J. prakt. Chem. 339 (1997) 467–472

Journal für praktische Chemie
Chemiker-Zeitung

Synthesis of Tripeptide Fragments of 14-Membered Cyclopeptide Alkaloids

Marco Aurélio Mostardeiro, Eduardo Miranda Ethur and Ademir Farias Morel

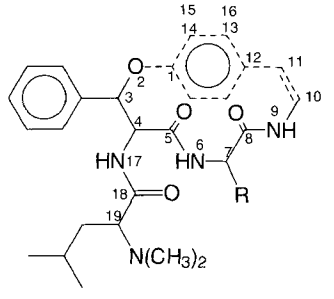
Santa Maria (RS, Brazil), Universidade Federal de Santa Maria, Departamento de Química

Ludger A. Wessjohann

München, Ludwig–Maximilians-Universität, Institut für Organische Chemie

Received March 20th, respectively October 16th, 1996

Cyclopeptide alkaloids of the discarine type (Scheme 1) are polyamidic bases encountered in many families of plants, mainly in Rhamnaceae species [1, 2]. The core of these molecules are 13-, 14- or 15-membered cyclic ethers. It is composed of a tyrosine-derived *p*-hydroxystyrylamine moiety, a common amino acid (Phe, Leu, Ile) and a *β*-hydroxy amino acid (usually 3-hydroxyproline, 3-hydroxyleucine or 3-phenylserine) which is connected to the styryl unit *via* ether bridge. Attached to the amino group of the latter component is a side chain, usually a peptidogenic amino acid with a dimethylated *N*-terminus.

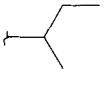


ALKALOIDS R

Discarine-C (**1**)

Discarine-D (**2**)

Myriantine-A (**3**)



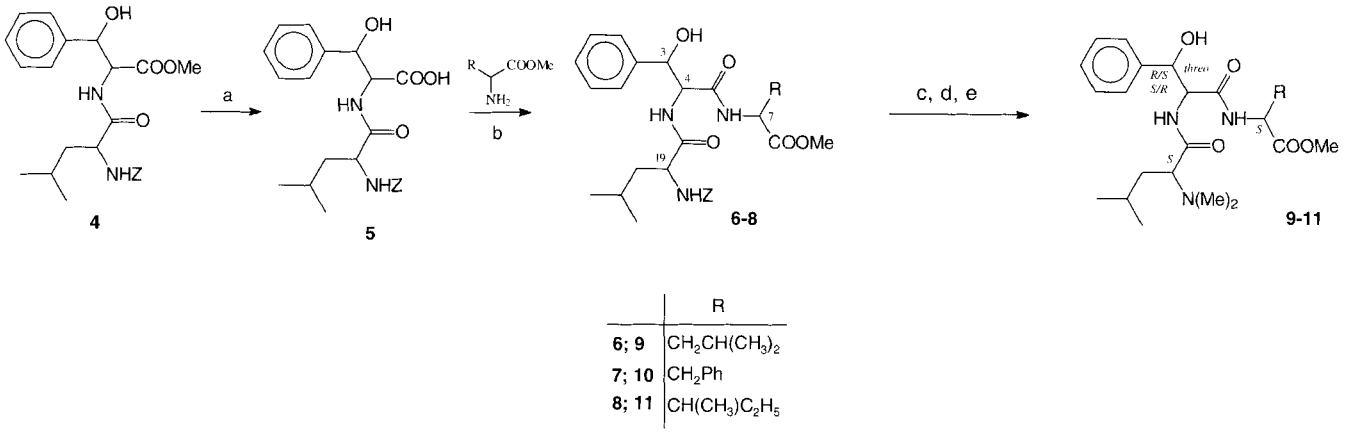
Scheme 1 Natural 14-membered cyclopeptide alkaloids

In recent years we were able to contribute considerably to the isolation and characterization of several classes of these compounds [3]. Now, we wish to report our synthetic studies of tripeptide-fragments of the title compounds, i.e. syntheses of discarine-C (**1**), discarine-D (**2**) and myriantine-A (**3**) with the styrylene bridge omitted. These initial studies eventually shall not only lead to total syntheses of those compounds but also should help to improve the interpretation of spectroscopic data of the various stereoisomers found in nature.

The general route towards the tripeptides is depicted in Scheme 2. The classical approach towards the syntheses was chosen because it will easily allow introduction of uncommon amino acids coupled with the ability to produce sufficient amounts for later studies in chemistry and biology. Thus the methyl esters of *(S)*-leucine, *(S)*-isoleucine and *(S)*-phenylalanine were utilized for coupling at the amino end.

