Multicomponent synthesis of tripeptides containing pipecolic acid derivatives: selective induction of *cis*- and *trans*-imide bonds into peptide backbones

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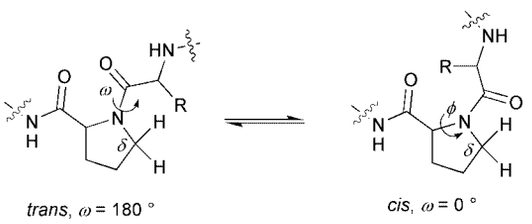
A simple approach to several tripeptides consisting of two terminal glycine fragments and a central pipecolic acid derivative was found *via* a multicomponent reaction starting from tetrahydropyridines. The protected peptides **2a**–**g** were formed in high yields and with different substitution patterns of the central heterocyclic amino acid. In cases where chiral imines were used the target compounds were obtained with remarkable diastereoselectivity. The influence of different substituents attached to the pipecolic acid fragment on *N*-terminal amide isomerism was investigated using X-ray crystallography and NMR spectroscopic methods.

Introduction

Replacement of proline for its higher homologue pipecolic acid in peptides is reported to go along with a significant change in bioactivity and leads to interesting model compounds for studies on peptide conformations,[1] where derivatives of pipecolic acid often find a role as β-turn mimics.[2] Comparative investigations of pipecolic acid residues to other cyclic amino acids, especially proline, incorporated in peptides give detailed insight into local mechanisms of peptide folding processes according to ring size.[3]

The proline residue plays a peculiar role in peptide and protein secondary structure and thereby represents an important means of directing peptide chains into favourable topologies.[4] The cyclic structure of proline confers unique conformational properties on the peptide backbone in comparison to other proteinogenic amino acids. The dihedral angle φ (Scheme 1) for instance is restricted to around −60° and the



Scheme 1 *cis*/*trans* imide isomerization *N*-terminal to proline and definition of torsional angles ω and φ.

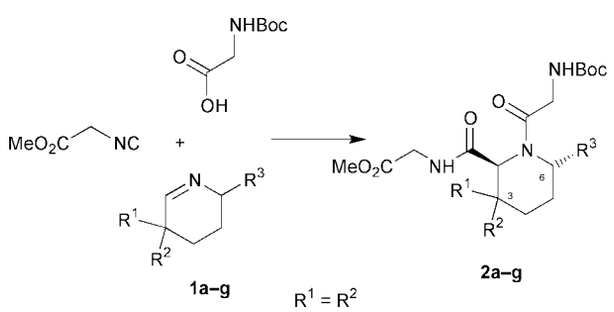
imidic bond formed with the preceding *N*-terminal amino acid is readily subject to *cis*/*trans*-isomerization. Accordingly, proline residues are often encountered in loop or turn structures.[4] In addition *cis*/*trans* amide isomerism next to prolyl residues is supposed to be a rate limiting step in protein folding.[5]

Due to the fact that proline residues play key roles in many biological processes such as protein folding and protein recognition, numerous mimetics and substituted proline analogues were developed in order to constrain and control the peptide backbone in reverse turn motifs[6] or to alter the imide *cis*:*trans* ratio.[7] However, little is known about pipecolic acid residues and their influence on peptide conformation. In a recent report, Raleigh and Wu revealed that pipecolic acid residues accelerate the rate of *cis*/*trans* imide isomerism and observed a higher preference for *cis*-imide bonds *N*-terminal to pipecolic acid in comparison to proline residues.[1] This prompted us to investigate 3- and 6-substituted pipecolic acid derivatives in order to design probes for the selective induction of *cis*- and *trans*-imide bonds into peptide backbones.

Results and discussion

Recently we presented an efficient approach to pipecolic acid analogues *via* Ugi multicomponent condensation.[8] Herein, we wish to extend this methodology to the synthesis of tripeptides with a central cyclic amino acid fragment and two terminal glycine units. These new tripeptides as well as some acylated pipecolic acid derivatives should provide simple model systems for investigations directed to tertiary amide isomerism *N*-terminal to substituted pipecolic acid residues.

The synthesis of tripeptides **2a**–**g** started from cyclic imines **1a**–**g**, Boc-protected glycine and isocyanoacetic acid methyl ester in a three-component condensation (Scheme 2).



Scheme 2 Multicomponent synthesis of tripeptides **2a**–**g** (only one enantiomer of racemic compounds is shown).

