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Efficient synthesis of 2,5-diketopiperazines using microwave assisted heating

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Abstract—In this study a general, efficient and environmentally benign solution phase synthesis of 2,5-diketopiperazines (DKPs) using microwave assisted heating in water is described. A series of 11 structurally different DKPs have been synthesized from dipeptide methyl esters. A range of common laboratory solvents have been tested as well as different reaction times and temperatures. Both classic thermal and microwave assisted heating have been investigated. Microwave assisted heating for 10 min using water as solvent proved, by far, to be the most efficient method of cyclization giving moderate to excellent yields (63–97%) of DKPs. In contrast to other published procedures, this method seems independent of the amino acid sequence.

1. Introduction

2,5-Diketopiperazines (DKPs) are cyclic dipeptide derivatives, which show a multitude of interesting biological activities,[1] e.g., efficient interactions with opioid receptors,[2] potent cytotoxic effects via a variety of mechanisms[3–5] and neuroprotective effects.[6] Recently, they have also been associated with blockade of L-type calcium channels,[7] tryptase inhibition,[8] oxytocin receptor antagonism[9] and plasminogen activator inhibition.[10] For some time we have been working on the use of suitable scaffolds for the development of novel peptidomimetics and in that context we have become interested in DKP derivatives. However, to be able to synthesize and investigate the properties of large numbers of DKPs an efficient, robust and reproducible method for their synthesis is needed.

DKPs can be synthesized from the corresponding dipeptides both in solution and on solid phase. There are many reports in the literature of general methods for solid phase synthesis.[11] They are all in small scale and due to the problems with scaling-up of solid phase reactions, mainly for economic reasons, this is a less useful procedure for large-scale synthesis of DKPs.[12] In contrast, for syntheses in the solution phase there are no general procedures available in the literature which lead to high yields of DKPs independent of the amino acid composition. In solution, the methods are generally based on cyclization of dipeptide methyl esters,[11] or direct cyclization of unprotected dipeptides.[11–13] Several of the reported methods have shortcomings, e.g., the Fischer method,[14] in which the dipeptide methyl esters are subjected to excess ammonia, has been reported to cause epimerization to a varying extent.[15,16a] A method based on the cyclization of the dipeptide methyl ester in toluene/2-butanol (1:4) has been reported[16a] to give generally good yields. However, there have also been reports of low yielding reactions using this method.[15,16b] Unfortunately, when using any of these or similar reaction conditions for the synthesis of DKPs we were not able to reproduce the reported results, even if high temperatures and long reaction times were used. In our hands, only dipeptides containing the conformationally restricted amino acid proline cyclized successfully. We therefore set out to identify the optimal reaction conditions for an efficient and general synthetic procedure for DKPs. To accomplish that we have used a series of 11 dipeptide methyl esters as starting materials and investigated the influence of amino acid composition, solvent, reaction time and reaction temperature, the latter using both thermal and microwave heating. During the last decade microwave assisted heating has proven to be highly successful in speeding up reactions otherwise run for long periods of time, but to the best of our knowledge the use of microwave heating for the formation of DKPs has only been investigated to a limited extent.[17,18]

In the present study it was found that cyclization of any of the tested dipeptide methyl ester hydrochlorides in water using microwave assisted heating reproducibly resulted in high to excellent yields of the corresponding DKPs.

Keywords: Diketopiperazines; Microwave heating; Dipeptide synthesis; Peptide cyclization.
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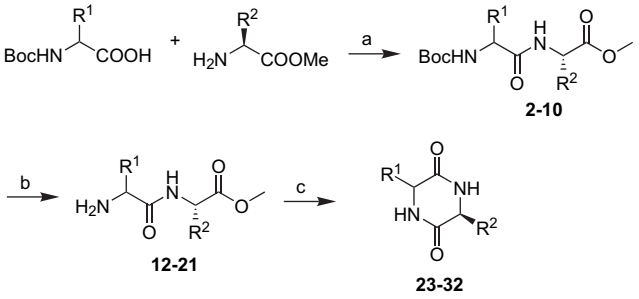
2. Results and discussion

Our synthetic procedure to obtain DKPs involved three steps (Scheme 1). First, the dipeptide methyl ester derivatives were formed via coupling of an *N*-Boc-protected amino acid with an amino acid methyl ester using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). EDC was chosen as it can easily be removed from the crude reaction mixture by extraction with 10% aqueous citric acid. In the second step, the Boc-group was cleaved using HCl–saturated methanol to afford the dipeptide methyl esters as their hydrochloride salts. Both the coupling reactions and the Boc-deprotection reactions proceeded in high yields (84–94% and 80–94% isolated yield, respectively). (For experimental procedures and structural characterization of compounds **1**–**22**, see Supplementary data.) In the final step, the dipeptide was cyclized in the presence of triethylamine as the base. For this step several different reaction conditions were tested and the results are discussed below.



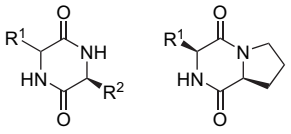
Scheme 1. Reagents: (a) EDC/NMM, CH_2Cl_2; (b) HCl (g)/MeOH; (c) H_2O, 2.5 equiv Et_3N/thermal or microwave heating.