The amino group of *(S)*-leucine was protected as benzyloxycarbonyl derivative (Cbz-Leu). Coupling with *(u)*-PheSer-OMe by the DCC-method gave 37% of the diastereomeric mixture of Cbz-*(S)*-Leu-(2*S*,3*R*)-PheSer-OMe and Cbz-*(S)*-Leu-(2*R*,3*S*)-PheSer-OMe (**4**). Basic hydrolysis of compounds **4** with 1N sodium hydroxide in methanol and acidification gave the corresponding acids **5** in 66% yield. The dipeptide mixture **5** then again could be coupled at the carboxyl terminus to *(S)*-Leu-OMe, *(S)*-Phe-OMe and *(S)*-Ile-OMe (natural enantiomer) to give the corresponding pairs of tripeptide methyl esters **6**–**8** in 70%, 76% and 75% yield, respectively.

Each of the three tripeptides, consisting of a 1:1 diastereomeric mixture regarding the stereochemistry at carbons 3/4 (numbering of discarines is applied, cf. schemes), could be separated by HPLC into the pure diastereomers. Thus both (3/4-*u*)-diastereomers of the tripeptide fragments of discarine-C (**1**), discarine-D (**2**) and myriantine-A (**3**) were available from **6**–**8**, respectively. However, the stereochemistry of these alkaloids on the basis of their 3-H, 4-H coupling constant (6 Hz) [4] of C-3 and C-4 seems to be *erythro* (≙3,4-*l*) unless ring constraint reduces the *threo* coupling constant through angle distortion in the 14-membered cycle. Thus, full clarification of the stereochemistry of the peptide alkaloids derived from the coupling constant will only be possible after their total synthesis. The stereochemical identity of each peptide was checked against synthetic samples obtained from enzymatic racemate resolution utilizing L-amino acid oxidase [5]. Acidic hydrolysis of **6**–**8** (6N HCl, 110 °C, 24 h) and



	R
6; 9	$CH_2CH(CH_3)_2$
7; 10	CH_2Ph
8; 11	$CH(CH_3)C_2H_5$

Scheme 2 a) 1N NaOH, MeOH; b) *N*-methylmorpholine, DCC, HOBt, THF; c) Chromatographic separation of LDL- and LLL-diastereomers, d) H_2, 10% Pd/C, CH_3OH, AcOH; e) CH_2O/H_2O, $NaBH_3CN$, CH_3OH.

derivatization with MeOH, HCl/TFAA gave the volatile amino acid derivatives which were analyzed by GC, GC/MS and chiral GC on a suitable cyclodextrine phase. Correlation of the latter data with the HPLC-retention times allowed the unequivocal assignment of each fractions stereochemistry, i.e. correlation with either (3*S*,4*R*,7*S*,19*S*)-tripeptides **6a–8a** or with (3*R*,4*S*,7*S*,19*S*)-tripeptides **6b–8b**.

Finally, removal of the benzyloxycarbonyl group was accomplished by catalytic hydrogenation in the presence of palladium on carbon. Reductive methylation of the resulting amines with formaldehyde/sodium cyanoborohydride afforded all six desired tripeptides as methyl esters **9a–11a** and **9b–11b** in 64–87% yield.

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPERGS (Fundação de Amparo a Pesquisa do Rio Grande do Sul) and the GTZ (Gesellschaft für technische Zusammenarbeit).

Experimental

Melting points were determined on a Kofler hot plate coupled to a Reichert microscope and are uncorrected. The onset decomposition temperature of some compounds depended on the heating characteristics (range given). Thin layer chromatography (TLC) was performed on precoated TLC plates (Merck, silica gel 60 F-254). The following solvent systems were used: chloroform/acetone (95:5), chloroform/methanol (95:5). The spots were detected using one or more of the following methods: UV (254nm), ninhydrin (0.1% in ethanol) and *o*-toluidine. The ^1H NMR and ^{13}C NMR spectra were registered on a Bruker AC80 operating at 80.13 and 20.15 MHz, respectively, and on a Varian VXR 400 at 400 MHz and 100.6 MHz, respectively. Chemical shifts are given in δ (ppm) using TMS as internal standard. Low resolution mass spectra were obtained on a GC/MS Finnigan Ion Trap Detector ITD 80A coupled to a Varian 3400 GC. FAB-MS spectra were obtained on a VG Analytical 70-150-S mass spectrometer equipped with a FAB ion source from 3-nitrobenzylalcohol matrix. HPLC/MPLC was carried out using a preparative liquid chromatograph (Shimadzu LC-8A) with a spherosorb CN 250×20 mm i.d. (LATEC Co.) column. Chiral GLC analyses were carried out on a Carlo Erba HRGC 5300 Mega Series, equipped with a flame ionization detector using 0.25 i.d. fused silica capillaries coated with 2,6-methyl-3-pentyl-β-cyclodextrine, diluted with the polysiloxane OV 1701. All amino acids used, except β-phenylserine (obtained according to ref. [6]) possess *S*-configuration (L-configuration) and are commercially available (Degussa/Aldrich/Sigma).