The reactions proceeded with good to excellent yields for a variety of different substituted tetrahydropyridines **1** and with remarkable diastereoselectivity for the chiral imines **1d**–**g** to give the diastereomeric pure tripeptides **2d**–**g** (Table 1). The relative configuration of substituents attached to C3 and C6 of the six-membered heterocycle was assigned by X-ray crystallography for **2g** and 2D-NOESY NMR experiments for **2d** and

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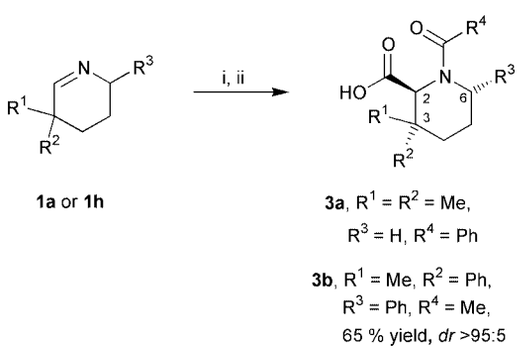
Table 1 Yield and diastereomeric ratios for the formation of tripeptides **2a**–**g**

Tripeptide	Reactant	R^1	R^2	R^3	Dr[a]	Yield (%)
2a	**1a**	Me	Me	H	—	85
2b	**1b**	Et	Et	H	—	78
2c	**1c**	$-(CH_2)_5-$		H	—	97
2d	**1d**	Me	Me	Me	—[b]	83[c]
2e	**1e**	Me	Me	Et	>95:5	89[c]
2f	**1f**	Me	Me	*i*-Pr	>95:5	96[c]
2g	**1g**	Me	Me	Ph	>95:5	82[c]

[a] Diastereomeric ratio dr determined by ^{1}H-NMR spectroscopy from the crude product. [b] Diastereomeric ratio could not be determined unambiguously from the crude product. [c] Yields are given for the diastereomerically pure *trans*-configured product after purification by column chromatography.

2g, indicating an axial arrangement of the carboxy terminus and an equatorial arrangement of substituents attached to C6 of the cyclic amino acid.

Two more derivatives of pipecolic acid (**3a** and **3b**) were synthesized in a two step procedure (Scheme 3) as reported



Scheme 3 Two-step synthesis of acylated pipecolic acid derivatives **3a**[9] and **3b**. *Reagents and conditions*: i, R^4-CO_2H, cyclohexenyl isocyanide, MeOH, 12 h, rt; ii: THF–HCl, 12 h. Only one enantiomer of racemic compounds is shown. The diastereomeric ratio (dr) of **3b** has been measured by ^{1}H-NMR spectroscopy.

previously by us.[8] In contrast to most of the tripeptides **2** these simple *N*-acylated derivatives gave suitable crystals for X-ray crystallographic investigations and were therefore interesting model compounds for investigations on *cis*/*trans* imide isomerism.

All products **2** and **3** were purified by column chromatography or crystallisation and gave satisfactory analytical data.

The chiral imines **1d**–**h** gave rise to the synthesis of 3,6-substituted pipecolic acid derivatives **2d**–**g** and **3b** (structural analogues to **D** in Fig. 1). These were of special interest to us because the corresponding δ-substituted proline[9] (**A** in Fig. 1) or pseudo proline[10] (**B** and **C** in Fig. 1) derivatives are known to induce selectively *cis*-imide bonds into peptide backbones, as demonstrated by the groups of Lubell and Mutter.

NMR spectroscopic investigations revealed that 3-substituted pipecolic acid derivatives **2a**–**c** exist in the expected mixture of *cis* and *trans* rotamers at room temperature in CD_3OD with quite a high content (18–25%) of the *cis*-imide isomer. However, the rotameric ratios depicted in Table 2 suggest that the *trans*-isomers are generally energetically favoured in solution. These findings correlate well with observations of Raleigh and Wu for small model peptides of unsubstituted pipecolic acid.[1]

While the *trans*-isomer predominates for derivative **3a** in solution, the *cis*-imide is favoured in the solid state, as is obvious from its crystal structure (Fig. 2).

In contrast to 3-substituted cyclic imino acid derivatives like **2a** and **3a**, all NMR spectra of 3,6-substituted derivatives of pipecolic acid (compounds **2d**–**g** and **3b**) show no evidence for a

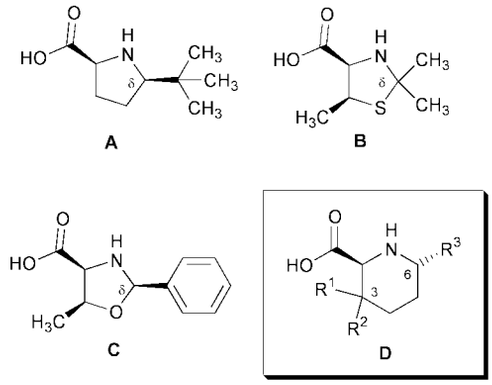


Fig. 1 Cyclic imino acids that favour *cis*-imide bonds in peptides.