As there are reports in the literature on microwave assisted Boc-removal[17–19] it was first investigated whether the Boc-protected dipeptides could be directly used for DKP formation both in thermally and microwave heated reactions. Dipeptide **2** (Boc-Phe–TrpOMe)[20] was therefore heated to reflux overnight in toluene/2-butanol (1:4), water, toluene and in *tert*-butanol, but unfortunately incomplete removal of the Boc-group was observed in all the reactions. Therefore, to allow comparisons of classical thermal heating and microwave assisted heating, the hydrochloride salts of the dipeptide methyl esters were used as starting materials in all cyclization reactions.

2.1. Optimization of individual reaction parameters

2.1.1. Choice of amino acids. To identify the optimal reaction conditions for the cyclization of dipeptides to DKPs a series of 11 dipeptide methyl ester hydrochlorides (**12**–**22**) were synthesized and cyclized to form the corresponding DKPs (**23**–**33**, Table 1). The amino acids were chosen to cover a range of physico-chemical properties such as polarity, conformational flexibility, steric and electrostatic properties. Thus, DKPs containing two sterically demanding aromatic side chains (**23**) or flexible aliphatic and/or aromatic side chains of varying size (**26**, **27**, **29**–**32**) have been synthesized (Scheme 1). In addition, DKPs containing functionalized amino acids (**24**, **25**, **28** and **32**) have also been investigated.

Table 1. Structures of the synthesized diketopiperazines



Compound	R^1	R^2
23	Benzyl	(3-Indolyl)–CH_2
24	(3-Indolyl)–CH_2	CH_2OH
25	(3-Indolyl)–CH_2	CH_2OBn
26	Benzyl	Butyl
27	Benzyl	Isobutyl
28	(3-Indolyl)–CH_2	CH_2CONH_2
29	L-Isopropyl	Butyl
30	D-Isopropyl	Butyl
31	H	Butyl
32	Isobutyl	4-OH–benzyl
33	Benzyl	—

Compound **30** was synthesized in order to investigate the influence of a D-amino acid on the cyclization efficiency, using the L-diastereomer **29** as comparison (see below). Compound **33** was synthesized as a reference compound as it is well known that proline facilitates the formation of DKPs.[11]

2.1.2. Choice of solvent, heating method and optimization of reaction time and temperature. To investigate the influence of solvent properties on the DKP formation dipeptide **16** (Phe–LeuOMe)[21] was used as a test compound. A range of common laboratory solvents were tested (Table 2) including the solvent mixture toluene/2-butanol (1:4) previously used in DKP synthesis.[16a] The reactions were stirred efficiently as DKPs are known to easily form gels in some solvents.[22] The reaction rates for the thermally heated reactions were slow according to TLC, and the reactions had to be heated to reflux for 12 h. The isolated yields of the cyclized product **27** varied from 5–6% in highly polar solvents such as water, DMF and MeOH to 36–38% in *tert*-butanol and toluene (Table 2).

Using microwave assisted heating for the cyclization of **16** gave low yields of **27** in all solvents except for water

Table 2. Isolated yields of **27** obtained in the cyclization reactions using 50 mg of **16** in the presence of 2.5 equiv of triethylamine

Solvent	Yield (%)	
	Δ	MW
Toluene	38	8
tert-Butanol	36	12
Acetonitrile	33	5
1,2-Dichloroethane	33	6
Toluene/2-butanol (1:4)	33	10
1,2-Dimethoxyethane	25	6
tert-Butanol/H_2O (1:1)	24	9
Benzene	17	5
CCl_4	16	5
1,4-Dioxane	10	7
Methanol	6	5
DMF	5	6
H_2O	5	67

The thermally heated reactions (Δ) were heated to reflux for 12 h and the microwave assisted (MW) reactions were heated for 10 min at a temperature 40 °C above the boiling point of the solvent. Each reaction was performed at least twice.

(Table 2), which resulted in the formation of **27** in 67% yield. The other solvents tested gave only low yields (5–12%) of product and resulted in complex reaction mixtures according to TLC.

For the microwave assisted reactions the yields were highest when run at a temperature of 40 °C above the boiling point of the solvents. This was shown for the cyclization of **16** in water for which the yields of **27** increased from 5% at 120 °C, to 67% at 140 °C, 63% at 150 °C and then decreased to 44% at 170 °C. At 170 °C the reaction became yellowish in colour and proved to be difficult to purify as a complex mixture of products was formed, none of the desired product could be isolated from the reaction mixture.

The optimal reaction time in the microwave heated reactions was also investigated. Already after 5 min at 140 °C in water the cyclization of **16** afforded **27** in 53% yield. The yield improved to 67% when heated for 10 min at the same temperature. Longer reaction times such as 20 or 45 min gave no further increase in yield. Therefore the reaction time for all the microwave reactions was set to 10 min.

