Studies on the Synthesis of Cyanomethyleneamino Pseudopeptides

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Various cyanomethyleneamino pseudodipeptides were easily prepared in high yield by the Lewis acid catalyzed addition of trimethylsilyl cyanide to unstable aldimine intermediates, obtained from the reaction of *N*-protected α-amino aldehydes with *C*-protected amino acids. The two possible (*R*)- and (*S*)-epimers at the peptide bond surrogate chiral center were obtained. In this thermodynamically controlled synthesis, the absolute configurations of the α-amino aldehyde and the amino acid proved to be the main factors determining its stereoselectivity. The new *N*-Boc- and *N*-*Z*-protected pseudodipeptides were deblocked under standard conditions. In spite of the lability of the new peptide bond surrogate ψ[CH(CN)NH] in basic medium, high yields of *C*-deprotected pseudodipeptides were obtained by controlling the saponification conditions of the methyl esters. The use of free α-amino acids in the modified Strecker synthesis reported here can be employed as an alternative for the synthesis of *C*-deprotected cyanomethyleneamino pseudopeptides. The *N*- or *C*-elongation of these pseudodipeptides *via* the DCC method led to the corresponding ψ[CH(CN)NH] pseudotripeptides in high yield.

Introduction

Isosteric peptide bond replacements in biologically active peptides have been widely used to increase stability toward proteolytic enzymes, to achieve receptor selectivity, and/or to obtain peptide antagonists.[1] Some of these amide bond surrogates have also been incorporated into peptidase inhibitors to mimic the enzyme-bound tetrahedral transition state of the scissile amide bond.[2] Among these backbone modifications, one of the simplest is the reduced peptide bond ψ[CH_2NH],[3] which has been successfully used in the design of metabolically stable agonists[4]/antagonists[5] of natural peptides or enzyme inhibitors.[6] However, this surrogate causes an increase in flexibility of the peptide backbone and a decrease in the *H*-bonding properties by loss of the *H*-bonding acceptor amide carbonyl group.[1a,7]

In a preliminary paper,[8] we have reported that semiempirical quantum mechanic calculations for the cyanomethyleneamino [CH(CN)NH] and the methyleneamino [CH_2NH] groups indicate that the first one could be a better mimic of the amide peptide bond and of the tetrahedral transition state involved in the peptide hydrolysis than the second one. In this paper, an easy method for the preparation of ψ[CH(CN)NH] pseudopeptides by the Lewis acid catalyzed reaction of *N*-protected α-amino aldehydes with *C*-protected amino acids or dipeptides and trimethylsilyl cyanide (TMSCN) was also reported. With the aim of exploring the scope and limitations of this method, we have studied and describe herein some mechanistic implications of this synthesis, as well as the influence of reaction conditions and substrates on its stereochemical outcome. Studies on the incorporation of this new backbone modification into larger peptides are also described.

Results and Discussion

Asymmetric Strecker syntheses have been widely used for the stereoselective preparation of α-amino acids, *via* hydrolysis of the corresponding α-amino nitriles, obtained by hydrocyanation of chiral aldimines.[9-11] In our case, initial attempts to obtain aldimines **3** (Scheme I), from the amino aldehydes **1** and the α-amino acid methyl esters **2**, following usual methods for the preparation of imines, were unsuccessful, recovering the starting materials. Therefore, the α-(substituted alkyl)amino nitriles **5** were obtained by a modified Strecker synthesis,[12] which involved a $ZnCl_2$-catalyzed reaction of *N*-protected α-amino aldehyde **1** with the amino acid methyl ester **2** and TMSCN in MeOH. However, in this synthesis amino nitriles **5** were obtained in low yields (30–50%) along with 40–60% of the corresponding *O*-(trimethylsilyl)cyanohydrins **4**. This high proportion of cyanohydrins **4**, and the fact that the formation of aldimines **3** as reaction intermediates[9-11] was not observed either by TLC or by HPLC, suggested that in this reaction the cyanohydrins **4** were intermediates, in equilibrium with amino nitriles **5**, as it has been proposed in other modified Strecker syntheses.[12,13] Later, this

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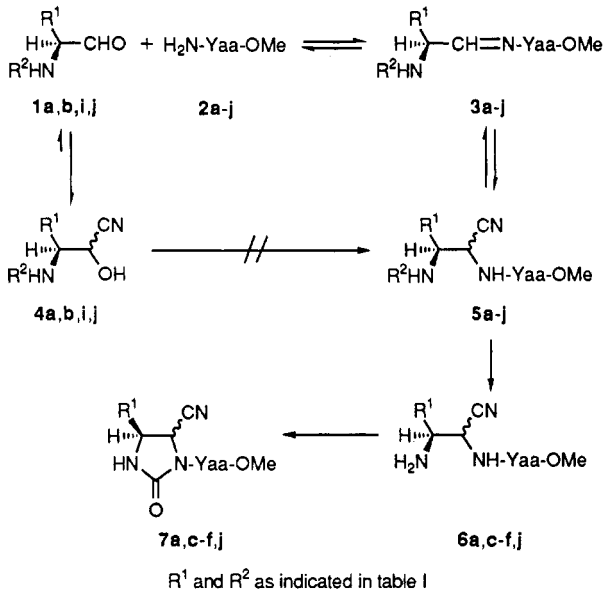
Table I. Protected R²Xaa Ψ[CH(CN)NH]Yaa-OMe Pseudopeptides 5 Synthesized

