Synthesis and Evaluation of Enantiomeric Purity of Protected α-Amino and Peptide Aldehydes[1])

by **Thomas Mindt** and **Urs Michel***

University of Applied Sciences, Department of Chemistry, Technikumstrasse 9, CH-8401 Winterthur

and **F. Dick***

Bachem AG, Hauptstrasse 144, CH-4416 Bubendorf

The synthesis of enantiomerically pure Ac-Tyr-Val-Ala-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**1**) is reported, a protected tetrapeptide C-terminal aldehyde belonging to a class of potent, reversible inhibitors of cysteine proteases (*e.g.*, interleukin-1β-converting enzyme (ICE), also called caspase-1). The coupling of the precursors Ac-Tyr-Val-Ala-OH ((*S*)-**8**) and H-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**6**) gave (*S*)-**1** in a yield of 85%, with epimerization of $< 2\%$ at the alanine and aspartic-acid residue. (*S*)-**6** itself was synthesized in four steps in an overall yield of 83% with an ee $> 98\%$.

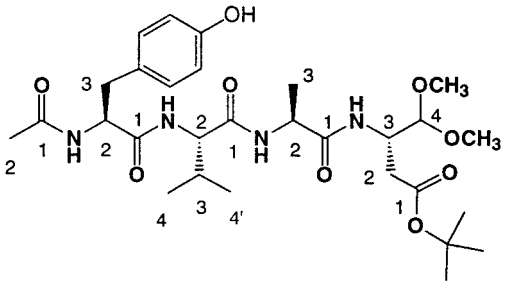
Introduction. – α-Amino and peptide aldehydes are useful synthetic intermediates [1], and some of them are potent inhibitors of proteases [2]. A range of interleukin-1β-converting enzyme (ICE) inhibitors is represented by small peptides with a modified C-terminus. Aspartic acid frequently appears as its α-semialdehyde at the C-terminus in such inhibitors. Especially the acetylated tetrapeptide aldehyde Ac-Tyr-Val-Ala-Asp-H has been reported to be a potent reversible inhibitor of ICE [2–4]. Since a few years ago, it is known that ICE is involved in apoptosis (programmed cell death), which is among the putative initiating factors in chronic and acute inflammatory diseases [5]. As a consequence, there is an increasing demand for such inhibitors, and, therefore, easy synthetic access is of some importance. Unprotected chiral α-amino and peptide aldehydes have the tendency to racemize (epimerize) at the C(α)-atom next to the aldehyde group [1]. The racemization (epimerization) rate depends on structure, temperature, and pH. Under the conditions of flash chromatography (silica gel), such aldehydes are especially prone to racemization (epimerization) [6]. Consequently, viable strategies of synthesis entail a suitably protected form of a given peptide aldehyde which can be transformed easily into the biologically active free form while keeping the stereogenic center at the C-terminal residue intact [2–4]. This is very important since the activity of such inhibitors depends on their enantiomeric purity [5]. Nevertheless, there is only little information available concerning the enantiomeric and diastereoisomeric purity of intermediates and end products, especially with respect to the aspartic acid C(α)-atom [2–4].

We now describe the synthesis of the protected, enantiomerically pure tetrapeptide aldehyde Ac-Tyr-Val-Ala-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**1**), with the main focus

---

[1]) Diploma Thesis of *Thomas Mindt*. Present address: Brown University, Providence RI 02912-1867, USA.

on the following: *i*) Preparation of the key building block (*S*)-**6** and determination of its enantiomeric purity by NMR spectroscopy (^{1}H- and ^{19}F-NMR of its MTPA-amides[2]) [9]), and *ii*) coupling of Ac-Tyr-Val-Ala-OH ((*S*)-**8**) and (*S*)-**6** and determination of the extent of epimerization at the aspartic-acid and alanine residue by ^{1}H-NMR spectroscopy, including full analytical characterization of (*S*)-**1**.