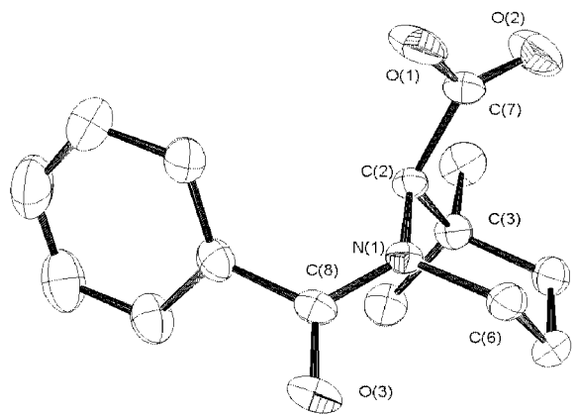


Fig. 2 Crystal structure of **3a**.

Table 2 Selected NMR spectroscopic data for *N*-acylated pipecolic acid derivatives **3a** and **3b** and tripeptides **2a**,**d**–**g**

	trans:*cis*[a]	δ^b H-2_{trans}	δ^b H-2_{cis}	δ^b $C2_{trans}$	δ^b $C2_{cis}$
3a	79:21	4.98	4.20	59.85	64.35
3b	>95:5[c]	5.13[c]	—	60.89[c]	—
2a	75:25	4.79	3.90	61.53	64.84
2d	<5:95	—	3.75	—	64.70
2e	<5:95	—	4.00[c]	—	63.03[c]
2f	<5:95	—	3.93	—	63.71
2g	<5:95	—	4.39	—	67.30

[a] The rotameric ratio (*trans*:*cis*) was determined by integration of peaks for 2-H in the ^{1}H-NMR spectra (CD_3OD) at room temperature. [b] Chemical shifts δ are reported in ppm from tetramethylsilane as internal standard. [c] DMSO-d_6.

second isomer at room temperature. The chemical shift values of 2-H and C2 for compounds **2d**–**g** indicate a strong preference for the *cis*-imide isomer that is observed exclusively in CD_3OD at room temperature. These findings are supported by the crystal structure of tripetide **2g** (Fig. 3).

The bulky phenyl substituent in the 6-position of compound **2g** skewed the neighbouring imidic bond away from planarity, as may be seen by the torsional angle around the imide bond ω of −39.2° (Table 3). This highly twisted situation leads to a substantive lengthening of the amide bond between C(8) and N(1) to 1.36 Å, compared to the average of 1.33 Å for similar amides, *e.g.* of **3a**. Similar observations have been reported by Halab and Lubell[9] for 5-*tert*-butylproline (structure **A** in Fig. 1), although the imide distortion is not as pronounced in the proline derivative as it is for **2g**.

Analysis of the chemical shift values for 2-H and C2 for compound **3b** in Table 2 reveals a *trans*-configuration of the imide bond in contrast to other 3,6-substituted derivatives **2d**–**g**, which prefer a *cis*-configuration of the corresponding *N*-terminal imide bond.

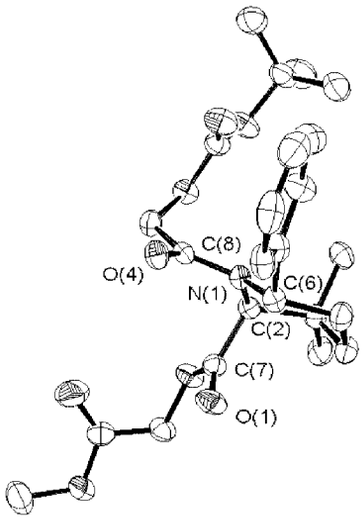


Fig. 3 Crystal structure of **2g**.

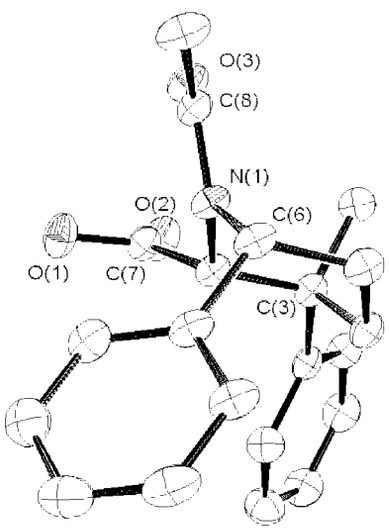


Fig. 4 Crystal structure of **3b**.