N-Benzyloxycarbonyl-(S)-leucyl-(u)-3-phenylserine methyl ester (Cbz-L-Leu-(u)-PheSer-OMe, **4**)

10.1 g (75 mmol) (*u*)-3-Phenylserine methyl ester [7] were coupled with 19.6 g (75 mmol) Cbz-L-leu-OH [8a] by the DCC-method according to ref. [8b] to give 12.3 g (37%) of the coupled product **4** as a yellow oil.

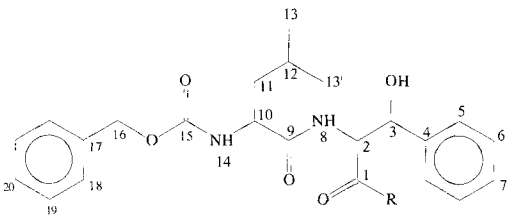
N-Benzyloxycarbonyl-(S)-leucyl-(u)-3-phenylserine (Cbz-L-Leu-(u)-PheSer-OH, **5**)

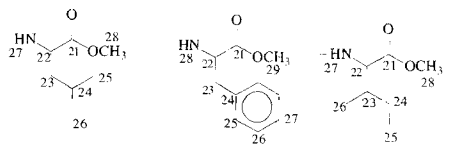
To 12.0 g (27.1 mmol) of the dipeptide Cbz-L-Leu-(*u*)-PheSer-OMe (**4**) in 60 ml methanol was added at room temp. 1N solution of sodium hydroxide according to the procedure described by Bodansky [8c]. After acidification the dipeptide acid **5** was obtained as a yellow oil (7.6 g) in 66% yield. – ^1H NMR (80 MHz, $CDCl_3$): δ 0.70 (d, 6H), 1.16 (m, 2H), 1.49 (m, 1H), 4.94 (m, 3H), 5.73 (bs, 3H), 7.19 (10H). – ^{13}C NMR: δ 21.39, 22.54, 24.38, 41.38, 53.41, 59.05, 66.72, 72.97, 73.13, 125.76, 127.76, 128.18, 136.03, 140.72, 156.50, (156.82), 173.50, 177.11, (177.52).

N-Benzyloxycarbonyl-(S)-leucyl-(u)-phenylseryl-(S)-leucine methyl ester (Cbz-L-Leu-(u)-PheSer-L-Leu-OMe, **6**)

0.72 g (5.5 mmol) (*S*)-Leu-OMe [7] were coupled with 2.35 g (5.3 mmol) of **5** by the DCC-method according to [8b] to give 2.05 g (70%) of **6** as an amorphous yellow solid. *M. p.* (dec.)

Table 1 Chemical shifts (δ) and coupling constants (Hz) of ^{1}H NMR spectra of *Z*-tripeptides **6–8** [a])



R = 

6 **7** **8**

Position	**6a**	**6b**	**7a** [b])	**7b** [b])	**8a**	**8b**
2	4.74 (d) *J* = 6.2	4.73 (m)	4.79 (m)	4.82 (m)	4.741(d) *J* = 7.64	4.72 (dd) *J* = 3.0, 7.8
3	5.54 (s)	5.34 (s)	5.38 (s)	5.21 (s)	5.49 (s)	5.33 (sa)
5–7	7.20–7.40 (m)	7.20–7.38 (m)	7.25 (sa)	7.20–7.28 (sa)	7.23–7.39 (m)	7.22–7.39 (m)
8	6.77 (d) *J* = 8.1	7.09 (d) *J* = 8.1	7.25 (sa)	7.28–7.46 (sa)	6.83 (d) *J* = 8.2	7.03 (d) *J* = 7.8
10	3.89 (m)	4.11 (m)	4.06 (m)	4.20 (m)	3.92 (m)	4.16 (m)
11	1.23, 1.33 (m)	1.63 (m)	1.27 (m)	1.45 (m)	1.26 (m)	1.33, 1.46 (m)
12	1.03 (m)	1.20 (m)	1.23 (m)	1.45 (m)	1.09 (m)	1.54 (m)
13, 13'	0.72 (d) *J* = 6.4	0.85 (m)	0.74 (sa)	0.83 (d) *J* = 4.96	0.72 (d) *J* = 6.6	0.84 (m)
14	5.13 (m)	5.07 (m)	–	5.57 (m)	5.19 (m)	5.12 (m)
16	4.97, 5.13 (dd)	5.07 (m)	4.95 (m)	5.04 (s)	5.11, 4.99 (dd)	5.10 (sa)
18–20	7.20–7.40 (m)	7.20–7.38 (m)	7.25 (sa)	7.20–7.28 (sa)		
22	4.58 (m)	4.54 (m)	4.19 (m)	4.73 (m)	4.51 (m)	4.50 (dd) *J* = 5.3, 8.2
23	1.71 (m)	1.51 (m)	3.03 (d) *J* = 6.13	3.02 (d) *J* = 5.98	1.87 (s)	1.83 (m)
24	1.71 (m)	1.29 (m)	–	–	1.36 (m)	1.15, 1.42 (m)
25	0.90 (d) *J* =5.1	0.85 (d) *J* =9.2	7.25 (s)	7.20–7.28 (sa)	0.90 (m)	
26	0.90 (d) *J* =5.1	0.85 (d) *J* =9.2	7.25 (s)	7.20–7.28 (sa)	0.90 (m)	
27	7.20–7.40 (m)	7.00 (d) *J* = 8.1	7.25 (s)	7.20–7.28 (sa)	7.23–7.39 (m)	7.20 (d) *J* = 8.2
28	3.71 (s)	3.74 (s)	7.25 (sa)	7.20–7.28 (sa)	3.69 (s)	3.73 (s)
29	–	–	3.58 (s)	3.60 (s)	–	–