(*S*)-**1** Ac-Tyr-Val-Ala-Asp(O^{t}Bu)-H dimethyl acetal

Results and Discussion. – When planning the synthesis of the target compound (*S*)-**1**, we realized that preparation of the key building block H-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**6**) would be a critical step. In particular, no reliable information about conservation/loss of the chiral integrity during the acetalization could be found. Based on theoretical considerations [7] as well as literature [8], we assumed a high risk of racemization at C(α) during the conversion of protected aspart-1-al in the corresponding dimethyl acetal. Therefore, we decided to synthesize H-(*R*)-Asp(O^{t}Bu)-H dimethyl acetal ((*R*)-**6**) and H-(*RS*)-Asp(O^{t}Bu)-H dimethyl acetal ((*RS*)-**6**) beside (*S*)-**6**, and to convert all three to the MTPA-amides (*S*)-MTPA-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**7**), (*S*)-MTPA-(*R*)-Asp(O^{t}Bu)-H dimethyl acetal ((*R*)-**7**), and (*S*)-MTPA-(*RS*)-Asp(O^{t}Bu)-H dimethyl acetal ((*RS*)-**7**), in order to establish a methodology for the determination of the enantiomeric purity by means of chromatography and/or spectroscopy [9].

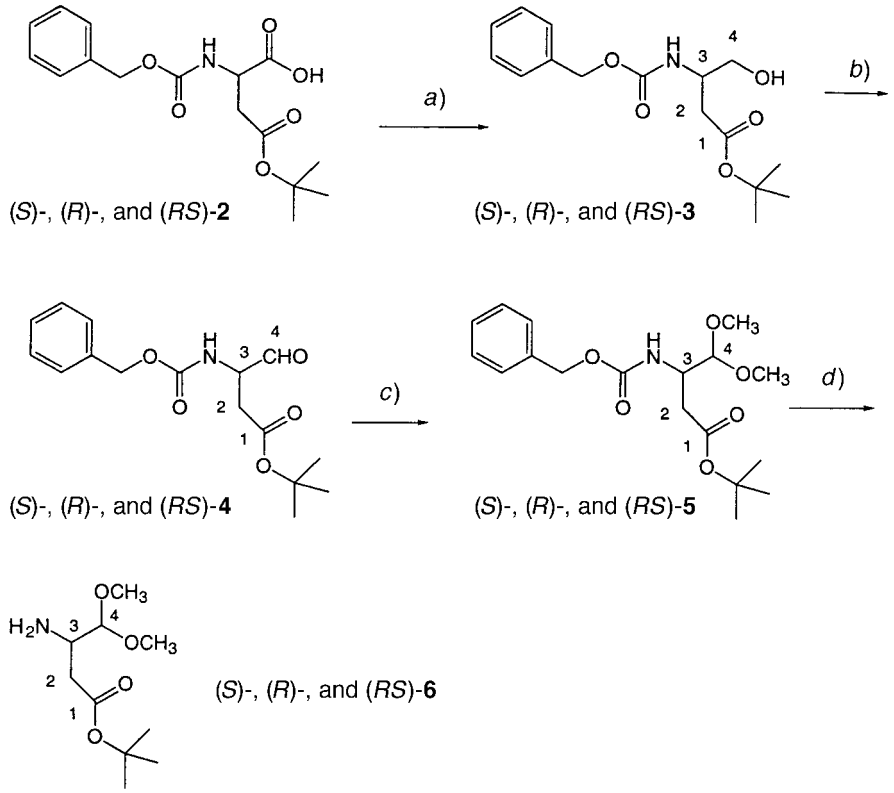
Thus, commercially available Z-Asp(O^{t}Bu)-OH ((*S*)-**2**) was activated to the mixed anhydride with isobutyl choroformate (= isobutyl carbonochloridate) and *N*-methylmorpholine and reduced to the corresponding alcohol Z–Asp(O^{t}Bu)–ol ((*S*)-**3**; purity >95%, yield 95%) [10][11] (*Scheme 1*). (*S*)-**3** was oxidized by mild *Swern* oxidation [12] to Z–Asp(O^{t}Bu)-H ((*S*)-**4**; purity > 95%, yield 95%), which was immediately converted with trimethyl orthoformate/MeOH/TsOH to the stable Z-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**5**; purity >95%, yield 83%) [13]. The (benzyloxy)carbonyl(Z) protecting group was finally removed by hydrogenolysis with 10% Pd/C in MeOH to give (*S*)-**6** (purity > 95%, yield 91%) [14]. According to the same procedure and with comparable yields and purities, (*R*)- and (*RS*)-**6** were synthesized.