2.2. Optimization of combined reaction parameters

Based on the results obtained in the test reactions four solvents were finally chosen in the cyclization reactions on all dipeptide methyl ester hydrochlorides synthesized (**12**–**22**): water as it proved to be the only suitable solvent in the microwave assisted reactions, *tert*-butanol and toluene as they usually gave the highest yields in the thermally heated reactions, and the toluene/2-butanol (1:4) mixture. Although giving moderate yields in this work, it has been previously proved to be suitable for the synthesis of DKPs.[16a] Both classical heating (refluxing temperature, 12 h) and microwave mediated heating (40 °C above the boiling point, 10 min) were used. All reactions were run at least twice to secure reproducibility.

The syntheses of **23**–**33** were accomplished with varying success (for isolated yields of products see Table 3). In most cases the DKPs were easy to isolate as they were not soluble to any great extent in any of the solvents tested and precipitated spontaneously upon formation. Thus the work-up procedure was straightforward; the reaction mixture was concentrated in vacuo, the residue was re-suspended in water and the solid product was filtered off, no further purification procedures were necessary (purity >98% according to NMR spectroscopy). Compounds **29**, **30** and **33**, which were partly soluble in the reaction solvent, were purified by flash chromatography as described in Section 4.

Despite the small volume of solvent used (3 mL), there were no signs of polymerization in any of the reactions nor were there any signs of epimerization at any of the temperatures or in any solvents chosen.[11]

Using classical thermal heating the yields of DKPs were generally low or moderate, with *tert*-butanol usually resulting in higher yields than in the other solvents (see Table 3), the only exception being **25**, which was formed in 57% yield in water but only in 10% yield in *tert*-butanol. Some combinations of solvent and dipeptide gave moderate to good yields, e.g., 62% yield of **23** in *tert*-butanol and 41% in water, or compound **31**, which was formed in 88% yield in *tert*-butanol and 51% in the toluene/2-butanol mixture. As described earlier in the literature diastereomeric dipeptides are generally cyclized in significantly different yields,[11] for **29** and **30** a facilitated cyclization to the D-valine containing derivative (**30**) was shown. In fact, **30** was formed considerably faster than **29**, showing signs of cyclization already after 5 min. Compounds **26**, **28** and **29** could not be obtained in yields higher than 10% independent of solvent used, whereas high yields of the proline containing DKP **33** were obtained in all solvents.[11] In conclusion, no general characteristics of the dipeptide that would result in high yields of DKPs in a given solvent could be observed in the thermally heated reactions.

Table 3. Isolated yields obtained in cyclization reactions (50 mg of dipeptide in the presence of 2.5 equiv of triethylamine) using different solvents and heating procedures, classical thermal heating (Δ) or microwave assisted heating (MW)

	Yield (%)/Δ[a]				Yield (%)/MW[b]			
	H_2O	Toluene/2-BuOH[c]	Toluene	*t*-BuOH	H_2O[d]	Toluene/2-BuOH[e]	Toluene[e]	*t*-BuOH[f]
23	41	8	20	62	73	14	18	10
24	<5	9	5	41	81	<5	10	5
25	57	6	5	10	70	6	<5	12
26	5	5	10	10	97	12	12	15
27	7	32	24	37	68	17	14	24
28	<5	<5	7	10	71	21	7	6
29	7	5	<5	8	63	15	8	8
30	35	7	22	41	84	12	<5	6
31	33	51	29	88	70	10	14	10
32	28	23	12	37	83	5	12	<5
33	89	88	83	93	93	73	70	76

Each reaction has been run at least twice with a difference in yield not higher than ±3%.
[a] The reactions were heated to reflux for 12 h.
[b] The reaction time was 10 min.
[c] The reaction temperature was 110 °C.
[d] The reaction temperature was 140 °C.
[e] The reaction temperature was 150 °C.
[f] The reaction temperature was 125 °C.

For the microwave assisted syntheses of **23**–**32** the only suitable solvent was water, giving moderate to excellent yields of cyclized products (63–97%). Reactions in the other solvents gave low yields, varying from <5 to 24% (Table 3), mainly due to the formation of complex mixtures according to TLC. Water on the other hand often gave a dazzling white reaction matrix from which the products were easily separated from the starting materials with no sign of any byproducts. Interestingly, the synthesis of **26**, **28** and **29**, which only produced traces of product using thermal heating, were formed in 97, 71 and 63% yield, respectively, using microwave heating in water.

It is notable that **27** was formed in higher yields than the structurally related **26** in *tert*-butanol, toluene and toluene/2-butanol (1:4) using thermal heating (37, 24 and 32% compared to 10, 10 and <5%) but the other way round in water when using microwave assisted heating (68 and 97%, respectively). This may be explained by the fact that **27** gave a solid gel instead of crystals in the microwave assisted reactions. The reaction mixture might therefore no longer have

the physical properties of a classic solvent. No formation of gels was detected in the thermally heated reactions.

In general, compound **30** was formed in much higher yields than its diastereomer **29** both in the microwave assisted and the classic thermal heated reactions. Dipeptides with different configuration at the α-carbons probably give less steric hindrance in the *cis*-amide conformation. In addition, the steric hindrance between the two side chains in **30** is lower than that in **29** because of its *trans*-configuration.[11] Interestingly, the solubility of **30** was shown to be higher than that of **29** in both water and MeOH.