[a]) Adapting a numbering in analogy to schemes 1 and 2, the following stereochemical assignments can be made: **a**: 7*S*,4*R*,19*S* (LDL); **b**: 7*S*,4*S*,19*S* (LLL) (Note: This numbering differs from the one used in the table!) [b]) 80 MHz-spectrometer

128–142 °C. – R_f ($CHCl_3$/MeOH 98:2) = 0.30. – IR (KBr): ν = 3300 cm^{-1} (NH, OH), 2954 (Ar-H), 1735 (OC=O), 1692, 1646 (NHC=O). – MS (FAB): *m/z* (%) = 556 (100) [M^+], 538 (39), 449 (12), 411 (30).

N-Benzyloxycarbonyl-(S)-leucyl-(u)-phenylseryl-(S)-phenylalanine methyl ester (Cbz-Leu-(u)-PheSer-L-Phe-OMe, **7**)

0.91 g (5.5 mmol) (*S*)-Phe-OMe [7] were coupled with 2.35 g (5.3 mmol) of **5** by the DCC method according to [8b] to give 2.34 g (75%) of **7** as an amorphous white solid. *M. p.* (dec.) 62–70 °C. – R_f ($CHCl_3$/MeOH 98:2) = 0.26. – IR (KBr): ν = 299 cm^{-1} (NH, OH), 2954 (Ar-H), 1730 (OC=O), 1682, 1645 (NHC=O). – MS (FAB): *m/z (%)* = 590 (100) [M^+], 572 (33), 483 (20), 411 (37).

N-Benzyloxycarbonyl-(S)-leucyl-(u)-phenylseryl-(S)-isoleucine methyl ester (Cbz-L-Leu-(u)-PheSer-L-Ile-OMe, **8**)

0.72 g (5.5 mmol) (*S*)-Ile-OMe [7] were coupled with 2.35 g (5.3 mmol) of **5** by the DCC method according to [8b] to give 2.26 g (77%) of **8** as an amorphous white solid. *M. p.* (dec.) 72–80 °C. – R_f ($CHCl_3$/MeOH 98:2) = 0.24. – IR (KBr): ν = 3290 cm^{-1} (NH, OH), 2960 (Ar-H), 1740 (OC=O), 1680, 1646 (NHC=O). – MS (FAB): *m/z* (%) = 556 (100) [M^+], 449 (15), 225 (20).

Separation of the respective (3*S*/4*R*)- and (3*R*/4*S*)-diastereomers of 6–8

The mixtures of the diastereomers of compounds **6–8**, the stereochemistry of which is *S* (*L*) at all α-stereocenters except for the phenylserine moiety, were separated by preparative MPLC on a spherosorb CN phase with dichloromethane/hexane (65:35). The two fractions resulting from each case were subjected to hydrolysis and chiral GC analyses (v. i.) in order to determine the respective absolute stereochemistry at C-4 (C-α of PheSer), the relative stereochemistry being unlike (*u*, *threo*) as determined by the starting material. The proton and carbon NMR data of the *Z*-tripeptides are compiled in tables 1 and 2, respectively.