compd	R²Xaa [(S)-R¹CH(NHR²)]	Yaa	yield[a] (%)	Ψ[CH(CN)NH] (R)-5:(S)-5 ratio[b]	t_R (R)/(S) (A:B)[c]
5a	Boc-L-Phe	L-Leu	95	1:1	16.45/15.10 (40:60)
5b	Z-L-Phe	L-Leu	90	1:1	17.60/16.35 (40:60)
5c	Boc-L-Phe	D-Leu	92	1:3[d]	15.66 (40:60)
5d	Boc-L-Phe	L-Val	80	2:1	20.48/19.43 (20:80)
5e	Boc-L-Phe	L-Phe	82	1:1	14.81/13.51 (40:60)
5f	Boc-L-Phe	D-Phe	85	1:4[e]	14.51 (40:60)
5g	Boc-L-Phe	L-Lys(Z)	76	1:1	11.65/11.36 (20:80)
5h	Boc-L-Phe	L-Glu(OMe)	88	1:1	14.51/14.11 (50:50)
5i	Z-L-Leu	L-Leu	85	1:1	11.31/10.50 (40:60)
5j	Z-L-Trp	L-Phe	75	1:1.5	16.33/15.66 (25:75)

[a] Overall yield of (R)- and (S)-epimers. [b] Determined by reversed-phase HPLC analysis, except for **5c** and **5f**. [c] A = 0.05% TFA in H_2O; B = CH_3CN. [d] Determined by measuring the respective OMe, 4-H, and 4-H′ integrals for (R)-**5c** and (S)-**5c** in the ^1H NMR spectrum of the mixture. [e] Determined by measuring the OMe and α-H integrals for (R)-**5f** and (S)-**5f** in the ^1H NMR spectrum of the mixture.

Scheme I. Synthesis of R²-XaaΨ[CH(CN)NH] Yaa-OMe Pseudodipeptides



suggestion was ruled out by the fact that the *O*-(trimethylsilyl)cyanohydrins **4a**, obtained by reaction of *N*-Boc-L-phenylalaninal (**1a**) with TMSCN,[14] did not lead to the α-amino nitriles **5a** upon reaction with the methyl ester of L-leucine in the above reaction conditions. This result indicated that aldimines **3** should be the reaction intermediates, although they were not stable enough to be detected by TLC or HPLC. These aldimines **3** are in equilibrium with amino aldehydes **1**, whose reaction with TMSCN to give cyanohydrins **4** is much faster. The formation of aldimines **3** is supported by the NMR spectra of a (1:1) mixture of *N*-Boc-L-phenylalaninal **1a** and Leu-OMe (**2a**). Thus, the ^1H NMR spectrum of this mixture in $CDCl_3$ showed the disappearance of a singlet at $\delta = 9.67$ ppm, corresponding to the aldehyde proton, and the appearance of a doublet ($J = 2.5$ Hz) at $\delta = 7.82$ ppm, attributed to the imine proton. The ^{13}C NMR spectrum also showed the presence of the imine carbon at $\delta = 166$ ppm. We deduced from these results that if the addition of TMSCN was performed after the formation of aldimines **3** an increase in the yield of the desired cyanomethyleneamino pseudodipeptide **5** should be obtained. In fact, as shown in Table I, compounds **5** were obtained in high yield when the α-amino aldehydes **1** and the amino acid methyl esters **2** were left to react for 1 h and then TMSCN was added.

The protected pseudodipeptides **5** were obtained in each case as an epimeric pair at the new CH(CN)NH chiral center, in the *R*:*S* ratio indicated in Table I. Only epimers (*R*)-**5e** (42%) and (*S*)-**5e** (40%) and (*R*)-**5j** (30%) and (*S*)-**5j** (45%) could be separated from the corresponding epimeric mixtures. Epimerization at the chiral center of the α-aldehyde was not observed either by ^1H NMR or by HPLC analysis when the formation of the peptide bond surrogate was carried out at 0 °C. However, up to 5% of epimerization was observed at room temperature.

Since the IR spectra of the cyanomethyleneamino pseudodipeptides **5** did not show the characteristic C≡N band at ~2250 cm^{-1}, the presence of the CH(CN) group was evidenced from their ^{13}C NMR spectra, which showed the resonances of the CN and CH carbons at δ 116–119 and δ 52–59 ppm, respectively.

The assignment of the absolute configuration at the new chiral center (C-2 in Xaa) in the separated epimers (*R*)- and (*S*)-**5e** and (*R*)- and (*S*)-**5j** was established by the $J_{4,5}$ value in the ^1H NMR spectra of the corresponding imidazolidin-2-ones (*R*)- and (*S*)-**7e** and (*R*)-**7j** (Scheme I), obtained from the respective *N*-deprotected pseudodipeptides (*R*)- and (*S*)-**6e** and (*R*)-**6j**, after reaction with bis(trichloromethyl) carbonate. The *threo* imidazolidin-2-ones (*R*)-**7e** and (*R*)-**7j** showed a $J_{4,5}$ value of 3–4 Hz consistent with a H_4,H_5 *trans* disposition, while in the *erythro* isomer (*S*)-**7e** the value of this coupling constant was 8 Hz.