Table 3 Torsion angles ω and bond lengths d of selected derivatives of pipecolic acid determined by X-ray analysis

	Torsion angle[a] ω (°)	Bond length[b] d/Å	Imide isomer
3a	−20.2	1.34	*cis*
2g	−39.2	1.36	*cis*
3b	174.9	1.35	*trans*

[a] The torsional angle ω (see also Scheme 1) is defined as described in the literature.[15] [b] Bond length d = distance between N(1) and C(8).

This initially surprising finding was also confirmed by the crystal structure of **3b** (Fig. 4). However, the unexpected *trans*-configured imide bond in **3b** can be rationalized as a consequence of the two bulky phenyl residues attached to C3 and C6 of the six-membered heterocycle, as is evident from its crystal structure. Destabilisation of the *trans*-imide geometry, imposed by steric interaction of the *N*-terminal acyl residue with a phenyl substituent attached to C6, is less effective for **3b** than it is for **2g**, for example, since the phenyl substituent at C6 is in an axial position in compound **3b**. By contrast, the equatorial carboxy group in **3b** destabilises the *cis*-imide configuration by steric interactions with the *N*-acyl group.

Conclusion

The synthesis of new tripeptides **2** with a central pipecolic acid derivative *via* Ugi multicomponent condensation has been described. This one-pot procedure led to the diastereoselective formation of tripeptides **2d–g** if chiral tetrahydropyridines were used as reactants. In addition two amide derivatives (**3a** and **3b**) of substituted pipecolic acids were prepared in a two step procedure.

These amides **3a** and **3b** as well as the tripeptides **2** were shown to be useful probes for investigating their effect on *cis*/*trans*-imide isomerism. NMR spectroscopic analysis and crystal structures of these compounds showed that imide isomerism *N*-terminal to pipecolic acid residues may be controlled by the substitution pattern at C3 and C6 of the heterocycle. Introduction of a bulky phenyl substituent at C6, as in compound **2g**, for example, resulted exclusively in a *cis*-configuration of the imide bond in CD_3OD solution and the solid state, whereas introduction of two bulky phenyl substituents at C3 and C6 resulted in a *trans*-configured imide bond for compound **3b**.

Experimental

General remarks

If indicated with 'abs.' methanol was treated with 5 g Mg-turnings for each litre and distilled prior to use. Thin layer chromatography (TLC) analyses were performed on Polygram® silica gel plates with fluorescence indicator from Macherey Nagel & Co., Düren. For preparative chromatography Merck silica gel 60, 230–400 mesh was used. Melting points were determined in open capillaries in a Dr. Lindström instrument and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker-Karlsruhe AM 300 or an Avance 300 spectrometer (300.1 MHz/75 MHz). 2D-NOESY spectra were recorded on a Bruker-Karlsruhe Avance 500 spectrometer (500.1 MHz/125.8 MHz). Chemical shifts are reported on the δ scale (ppm) relative to residual nondeuterated solvent or tetramethylsilane (TMS) in CD_3OD, $CDCl_3$, or DMSO-d_6. Coupling constants, J, are given in hertz (Hz). Mass spectra were taken on a Finnigan-MAT 212 instrument in CI mode with isobutane as reactant gas. Elemental analyses were performed with a C, H, N Analyser EA 1108 from Fisons Instrument. The following compounds were synthesized according to literature procedures: 3,3-dimethyl-3,4,5,6-tetrahydropyridine (**1a**),[11] 3,3-diethyl-3,4,5,6-tetrahydropyridine (**1b**),[12] 2-azaspiro[5.5]undec-1-ene (**1c**),[13] 3,3,6-trimethyl-3,4,5,6-tetrahydropyridine (**1d**),[12] 3,3-dimethyl-6-ethyl-3,4,5,6-tetrahydropyridine (**1e**),[12] 3,3-dimethyl-6-isopropyl-3,4,5,6-tetrahydropyridine (**1f**),[12] 3,3-dimethyl-6-phenyl-3,4,5,6-tetrahydropyridine (**1g**),[12] (3*SR*,6*RS*)-3,6-diphenyl-3-methyl-3,4,5,6-tetrahydropyridine (**1h**),[8] cyclohex-1-enyl isocyanide,[14] 1-benzoyl-3,3-dimethylpiperidine-2-carboxylic acid (**3a**).[8]

General procedure for the preparation of tripeptides 2 (GP1)

The appropriate imine **1** (5 mmol) and 0.88 g (5 mmol) *N*-Boc-glycine were dissolved in 10 ml abs. MeOH. 0.50 g (5 mmol) Isocyanoacetic acid methyl ester was added and the solution was stirred for 12 hours at room temperature. The solvent was removed *in vacuo* and the resulting crude product purified by column chromatography on silica gel (dichloromethane–methanol, 98 : 2 eluent).

