A Simple Method for *N*-Amination of Peptide Derivatives

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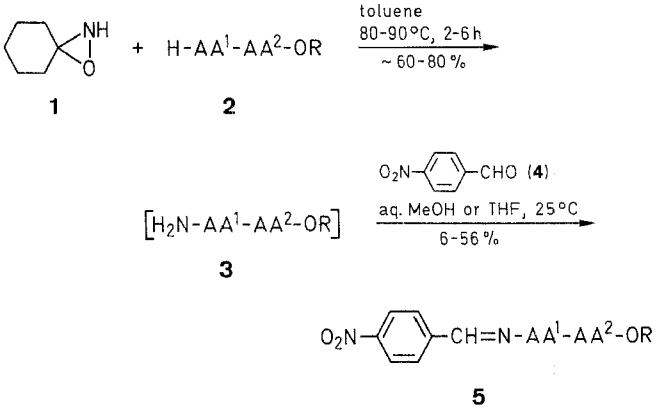
A smooth and mild procedure is reported for the *N*-amination of α-amino acid and peptide derivatives with cyclohexanespiro-3'-oxaziridine; the chiral integrity is preserved throughout.

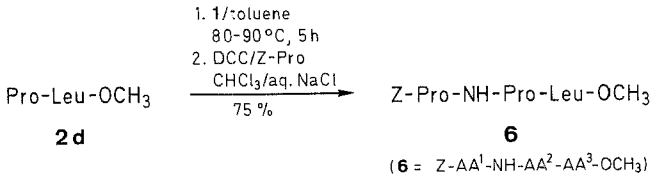
Since 1960 great interest has been focussed on the synthesis[1-6] of α-hydrazino carboxylic acids due to their diverse biological activity.[5-7] Related compounds serve as chiral auxiliaries in asymmetric syntheses.[8,9] Nevertheless, no simple and mild method for *N*-amination of amino acid and peptide derivatives has been published up to now. Difficulties have been encountered[2] in such an attempt with hydroxylamine-*O*-sulfonic acid. The amination by chloramine of a deprotonated *N*-acyl aminonitrile was reported,[2] but this method required extremely strong base (NaH/DMSO).[2] A third procedure,[2,3,6,9] preparation of an urea from the appropriate amino acid (derivative) followed by treatment with alkaline hypochlorite solution to bring about N–N bond formation, has recently been significantly improved.[6,9] However, this process[6,9] is unsuitable for substrates sensitive to the harsh reaction conditions of the Hofmann (Shestakov) rearrangement.[6] The reported[4] multistep approach is disfavored, because of the mutagenic nature of the *N*-nitroso intermediates.[9] Asymmetric syntheses[5] seem to be attractive mainly for producing hydrazino acids corresponding to unnatural α-amino acids.

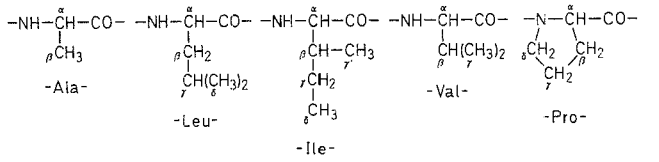
Now we wish to report here, that our method for the synthesis of α-hydrazino carboxylic acid derivatives by *N*-amination with oxaziridines[10-12] also proved to be useful for the amination of the free *N*-terminus of peptides. The readily available cyclohexanespiro-3'-oxaziridine **1**[12] was applied as aminating

agent in the process. The starting materials,[13,14] amino acid and dipeptide esters **2**,[15,16] were treated with a toluene[17] solution of reagent **1** in slight excess, usually at 80–90 °C for several hours. Hydrazino compound **3** was extracted with cold diluted aqueous acid, and conveniently derivatized for characterization by admixing the buffered aqueous solution with 4-nitrobenzaldehyde (**4**) dissolved in methanol or tetrahydrofuran. The yellow hydrazones **5** were obtained in 6–56% yield[15] by flash chromatography[18] and/or crystallization. Physical and spectral data are collected in Tables 1 and 2.[19] Racemization was not observed. Thus, **5c** could not be detected by TLC in crude **5b** prepared from **2b**,[20] although a mixture of the corresponding diastereoisomers **5b** and **5c**, synthesized starting from Boc-(*R*,*S*)-Ala-(*S*)-Pro-OBzl was clearly separated by chromatography.[21,22]



The moderate yield of the hydrazones **5a–g** are partly the consequence of incomplete condensation reaction of hydrazines **3** with the aldehyde **4**. The amination step itself often proceeds with high yield.[23] This statement, as well as the preparative value of this method, is demonstrated by a straightforward synthesis of tripeptide **6**, in which *N*-aminated proline is incorporated. In this reaction sequence the amination of dipeptide ester **2d** was followed by the DCC-mediated acylation of hydrazino compound **3d** with Z-proline to afford hydrazide **6** in fair yield.