Methanolic HCl treatment of the *N*-Boc-protected pseudodipeptides **5a** and **5c-f** yielded the corresponding *N*-deprotected analogues **6a** and **6c-f** as dihydrochlorides. The *N*-*Z*-deprotected tryptophan derivatives **6j** were obtained by catalytic hydrogenation of the protected pseudodipeptides **5j**. These *N*-deprotected tryptophan derivatives **6j** were unstable and could not be isolated as pure compounds. Therefore, the imidazolidin-2-one **7j** was obtained from the crude compound **6j** after the hydrogenation of **5j**.

In compounds **5c**, **5d**, and **5f**, where (*R*)- and (*S*)-epimers could not be separated, the assignment of configuration was made by conversion into the corresponding mixture of (5*R*)- and (5*S*)-imidazolidin-2-ones and relating the major and minor imidazolidin-2-ones **7** with the respective major and minor amino nitriles **5**.

In the ^1H NMR spectra of *N*-protected and -deprotected pseudodipeptides **5** and **6** (**d**, **e**, and **j**) the α-protons of Xaa and Yaa residues appeared ≈ 0.1–0.3 and 0.2–0.3 ppm, respectively, at lower field in the (*S*)-isomer than in the

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corresponding (*R*)-epimer. This criterium was used for the tentative assignment of compounds **5a**, **5b**, and **5g-i**, obtained as 1:1 mixtures of epimers which could not be separated.

With the aim of improving the stereoselectivity in the synthesis of α-(substituted alkyl)amino nitriles **5**, the factors which could contribute to control the stereochemistry of this synthesis were studied. Firstly, concerning the influence of solvent, Kunz *et al.* have found an interesting solvent effect of reversing the stereoselectivity of the TMSCN addition to Shiff bases in asymmetric Strecker syntheses using 1-amino-2,3,4,6-tetra-*O*-pivaloyl-β-D-galactose as chiral matrix.[15] When the addition was carried out in $CHCl_3$ solution, a preponderance of the corresponding (*S*)-amino nitrile resulted, while the TMSCN addition to the same substrates in either THF or 2-propanol exhibited selectivity for the corresponding (*R*)-isomers. In our case, a similar solvent effect upon the synthesis of amino nitriles **5** was not observed when the reaction solvent was changed from MeOH to $CHCl_3$ or THF.

On the other hand, the induction of asymmetry under thermodynamic control conditions in the synthesis of cyanohydrins obtained by addition of TMSCN to α-amino aldehydes,[14] as well as in the synthesis of some α-(substituted alkyl)amino nitriles,[11] has been reported. On the basis of these reports, the possibility of improving the stereoselectivity in the preparation of amino nitriles **5** acting upon the time and the temperature of reaction was studied for compounds **5a**, **5e**, and **5j**. In the case of **5a** and **5e**, no stereoselectivity was observed either under kinetic control conditions (30 min at 0 °C, 8% yield) or under thermodynamic control conditions (3 days at rt, 95% yield). However in the case of amino nitriles **5j**, after 30 min of reaction at 0 °C, the *R*:*S* epimeric ratio was 1:1, with a yield lower than 10%, while, after 1 h of reaction at 65 °C, 8% ee in the (*R*)-**5j** epimer was observed, which increased up to 50% when complete equilibration was attained within 24 h at this temperature. In compounds **5j**, the configurational stability of both separated epimers (*R*)-**5j** and (*S*)-**5j** was also studied. Both diastereoisomers were stable in MeOH solution at rt for, at least, 2 months. However, under the above reaction conditions both stereoisomers separately dissolved in MeOH, and in the presence of TMSCN and $ZnCl_2$, epimerized at 0 °C, attaining complete equilibration after 2 months, with a *R*:*S* ratio of 3:1. In this case, the formation of ~10% of cyanohydrins **4j** was also observed.

Concerning the infuence of the Xaa and Yaa residues on the stereochemistry of compounds **5**, although this study is not complete to draw conclusions, from the data indicated in Table I, it seems that the relative configuration of Xaa and Yaa residues is the main factor determining the diastereoisomeric ratio.

Studies on *N*- and *C*-chain extension of cyanomethyleneamino pseudopeptides were carried out with the 1:1 epimeric mixture of pseudodipeptides **5a**. In the first case, coupling of the 1:1 mixture of *N*-deprotected pseudodipeptides **6a** with *N*-Boc-L-phenylalanine (Scheme II), *via* the DCC method[16] in the presence of 1-hydroxybenzotriazole (1-HOBt) gave the 1:1 diastereoisomeric mixture of pseudotripeptides Boc-Phe-Pheψ[(*R*)CH(CN)NH]Leu-OMe [(*R*)-**8a**] and Boc-Phe-Pheψ[(*S*)CH(CN)NH]Leu-OMe [(*S*)-**8a**] in 86% overall yield, which were separated by preparative column chromatography. No acylation of the secondary NH of the ψ[CH(CN)NH] was observed in this reaction.