Samples of (*S*)-, (*R*)-, and (*RS*)-**6** were subsequently reacted with (–)-(2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride ((*R*)-MTPA-Cl) to furnish the dia-

---

[2]) MTPA = 2-*M*ethoxy-2-(*t*rifluoromethyl)-2-*p*henyl*a*cetyl (= 3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl).

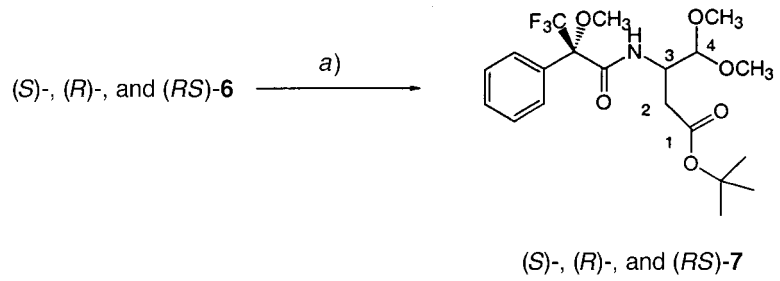
Scheme 1



a) Isobutyl chloroformate/*N*-methylmorpholine/THF, $NaBH_4$, −78°. *b*) $(COCl)_2$/DMSO/CH_2Cl_2, −45°, $^{i}Pr_2EtN$. *c*) MeOH/TsOH/$CH(OMe_3)_3$, r.t. *d*) H_2, 10% Pd/C, MeOH, 48 h, r.t.

stereoisomeric MTPA-amides as individual compounds (*S*)-MTPA-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**7**), (*S*)-MTPA-(*R*)-Asp(O^{t}Bu)-H dimethyl acetal ((*R*)-**7**) and as diastereomer mixture (*S*)-MTPA-(*RS*)-Asp(O^{t}Bu)-H dimethyl acetal ((*RS*)-**7**) (*Scheme 2*).

Scheme 2



a) (*R*)-MTPA-Cl, pyridine/CCl_4, 30 min, r.t., *N*,*N*-dimethylpropane-1,3-diamine [9].

Attempts to separate the diastereoisomers by TLC or HPLC were unsuccessful. Careful spectroscopic analyses, however, allowed us to unambiguously distinguish between (*S*)- and (*R*)-**7**. Determination of the (*R*/*S*) ratio with respect to aspart-1-al was accomplished by comparing selected ^{1}H- and ^{19}F-NMR data (see *Table 1*) of compounds (*S*)-, (*R*)-, and (*RS*)-**7** with corresponding data of artificial mixtures of (*S*)- and (*R*)-**7** (90 : 10, 99 : 1; data not shown). The results can be summarized as follows: *i*) The method of choice for checking enantiomeric purity is ^{1}H-NMR spectroscopy using the *s* of the Boc group (^{19}F-NMR is slightly less sensitive but suitable as well). *ii*) The detection limit of (*S*)-**7** in (*R*)-**7** (and *vice versa*) is 1% or better. *iii*) The prepared (*S*)- and (*R*)-**7** have enantiomeric purities of > 99% (*i.e.*, ee > 98%). These results suggest that acetalization of an α-amino aldehyde – at least in the case of aspartic acid – is possible without concomitant racemization. The behavior of other α-amino aldehydes derived from amino acids is currently under investigation.