As expected for cyclization of dipeptides containing a proline residue and thereby a higher *cis*-amide content[23] the yields of **33** were excellent in all solvents using both heating methods (70–96%). However, using thermal heating the formation of **33** was still quite slow so the reaction had to be heated to reflux for 12 h.

The dipeptides containing serine or benzyl-protected serine residues (**13** and **14**, respectively) were possible to cyclize in acceptable yields using different solvents: unprotected **13** gave 41% yield of **24** in *tert*-butanol whereas **14** preferentially cyclized in water producing **25** in 57% yield. Both dipeptides cyclized efficiently in water using microwave heating, producing **24** and **25** in 81 and 70% yield, respectively. Debenzylation of **25** using catalytic hydrogenation (5% Pd/C in EtOH) proceeded smoothly and **24** could be isolated in high yields (96%) (data not shown).

2.3. Conformational effects observed in 23, 26 and 32

During the structural characterization of the DKPs several derivatives showed extraordinary chemical shifts in ^{1}H NMR spectra. To explain these unexpected results computer assisted molecular modelling was performed using the MacroModel program (v 7.1) and the Amber 94 force field. It is known from the literature that DKPs containing aromatic side chains can adopt extraordinary conformations in solution as the otherwise rather flexible aromatic rings often choose severely restricted conformations.[2b,24] This was also observed for some of the DKPs obtained in this study, e.g., strong shielding effects in ^{1}H NMR spectra of compound **23** indicated that one of the benzylic protons in phenylalanine was affected by the aromatic ring of the tryptophane residue.[25] The chemical shift for this proton was δ 1.40 compared to δ 2.60 for the other β-proton.

Computer assisted conformational searches of compound **23** (performed both in vacuum and in simulated water or chloroform environments) corroborated this finding as the global minimum and other low energy conformers (ΔE<3.7 kJ/mol) showed that either one of the four β-protons could be shielded (Fig. 1, above left).

Also in the ^{1}H NMR spectra of compound **32** a similar shielding effect was observed on the signal from the CH_2-group in the leucine side chain. This resulted in a chemical shift of δ 0.10 compared to δ 1.54 for the same signal in **27** where no shielding effect was observed. Conformational analysis of **32** showed a global minimum conformation and low energy conformations in which the hydrogen atoms of the CH_2-group were directed towards the aromatic ring of tyrosine (Fig. 1, above right). Interestingly, the published X-ray crystal structure of **32**[26] shows the same conformational effects as those observed in solution and in the computer calculations in this study.

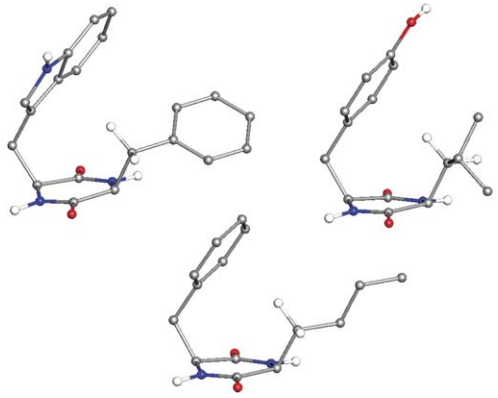


Figure 1. Global minimum conformations (Amber 94 force field, MacroModel v 7.1) of **23** (above left), **32** (above right) and **26** (below). In **23** the shielded benzylic hydrogen atom in phenylalanine is shown. In **32** and **26** the shielded β-hydrogens in leucine and norleucine, respectively, are shown. The other hydrogens in the structures have been omitted for clarity.

A similar shielding effect was also observed in the ^{1}H NMR spectra of **26** in which the β-methylene protons of norleucine were experiencing different magnetic environments. The shielding effect was also observed in the computer modelling (Fig. 1, lower middle). No shielding effects were observed in the NMR spectra of **27**, this is especially interesting as compound **27** is an isomer of **26**.

3. Conclusions

In this study we have developed a general, efficient and environmentally benign synthetic procedure for the formation of DKPs from the corresponding dipeptide methyl ester hydrochlorides. The results show that the highest yielding way to synthesize DKPs is to use microwave assisted heating with water as solvent. The reactions were run only for short times (10 min), and as most products precipitated during the reaction the work-up procedure was simple, and the products isolated in moderate to excellent yields (63–97%). Although classic thermal heating provided good yields for some derivatives, the optimal conditions for a certain dipeptide could not be predicted. The observations of constrained conformations of the DKPs in ^{1}H NMR spectra have been confirmed by computational analyses.