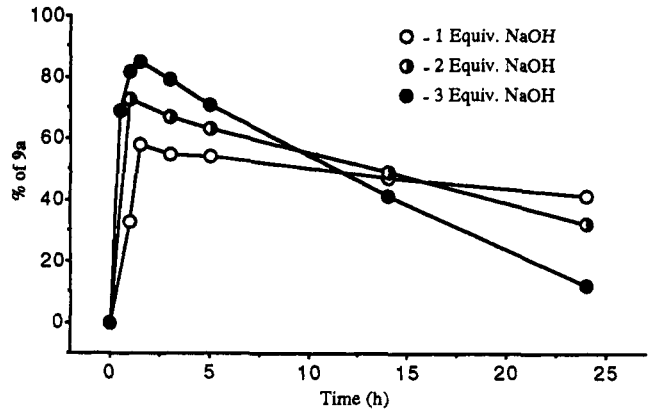
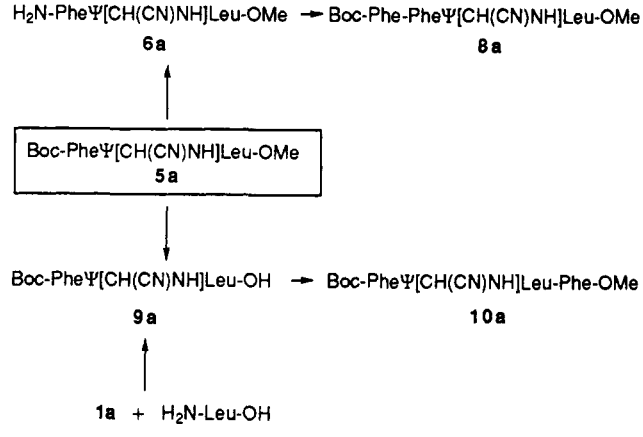


Figure 1. Synthesis of **9a** by Saponification of **5a**.

Scheme II. Chain Extension of ψ[CH(CN)NH] Pseudodipeptides



Initial attempts to saponify the methyl ester of **5a**, as in the previous step for the *C*-terminal elongation, were made using an equivalent amount of 1 N NaOH in 1:1 dioxane–water at rt for 24 h. Although the starting material disappeared under these conditions, the yield of *C*-deprotected pseudodipeptides **9a** was lower than 45%, due to the scission of the [CH(CN)NH] peptide bond surrogate to provide **9a** along with Boc-phenylalaninal and leucine. In order to minimize this scission, the saponification was studied by HPLC using different amounts of NaOH and reaction times. From this study, it appeared that the ψ[CH(CN)NH] bond cleavage was time dependent, while the saponification was NaOH concentration dependent. As shown in Figure 1, the best results were obtained by using 3 equiv of NaOH for 90 min to give the 1:1 epimeric mixture of **9a** in 83% yield.

The direct prepation of *C*-deprotected ψ[CH(CN)NH] pseudodipeptides was also explored as a way of circumventing the lability of this peptide bond surrogate to the basic saponification medium. Thus, as indicated in Scheme II, reaction of *N*-Boc-L-phenylalaninal (**1a**) with L-leucine and TMSCN, under the conditions above indicated for the formation of the [CH(CN)NH] bond, yielded 75% of a 1:1 mixture of the (*R*)- and (*S*)-epimers **9a**, which could not be separated. Finally, coupling of **9a**

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Table II. Analytical and Significant Spectroscopic Data for Protected Pseudodipeptides R^2XaaΨ[CH(CN)NH]Yaa-OMe 5

					^{1}H NMR ($CDCl_3$, δ, ppm)						
			^{13}C NMR		Xaa				Yaa		
compd	formula[a]	λ_{max} (MeOH) (nm (ε))	C-2 (Xaa)	C≡N	2-H	3-H	4-H	4-H′	NH	α-H	OCH_3
(*R*)-**5a**					3.73	4.10	2.83	3.10	1.90	3.30	3.66
+	$C_{22}H_{33}N_3O_4$	213 (6411)	52.95; 53.41	117.99; 118.46							
(*S*)-**5a**					3.77	4.23	2.96		2.00	3.50	3.79
(*R*)-**5b**					3.70	4.15	2.83	3.10	1.55	3.26	3.63
+	$C_{25}H_{31}N_3O_4$	215 (7676)	*b*	*b*							
(*S*)-**5b**					3.75	4.26	2.96		1.55	3.48	3.76
(*R*)-**5c**					3.75	4.13	2.85	3.12	2.15	3.55	3.72
+	$C_{22}H_{31}N_3O_4$	213 (6673)	52.46; 53.02	117.84; 118.23							
(*S*)-**5c**					3.59	4.13	2.93		2.15	3.28	3.75
(*R*)-**5d**					3.63	4.11	2.83	3.10	1.66	3.04	3.66
+	$C_{21}H_{31}N_3O_4$	213 (7883)	53.60	117.99; 118.56							
(*S*)-**5d**					3.72	4.22	2.95		2.16	3.29	3.70
(*R*)-**5e**	$C_{25}H_{31}N_3O_4$	214 (6834)	52.51	118.38	3.55	3.99	2.86		2.05	3.55	3.72
(*S*)-**5e**	$C_{25}H_{31}N_3O_4$	213 (6939)	52.87	117.61	3.70	4.10	2.79		2.01	3.70	3.62
(*R*)-**5f**					3.50	4.09	2.80		2.16	3.79	3.70
+	$C_{25}H_{31}N_3O_4$	215 (6605)	54.71	118.75; 119.25							
(*S*)-**5f**					3.36	4.09	2.80		2.16	3.50	3.67
(*R*)-**5g**					3.70	4.08	2.79	3.07	2.08	3.25	3.74
+	$C_{30}H_{40}N_4O_6$	214 (7189)	53.28; 53.23	118.04; 118.47							
(*S*)-**5g**					3.70	4.18	2.91		2.00	3.41	3.63
(*R*)-**5h**					3.74	4.10	2.82	3.07	2.10	3.37	3.67
+	$C_{22}H_{31}N_3O_6$	213 (5740)	53.37; 52.86	117.85; 118.34							
(*S*)-**5h**					3.77	4.21	2.92		2.12	3.48	3.78
(*R*)-**5i**					3.71	3.88	1.43		1.60	3.28	3.68
+	$C_{22}H_{33}N_3O_4$	212 (5388)	51.93	117.98; 118.36							
(*S*)-**5i**					3.71	4.09	1.43		1.60	3.50	3.74
(*R*)-**5j**	$C_{30}H_{30}N_4O_4$	224 (12 366) 280 (4604)	52.47	118.53	3.48	4.21	2.96	3.10	1.80	3.48	3.72
(*S*)-**5j**	$C_{30}H_{30}N_4O_4$	224 (11 914) 280 (5811)	52.70	117.66	3.79	4.30	2.99		1.95	3.75	3.56