Table 1. *Relevant ^{19}F- and ^{1}H-NMR Signals* ($CDCl_3$) *of* (S)*-MTPA-Asp(O^{t}Bu)-H Dimethyl Acetals*

	δ(F) [ppm]	δ(H) [ppm]
	s of CF_3	*s* of Asp (O^{t}Bu)
(*S*)-**7**	– 69.45	1.39
(*R*)-**7**	– 69.35	1.45
(*RS*)-**7**	– 69.35, – 69.45	1.45, 1.39

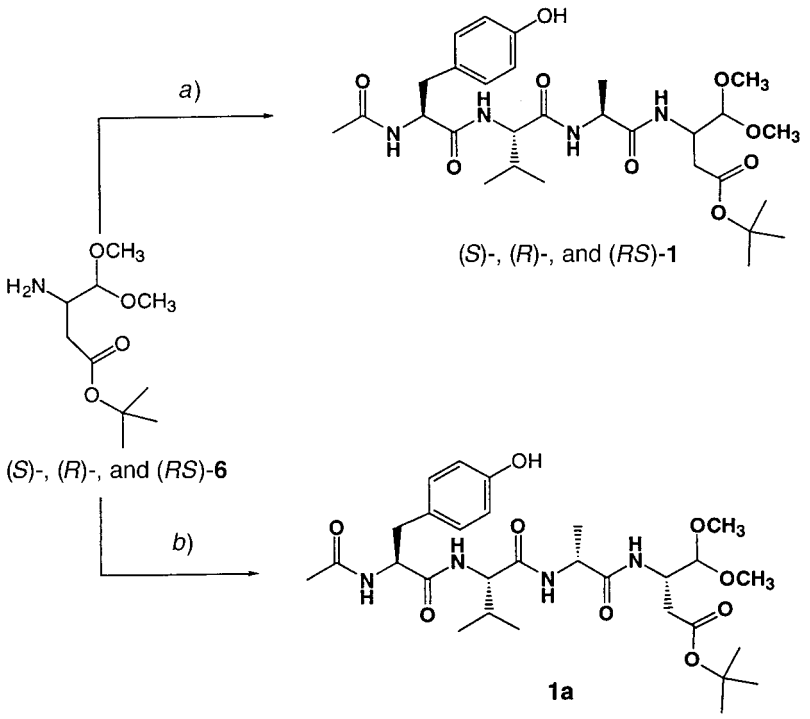
After having verified the quality of the key building block (*S*)-**6**, we concentrated on the final coupling to the target compound (*S*)-**1**. Thus, (*S*)-**8** and (*S*)-**6** were coupled in CH_2Cl_2/DMF with EDCI[3])/*N*-methylmorpholine and 1-hydroxybenzotriazole (HOBt) as additive, conditions which cleanly afforded (*S*)-**1** (purity > 95%, yield 85%; *Scheme 3*).

Since one of the most serious problems in peptide synthesis remains epimerization of the C-terminal residue of the carboxy component during fragment couplings [15] – and the reaction in discussion represents such a coupling – possible epimerization at the alanyl residue in (*S*)-**8** had to be kept in mind. To quantify the extent of epimerization, a similar approach as described for compound (*S*)-**6** was chosen, *i.e.*, diastereoisomers with opposite or mixed configuration at the stereogenic centers in Asp and Ala were prepared and analyzed by ^{1}H-NMR spectroscopy (*Scheme 3*).

Table 2 lists characteristic NMR data of Ac-Tyr-Val-Ala-Asp(O^{t}Bu)-H dimethyl acetal ((*S*)-**1**), Ac-Tyr-Val-Ala-(*R*)-Asp(O^{t}Bu)-H dimethyl acetal ((*R*)-**1**), Ac-Tyr-Val-Ala-(*RS*)-Asp(O^{t}Bu)-H dimethyl acetal ((*RS*)-**1**), and Ac-Tyr-Val-(*R*)-Ala-Asp-(O^{t}Bu)-H dimethyl acetal (**1a**), which were prepared analogously to (*S*)-**1** (for details, see *Exper. Part*). Again, differences in chemical shifts (*s* of ^{t}BuO in Asp and/or *d* of Me(3) in Ala) enabled us to differentiate between individual diastereoisomers. The results of this study are as follows: *i*) The chiral integrity of the Asp(O^{t}Bu)-H dimethyl acetal part is not affected at all, as expected. *ii*) The detection limit of **1a** in (*S*)-**1**, which is the most important in this context, is 2% or better. *iii*) Our preparation of (*S*)-**1** contains less than 2% of **1a**.