Products of this type may serve as versatile building blocks for larger peptides containing hydrazino acid residues. The scope of the recorded procedure is under investigations.[24]

Table 1. Hydrazones **5** Prepared

Product	Amino Acid, Peptide, Moieties	Method	Reaction Time (h)	Yield[a] (%)	mp (°C) (solvent)	Molecular Formula[b]	$[\alpha]_D^{25}$ ($CHCl_3$)	IR (neat) ν (cm^{-1})	^{1}H-NMR ($CDCl_3$/TMS)[c] δ, J (Hz)	MS (70 eV) *m/z* (%)
5a	Ala-Pro-OBu-*t*	B	4	39	oil	$C_{19}H_{26}N_4O_5$ (390.4)	−52° ($c = 2$)	3240, 1735, 1642, 1596, 1512, 1338, 850	1.43 (d, 3H, $J = 7$, $CHCH_3$); 1.45 (s, 9H, t-C_4H_9); 1.9–2.2 (m, 4H, β^2-CH_2, γ^2-CH_2); 3.63, 3.74 (m, 2H, δ^2-CH_2); 4.40 (dd, 1H, $J = 8,4$, α^2-CH); 4.41 (m, 1H, $J = 3 \times 7$, 9, α^1-CH); 6.48 (d, 1H, $J = 9$, NH); 7.65 (d, 2H, $J = 9$, H-2, H-6); 7.68 (s, 1H, CH=N); 8.18 (d, 2H, $J = 9$, H-3, H-5)	390 (M^+, 4); 334 (6); 289 (2); 192 (100); 174 (4)
5b	Ala-Pro-OBzl	C[d]	3	23[e]	oil	$C_{22}H_{24}N_4O_5$ (424.5)	−95° ($c = 1$)	3230, 1742, 1642, 1596, 1515, 1339, 850, 750, 695	1.38 (d, 3H, $J = 7$, $CHCH_3$); 1.95–2.26 (m, 4H, β^2-CH_2 + γ^2-CH_2); 3.67, 3.76 (m, 2H, δ^2-CH_2); 4.44 (m, 1H, $J = 3 \times 7$, 6, α^1-CH); 4.60 (dd, 1H, $J = 8$, 3.5, α^2-CH); 5.10, 5.22 (2d, 2H, $J_{gem} = 12.5$, CO_2CH_2); 6.39 (d, 1H, $J = 6$, NH); 7.35 (m, $5H_{arom}$); 7.62 (d, 2H, $J = 9$, H-2, H-6); 7.69 (s, 1H, CH=N); 8.15 (d, 2H, $J = 9$, H-3, H-5)	424 (M^+, 6); 394 (< 1); 316 (2); 204 (3); 192 (100); 168 (11); 149 (2); 108 (11); 107 (9); 91 (34); 70 (53)
5c	(*R*)-Ala-(*S*)-Pro-OBzl	C[d]	3	6[f]	oil	$C_{22}H_{24}N_4O_5$ (424.5)	+27° ($c = 2$)	3230, 1747, 1647, 1599, 1518, 1342, 850, 752, 698	1.41 (d, 3H, $J = 7$, $CHCH_3$); 1.9–2.3 (m, 4H, β^2-CH + γ^2-CH_2); 3.60, 3.93 (m, 2H, δ^2-CH_2); 4.46 (q, 1H, $J = 7$, α^1-CH); 4.56 (dd, 1H, $J = 8.5$, 3.5, α^2-CH); 5.07 (s, 2H, CO_2CH_2); 6.40 (d, 1H, $J = 7$, NH); 7.35 (m, $5H_{arom}$); 7.60 (d, 2H, $J = 9$, H-2, H-6); 7.63 (s, 1H, CH=N); 8.12 (d, 2H, $J = 9$, H-3, H-5)	424 (M^+, 6); 394 (< 1); 316 (1); 204 (2); 192 (100); 168 (7); 149 (2); 108 (8); 107 (6); 91 (30); 70 (43)