***rac*-*N*-(Methoxycarbonylmethyl)-1-(*tert*-butoxycarbonylaminoacetyl)-3,3-dimethylpiperidine-2-carboxamide 2a.** The title compound was prepared according to **GP1** from 0.55 g (5 mmol) imine **1a**. Two rotamers in a 75 : 25 ratio were observed by ^{1}H-NMR spectroscopy in CD_3OD at room temperature. The tripeptide **2a** (1.63 g, 85%) was obtained as a colourless solid, mp 89–90 °C; R_f 0.42 (dichloromethane–methanol, 98 : 2) [Found C, 56.12; H, 8.05; N, 10.83; $C_{18}H_{31}N_3O_6$ (385.5) requires C, 56.09; H, 8.11; N, 10.90%]; δ_H (CD_3OD) 1.05 [2.25H, s, $C(CH_3)_2$], 1.13 [3H, s, $C(CH_3)_2$],

1.14 [0.75H, s, $C(CH_3)_2$], 1.35 (1H, m, 4-H), 1.44 (9H, s, Boc-CH_3), 1.6–1.9 (2H, m, 5-H), 2.11 (1H, m, 4-H), 3.44 (0.25H, m, 6-H), 3.74 (1.50H, m, 6-H), 3.75 (3H, s, OCH_3), 3.87 (1H, d, NCH_2, ^3J = 17.5 Hz), 3.90 (0.25H, s, 2-H), 3.92 (1H, d, NCH_2, ^3J = 16.7 Hz), 4.01 (1H, d, NCH_2, ^3J = 17.5 Hz), 4.14 (1H, d, NCH_2, ^3J = 16.7 Hz), 4.48 (0.25H, s, 6-H), 4.79 (0.75H, s, 2-H); δ_C (CD_3OD, two rotamers) 21.46, 22.23, 26.24, 26.51, 27.99, 28.71, 33.89, 39.77, 41.57, 42.39, 43.41, 52.47, 61.53, 64.84, 80.42, 157.98, 170.94, 171.38, 171.84, 172.41; *m*/*z* (CI-isobutane) 386 (100%) [MH^+].

***rac*-*N*-(Methoxycarbonylmethyl)-1-(*tert*-butoxycarbonylaminoacetyl)-3,3-diethylpiperidine-2-carboxamide 2b.** The title compound was prepared according to **GP1** from 0.70 g (5 mmol) imine **1b**. Two rotamers in a 82 : 18 ratio were observed by ^{1}H-NMR spectroscopy in CD_3OD at room temperature. The tripeptide **2b** (1.62 g, 78%) was obtained as a colourless solid, mp 85–87 °C; R_f 0.54 (dichloromethane–methanol, 98 : 2) [Found C, 58.34; H, 8.61; N, 10.24; $C_{20}H_{35}N_3O_6$ (413.5) requires C, 58.09; H, 8.53; N, 10.16%]; δ_H (CD_3OD) 0.76 (3H, t, CH_3CH_2, ^3J = 7.7 Hz), 0.85 (3H, t, CH_3CH_2, ^3J = 7.7 Hz), 1.30–1.58 (6H, m, 4-H + CH_2CH_3), 1.44 (9H, s, Boc-CH_3), 1.72 (1H, m, 5-H), 2.02 (1H, m, 4-H), 3.38 (0.18H, m, 6-H), 3.64 (1.64H, m, 6-H), 3.69 (2.46H, s, OCH_3), 3.72 (0.54H, s, OCH_3), 3.82 (1H, d, NCH_2, ^3J = 17.6 Hz), 3.86 (1H, d, NCH_2, ^3J = 16.9 Hz), 3.93 (0.18H, s, 2-H), 3.95 (1H, d, NCH_2, ^3J = 17.6 Hz), 4.08 (1H, d, NCH_2, ^3J = 16.9 Hz), 4.40 (0.18H, s, 6-H), 4.86 (0.82H, s, 2-H); δ_C ($CDCl_3$, major rotamer) 6.85, 6.97, 20.37, 23.29, 26.66, 27.52, 28.21, 37.01, 40.63, 41.30, 42.57, 52.04, 57.70, 79.40, 155.59, 168.97, 169.53, 169.93; *m*/*z* (CI-isobutane) 414 (100%) [MH^+].