4. Experimental

4.1. General

All reagents and solvents were of analysis or synthesis grade. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL JNM-EX 400-spectrometer at 400 and 100 MHz, respectively. The designations of atoms for interpretation of NMR spectra are given in Figure 2. The reactions were monitored by thin-layer chromatography (TLC), on silica plated aluminium

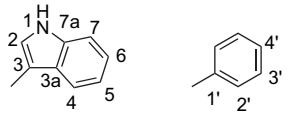


Figure 2. Designation of atoms for interpretation of NMR data.

sheets (silica gel 60 F254, E. Merck), detecting spots by UV and/or 2% ninhydrin in ethanol followed by heating. Column chromatography was performed on wet packed silica (silica gel 60 (0.040–0.063 mm), E. Merck) using flash chromatography. Melting points were measured in a Büchi Melting Point B-540 apparatus and are uncorrected. Optical rotations were measured at room temperature with a Perkin–Elmer 341 LC polarimeter. The microwave reactions were carried out in a Biotage Initiator instrument with a fixed hold time. The IR spectra were obtained on a Perkin–Elmer 16 PC spectrometer. Elemental analyses were performed at Mikrokemi AB, Uppsala, Sweden and at Kolbe Mikroanalytisches Laboratorium, Mülheim and der Ruhr, Germany. Conformational analyses were performed using the Amber 94 force field as implemented in the MacroModel program 7.1 run on a Silicon Graphics Octane workstation.

The synthetic procedure and characterization of compounds **1**–**22** are found in the Supplementary data. Compounds **23**, **27** and **33** are commercially available.

4.2. General procedure for coupling reactions using EDC (Scheme 1)

The methyl ester of the C-terminal amino acid was dissolved in dry solvent (CH_2Cl_2 or DMF) (10 mL), followed by addition of NMM. The reaction mixture was stirred for 40 min at 0 °C whereupon EDC and the Boc-protected N-terminal amino acid were added. The reaction was thereafter stirred for 3 h at 0 °C and then overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and extracted with 10% aqueous citric acid. The organic layer was dried ($MgSO_4$), concentrated in vacuo and the crude product was purified by flash chromatography.

4.3. General procedure for dipeptide cyclization (Scheme 1)

The hydrochloride salt of the deprotected dipeptide was dissolved in the solvent (H_2O, *tert*-butanol, toluene/2-butanol (1:4) or toluene) (3 mL) and 2.5 equiv of triethylamine was added. In each reaction 50 mg of each compound was used. The microwave assisted heated reactions were run for 10 min and the classic thermally heated reactions were heated to reflux overnight. The crude product precipitated spontaneously and the reaction mixture was concentrated in vacuo, suspended in H_2O and filtered. In the microwave assisted reaction the procedure was the same with the exceptions that the reaction temperature was set at 40 °C above the boiling point of the solvent. For choice of solvents and the isolated yields of the reactions see Table 3.

4.3.1. c(L-Phenylalaninyl-L-tryptophanyl) (23). Compound **12** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **23** was isolated as white crystals.

Mp 284–286 °C (lit.[27] mp 284 °C). $[\alpha]_D$ −174.4 (*c* 0.3, CH_3OH) (lit.[28] $[\alpha]_D$ −245.9 (*c* 1, CH_3OH)). IR (KBr) ν_{max} 3420, 3050, 1670, 1456, 1328 cm^{-1}.[28,29] ^1H NMR (CD_3OD) δ 7.59 (d, *J*=7.3 Hz, 1H, indole), 7.34 (d, *J*=7.3 Hz, 1H, indole), 7.19–7.01 (m, 6H, indole and Ph-*H*), 6.61–6.57 (m, 2H, indole and Ph-*H*), 4.19–4.16 (m, 1H, α-C*H*), 3.98–3.88 (m, 1H, α-C*H*), 3.03 (dd, *J*=13.4, 3.5 Hz, 1H, CH_2-indole), 2.85–2.80 (m, 1H, CH_2-Ph), 2.60 (dd, *J*=13.4, 3.5 Hz, 1H, CH_2-indole), 1.44–1.38 (m, 1H, CH_2-Ph). ^{13}C NMR (CD_3OD) δ 165.6, 164.7 (C═O, amides), 136.3, 135.9 (C-1′ and C-7a), 129.5, 128.2 (C-2′ and C-3′), 127.7 (C-3a), 126.6 (C-4′), 124.5 (C-2), 121.4 (C-6), 118.9, 118.7 (C-4 and C-5), 115.0 (C-7), 111.2 (C-3), 56.4, 55.8 (α-*C*H), 40.2 (*C*H_2-Ph), 29.8 (*C*H_2-indole). Anal. Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60; Found C, 72.0; H, 5.9; N, 12.6.

4.3.2. c(L-Tryptophanyl-L-serinyl) (24). Compound **13** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **24** was isolated as white crystals.

Mp 268–269 °C. $[\alpha]_D$ −100 (*c* 0.5, CH_3OH). IR (KBr) ν_{max} 3412, 3349, 3204, 1733, 1635 cm^{-1}. ^1H NMR (CD_3OD) δ 7.60 (d, *J*=8.1 Hz, 1H, H-6-indole), 7.34 (d, *J*=8.1 Hz, 1H, H-3-indole), 7.13-7.05 (m, 2H, indole), 7.04–6.98 (m, 1H, indole), 4.24–4.19 (m, 1H, α-C*H*), 3.83–3.81 (m, 1H, α-C*H*), 3.41–3.33 (m, 2H, CH_2-Ser), 3.30–3.26 (m, 1H, CH_2-indole), 2.91–2.85 (m, 1H, CH_2-indole). ^{13}C NMR (CD_3OD) δ 168.8, 163.5 (C═O, amides), 136.7 (C-7a), 127.6 (C-3a), 124.1 (C-2), 121.2 (C-6), 118.8, 118.4 (C-4 and C-5), 111.0 (C-7), 108.5 (C-3), 63.4 (*C*H_2OH), 57.5, 56.1 (α-*C*H), 30.6 (*C*H_2-indole). Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38; Found C, 61.4; H, 5.6; N, 15.6.