5d	Pro-Leu-OCH_3	A	5	56	oil	$C_{19}H_{26}N_4O_5$ (390.4)	$-335°$ ($c = 2$)	3380, 3310, 1747, 1670 (br), 1597, 1553, 1512, 1338, 846	0.90 (d, 6H, $J = 6$, $(CH_3)_2$); 1.4–2.4 (m, 7H, β^1-CH_2, β^2-CH_2, γ^2-CH, γ^1-CH_2); 3.2–3.6 (m, 2H, δ^1-CH_2); 3.69 (s, 3H, OCH_3); 4.14 (t, 1H, $J = 7$, α^1-CH); 4.5–4.8 (m, 1H, α^2-CH); 6.83 (br, 1H, NH); 7.27 (s, 1H, CH=N); 7.68 (d, 2H, $J = 9$, H-2, H-6); 8.17 (d, 2H, $J = 9$, H-3, H-5)	390 (M^+, 2); 360 (1); 331 (1); 241 (4); 218 (100); 172 (5)
5e	Pro-Ile-OCH_3	A	2	50	87–89 (ether/hexane)	$C_{19}H_{26}N_4O_5$ (390.4)	$-290°$ ($c = 2$)	3305, 1748, 1660, 1595, 1547, 1508, 1334, 850[g]	0.84 (t, 3H, $J = 7$, CH_2CH_3); 0.86 (d, 3H, $J = 7$, $CHCH_3$); 1.28 (m, 2H, γ^2-CH_2); 2.05–2.24 (m, 5H, β^2-CH, β^1-CH_2, γ^1-CH_2); 3.66 (m, 2H, δ^1-CH_2); 3.68 (s, 3H, OCH_3); 4.15 (t, 1H, $J = 6.5$, α^1-CH); 4.62 (dd, 1H, $J = 8.5$, 5, α^2-CH); 6.96 (d, 1H, $J = 8.5$, NH); 7.27 (s, 1H, CH=N); 7.68 (d, 2H, $J = 9$, H-2, H-6); 8.16 (d, 2H, $J = 9$, H-3, H-5)	390 (M^+, 2); 360(1); 331 (1); 241 (4); 218 (100); 149 (1)
5f	Pro-OBu-*t*	B	6	48	110–111 (ether/hexane)	$C_{16}H_{21}N_3O_4$ (319.4)	$-206°$ ($c = 1$)	1730, 1597, 1506, 1330, 850[g]	1.44 (s, 9H, t-C_4H_9); 2.15 (m, 4H, β-CH_2, γ-CH_2); 3.5 (m, 2H, δ-CH_2); 4.28 (t, 1H, $J = 5.5$, α-CH); 7.12 (s, 1H, CH=N); 7.62 (d, 2H, $J = 9$, H-2 + H-6); 8.14 (d, 2H, $J = 9$, H-3, H-5)	319 (M^+, 6); 263 (1); 218 (100); 172 (5); 149 (1)
5g	Val-Leu-OCH_3	A	2	35	oil	$C_{19}H_{28}N_4O_5$ (392.5)	$-47°$ ($c = 1$)	3240, 1747, 1670 (br), 1596, 1550, 1513, 1338, 850	0.80–1.10 [4d, 12H, $2 \times CH(CH_3)_2$]; 1.35–2.45 (m, 4H, β^2-CH_2 + β^1-CH + γ^2-CH); 3.67 (s, 3H, OCH_3); 4.5–4.8 (m, 2H, α^1-CH, α^2-CH); 6.50 (d, 1H, $J = 8$, NH); 7.2 (br, 1H, NHN); 7.28 (s, 1H, CH=N); 7.66 (d, $2H_{arom}$, $J = 9$, H-2, H-6); 8.18 (d, $2H_{arom}$, $J = 9$, H-3, H-5)	392 (M^+, 4); 349 (4); 220 (100); 151 (7); 149 (5)

[a] Isolated yields, not optimized.
[b] Satisfactory microanalyses obtained: C ±0.35, H ±0.29, N ±0.26.
[c] Compounds **5a–c** were recorded at 400 MHz and compounds **5d–g** at 100 MHz.
[d] Reaction carried out in toluene/$CHCl_3$ (3 : 1) at 60 °C.
[e] Based on the hydrochloride of compound **2b**. Starting material **2b** was liberated *in situ* in $CHCl_3$ solution that was used in this experiment.
[f] Based on Boc-(*R*,*S*)-Ala-(*S*)-Pro-OBzl. Hydrazone **5b** (10%) was also isolated in this reaction, cf. experimental part.
[g] KBr pellet.