***rac*-*N*-(Methoxycarbonylmethyl)-2-(*tert*-butoxycarbonylaminoacetyl)-2-azaspiro[5.5]undecane-1-carboxamide 2c.** The title compound was prepared according to **GP1** from 0.76 g (5 mmol) imine **1c**. Two rotamers in a 78 : 22 ratio were observed by ^{1}H-NMR spectroscopy in CD_3OD at room temperature. The tripeptide **2c** (2.06 g, 97%) was obtained as a colourless solid, mp 73–74 °C; R_f 0.50 (dichloromethane–methanol, 98 : 2) [Found C, 59.51; H, 8.30; N, 9.93; $C_{21}H_{35}N_3O_6$ (425.5) requires C, 59.27; H, 8.29; N, 9.88%]; δ_H (CD_3OD) 1.28–2.08 (14H, m, 4-H + cyclohexyl-CH_2 + 5-H), 1.43 (9H, s, Boc-CH_3), 3.41 (0.22H, m, 3-H), 3.65 (1.56H, m, 6-H), 3.68 (2.34H, s, OCH_3), 3.70 (0.66H, s, OCH_3), 3.80 (1H, d, NCH_2, ^3J = 17.4 Hz), 3.84 (1H, d, NCH_2, ^3J = 16.6 Hz), 3.94 (0.22H, s, 1-H), 3.96 (1H, d, NCH_2, ^3J = 17.4 Hz), 4.05 (1H, d, NCH_2, ^3J = 16.6 Hz), 4.38 (0.22H, s, 3-H), 5.03 (0.78H, s, 1-H); δ_C ($CDCl_3$, major rotamer) 20.16, 21.22, 21.27, 26.14, 28.24, 28.90, 32.68, 34.49, 35.33, 40.69, 41.46, 42.57, 52.10, 58.14, 79.44, 155.62, 168.87, 169.45, 169.97; *m*/*z* (CI-isobutane) 426 (100%) [MH^+].

(2*RS*,6*SR*)-*N*-(Methoxycarbonylmethyl)-1-(*tert*-butoxycarbonylaminoacetyl)-3,3,6-trimethylpiperidine-2-carboxamide 2d. The title compound was prepared according to **GP1** from 0.63 g (5 mmol) imine **1d**. The tripeptide **2d** (1.65 g, 83%) was obtained as a colourless solid, mp 104–105 °C; R_f 0.27 (dichloromethane–methanol, 98 : 2) [Found C, 57.35; H, 8.41; N, 10.47; $C_{19}H_{33}N_3O_6$ (399.5) requires C, 57.12; H, 8.33; N, 10.52%]; δ_H (CD_3OD) 1.05 [3H, s, $C(CH_3)_2$], 1.13 [3H, s, $C(CH_3)_2$], 1.31 (3H, d, $CHCH_3$, ^3J = 6.8 Hz), 1.40–1.61 (3H, m, 4-H + 5-H), 1.44 (9H, s, Boc-CH_3), 2.05 (1H, m, 4-H), 3.69 (3H, s, OCH_3), 3.75 (1H, s, 2-H), 3.78 (1H, d, NCH_2, ^3J = 17.8 Hz), 3.90 (1H, d, NCH_2, ^3J = 16.9 Hz), 3.98 (1H, d, NCH_2, ^3J = 17.8 Hz), 4.00 (1H, d, NCH_2, ^3J = 16.9 Hz), 4.16 (1H, m, 6-H); δ_C (CD_3OD) 19.59, 24.73, 27.84, 29.01, 29.71, 34.65, 42.32, 44.71, 50.44, 64.70, 80.87, 158.67, 172.27, 172.84, 174.34; *m*/*z* (CI-isobutane) 400 (100%) [MH^+].