[3]) EDCI = 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide.

Scheme 3



a) Ac-Tyr-Val-Ala-OH ((*S*)-**8**), HOBt, *N*-methylmorpholine, EDCl, CH_2Cl_2/DMF. *b*) Ac-Tyr-Val-(*R*)-Ala-OH ((*R*)-**8**), HOBt, *N*-methylmorpholine, EDCl, CH_2Cl_2/DMF; reaction carried out with (*S*)-**6** only.

Table 2. *Relevant ^{1}H-NMR Signals* ((D_6)DMSO) *of the Diastereoisomeric Tetrapeptides*

	δ(H) [ppm]	
	d of Me(3) (Ala)	*s* of ^{t}BuO (Asp)
(*S*)-**1**	1.19	1.35
(*R*)-**1**	1.17	1.36
(*RS*)-**1**	1.17, 1.19	1.35, 1.36
1a	1.15	1.36

In parallel, further trials to separate diastereoisomers by anal. HPLC were carried out with varying degrees of success (data not shown), *i.e.*, the mixtures (*S*)-**1**/**1a** and Ac-Tyr-Val-Ala-Asp-H/Ac-Tyr-Val-(*R*)-Ala-Asp-H were separable, whereas Ac-Tyr-Val-Ala-Asp-H/Ac-Tyr-Val-Ala-(*R*)-Asp-H was not.

The *Figure* illustrates the 2D-COSY spectrum of compound (*S*)-**1**, which is fully consistent with expectation and thus confirms the correct structure, together with the ^{13}C-NMR and FAB-MS data.

In summary, we have developed a five-step synthesis of the protected peptide aldehyde (*S*)-**1** (overall yield *ca.* 70%) which may be of general practicability. In particular, racemization- and epimerization-endangered key steps – especially the