[a] Satisfactory analyses for C, H, N. [b] Not registered.

with L-phenylalanine methyl ester led to the corresponding 1:1 mixture of pseudotripeptides (*R*)- and (*S*)-**10a** in 80% yield, which could not be separated.

In conclusion, from the mechanistic studies reported here, it appears that protected Xaaψ[CH(CN)NH]Yaa pseudodipeptides are easily prepared in high yield by addition of TMSCN to unstable aldimine intermediates obtained from the corresponding *N*-protected amino aldehyde and *C*-protected amino acid. Although general conclusions cannot be drawn, it seems that the relative stereochemistry of Xaa and Yaa residues is the main factor determining the stereoselectivity of this thermodynamically controlled synthesis. In spite of the lability of the new peptide bond surrogate ψ[CH(CN)NH] to basic medium, high yields of *C*-deprotected pseudodipeptides can be obtained by controlling the saponification conditions of the corresponding methyl esters. The use of *C*-deprotected α-amino acids in the modified Strecker synthesis reported here can be an alternative route for the direct synthesis of *C*-deprotected ψ[CH(CN)NH] pseudopeptides. The application of this new backbone modification to the search of peptidase inhibitors and agonist/antagonist of several neuropeptides will be reported elsewhere.

Experimental Section

General. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were obtained from Bachem Feinchemikalien AG. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254}, Merck. Silica gel 60 (230–400 mesh), Merck, was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. NMR spectra were recorded at 200 or 300 MHz, using TMS as reference, except for the spectra recorded in D_2O, where this solvent was the reference. Reversed-phase HPLC analyses were performed on a Lichrosorb RP-18 (5 μm) stainless steel, Merck, column (4 × 250 mm), with a flow rate of 1 mL/min, and using a tunable UV detector set at 214 nm. Mixtures of 0.05% TFA in H_2O (solvent A) and CH_3CN (solvent B) were used as mobile phase.

General Procedure for the Synthesis of Protected ψ[CH(CN)NH] Pseudodipeptides 5. Triethylamine (0.55 mL, 4 mmol) was added to a solution of the corresponding α-amino acid methyl ester hydrochloride **2** (4 mmol) in MeOH (25 mL). After being stirred at rt for 15 min, the reaction mixture was cooled at −20 °C, and then $ZnCl_2$ (0.27 g, 2 mmol) and the appropriate *N*-protected α-amino aldehyde **1** (2 mmol), freshly prepared,[17] were added, and the stirring at this temperature was continued for 1 h. Finally, TMSCN (0.30 mL, 2.4 mmol) was added, and the mixture was stirred at 0 °C for 24 h. Then, the solvent was removed, the residue dissolved in ethyl acetate (25 mL), and the resulting solution washed with H_2O (20 mL) and brine (20 mL) and dried over Na_2SO_4. Evaporation and flash chromatography afforded the protected pseudodipeptides **5**, as foams, whose analytical and spectroscopic data are summarized in Tables I and II.

General Procedure for the Removal of the *N*-Boc Protecting Group. Synthesis of *N*-Deprotected ψ[CH(CN)NH] Pseudodipeptides 6a,c-f. The corresponding *N*-Boc-protected pseudodipeptide **5a,c-f** (1 mmol) was dissolved in 1 N HCl methanolic solution (25 mL). After 5 h at rt, removal of the solvent and subsequent lyophilization of the residue dissolved in H_2O gave quantitatively the *N*-deprotected pseudodipeptide dihydrochlorides **6a,c-f** as white amorphous solids. The analytical and spectroscopic data of these compounds are summarized in Table III.

General Procedure for the Synthesis of Imidazolidin-2-ones 7a,c-f. Triethylamine (0.28 mL, 2 mmol) was added to a suspension of the dihydrochloride of the corresponding *N*-deprotected pseudodipeptide **6a,c-f** (1 mmol), and the mixture was

(17) Fehrentz, J. A.; Castro, B. *Synthesis* **1983**, 676.