(2*RS*,6*SR*)-*N*-(Methoxycarbonylmethyl)-1-(*tert*-butoxycarbonylaminoacetyl)-6-ethyl-3,3-dimethylpiperidine-2-carboxamide 2e. The title compound was prepared according to **GP1** from 0.70 g (5 mmol) imine **1e**. The tripeptide **2e** (1.85 g, 89%) was obtained as a colourless solid, mp 93–94 °C; R_f 0.52 (dichloromethane–methanol, 98 : 2) [Found C, 58.17; H, 8.61; N, 10.23; $C_{20}H_{35}N_3O_6$ (413.5) requires C, 58.09; H, 8.53; N, 10.16%]; δ_H (DMSO-d_6) 0.98 (3H, t, CH_2CH_3, ^3J = 7.5 Hz), 1.05 [3H, s, $C(CH_3)_2$], 1.14 [3H, s, $C(CH_3)_2$], 1.43 (9H, s, Boc-CH_3), 1.4–1.6 (3H, m), 1.74 (2H, m), 2.00 (1H, m, 4-H), 3.70 (3H, s, OMe), 3.76 (1H, m, 6-H), 3.80 (1H, dd, NCH_2, ^2J = 18.5 Hz, ^3J = 4.9 Hz), 3.85 (2H, m, NCH_2), 3.96 (1H, dd, NCH_2, ^2J = 18.5 Hz, ^3J = 5.3 Hz), 4.00 (1H, s, 2-H), 6.69 (1H, br, Boc-NH), 8.00 (1H, br, CONH); δ_C (DMSO-d_6) 11.79, 24.21, 25.40, 28.72, 29.54, 34.34, 41.65, 43.55, 52.59, 55.90, 63.03, 79.98, 156.26, 169.86, 170.57, 171.00; *m*/*z* (CI-isobutane) 414 (100%) [MH^+].

(2*RS*,6*SR*)-*N*-(Methoxycarbonylmethyl)-1-(*tert*-butoxycarbonylaminoacetyl)-6-isopropyl-3,3-dimethylpiperidine-2-carboxamide 2f. The title compound was prepared according to **GP1** from 0.77 g (5 mmol) imine **1f**. The tripeptide **2f** (2.05 g, 96%) was obtained as a colourless solid, mp 92–93 °C; R_f 0.57 (dichloromethane–methanol, 98 : 2) [Found C, 59.11; H, 8.93; N, 9.87; $C_{21}H_{37}N_3O_6$ (427.5) requires C, 59.00; H, 8.72; N, 9.83%]; δ_H (CD_3OD) 0.82 [3H, d, $CH(CH_3)_2$, ^3J = 6.4 Hz], 0.90 [3H, d, $CH(CH_3)_2$, ^3J = 6.4 Hz], 0.95 [6H, s, $C(CH_3)_2$], 1.24 (9H, s, Boc-CH_3), 1.20–1.70 (4H, m, 4-H + 5-H), 2.17 [1H, m, $CH(CH_3)_2$], 3.32 (1H, m, NCH_2), 3.45 (3H, s, OMe), 3.60 (1H, d, NCH_2, ^2J = 18.5 Hz,), 3.76 (2H, m, NCH_2), 3.84 (1H, d, NCH_2, ^2J = 18.5 Hz), 3.93 (1H, s, 2-H); δ_C (CD_3OD) 20.39, 20.89, 23.85, 27.40, 28.33, 28.68, 35.49, 37.07, 41.81, 43.37, 52.61, 61.65, 63.71, 79.95, 156.18, 168.98, 169.17, 171.16; *m*/*z* (CI-isobutane) 428 (100%) [MH^+].

(2*RS*,6*SR*)-*N*-(Methoxycarbonylmethyl)-1-(*tert*-butoxycarbonylaminoacetyl)-3,3-dimethyl-6-phenylpiperidine-2-carboxamide 2g. The title compound was prepared according to **GP1** from 0.94 g (5 mmol) imine **1g**. The tripeptide **2g** (1.89 g, 82%) was obtained as a colourless solid, mp 73 °C; R_f 0.35 (dichloromethane–methanol, 98 : 2) [Found C, 62.56; H, 7.71; N, 9.08; $C_{24}H_{35}N_3O_6$ (461.6) requires C, 62.45; H, 7.64; N, 9.10%]; δ_H (CD_3OD) 1.15 [3H, s, $C(CH_3)_2$], 1.21 [3H, s, $C(CH_3)_2$], 1.44 (10H, s, 4–H + Boc-CH_3), 1.65–1.84 (2H, m, 5-H), 2.25 (1H, m, 4-H), 3.40 (1H, m, NCH_2), 3.74 (3H, m, OMe), 3.90 (2H, m, NCH_2), 3.98 (1H, d, NCH_2, ^2J = 18.2 Hz), 4.39 (1H, s, 2-H), 5.13 (1H, m, 6-H), 7.2–7.4 (5H, m, ArH); δ_C (CD_3OD) 27.10, 27.78, 28.70, 30.23, 32.24, 32.84, 41.55, 45.02, 52.60, 57.71, 67.30, 79.74, 126.36, 127.71, 129.42, 143.94, 156.01, 170.74, 171.23, 173.70; *m*/*z* (CI-isobutane) 462 (100%) [MH^+].