Table 2. ^{13}C-NMR Data of Compounds **5** ($CDCl_3$/TMS), δ

Compound	C_{arom}					AA[1]					AA[2]					R-Group
	C-1	C-2 C-6	C-3 C-5	C-4	CH=N	C_α	C_β	C_γ	C_δ	CO	C_α	C_β	C_γ	C_δ	CO	
5a	142.0	126.2	123.9	147.0	136.7	56.6	17.1	–	–	171.0[a]	59.7	29.0	24.9	46.7	172.2[a]	81.3 [$\underline{C}(CH_3)_3$]; 28.0 [$C(\underline{C}H_3)_3$]
5b	141.8	126.3	123.9	147.1	136.9	56.3	17.0	–	–	171.8[a]	58.9	28.9	24.9	46.6	172.5[a]	67.0 ($\underline{C}H_2C_6H_5$); 135.4, 128.2, 128.3, 128.6 (C_{arom})
5c	142.1	126.2	123.8	146.9	136.0	56.1	17.2	–	–	171.6[a]	59.2	29.0	24.8	46.8	172.3[a]	66.7 ($\underline{C}H_2C_6H_5$); 135.7, 127.8, 128.2, 128.5 (C_{arom})
5d	142.9	125.8	124.0	146.6	131.7	66.6	28.8	21.9	50.7	173.0[a]	50.7	41.4	25.1	22.9	172.2[a]	52.3 (CH_3)
5e	142.8	125.8	124.0	146.6	131.6	66.7	28.6	22.9	50.4	172.0[a]	56.4	37.9	25.2 15.6[b]	11.5	172.1[a]	52.1 (CH_3)
5f	143.8	125.2	124.0	145.9	128.4	–	–	–	–	–	65.5	22.5	28.3	48.7	172.2	81.4 [$\underline{C}(CH_3)_3$]; 28.0 [$C(\underline{C}H_3)_3$]
5g	141.9	126.3	123.9	147.1	128.5	69.0	30.2	18.7 19.4	–	173.1[a]	50.6	41.2	24.9	22.8 21.5	172.3[a]	52.3 (CH_3)

[a] Assignments of CO within a line may be interchanged.
[b] Assigned to $C_{\gamma'}$.

Table 3. ^{13}C-NMR Data of **6** ($CDCl_3$/TMS), δ[a]

Amino Acid Component	Assignments				
	C_α	C_β	C_γ	C_δ	CO
AA^1 (Pro)	59.8	29.0	22.4	47.3	171.5[b]
AA^2 (Pro)	68.5	24.9	21.7	55.9	173.4[b]
AA^3 (Leu)	50.9	40.3	25.1	22.9	172.9[b]

[a] Other signals: δ = 51.8 (OCH_3); 67.5 ($\underline{C}H_2C_6H_5$); 156.5 (CO_2CH_2); 127.9, 128.2, 128.6, 136.3 (C_{arom}).
[b] Assignments may be interchanged.

Melting points are uncorrected, $[\alpha]_D^{25}$ values were measured on a POLAMAT A polarimeter. IR spectra were taken on a Nicolet 170SX FTIR spectrophotometer. Mass spectra were measured on an AEI MS902 apparatus (70 eV, direct inlet). ^{1}H-NMR spectra were recorded on Varian XL 100 (100 MHz) and Varian XL 400 (400 MHz) spectrometers at 60 °C; ^{13}C-NMR (25 MHz) spectra were measured using a Varian XL 100 instrument. Flash chromatography was performed according to the literature[18] on silica gel columns (Merck's Kieselgel 60, No. 9385, 40–63 μm). Analytical TLC plates were purchased from Merck (No. 5554).