Table III. Analytical and Significant Spectroscopic Data for *N*-Deprotected Pseudodipeptides H_2N-XaaΨ[CH(CN)NH]Yaa-OMe 6

		found (%) (required)			^1H NMR (D_2O, δ, ppm)					
					Xaa				Yaa	
compd	formula	C	H	N	2-H	3-H	4-H	4-H′	α-H	OCH_3
(*R*)-**6a** + (*S*)-**6a**	$C_{17}H_{25}N_3O_2.2HCl$	53.95 (54.26)	7.36 (7.18)	10.89 (11.17)	4.06 4.15	3.84 3.95	3.05 3.20	3.37	3.52 3.69	3.80 3.74
(*R*)-**6c** + (*S*)-**6c**	$C_{17}H_{25}N_3O_2.2HCl$	54.14 (54.26)	7.42 (7.18)	11.25 (11.17)	3.87 4.06	3.78 3.78	2.89 2.95	3.40 3.12	3.60 3.38	3.64 3.65
(*R*)-**6d** + (*S*)-**6d**	$C_{16}H_{23}N_3O_2.2HCl$	52.87 (53.04)	7.08 (6.91)	11.53 (11.60)	3.74 3.83	3.64 3.64	2.84 2.93	3.11	3.03 3.29	3.70 3.67
(*R*)-**6e**	$C_{20}H_{23}N_3O_2.2HCl$	58.60 (58.54)	6.23 (6.10)	10.15 (10.24)	3.72	3.47–3.60	2.60–3.10		3.47–3.60	3.55
(*S*)-**6e**	$C_{20}H_{23}N_3O_2.2HCl$	58.48 (58.54)	6.09 (6.10)	10.32 (10.24)	4.00	3.73	2.79–3.14		3.73	3.60
(*R*)-**6f** + (*S*)-**6f**	$C_{20}H_{23}N_3O_2.2HCl$	58.37 (58.54)	6.14 (6.10)	10.30 (10.24)	3.80 3.85	3.62 3.59	2.70–3.16		3.78 3.59	3.52 3.52

Table IV. Analytical and Significant Spectroscopic Data for the Imidazolidin-2-ones 7

			found (%) (required)			^1H NMR (δ, ppm)[a]						
						imidazolidin-2-one					Yaa	
compd	yield %	formula	C	H	N	4-H	5-H	$J_{4,5}$	4-CH_2	NH	α-H	OCH_3
(*R*)-**7a**	35	$C_{18}H_{23}N_3O_3$	65.60 (65.65)	7.11 (6.99)	12.50 (12.76)	4.10	4.69	3.8	2.93, 3.02	5.18	4.77	3.76
(*S*)-**7a**	35	$C_{18}H_{23}N_3O_3$	65.80 (65.65)	7.30 (6.99)	12.58 (12.76)	4.07	4.59	7.9	3.05, 3.18	4.66	4.49	3.79
(*R*)-**7c** + (*S*)-**7c**	61	$C_{18}H_{23}N_3O_3$	65.80 (65.65)	7.10 (6.99)	12.66 (12.76)	4.19 4.19	4.19 4.96	3.8 8.2	2.91 3.01, 3.14	5.30 4.84	4.35 4.72	3.69 3.72
(*R*)-**7d**	47	$C_{17}H_{21}N_3O_3$	64.92 (64.76)	6.83 (6.67)	13.27 (13.33)	4.17	4.72	3.2	2.77, 2.87	5.51	4.27	3.75
(*S*)-**7d**	23	$C_{17}H_{21}N_3O_3$	64.58 (64.76)	6.72 (6.67)	13.15 (13.33)	4.33	4.90	7.3	3.14, 3.20	5.19	4.16	3.77
(*R*)-**7e**	80	$C_{21}H_{21}N_3O_3$	69.54 (69.42)	5.92 (5.79)	11.38 (11.57)	3.99	4.29	4.1	2.74	4.98	4.69	3.74
(*S*)-**7e**	78	$C_{21}H_{21}N_3O_3$	69.47 (69.42)	5.83 (5.79)	11.60 (11.57)	3.65	4.46	7.9	2.72, 2.93	4.63	4.62	3.74
(*R*)-**7f** + (*S*)-**7f**	83	$C_{21}H_{21}N_3O_3$	69.19 (69.42)	5.73 (5.79)	11.52 (11.57)	3.98 3.98	4.06 4.67	4.0 8.0	2.29, 2.50 2.88, 3.07	5.14 4.66	4.85 4.72	3.80 3.72
(*R*)-**7j**	40[b]	$C_{23}H_{22}N_4O_3$	68.54 (68.66)	5.81 (5.47)	13.62 (13.93)	4.22	4.41	3.2	2.86, 3.06	5.54	4.79	3.76

[a] In $CDCl_3$ except for (*R*)-**7j** which was registered in $(CD_3)_2CO$. [b] From (*R*)-**5j**.

stirred for 15 min at rt. Then, bis(trichloromethyl) carbonate (0.119 g, 0.4 mmol) and triethylamine (0.34 mL, 2.4 mmol) were added at 0 °C, and stirring was continued at this temperature for 5 h. Afterwards, the reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with water (20 mL) and brine (20 mL), and dried over Na_2SO_4. After removal of the solvent, flash chromatography of the residue, using hexane–ethyl acetate mixtures as eluants, yielded the imidazolidin-2-ones **7a,c-f** as foams. In this chromatography, epimeric (5*R*)- and (5*S*)-imidazolidin-2-ones **7a** and **7d** were separated. The analytical and spectroscopic data of imidazolidin-2-ones **7** are summarized in Table IV.