(2*RS*,3*SR*,6*RS*)-1-Acetyl-3-methyl-3,6-diphenylpiperidine-2-carboxylic acid 3b

1.25 g (5 mmol) **1h**, 0.54 g (5 mmol) cyclohex-1-enyl isocyanide and 0.30 g (5 mmol) acetic acid were dissolved in 10 ml abs. MeOH and stirred for 12 h at room temperature. The solvent was removed *in vacuo* and the residue purified by column chromatography on silica gel (dichloromethane–methanol, 98 : 2 eluent) to give a bisamide (1.72 g, 83%) as a colourless solid, mp 203–204 °C. The solid was dissolved in a mixture of 19 ml THF and 1 ml conc. HCl and stirred for 12 h at room temperature. Solid Na_2CO_3 was added and the suspension was filtered. After removal of the solvent *in vacuo* the solid residue was dissolved in dichloromethane and extracted with water (pH 10). The aqueous phase was washed twice with dichloromethane and then acidified to pH 1 by adding 4 M HCl. Extraction with dichloromethane (3 × 100 ml), drying of the combined extracts over $MgSO_4$ and evaporation of the solvent *in vacuo* gave the carboxylic acid **3b** (1.10 g, 65% overall yield) as a colourless solid in diastereomerically pure form, mp 177–178 °C [Found C, 75.03; H, 6.89; N, 4.21; $C_{21}H_{23}NO_3$ (337.4)

requires C, 74.75; H, 6.87; N, 4.15%]; δ_H (DMSO-d_6) 1.52 (3H, s, CCH_3), 1.6–2.1 (4H, m, 4-H + 5-H), 1.86 (3H, s, $COCH_3$), 5.03 (1H, m, 6-H), 5.13 (1H, s, 2-H), 7.1–7.4 (10H, m, ArH); δ_C (DMSO-d_6) 23.10, 28.10, 34.68, 39.51, 54.84, 57.07, 60.89, 125.45, 125.66, 125.81, 126.56, 128.22, 128.63, 147.17, 170.89, 172.09; *m*/*z* (CI-isobutane) 338 (100%) [MH^+].

Crystal structure determination of tripeptide 2g †

Single crystals of **2g** were crystallized from CD_3OD, mounted in inert oil and transferred to the cold gas stream of the diffractometer.

Crystal data. $C_{24}H_{35}N_3O_6$, $M = 461.55$, monoclinic, $a = 22.179(2)$, $b = 10.9087(8)$, $c = 10.7255(9)$ Å, $U = 2519.5(4)$ Å, $T = 193(2)$ K, space group Cc, $Z = 4$, absorption coefficient = 0.088 mm^{-1}, 9154 reflections measured, 4539 unique ($R_{int} = 0.0557$) which were used in all calculations. The final $\omega R(F^2)$ was 0.0748 (all data).

Crystal structure determination of 3a †

Single crystals of **3a** were crystallized from dichloromethane, mounted in inert oil and transferred to the cold gas stream of the diffractometer.

Crystal data. $C_{15}H_{19}NO_3$, $M = 261.31$, monoclinic, $a = 7.1046(7)$, $b = 7.2949(5)$, $c = 13.3142(14)$ Å, $U = 689.07(11)$ Å, $T = 213(2)$ K, space group $P2_1$, $Z = 2$, absorption coefficient = 0.087 mm^{-1}, 5346 reflections measured, 2511 unique ($R_{int} = 0.0379$) which were used in all calculations. The final $\omega R(F^2)$ was 0.0837 (all data).

Crystal structure determination of 3b †

Single crystals of **3b** were crystallized from methanol, mounted in inert oil and transferred to the cold gas stream of the diffractometer.

Crystal data. $C_{21}H_{23}NO_3$, $M = 337.40$, monoclinic, $a = 11.1496(7)$, $b = 8.7609(6)$, $c = 18.8400(12)$ Å, $U = 1773.8(2)$ Å, $T = 193(2)$ K, space group $P2_1/c$, $Z = 4$, absorption coefficient = 0.084 mm^{-1}, 12820 reflections measured, 3465 unique ($R_{int} = 0.1085$) which were used in all calculations. The final $\omega R(F^2)$ was 0.0980 (all data).

† CCDC reference number 207/430. See http://www.rsc.org/suppdata/p1/b0/b002258f/ for crystallographic files in .cif format.

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