds **1–7**, several side reactions occurred and only small amounts of the final products could be isolated. For that reason, an alternative strategy was developed. We chose a reaction sequence in solution. This led to much better results, as most of the reactions are standard procedures such as peptide coupling reactions and deprotection of functional groups. The isolated products were subjected to NMR and x-ray investigations.

The purified compounds showed high stability in DMSO at room temperature for several weeks.

Measurement of optical rotation and analysis on chiral HPLC-columns showed that the stereo centre of the α-amino acid had racemized. This happened probably during the basic saponification step.

The configurations and conformations of the products were determined by NMR and x-ray experiments. Except for compound **1**, the molecules show high rigidity, depending on their substitution pattern. These properties are useful for their potential use as pharmaceutical active compounds. Consistent with modelling studies, which had been performed before, the two cisoid amide bonds in the 7-membered ring are planar. They define two planes

in which all atoms of the ring can be found. The relative configurations of the two stereocentres could be clearly defined by evaluation of the NOE signals. The residues located around the C^{α}-C^{β}-bond of the β-amino acid prefer staggered conformations and thus determine the ring structure.

The conformations of compounds **2** and **7** have been investigated in solution as well as in the crystal state by single-crystal x-ray diffraction analysis. The conformation determined by NMR could very well be confirmed by the x-ray method.

Acknowledgement

We are very grateful to M.A. Burget, H.U. Gremlich, H. Walter, G. Rihs, F. Houdiere, E. Jacoby and E. Francotte for numerous analyses.

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