4.3.3. c(L-Tryptophanyl-*O*-benzyl-L-serinyl) (25). Compound **14** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **25** was isolated as white crystals.

Mp 250–251 °C. $[\alpha]_D$ −52.4 (*c* 0.45, DMSO). IR (KBr) ν_{max} 3338, 2947, 1725, 1674 cm^{-1}. ^1H NMR (DMSO-d_6) δ 10.92 (s, 1H, N*H*, indole), 8.05 (dd, *J*=2.6 Hz, 2H, N*H*, amides), 7.36–6.92 (m, 10H, Ar-*H*), 4.18 (d, *J*=12.0 Hz, 1H, CH_2-Ph), 4.10 (d, *J*=12.0 Hz, 1H, CH_2-Ph), 4.08–4.05 (m, 1H, α-C*H*), 3.79 (ddd, *J*=6.0, 2.8, 2.6 Hz, 1H, α-C*H*), 3.24–3.16 (m, 2H, CH_2-Ser), 3.07 (dd, *J*=14.3, 4.4 Hz, 1H, CH_2-indole), 2.54–2.50 (m, 1H, CH_2-indole). ^{13}C NMR (DMSO-d_6) δ 167.7, 165.5 (C═O, amides), 138.4, 136.5, 128.7, 128.2 (C-2′ and C-3′), 128.0, 124.9, 121.4, 119.3, 119.0 (Ar), 111.8 (C-7), 109.4 (C-3), 72.7, 72.2 (*C*H_2-Ser and *C*H_2-Ph), 56.0, 55.7 (α-*C*H), 30.6 (*C*H_2-indole). Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56; Found C, 69.3; H, 5.8; N, 11.5.

4.3.4. c(L-Phenylalaninyl-L-norleucinyl) (26). Compound **15** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **26** was isolated as white crystals.

Mp 269 °C. $[\alpha]_D$ −20 (*c* 0.2, CH_3OH). IR (KBr) ν_{max} 3425, 3057, 1976, 1578, 1425 cm^{-1}. ^1H NMR (CD_3OD) δ 7.30–7.17 (m, 5H, Ph-*H*), 4.32–4.29 (m, 1H, α-C*H*), 3.68–3.64

(m, 1H, α-C*H*), 2.97–2.91 (m, 2H, CH_2-Ph), 1.15–1.04 (m, 3H, CHCH_2-Nle and CH_2CH$_3$-Nle), 0.91–0.82 (m, 2H, CH$_2$CH_2-Nle), 0.78 (dt, *J*=7.3, 1.8 Hz, 3H, CH_3-Nle), 0.53–0.47 (m, 1H, CHCH_2-Nle). ^{13}C NMR (CD$_3$OD) δ 168.8, 167.6 (C=O, amides), 135.4 (C-1′), 130.4, 128.2 (C-2′ and C-3′), 127.1 (C-4′), 56.0, 54.6 (α-*C*H), 38.7 (*C*H$_2$-Ph), 33.8 (CH*C*H$_2$-Nle), 26.4 (CH$_2$*C*H$_2$-Nle), 21.9 (*C*H$_2$CH$_3$-Nle), 12.9 (*C*H$_3$-Nle). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; Found C, 68.9; H, 7.6; N, 10.9.

4.3.5. c(L-Phenylalaninyl-L-leucinyl) (27). Compound **16** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **27** was isolated as white crystals.

Mp 282–284 °C. [α]$_D$ −8.0 (*c* 0.3, CH$_3$OH). IR (KBr) ν_{max} 3430, 3012, 1666, 1570, 1389 cm^{-1}.[21,30] ^{1}H NMR (CDCl$_3$) δ 7.36–7.18 (m, 5H, Ph-*H*), 4.28–4.24 (m, 1H, α-C*H*), 3.90–3.85 (m, 1H, α-C*H*), 3.28–3.22 (m, 1H, CH_2-Ph), 3.09–3.02 (m, 1H, CH_2-Ph), 1.54 (app s, 2H, CH_2-Leu), 1.24 (app s, 1H, C*H*-Leu), 0.86 (app t, *J*=6.2 Hz, 6H, CH_3-Leu). ^{13}C NMR (CDCl$_3$) δ 171.6, 168.0 (C=O, amides), 135.1 (C-1′), 129.9, 129.2 (C-2′ and C-3′), 127.7 (C-4′), 56.3, 53.3 (α-*C*H), 42.9 (*C*H$_2$-Leu), 40.3 (*C*H$_2$-Ph), 24.1 (*C*H-Leu), 23.2, 20.9 (*C*H$_3$-Leu). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; Found C, 69.0; H, 7.8; N, 10.5.

4.3.6. c(L-Tryptophanyl-L-asparginyl) (28). Compound **17** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **28** was isolated as off-white crystals.