Hydrolysis of the (3*S*,4*R*,7*S*,19*S*)- and (3*R*,4*S*,7*S*,19*S*)-tripeptides 6a–8a and 6b–8b

The hydrolyses of the individual diastereomers of the tripeptides **6–8** were performed in a sealed tube at 90–120 °C with 6N HCl for 12–24 h. The aqueous acidic solutions

Table 2 Chemical shifts (δ) and coupling constants (Hz) of ^{13}C NMR spectra of Z-tripeptides **6–8** [a,b])

Carbon	6a	6b	7a	7b	8a	8b
1	173.08	172.65	171.89	171.29	172.07	171.62
2	58.79	57.86	58.46	58.06	58.70	57.48
3	71.80	72.97	71.70	72.33	71.78	71.83
5–7	–	–	125.6, 127.0, 127.5,	126.1, 126.9, 127.6,	–	–
18–20			127.9, 128.1, 128.2,	127.8, 128.0, 128.3,		
25–27			128.4, 128.5, 129.1	129.1		
4	139.72	139.21	139.51	139.17	139.68	138.97
9	173.44	172.95	173.00	172.94	173.35	173.03
10	54.10	53.86	53.99	53.58	53.98	53.71
11	40.66	41.11	41.02	40.97	40.86	40.98
12	24.60	24.67	24.27	24.46	24.23	24.61
13, 13'	21.67, 22.57	21.82, 22.69	22.11, 22.50	21.63, 22.74	21.99, 22.46	21.81, 22.75
15	156.30	156.16	156.19	156.21	156.16	156.08
16	66.92	66.97	67.01	66.93	66.81	67.02
17	135.96	136.09	136.06	136.08	136.05	136.00
21	170.45	169.83	170.33	169.47	170.35	170.30
22	51.17	50.95	53.99	53.58	56.91	56.75
23	40.39	41.11	37.54	37.66	37.08	37.64
24	24.16	24.53	136.06	135.79	25.06	25.01
25	21.69	21.82	–	–	11.31	11.47
26	22.42	22.57	–	–	15.29	15.38
28	52.18	52.10	–	–	51.86	52.11
29	–	–	52.21	52.66	–	–

[a]) Adapting a numbering in analogy to schemes 1 and 2, the following stereochemical assignments can be made: **a**: 7*S*,4*R*,19*S* (LDL); **b**: 7*S*,4*S*,19*S* (LLL) (Note: This numbering differs from the one used in the table!)

[b]) For numbering see table 1

were concentrated and the residues derivatized as described below.

Preparation of a sample of known absolute configuration of (*u*)-PheSer with L-amino acid oxidase [9]

L-Amino acid oxidase (5 mg) from *C. atrox* (Sigma) was dissolved in 2 ml of 0.005 M Tris–HCl buffer of pH 7.2, and 5 mg of (*u*)-3-phenylserine was added and incubated under oxygen atmosphere (18 h; 37 °C). The solvent was removed and the residue derivatized as described below to give a sample of volatile D-*threo*-phenylserine (2*R*,3*S*-PheSer-OH) as a reference for chiral GC.

Derivatization of the hydrolysates (amino acids) [10]

Acid catalyzed esterifications were carried out by addition of a 1.5N anhydrous solution of HCl (gas) in methanol and leaving the mixture at room temp. for 30 min. After removal of the reagents in a stream of dry nitrogen, the samples were taken up in 200 µl of CH_2Cl_2 and 50 µl of trifluoroacetic anhydride. The mixture was kept for 30 min at room temp., then the reagent was removed in a stream of dry nitrogen.

GC-Analysis of (*u*)-3-phenylserine residues of the tripeptide hydrolysates

The absolute stereochemistry of the 3-phenylserine residues of the hydrolysates of both fractions of tripeptides **6–8** were unambiguously established by GC/MS and GLC-analyses employing modified cyclodextrins as chiral stationary phases (2,6-Me-3-Pe-β-CD) and utilizing the enzymatically enriched enantiomer as reference. It could be shown, that the diastereomer with LDL-configuration (α-C-atoms, i.e. the (3*S*,4*R*, 7*S*,19*S*)-diastereomer) is eluted prior to the *LLL*-diastereomer (i.e. the 3*R*,4*S*,7*S*,19*S*-diastereomer).

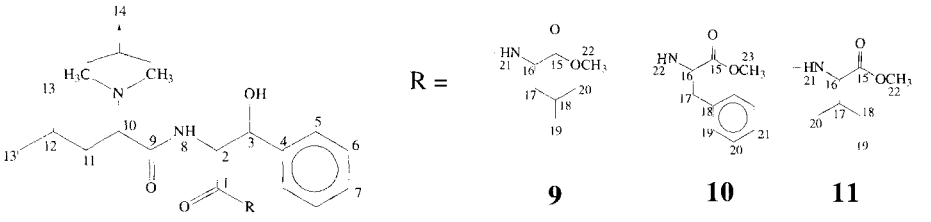
General procedure for the synthesis of *N*, *N*-dimethyltripeptides 9–11