N-Amination of Amino Acid and Peptide Derivatives, Typical Procedures:

Method A. *Methyl N-amino-(S)-prolyl-(S)-leucinate 4-Nitrophenylhydrazone* (**5d**): A 0.1 M toluene solution of cyclohexanespiro-3′-oxaziridine (**1**; 45 mL, 4.5 mmol) is added to methyl (*S*)-prolyl-(*S*)-leucinate (0.73 g, 3.0 mmol) and stirred under an inert atmosphere at 80–90 °C for 5 h. The reaction is monitored with TLC. The consumption of the reagent **1** can be followed by iodometric titration.[12] A 10% aq. H_2SO_4 (7 mL) is added to the ice cooled, stirred mixture. The toluene phase is separated and extracted again with ice-cold 10% H_2SO_4 (3 × 4 mL). The combined cold water layer is washed with ether (2 × 5 mL), and cautiously concentrated (foaming!) *in vacuo* using an oil pump (< 20 °C) to remove the remaining cyclohexanone. Crushed ice (20 g) is added and the stirred acidic solution is neutralized by dropwise addition of 10% aq. $KHCO_3$ (about 40 mL) at 5–10 °C, poured slowly into a stirred solution of 4-nitrobenzaldehyde (**4**; 0.45 g, 3.0 mmol) in MeOH (150 mL) preheated to 45 °C. The mixture is stirred at room temperature for 2 h, and diluted with water (500 mL). The product is extracted with $CHCl_3$ (1 × 100 mL, 5 × 20 mL), the collected organic layer is then washed with brine (15 mL), dried (Na_2SO_4) and stripped off the solvent. Flash chromatography (cyclohexane/EtOAc, 1:1, column dimension: 25 × 4 cm) affords **5d** as a yellow oil; yield: 0.66 g (56%).

Several related simple peptide derivatives (e.g. **2e, g**) have succesfully been aminated analogously. Details are given in Tables 1 and 2. In special cases some modifications are advisable to minimize certain side reactions.

Method B (*for moderately acid sensitive substrates*): *tert*-Butyl ester group is stable enough to survive a brief exposure to ice-cold diluted strong mineral acid. Hence, compounds **2a** and **2f** are treated as above but the concentration of the obtained aqueous solutions is omitted, and the derivatization with aldehyde **4** is performed immediately.[25]

Method C (*for substrates prone to form piperazinediones*): *Amination of benzyl (R,S)-alanyl-(S)-prolinate:* CF_3CO_2H (10 mL) is added to an ice cooled, stirred solution of benzyl *N-tert*-butyloxycarbonyl-(*R,S*)-alanyl-(*S*)-prolinate (3.77 g, 10.0 mmol) in $CHCl_3$ (10 mL). The mixture is stirred for 1 h at 0 °C, concentrated *in vacuo*, then poured into a stirred mixture of crushed ice (100 g) and sat. aq. $NaHCO_3$ solution (200 mL). The organic phase is separated, and the aqueous solution is extracted with $CHCl_3$ (4 × 15 mL). The combined organic layer is quickly dried (Na_2SO_4) in a refrigerator, and admixed with a 0.1 M toluene solution of cyclohexanespiro-3′-oxaziridine (180 mL, 18 mmol). The mixture is stirred at 60 °C under argon overnight, and worked up as described above (Method A), but 4-nitrobenzaldehyde is dissolved in THF instead of MeOH. Purification of the crude product by flash chromatography (cyclohexane/EtOAc, 1.5:1, column dimension: 30 × 5 cm) results in two epimeric products **5b** and **5c**.

5b yield (based on 10.0 mmol protected dipeptide): 0.43 g (10%); R_f = 0.4 (ether); $[\alpha]_D^{25}$ = −91° (*c* = 1, $CHCl_3$).

$C_{19}H_{28}N_4O_5$ (392.5)	calc.	C 58.15	H 7.19	N 14.28
	found	57.82	7.44	14.39

The same compound with identical spectral properties is prepared from **2b** freshly liberated from HCl·Ala-Pro-OBzl in $CHCl_3$.

5c; yield: 0.26 g (6%); R_f = 0.3 (ether). Physical and spectral characteristics are listed in Tables 1 and 2.