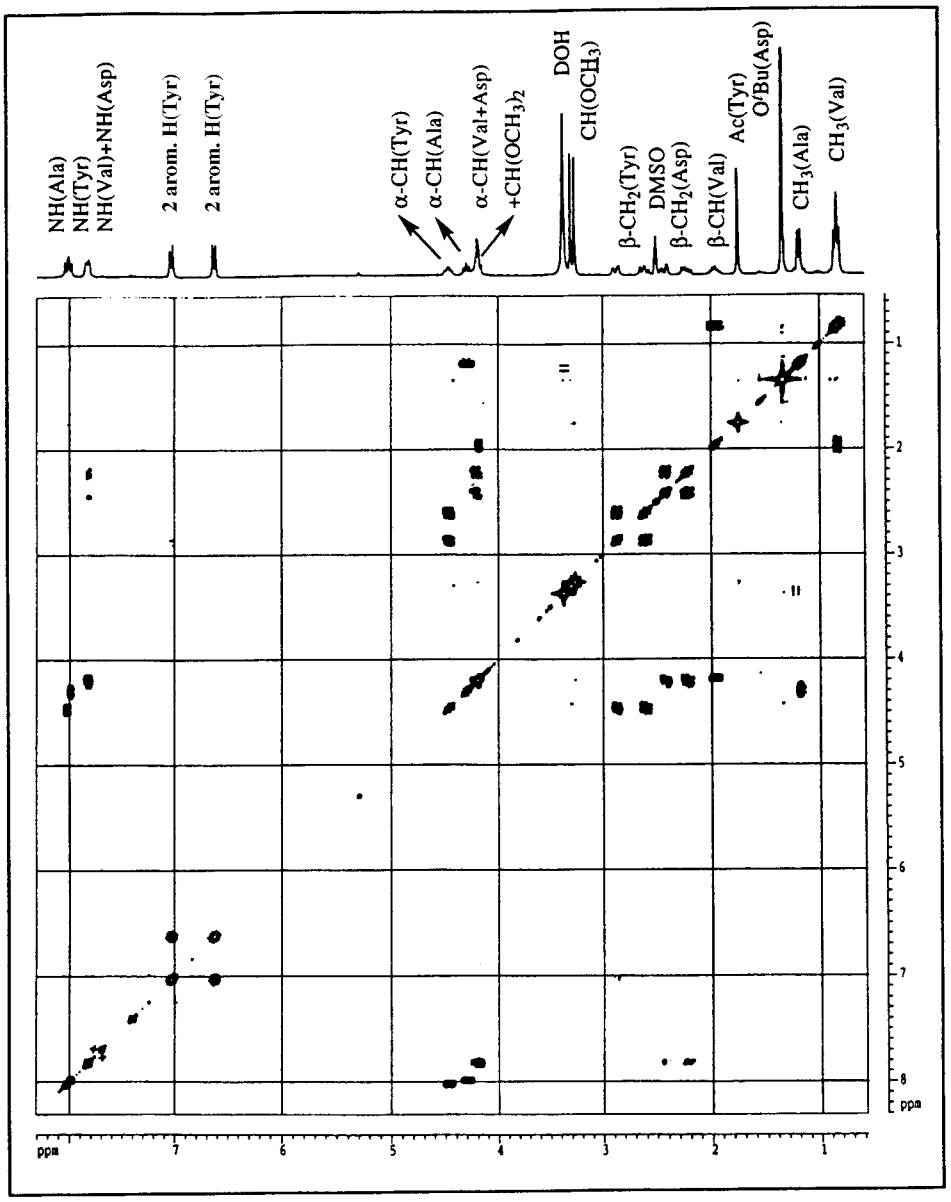


Figure. *2D-COSY(45) of Ac-Tyr-Val-Ala-Asp(O^{t}Bu)-H dimethyl acetal* ((*S*)-**1**) *in* (D_6)*DMSO at 300 MHz*

acetalization of the protected amino aldehyde (*S*)-**4** – were carefully looked at and shown to proceed with almost complete conservation of the chiral integrity at the critical stereocenters.

We thank *F. Nydegger* for MS, *F. Fehr* for NMR spectra, and *A. Ruf* and *M. Vock* for skillful technical assistance.

Experimental Part

General. Reagents were reagent-grade commercials and used without further purification. Amino-acid derivatives and the tripeptides Ac-Tyr-Val-Ala-OH ((*S*)-**8**) and Ac-Tyr-Val-(*R*)-Ala-OH ((*R*)-**8**) were from *Bachem AG*, CH-4416 Bubendorf. Pyridine was distilled over KOH. Other solvents were dried over activated molecular sieves for at least 12 h. Evaporation was done at water-aspirator pressure. Flash chromatography (FC): SiO_2 *60* (230–400 mesh, 0.040–0.063 mm) from *Bachem* and *Fluka*. TLC: precoated plates SiO_2- *60* F_{254} from *Fluka*; visualization by UV light. M.p. *Büchi Smp-20*; uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. IR Spectra (cm^{-1}): *Perkin-Elmer FT-IR 16 PC*. NMR Spectra: *Bruker Avance DPX-300*, *DRX-500*; δ in ppm rel. to internal $SiMe_4$ (^1H) or $CFCl_3$ (^{19}F; unless mentioned otherwise); *J* in Hz. MS: *VG Micromass 70/70e*; FAB (fast-atom bombardment): Ar atoms and 3-nitrobenzyl alcohol matrix.