The individual diastereomeric tripeptides **6a–8a** and **6b–8b** were *N*-deprotected in absolute methanol with 1 mmol glacial acetic acid by hydrogenation under normal pressure on 10% palladium/carbon according to [8d]. The catalyst was removed by filtration through celite and the filtrate concentrated *in vacuo*. The resulting crude product was dissolved in 5 ml of methanol and 0,1 ml of 37% aqueous formaldeyde solution (1 mmol) was added. The reaction mixture was stirred and then cooled to 0 °C followed by addition of 0.18 mg (3 mmol) sodium cyanoborohydride. The reaction mixture then was warmed to room temp. and stirring continued for 3–6 h. The solvent was removed *in vacuo* and the residue redissolved in ethyl acetate and washed first with saturated aqueous $NaHCO_3$ solution and then with brine. The organic layer was dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatographyon silica gel 60 (Merck 230–400 mesh) using chloroform as solvent, afforded the pure *N,N*-dimethyl tripeptides **9–11**. The proton and carbon NMR data of the target tripeptides are compiled in tables 3 and 4, respectively.

N,N-Dimethyl-(S)-leucyl-(S,R)-phenylseryl-(S)-leucine methyl ester [$(CH_3)_2$-L-Leu-D-PheSer-L-Leu-OMe] (**9a**)

0.31 g (0.56 mmol) of **6a**, according to the general procedure for reductive *N,N*-dimethylation given above, yielded 0.16 g (66%) of **9a** as a yellow oil which decomposes upon heating. – R_f ($CHCl_3$/MeOH 98:2) = 0.32. – IR (KBr): ν = 3321 cm^{-1}

Table 3 Chemical shifts (δ) and coupling constants (Hz) of ^{1}H NMR spectra of *N,N*-dimethyl tripeptides **9**–**11**[a])



9 **10** **11**

Position	**9a**	**9b**	**10a** [b])	**10b**	**11a**	**11b**
2	4.67 (m)	4.65 (m)	4.67 (m)	4.66 (m)	4.74 (m)	4.63 (dd)
						J = 2.06, 7.6 3
5.39 (d)	5.38 (d)	5.35 (d)	5.34 (s)	5.39 (d)	5.42 (d)	
	J = 2.67	J = 2.36	J = 2.33		J = 2.6	J = 2.06
5–7	7.14–7.40 (m)	7.27–7.39 (m)	7.17–7.31 (m)	7.28 (s)	7.20–7.34 (m)	7.22–7.39 (m)
8	7.57 (d)	7.20–7.63 (m)	7.07–7.65 (m)	7.28 (s)	7.58 (d)	7.71 (d)
	J = 7.7				J = 7.0	J = 7.6
10	2.75 (m)	2.79 (m)	2.69 (m)	2.74 (m)	2.76 (m)	2.79 (m)
11	1.39 (m)	1.25 (m)	1.31 (m)	1.27 (m)	1.27 (m)	1.23 (m)
12	1.31 (m)	1.25 (m)	1.31 (m)	1.27 (m)	1.37 (m)	1.39 (m)
13, 13'	0.80 (m)	0.80 (m)	0.77 (d)	0.80 (s)	0.81 (m)	0.80, 0.81 (d)
			J = 4.92			J = 6.6
14	2.09 (s)	2.03 (s)	2.02 (s)	1.98 (s)	2.10 (s)	2.03 (s)
16	4.44 (m)	4.57 (m)	4.73 (m)	4.84 (m)	4.49 (m)	4.52 (dd)
						J = 5.15, 8.4
17	1.60 (m)	1.62 (m)	3.04 (m)	3.08 (m)	1.86 (m)	1.89 (m)
18	1.58 (m)	1.62 (m)	–	–	1.42 (m)	1.39, 1.19 (m)
19	0.91 (m)	0.86 (m)	7.17–7.31 (m)	7.28 (m)	0.91 (m)	0.89 (m)
20	0.91 (m)	0.86 (m)	7.17–7.31 (m)	7.28 (m)	0.91 (m)	0.89 (m)
21	7.14–7.40 (m)	7.20–7.63 (m)	7.17–7.31 (m)	7.28 (m)	7.20–7.34 (m)	7.47 (d)
						J = 8.3
22	3.69 (m)	3.73 (s)	7.28 (m)	–	3.68	3.76 (s)
23	–	–	3.64 (s)	3.64 s)	–	–

[a]) Adapting a numbering in analogy to schemes 1 and 2, the following stereochemical assignments can be made: **a**: 7*S*,4*R*,19*S* (LDL); **b**: 7*S*,4*S*,19*S* (LLL) (Note: This numbering differs from the one used in the table!)

[b]) 80 MHz-spectrometer

(NH, OH), 2962 (Ar-H), 2876 (N-CH_3), 1730 (OC=O), 1680 (NHC=O). – MS (FAB): *m/z* (%) = 450 (40) [M^+], 114 (100).