Methyl *N*-(*N*-Benzyloxycarbonyl-(*S*)-prolyl-amino)-(*S*)-prolyl-(*S*)-leucinate (6):

The amination of (*S*)-prolyl-(*S*)-leucine methyl ester (0.73 g, 3.0 mmol) is carried out as given under Method A. The neutralized aqueous solution of hydrazine **3d** is added dropwise to a vigorously stirred mixture of *N,N′*-dicyclohexylcarbodiimide (0.69 g, 3.3 mmol), *N*-carbobenzyloxy-(*S*)-proline (0.75 g, 3.0 mmol) dissolved in $CHCl_3$ (75 mL) and brine (75 mL) at + 5 °C under argon during a period of 10 min. The stirring is continued at + 5 °C for 2 h and at ambient temperature overnight. The mixture is diluted with sat. aq. $NaHCO_3$ (50 mL), filtered, and the organic layer is separated. The water phase is then extracted with $CHCl_3$ (4 × 10 mL), the collected organic layer is washed with sat. aq. $NaHCO_3$ (10 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residue is taken up in ether (70 mL), filtered and evaporated. Flash chromatography ($CHCl_3$/MeOH, 30:1 → 10:1, column dimension: 30 × 5 cm) gives **6** as a colorless oil; yield: 1.10 g (75%); $[\alpha]_D^{22}$ = −109° (*c* = 2.5, $CHCl_3$).

$C_{25}H_{36}N_4O_6$ (488.6)	calc.	C 61.46	H 7.43	N 11.47
	found	61.13	7.68	11.20

MS: *m/z* (%): 488 (0.9, M^+); 316 (80); 241 (13); 208 (100); 204 (7); 111 (52); 108 (13); 107 (10); 91 (86).

IR (neat): ν = 3260, 3060, 3035, 1749, 1675 (v br), 1538, 698 cm^{-1}.

^{1}H-NMR ($CDCl_3$/TMS, 400 MHz): δ = 0.93, 0.98 [2 d, 6 H, *J* = 7 Hz each, $CH(C\underline{H}_3)_2$]; 1.4–2.5 (m, 11 H, β^1-CH_2, β^2-CH_2, β^3-CH_2, γ^1-CH_2, γ^2-CH_2, γ^3-CH); 3.31 (dd, 1 H, *J* = 9, 6 Hz, α^2-CH); 3.47–3.80 (m, 4 H, δ^1-CH_2, δ^2-CH_2); 3.64 (s, 3 H, OCH_3); 4.32 (dd, 1 H, *J* = 8.5, 2.5 Hz, α^1-CH); 4.43 (q, 1 H, *J* = 3 × 7.5 Hz, α^3-CH); 5.14 + 5.20 (2d, 2 H, J_{gem} = 12.5 Hz, CO_2CH_2); 7.36 (s, 5 H_{arom}); 7.7 (br, 1 H, NHN); 8.38 (d, 1 H, *J* = 7.5 Hz, NH).

^{13}C-NMR: see Table 3.

Similar coupling reaction of **3d** with mixed anhydride Z-Pro-O-CO_2Et offers no advantage over the reported method.

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(1) Carmi, A., Pollack, G., Yellin, H. *J. Org. Chem.* **1960**, *25*, 44.
(2) Karady, S., Ly, M.G., Pines, S.H., Sletzinger, M.S. *J. Org. Chem.* **1971**, *36*, 1949, and references cited therein.
(3) Gustaffson, H. *Acta Chem. Scand. Ser. B* **1975**, *29*, 93.
(4) Achiwa, K., Yamada, S.-I. *Tetrahedron Lett.* **1975**, 2701.
(5) Gennari, C., Colombo, L., Bertolini, G. *J. Am. Chem. Soc.* **1986**, *108*, 6394.
Evans, D.A., Britton, T.C., Dorow, R.L., Dellaria, J.F. *J. Am. Chem. Soc.* **1986**, *108*, 6395.
Trimble, L.A. Vederas, J.C. *J. Am. Chem. Soc.* **1986**, *108*, 6397.
(6) Viret, J., Gabard, J., Collet, A. *Tetrahedron* **1987**, *43*, 891, and references cited therein.
(7) Grupe, R., Niedrich, H. *Chem. Ber.* **1967**, *100*, 3283.
Wang, Y.-F., Izawa, T., Kobayashi, S., Ohno, M. *J. Am. Chem. Soc.* **1982**, *104*, 6465, and references cited therein.
Tanase, S., Guirard, B.M., Snell, E.E. *J. Biol. Chem.* **1985**, *260*, 6738.
Ujváry, I., Hiruma, K., Riddiford, L.M., Matolcsy, G., Roseland, C.R., Kramer, K.J. *Insect Biochem.* **1987**, *17*, 389.