*(*S*)-4-Hydroxy-3-{[(phenylmethoxy)carbonyl]amino}butanoic Acid 1,1-Dimethylethyl Ester* ((*S*)-**3**; Z-Asp(O^{t}Bu)-ol). At 0–5°, *N*-methylmorpholine (2.80 g, 27.5 mmol) and isobutyl carbonochloridate (3.60 g, 26.25 mmol) were added to a soln. of (*S*)-**2** (8.10 g, 25 mmol) in THF (150 ml). After 15 min, the white suspension was added dropwise at −78° to a suspension of $NaBH_4$ (3.8 g, 50 mmol) in THF/MeOH 3:1 (200 ml) (immediate gas production). After 20 min at −78°, the mixture was quenched with 10% AcOH/H_2O (100 ml), most of the solvent evaporated, the residue extracted with AcOEt (3 × 200 ml), the org. layer washed with 5% $NaHCO_3$ soln. (2 × 200 ml) and H_2O (100 ml), dried (Na_2SO_4), and evaporated, and the residue (9.5 g) purified by FC (SiO_2 (300 g), CH_2Cl_2/MeOH 20:1): (*S*)-**3** (7.7 g, 95%; purity >95% by ^1H-NMR). Slightly yellow oil. $[\alpha]_D^{20} = -9.7$ ($c = 1.2 \cdot 10^{-2}$, MeOH). IR (1% in CCl_4): 3450*m* (OH, NH), 2991*m* (CH), 1733*vs* (C=O), 1513*m* (amide II), 1375*m* (Me), 1261*m* (C–O), 1068*m*. ^1H-NMR (300 MHz, $CDCl_3$): 1.40 (*s*, ^{t}Bu); 2.40–2.55 (*m*, 2 H–C(2)); 3.52 (br. *s*, OH); 3.58–3.70 (*m*, 2 H–C(4)); 3.98–4.11 (*m*, H–C(3)); 5.02, 5.08 (2*d*, ^2J = 12.2, PhCH_2); 5.82 (*d*, ^3J = 8.6, NH); 7.35–7.23 (*m*, 5 arom. H). ^{13}C-NMR (DEPT; 75 MHz, $CDCl_3$): 27.7 (Me); 37.0, 63.8, 66.5 (CH_2); 49.8, 127.8, 128.2, 128.22 (CH); 80.9, 136.2, 156.1, 170.8 (C).

*(*S*)-4-Oxo-3-{[(phenylmethoxy)carbonyl]amino}butanoic Acid 1,1-Dimethylethyl Ester* ((*S*)-**4**; Z-Asp(O^{t}Bu)-H). Dry DMSO (3.95 g, 50.5 mmol) was dissolved under Ar at −45° in CH_2Cl_2 (60 ml), and oxalyl chloride (3.5 g, 27.5 mmol) was added dropwise (immediate gas production). After 5 min, a soln. of (*S*)-**3** (7.1 g, 22.9 mmol) in CH_2Cl_2 (35 ml) was added dropwise at −45° and the white suspension stirred 30 min at this temp. Then ^{i}Pr$_2$EtN (9.2 g, 71 mmol) was added, the mixture allowed to warm up to −20°, and stirring continued for 30 min at −20°. The soln. was diluted with CH_2Cl_2 (200 ml) and extracted with H_2O (50 ml), 1N $NaHSO_4$ (50 ml), and again with H_2O (3 × 50 ml). The org. phase was dried (Na_2SO_4) and evaporated: (*S*)-**4** (7.0 g, 95%; purity >95% by ^1H-NMR). White crystals. M.p. 63–64°. $[\alpha]_D^{20} = -23.4$ ($c = 1.3 \cdot 10^{-2}$, MeOH). IR (1% in CCl_4): 3455*m* (NH), 2996*w* (C–H), 2837*w* and 2717*w* (C–H, aldehyde), 1736*vs* (C=O), 1511*s* (amide II), 1381*m* (Me), 1247*m* (C–O), 1172*m*, 1062*m*, 848*w*. ^1H-NMR (300 MHz, $CDCl_3$): 1.41 (*s*, ^{t}Bu); 2.75 (*dd*, ^2J = 17.1, ^3J = 5.0, 1 H–C(2)); 2.89 (*dd*, ^2J = 17.1, ^3J = 5.0, 1 H–C(2)); 4.37 (*ddd*, ^3J = 8.1, 5.0, 5.0, H–C(3)); 5.12 (*s*, PhCH_2); 6.09 (*d*, ^3J = 8.1, NH); 7.39–7.26 (*m*, 5 arom. H); 9.60 (*s*, H–C(4)). ^{13}C-NMR (DEPT; 75 MHz, $CDCl_3$): 27.8 (Me); 35.4, 67.0 (CH_2); 56.4, 128.0, 128.1, 128.37, 198.8 (CH); 81.8, 135.9, 156.0, 169.9 (C).