$C_{24}H_{39}N_3O_5$: calcd.: C 64.12 H 8.74 N 9.35,
(449.59) found: C 64.09 H 8.70 N 9.33.

N,N-Dimethyl-(S)-leucyl-(R,S)-phenylseryl-(S)-leucine methyl ester [$(CH_3)_2$-L-Leu-L-PheSer-L-Leu-OMe] **(9b)**

0.23 g (0.41 mmol) of **6b**, according to the general procedure for reductive *N,N*-dimethylation given above, yielded 0.16 g (79%) of **9b** as a yellow oil which decomposes upon heating. – R_f ($CHCl_3$/MeOH 98:2) = 0.31. – IR (KBr): ν = 3330 cm^{-1} (NH, OH), 2960 (Ar-H), 2880 (N-CH_3), 1740 (OC=O), 1690 (NHC=O). – MS (FAB): *m/z* (%) = 450 (48) [M^+], 114 (100).

$C_{24}H_{39}N_3O_5$: calcd.: C 64.12 H 8.74 N 9.35,
(449.59) found: C 64.10 H 8.72 N 9.32.

N,N-Dimethyl-(S)-leucyl-(S,R)-phenylseryl-(S)-phenylalanine methyl ester [$(CH_3)_2$-L-Leu-D-PheSer-L-Phe-OMe] **(10a)**

0.23 g (0.38 mmol) of **7a**, according to the general procedure for reductive *N,N*-dimethylation given above, yielded 0.19 g (78%) of **10a** as an amorphous white solid. – *M. p.* (dec.) 95–105 °C. – R_f ($CHCl_3$/MeOH 98:2) = 0.14. – IR (KBr): ν = 3280 cm^{-1} (NH, OH), 2955 (Ar-H), 2840 (N-CH_3), 1748 (OC=O), 1680 (NHC=O). – MS (FAB): *m/z* (%) = 484 (38) [M^+], 114 (100).

$C_{27}H_{37}N_3O_5$: calcd.: C 67.06 H 7.71 N 8.69,
(483.61) found: C 67.03 H 7.68 N 8.67.

N,N-Dimethyl-(S)-leucyl-(R,S)-phenylseryl-(S)-phenylalanine methyl ester [$(CH_3)_2$-L-Leu-L-PheSer-L-Phe-OMe] **(10b)**

0.25 g (0.38 mmol) of **7b**, according to the general procedure for reductive *N,N*-dimethylation given above, yielded 0.18 g (70%) of **10b** as an amorphous white solid. – *M. p.* (dec.) 91–98 °C. – R_f ($CHCl_3$/MeOH 98:2) = 0.13. – IR (KBr): ν = 3310 cm^{-1} (NH, OH), 2970 (Ar-H), 2845 (N-CH_3), 1740 (OC=O), 1675 (NHC=O). – MS (FAB): *m/z* (%) = 484 (38) [M^+], 114 (100).

$C_{27}H_{37}N_3O_5$: calcd.: C 67.06 H 7.71 N 8.69,
(483.61) found: C 67.04 H 7.70 N 8.66.

N,N-Dimethyl-(S)-leucyl-(S,R)-phenylseryl-(S)-isoleucine methyl ester [$(CH_3)_2$-L-Leu-D-PheSer-L-Ile-OMe] **(11a)**

0.29 g (0.41 mmol) of **8a**, according to the general procedure for reductive *N,N*-dimethylation given above, yielded 0.16 g (69%) of **11a** as a yellow oil which decomposes upon heating. – R_f ($CHCl_3$/MeOH 98:2) = 0.13. – IR (KBr): ν = 3325 cm^{-1} (NH, OH), 2970 (Ar-H), 2870 (N-CH_3), 1740 (OC=O), 1675

Table 4 Chemical shifts (δ) of ^{13}C NMR spectra of *N,N*-dimethyl tripeptides **9**–**11**[a,b])

Carbon	9a	9b	10a	10b	11a	11b
1	174.74	174.89	171.30	171.36	174.55	174.48
2	57.95	56.91	57.74	57.55	57.95	57.17
3	71.72	71.38	71.53	71.65	71.50	71.41
4	139.61	139.26	139.69	139.40	139.72	139.78
5–7	125.68, 127.39, 128.13	125.62, 127.47, 128.20	125.60, 127.34, 128.46 (*)	125.62, 127.52, 128.19 (*)	125.55, 127.24, 127.98	125.55, 127.21, 127.92
9	172.85	172.64	174.55	174.55	171.60	171.47
10	67.26	67.42	67.04	67.22	67.10	67.36
11	37.10	36.68	36.96	36.59	37.12	36.73
12	24.63	24.74	25.33	25.50	24.95	25.33
13, 13'	21.70, 22.61	21.70, 22.65	21.92, 22.96	21.79, 23.10	21.80, 22.97	21.55, 23.05
14	41.98	41.95	41.67	41.89	41.84	41.83
15	171.00	171.49	170.80	170.86	170.77	171.00
16	50.88	50.91	53.50	53.48	56.60	56.56
17	40.97	40.99	37.67	37.65	37.28	37.27
18	25.43	25.59	135.61	135.67	25.26	24.80
19*	23.10	23.21	–	–	11.28	11.19
20*	21.89	21.70	–	–	15.24	15.19
19–21*	–	–	126.95, 128.80, 129.11	126.96, 128.40, 129.04	–	–
22	52.08	52.19	–	–	51.71	51.74
23	–	–	52.03	52.27		