(8) Enders, D., Eichenauer, H. *Chem. Ber.* **1979**, *112*, 2933.
ApSimon, J.W., Collier, T.L. *Tetrahedron* **1986**, *42*, 5157.
(9) Enders, D., Fey, P., Kipphardt, H. *Org. Prep. Proc. Int.* **1985**, *17*, 1.
(10) Schmitz, E., Andreae, S., Schramm, S., Albert, F.-M., Lohmann, D. *DDR Patent* 230865 (1982); *C.A.* **1986**, *105*, 227301.
(11) A preliminary account of this work was given at the *Fourth International Conference on Chemistry and Biotechnology of Biologically Active Natural Products* FECS, Budapest, 1987.
(12) Schmitz, E., Ohme, R., Schramm, S. *Chem. Ber.* **1964**, *97*, 2521.
Improvement of the described procedure: toluene is substituted for ether as solvent while the temperature is kept at 5–10°C; the required toluene solution of reagent **1** is carefully dried with Na_2SO_4 and then by evaporation of a minor part of the solvent *in vacuo*.
Schmitz, E., Schramm, S., Ohme, R. *J. Prakt. Chem.* **1967**, *306*, 86.
Schmitz, E., Ohme, R., Schramm, S., Striegler, H., Heyne, H.-U., Rusche, J. *J. Prakt. Chem.* **1977**, *319*, 195.
Andreae, S., Schmitz, E., Sonnenschein H., Dörnyei G., Szántay Cs., Tamás, J. *J. Prakt. Chem.* **1985**, *327*, 445.
(13) Standard methods[14] were used for deblocking the corresponding *N*-protected Z- and Boc-derivatives to obtain compounds **2**, unless otherwise stated.
(14) Anwer, M., Spatola, A. *Synthesis* **1980**, 929.
Gutte, B., Merrifield, R. *J. Am. Chem. Soc.* **1969**, *91*, 501.
Karlsson, S., Lindeberg, G., Porath, J., Ragnarsson, U. *Acta Chem. Scand.* **1970**, *24*, 1010.
(15) The application of *tert*-butyl esters may be advantageous in case of certain amino acids or dipeptide sequences (e.g. **2a, 2f**) to avoid concurrent formation of piperazinediones (cf, ref. 16). Apparently, this side reaction resulted in diminished yields of **5b** and **5c**. (*R*)-Ala-(*S*)-Pro-OBzl showed especially high propensity to cyclize.[16]
(16) Bodanszky, M., Martinez, J. *Synthesis* **1981**, 333.
Bodanszky, M., in: *Principles of Peptide Synthesis*, Springer-Verlag, Heidelberg, 1984, p. 173, 174.
(17) For substrates **2** sparingly soluble in warm toluene, an inert consolvent, e.g. $CHCl_3$, can be added.
(18) Still, W.C., Kahn, M., Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(19) Amino acid residues have (*S*)-configuration unless otherwise indicated.
(20) The crude product **5b** was contaminated with $\leq 1\%$ of **5c** revealed by HPLC. The Gilson HPLC system used in these studies was assembled with Model 303 pump and Model 116 UV detector at 360 nm. The mobile phase, 100% $CHCl_3$ (1.00 mL/min) was pumped through a LiChrosorb Si-60 (7 µm) column with the dimension of 250 × 0.4 mm. The samples for analyses were freshly dissolved in the mobile phase. The quantitative evaluation was carried out with the Waters 740 Data Modul recording integrator. The retention times of epimers **5b** and **5c** were 5.5 and 6.9 min, respectively.
(21) Routine coupling (DCC/1-hydroxybenztriazole/THF, according to Ref. 22) of Boc-(*R,S*)-Ala and Pro-OBzl afforded this protected dipeptide.
(22) König, W., Geiger, R. *Chem. Ber.* **1970**, *103*, 788, 2024.
(23) Unstable hydrazino compounds **3** could be isolated in the form of salts (e.g. with HCl), however these derivatives proved to be oily, amorphous or strongly hygroscopic. A related work in progress deals with the synthesis of crystalline α-hydrazino acids and their salts.
(24) Attempted amination of Ser-Ile-OCH_3 resulted in a rather contaminated form of the corresponding hydrazone **5**, probably due to side reactions at the hydroxy group of serine.
(25) Cold 0.5 M aq. citric acid can also be applied for extraction of acid labile products **3** giving no higher yield in the case of **5a**.