Mp 272–274 °C. [α]$_D$ −36 (*c* 0.2, DMSO). IR (KBr) ν_{max} 3450, 3207, 3048, 1672, 1559, 1507 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H, N*H*, indole), 7.94 (d, *J*=1.8 Hz, 1H, N*H*, amide), 7.67 (d, *J*=1.5 Hz, 1H, N*H*, amide), 7.57 (d, *J*=7.7 Hz, 1H, indole), 7.12 (s, 1H, indole), 7.05 (t, *J*=7.0 Hz, 1H, indole), 6.96 (t, *J*=7.5 Hz, 1H, indole), 6.91 (d, *J*=7.7 Hz, 1H, indole), 4.13 (t, *J*=4.2 Hz, 1H, α-C*H*), 3.99 (dd, *J*=7.0, 3.7 Hz, 1H, α-C*H*), 3.21 (dd, *J*=14.5, 4.6 Hz, 1H, CH_2-indole), 3.10 (dd, *J*=14.5, 4.6 Hz, 1H, CH_2-indole), 2.18 (dd, *J*=15.7, 4.4 Hz, 1H, CH_2-Asn), 1.48 (dd, *J*=15.7, 8.1 Hz, 1H, CH_2-Asn). ^{13}C NMR (DMSO-d_6) δ 172.0 (C=O, Asn), 167.8, 167.5 (C=O, amides), 136.5 (C-7a), 128.2 (C-3a), 125.0 (C-2), 121.4, 119.4, 119.0 (C-4, C-5 and C-6), 111.8, 109.4 (C-3 and C-7), 55.8 (α-*C*H, Trp), 51.9 (α-*C*H, Asn), 38.8 (*C*H$_2$-Asn), 29.2 (*C*H$_2$-indole). Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66; Found C, 59.9; H, 5.6; N, 18.3.

4.3.7. c(L-Valinyl-L-norleucinyl) (29).[31] Compound **18** and triethylamine were reacted as described in the general procedure (Section 4.3). The crude product had to be purified by flash chromatography using CH$_2$Cl$_2$/CH$_3$OH (95:5) as eluent. Pure **29** was isolated as white crystals.

Mp 254–256 °C. [α]$_D$ −87.7 (*c* 0.4, CH$_3$OH). IR (KBr) ν_{max} 3310, 3019, 1800, 1640 cm^{-1}. ^{1}H NMR (CD$_3$OD) δ 3.96–3.93 (m, 1H, α-C*H*), 3.83–3.81 (m, 1H, α-C*H*), 2.30–2.22 (m, 1H, C*H*-Val), 1.87–1.82 (m, 1H, CHCH_2-Nle), 1.79–1.74 (m, 1H, CHCH_2-Nle), 1.43–1.32 (m, 4H, CH_2-Nle), 1.04 (d, *J*=7.3 Hz, 3H, CH_3-Val), 0.94 (d, *J*=7.3 Hz, 3H, CH_3-Val), 0.93 (t, *J*=7.0 Hz, 3H, CH_3-Nle). ^{13}C NMR (CD$_3$OD) δ 169.5, 168.4 (C=O, amides), 60.1, 54.6 (α-*C*H), 34.1, 32.0 (*C*H-Val and CH*C*H$_2$-Nle), 27.1 (CH$_2$*C*H$_2$-Nle), 22.1 (*C*H$_2$CH$_3$-Nle), 17.9, 16.3 (*C*H$_3$-Val), 12.9 (*C*H$_3$-Nle). Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.23; H, 9.50; N, 13.20; Found C, 62.4; H, 9.8; N, 13.5.

4.3.8. c(D-Valinyl-L-norleucinyl) (30).[31] Compound **19** and triethylamine were reacted as described in the general procedure (Section 4.3). The crude product had to be purified by flash chromatography using CH$_2$Cl$_2$/CH$_3$OH (95:5) as eluent. Pure **30** was isolated as white crystals.

Mp 263–265 °C. [α]$_D$ −6.6 (*c* 0.3, CH$_3$OH). IR (KBr) ν_{max} 3324, 3019, 1776, 1589 cm^{-1}. ^{1}H NMR (CD$_3$OD) δ 4.04 (td, *J*=4.8, 1.1 Hz, 1H, α-C*H*), 3.79 (dd, *J*=3.7, 1.1 Hz, 1H, α-C*H*), 2.32–2.24 (m, 1H, C*H*-Val), 1.96–1.86 (m, 1H, CHCH_2-Nle), 1.80–1.71 (m, 1H, CHCH_2-Nle), 1.39–1.27 (m, 4H, CH_2-Nle), 1.03 (d, *J*=7.0 Hz, 3H, CH_3-Val), 0.94 (d, *J*=7.0 Hz, 3H, CH_3-Val), 0.92 (t, *J*=7.0 Hz, 3H, CH_3-Nle). ^{13}C NMR (CD$_3$OD) δ 169.6, 169.0 (C=O, amides), 60.2, 54.0 (α-*C*H), 32.8, 32.2 (*C*H-Val and CH*C*H$_2$-Nle), 25.7 (CH$_2$*C*H$_2$-Nle), 22.2 (*C*H$_2$CH$_3$-Nle), 17.5, 15.7 (*C*H$_3$-Val), 12.9 (*C*H$_3$-Nle). Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.23; H, 9.50; N, 13.20; Found C, 62.0; H, 9.8; N, 13.5.