[a]) Adapting a numbering in analogy to scheme 1, the following stereochemical assignments can be made: **a**: 7*S*,4*R*,19*S* (LDL); **b**: 7*S*,4*S*,19*S* (LLL) (Note: This numbering differs from the one used in the table!) [b])For numbering see table 3

(NHC=O). – MS (FAB): *m/z* (%) = 450 (40) [M$^+$], 114 (100).
$C_{24}H_{39}N_3O_5$: calcd.: C 64.12 H 8.74 N 9.35,
(449.59) found: C 64.08 H 8.73 N 9.31.

N,N-Dimethyl-(S)-leucyl-(R,S)-phenylseryl-(S)-isoleucine methyl ester [$(CH_3)_2$-L-Leu-L-PheSer-L-Ile-OMe] (**11b**)

0.46 g (0.84 mmol) of **8b**, according to the general procedure for reductive *N,N*-dimethylation given above, yielded 0.29 g (66%) of **11b** as a yellow oil which decomposes upon heating. – R_f ($CHCl_3$/MeOH 98:2) = 0.12. – IR (KBr): ν = 3330 cm^{-1} (NH, OH), 2972 (Ar-H), 2869 (N–CH_3), 1738 (OC=O), 1676 (NHC=O). – MS (FAB): *m/z* (%) = 450 (52) [M$^+$], 114 (100).
$C_{24}H_{39}N_3O_5$: calcd.: C 64.12 H 8.74 N 9.35,
(449.59) found: C 64.10 H 8.71 N 9.30.

References

[1] M. M. Joullié, R. F. Nutt: in Alkaloids: Chemical and Biological Perspectives, (Pelletier, S. W., ed.) Vol. 3, Wiley, New York 1985, p. 113

[2] A. F. Morel (1985) PhD dissertation. University of Tübingen, Tübingen (Germany)

[3] a) M. Digel, A. F. Morel, J. Biermann, W. Voelter, Hoppe–Seyler's Z. Physiol. Chem. **364** (1983) 1641; b) W. Voelter, A. F. Morel, R. Herzog, J. Biermann, Z. Naturforsch. **39b** (1984) 1825; c) W. Voelter, A. F. Morel, R. Herzog, J. Biermann, Hoppe–Seyler's Z. Physiol. Chem. **108** (1984) 406; d) W. Voelter, A. F. Morel, R. Herzog, Chimia **4** (1985) 98; e) W. Voelter, A. F. Morel, Z. Naturforsch. **42b** (1987) 467; f) A. F. Morel, E. C. Machado, L. A. Wessjohann, Phytochemistry **39** (1995) 431; g) A. F. Morel, A. S. Meneses, M. A. Mostardeiro, N. Zanatta, Phytochemistry **38** (1995) 783; h) A. F. Morel, E. C. Machado, A. A. Filho, F. D. Monache, J. Nat. Prod. **58** (1995) 548

[4] J. Marchand, M. Pais, F.–X. Jarreau, Bull. Soc. Chim. Fr. **10** (1971) 3712

[5] R. Tschesche, R. Ammermann, Chem. Ber. **107** (1974) 2274

[6] K. N. F. Shaw, S. W. Fox, J. Am. Chem. Soc. **75** (1953) 3417

[7] M. Bremmer, W. Huber, Helv. Chim. Acta **36** (1953) 1109

[8] a) M. Bodanszky, A. Bodanszky, in: The Practice of Peptide Synthesis, Springer, New York 1984, p. 12; b) *ibid.*, p. 145; c) *ibid.*, p. 177; d) *ibid.*, p. 153

[9] R. Tschesche; D. Hillebrand, I. R. C. Bick, Phytochemistry **19** (1980) 1000

[10] E. Bayer, W. A. König, J. Chromatogr. Sci. **7** (1969) 95

Address for correspondence:
Prof. Dr. Ademir Farias Morel
Universidade Federal de Santa Maria - UFSM
Departamento de Química
Campus - Camobi
BR-97119-900 - Santa Maria, RS - Brazil