Synthesis of Methyl (2*S*,4′*S*,5′*R*)-2-[4′-(Indol-3″-ylmethyl)-5′-cyano-2′-oxoimidazolidin-1′-yl]propionate [(*R*)-7j]. A solution of the *N*-*Z*-protected pseudodipeptide (*R*)-**5j** (0.580 g, 1 mmol) in a 0.05 N HCl solution in methanol (50 mL) was hydrogenated in the presence of 10% Pd/C (0.058 g) at 2 atm of H_2 pressure and at rt for 1 h. The catalyst was filtered off and washed with MeOH (10 mL), and the removal of the solvent yielded the crude unstable *N*-deprotected pseudodipeptide (*R*)-**6j**. This crude (*R*)-**6j** was used for the synthesis of the imidazolidin-2-one (*R*)-**7j** following the general procedure above described. The analytical and more significant spectroscopic data of this compound are summarized in Table IV.

Synthesis of the Protected Pseudotripeptides *N*-Boc-Phe-PheΨ[CH(CN)NH]Leu-OMe (8a). Triethylamine (0.55 mL, 4 mmol) was added to a solution of the dihydrochloride of the *N*-deprotected pseudodipeptide **6a** (0.750 g, 2 mmol) in dry THF (10 mL) cooled at 0 °C, and the mixture was stirred for 15 min at this temperature. Then, *N*-Boc-L-Phe-OH (0.640 g, 2.4 mmol), 1-HOBt (0.320 g, 2.4 mmol), and DCC (0.500 g, 2.4 mmol) dissolved in dry CH_2Cl_2 (40 mL) were added successively. After 24 h of stirring at rt, the dicyclohexylurea was filtered off, and the solvents were removed. The residue was washed with H_2O (20 mL) and brine (20 mL) and finally dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by flash chromatography, using (6:1) hexane–ethyl acetate as eluant, yielding the two separated epimers (*R*)-**8a** and (*S*)-**8a**.

***N*-Boc-Phe-PheΨ[(*R*)-CH(CN)NH]Leu-OMe [(*R*)-8a].** White solid (0.474 g, 43%): mp 185–187 °C (benzene–CCl_4); ^1H NMR [300 MHz, $(CD_3)_2CO$] δ (ppm) 0.91 [d, 6H, J = 6 Hz, $2CH_3$(Leu)], 1.32 (s, 9H, Boc), 1.48 [m, 2H, β-H (Leu)], 1.82 [m, 1H, γ-H (Leu)], 2.53 [t, 1H, J = 10 Hz, NH (Leu)], 2.85 [m, 2H, γ-H (PheΨ[CH(CN)NH])] and β-H (Phe)], 3.04 [dd, 1H, J = 5 and 14 Hz, γ-H (PheΨ[CH(CN)NH])], 3.17 [dd, 1H, J = 5 and 14 Hz, β-H (Phe)], 3.36 [m, 1H, α-H (Leu)], 3.70 (s, 3H, OCH_3), 3.75 [dd, 1H, J = 4 and 10 Hz, α-H (PheΨ[CH(CN)NH])], 4.29 [m, 1H, α-H (Phe)], 4.37 [m, 1H, β-H (PheΨ[CH(CN)NH])], 5.90 (d, 1H, J = 8 Hz, NH-Boc), 7.22 (m, 10H, aromatics), 7.50 [d, 1H, J = 8 Hz, NH (Phe²)]. Anal. Calcd for $C_{31}H_{42}N_4O_5$: C, 67.63; H, 7.63; N, 10.18. Found: C, 67.25; H, 7.70; N, 10.31.

***N*-Boc-Phe-PheΨ[(*S*)-CH(CN)NH]Leu-OMe [(*S*)-8a].** White solid (0.473 g, 43%): mp 113–115 °C (CCl_4); ^1H NMR

[300 MHz, $(CD_3)_2CO$] δ (ppm) 0.91 [d, 3H, J = 7 Hz, CH_3 (Leu)], 0.92 [d, 3H, J = 7 Hz, CH_3 (Leu)], 1.31 (s, 9H, Boc), 1.52 [m, 2H, β-H (Leu)], 1.80 [m, 1H, γ-H (Leu)], 2.53 [t, 1H, J = 7 Hz, NH (Leu)], 2.78 [dd, 1H, J = 9 and 14 Hz, β-H (Phe)], 2.95 [dd, 1H, J = 9 and 14 Hz, γ-H (Pheψ[CH(CN)NH])], 3.08 [dd, 1H, J = 5 and 14 Hz, β-H (Phe)], 3.10 [dd, 1H, J = 5 and 14 Hz, γ-H (Pheψ[CH(CN)NH])], 3.52 [m, 1H, α-H (Leu)], 3.64 (s, 3H, OCH_3), 3.88 [dd, 1H, J = 6 and 7 Hz, α-H (Pheψ[CH(CN)NH])], 4.32 [m, 1H, α-H (Phe)], 4.49 [m, 1H, β-H (Pheψ[CH(CN)NH])], 5.95 [d, 1H, J = 8 Hz, NH-Boc), 7.23 (m, 10H, aromatics), 7.49 [d, 1H, J = 8 Hz, NH (Phe^2)]. Anal. Calcd for $C_{31}H_{42}N_4O_5$: C, 67.63; H, 7.63; N, 10.18. Found: C, 67.50; H, 7.66; N, 9.99.