4.3.9. c(Glycinyl-L-norleucinyl) (31).[32] Compound **20** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **31** was isolated as fluffy white crystals.

Mp 256 °C. [α]$_D$ −1.4 (*c* 1, DMSO). IR (KBr) ν_{max} 3200, 2954, 1682, 1468, 1337 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 8.18 (s, 1H, N*H*-amide), 7.98 (s, 1H, N*H*-amide), 3.81–3.63 (m, 3H, CH_2-Gly and α-C*H*), 1.71–1.61 (m, 2H, CHCH_2-Nle), 1.33–1.23 (m, 4H, CH_2-Nle), 0.87 (t, *J*=7.4 Hz, 3H, CH_3-Nle). ^{13}C NMR (DMSO-d_6) δ 168.6, 166.6 (C=O, amides), 54.7 (α-*C*H), 44.8 (*C*H$_2$-Gly), 33.2 (CH*C*H$_2$-Nle), 26.8 (CH$_2$*C*H$_2$-Nle), 22.5 (*C*H$_2$CH$_3$-Nle), 14.4 (*C*H$_3$-Nle). Anal. Calcd for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46; Found C, 56.5; H, 8.3; N, 16.1.

4.3.10. c(L-Leucinyl-L-tyrosinyl) (32). Compound **21** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **32** was isolated as white crystals.

Mp 301–303 °C (lit.[33] mp 295–296 °C). [α]$_D$ 33.3 (*c* 0.3, CH$_3$OH). IR (KBr) ν_{max} 3306, 3206, 2953, 1667, 1467 cm^{-1}. ^{1}H NMR (CD$_3$OD) δ 6.99 (d, *J*=8.4 Hz, 2H, Ph-*H*), 6.70 (d, *J*=8.4 Hz, 2H, Ph-*H*), 4.22 (t, *J*=4.0 Hz, 1H, α-C*H*), 3.65 (dd, *J*=10.0, 4.2 Hz, 1H, α-C*H*), 3.19 (dd, *J*=13.5, 3.7 Hz, 1H, CH_2-Ph), 2.81 (dd, *J*=13.5, 3.7 Hz, 1H, CH_2-Ph), 1.47–1.36 (m, 1H, C*H*-Leu), 0.87 (ddd, *J*=13.8, 9.6, 4.4 Hz, 1H, CH_2-Leu), 0.73 (app t, *J*=8.1 Hz, 6H, CH_3-Leu), 0.10 (ddd, *J*=13.8, 9.6, 4.4 Hz, 1H, CH_2-Leu). ^{13}C NMR (CD$_3$OD) δ 171.4, 167.6 (C=O, amides), 157.0 (C-4′), 131.4 (C-2′), 125.7 (C-1′), 115.1 (C-3′), 56.3, 52.8 (α-*C*H), 43.9 (*C*H$_2$-Leu), 38.1 (*C*H$_2$-Ph), 23.3 (*C*H-Leu), 22.1, 20.0 (*C*H$_3$-Leu). Anal. Calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14; Found C, 65.1; H, 7.3; N, 9.9.

4.3.11. c(L-Phenylalaninyl-L-prolinyl) (33). Compound **22** and triethylamine were reacted as described in the general procedure (Section 4.3). The crude product had to be purified by flash chromatography using CH_2Cl_2/CH_3OH (95:5) as eluent. Pure **33** was isolated as white crystals. For ^1H and ^{13}C NMR spectral data see Ref. 34.

Mp 130–132 °C (lit.[35] mp 132 °C). $[\alpha]_D$ −184 (*c* 0.3, CH_2Cl_2). IR (KBr) ν_{max} 3250, 3054, 1679, 1580, 1487 cm^{-1}.[36] ^1H NMR ($CDCl_3$) δ 7.33–7.25 (m, 5H, Ph-*H*), 5.80 (br s, 1H, N*H*, amide), 4.26 (dd, *J*=10.4, 3.5 Hz, 1H, α-C*H*, Phe), 4.05 (t, *J*=7.9 Hz, 1H, α-C*H*), 3.66–3.51 (m, 3H, CH_2-Ph, dd, *J*=14.3, 10.3 Hz, δ-CH_2), 2.78 (dd, *J*=14.3, 10.3 Hz, 1H, CH_2-Ph), 2.33–2.27 (m, 1H, β-CH_2), 2.03–1.83 (m, 3H, β-CH_2 and γ-CH_2). ^{13}C NMR (CD_3OD) δ 169.5, 165.1 (C=O, amides), 136.1 (C-1′), 129.3, 129.2 (C-2′ and C-3′), 127.6 (C-4′), 59.2, 56.3 (α-*C*H), 45.5 (δ-*C*H_2), 36.9 (*C*H_2-Ph), 28.4 (β-*C*H_2), 22.6 (γ-*C*H_2). Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; Found C, 68.7; H, 6.7; N, 11.2.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.05.010.

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