Saponification of Boc-Pheψ[CH(CN)NH]Leu-OMe (5a). Synthesis of *C*-Deprotected Pseudodipeptides 9a. NaOH (1 N) solution (6 mL, 6 mmol) was added to a solution of the 1:1 mixture of the methyl esters (*R*)- and (*S*)-**5a** (0.810 g, 2 mmol) in 1:1 1,4-dioxane–water (70 mL), and the mixture was stirred at rt for 90 min. Then, the reaction mixture was concentrated (~30 mL) and washed with CH_2Cl_2 (3 × 20 mL), and the aqueous phase was acidified to pH 3–4 with Dowex 50 W-X4 resin. The resin was filtered off and washed with ethyl acetate (20 mL). The aqueous phase was extracted with ethyl acetate (3 × 75 mL), and the combined organic phases were dried over Na_2SO_4 and evaporated to dryness to provide the (1:1) mixture of the corresponding acids (*R*)- and (*S*)-**9a** (0.650 g, 83%) as a colorless oil. This mixture was also obtained in 75% yield by the coupling of *N*-Boc-L-phenylalaninal (**1a**) with L-leucine and TMSCN, following the general procedure above described for the synthesis of protected pseudodipeptides **5**; ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ (ppm) 0.92 [d, 3H, J = 7 Hz, CH_3 (Leu)], 0.94 [d, 3H, J = 7 Hz, CH_3 (Leu)], 1.29 (s, 9H, Boc), 1.52 [m, 2H, β-H (Leu)], 1.87 [m, 1H, γ-H (Leu)], 2.89 [dd, 1H, J = 11 and 13 Hz, γ-H (Phe)], 3.12 and 3.17 [2dd, 1H, J = 4 and 14 Hz, γ-H (Phe)], 3.34 [t, 0.5H, J = 7 Hz, α-H (Leu), (*R*)-**9a**], 3.48 [dd, 0.5H, J = 5 and 9 Hz, α-H (Leu), (*S*)-**9a**], 3.83 [d, 0.5H, J = 4 Hz, α-H (Phe), (*R*)-**9a**], 3.89 [d, 0.5H, J = 6 Hz, α-H (Phe), (*S*)-**9a**], 4.08 [m, 1H, β-H (Phe)] 6.42 and 6.46 [2d, 1H, J = 8 Hz, NH(Phe)], 7.42 (m, 5H, aromatics). Anal. Calcd for $C_{21}H_{31}N_3O_4$: C, 64.78; H, 7.97; N, 10.80. Found: C, 64.90; H, 7.66; N, 10.45.

Synthesis of Pseudotripeptides Boc-Pheψ[CH(CN)NH]-Leu-Phe-OMe (10a). Triethylamine (0.017 mL, 0.125 mmol) was added to a solution of phenylalanine methyl ester hydrochloride (0.026 g, 0.125 mmol) in dry THF (1 mL) cooled at 0 °C, and the mixture was stirred for 15 min at this temperature. Then, the pseudodipeptides **9a** (0.041 g, 0.105 mmol), 1-HOBt (0.017 g, 0.125 mmol), and DCC (0.026 g, 0.125 mmol) dissolved in dry CH_2Cl_2 (3 mL) were added successively. After 24 h of stirring at rt the reaction mixture was worked out as for **8a**, affording a 1:1 mixture of epimeric (*R*)- and (*S*)-**10a** (0.047 g, 80%), which could not be separated: ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ (ppm) 0.87 [d, 3H, J = 6.5 Hz, CH_3 (Leu)], 0.90 [d, 3H, J = 6 Hz, CH_3 (Leu)], 1.32 (s, 9H, Boc), 1.37 [m, 2H, β-H (Leu)], 1.58 and 1.73 [2m, 1H, γ-H (Leu)], 2.59 and 2.71 [2t, 1H, J = 9 Hz, NH (Leu)], 2.75 and 2.81 [2dd, 1H, γ-H (Pheψ[CH(CN)NH])], 3.09 [m, 2H, β-H (Phe)], 3.35 [m, 1H, α-H (Leu)], 3.61 and 3.72 (2s, 3H, OCH_3), 3.62 and 3.74 [2dd, 1H, J = 5 and 9 Hz, α-H (Pheψ[CH(CN)-NH])], 4.01 and 4.15 [2m, 1H, β-H (Pheψ[CH(CN)NH])], 4.77 [m, 1H, α-H (Phe)], 6.30 and 6.36 (2d, 1H, NH-Boc), 7.23 (m, 10H, aromatics), 7.85 and 7.97 [2d, 1H, NH (Phe^3)]. Anal. Calcd for $C_{31}H_{42}N_4O_5$: C, 67.64; H, 7.63; N, 10.10. Found: C, 67.87; H, 7.65; N, 10.40.

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