*(*S*)-4,4-Dimethoxy-3-{[(phenylmethoxy)carbonyl]amino}butanoic Acid 1,1-Dimethylethyl Ester* ((*S*)-**5**; Z-Asp(O^{t}Bu)-H dimethyl acetal). At r.t., trimethyl orthoformate (11.2 g, 105 mmol) and TsOH (100 mg) were added to a soln. of (*S*)-**4** (6.50 g, 21.1 mmol) in MeOH (30 ml). After 30 min, the mixture was evaporated, the residue dissolved in CH_2Cl_2 (200 ml), and the soln. extracted with 5% $NaHCO_3$ soln. (50 ml) and H_2O (50 ml). The extraction of the aq. layer with CH_2Cl_2 (200 ml) was repeated. The combined org. phase was dried (Na_2SO_4) and evaporated and the residue purified by FC (SiO_2 (300 g), AcOEt/hexane 1:4 with 0.5% Et_3N): (*S*)-**5** (6.5 g, 83%; purity >95% by ^1H-NMR). Yellow oil. $[\alpha]_D^{20} = -17.7$ ($c = 10^{-2}$, MeOH). IR (1% in CCl_4): 3465*m* (NH), 2996*m* and 2947*m* (C–H), 2847*m* (acetal); 1740*vs* (C=O), 1516*s* (amide II), 1381*m* (Me), 1237*m* (C–O), 1173*m*, 1093*m*, 933*m*. ^1H-NMR (300 MHz, $CDCl_3$): 1.42 (*s*, ^{t}Bu); 2.43 (*dd*, ^2J = 15.6, ^3J = 6.9, 1 H–C(2)); 2.53 (*dd*, ^2J = 15.6, ^3J = 5.7, 1 H–C(2)); 4.28–4.13 (*m*, H–C(3)); 3.39 (*s*, (*Me*O)$_2$CH); 4.33 (*d*, ^3J = 4.0, (MeO)$_2$C*H*); 5.12, 5.06 (2*d*, ^2J = 12.3, PhCH_2); 5.40 (*d*, ^3J = 9.2, NH); 7.38–7.25 (*m*, 5 arom. H). ^{13}C-NMR (DEPT; 75 MHz, $CDCl_3$): 27.8 (Me); 35.5, 66.5 (CH_2); 49.5, 127.9, 128.30 (CH); 80.7, 136.4, 155.8, 170.0 (C).

*(*S*)-3-Amino-4,4-dimethoxybutanoic Acid 1,1-Dimethylethyl Ester* ((*S*)-**6**; H-Asp(O^{t}Bu)-H dimethyl acetal). A suspension of (*S*)-**5** (5.90 g, 17 mmol) and 10% Pd/C (300 mg) in MeOH (30 ml) was stirred for 48 h at r.t. under 1 atm H_2. The black suspension was filtered over *Hyflo*, the filtrate evaporated, and the residue triturated with hexane. The insoluble components were filtered off, and the filtrate was evaporated to give (*S*)-**6** (3.5 g, 91%; purity >95% by ^1H-NMR). Slightly yellow liquid. B.p. 160–165°. $[\alpha]_D^{20} = -20.3$ ($c = 10^{-2}$, MeOH). IR (1% in CCl_4): 3406*w* (N–H), 2996*m* (C–H), 2779*m* (C–O, acetal), 1735*s* (C=O), 1461*w*, 1381*m* (Me), 1167*s* (C–O), 1127*m*, 1087*m*, 933*w*, 863*w*. ^1H-NMR (300 MHz, $CDCl_3$): 1.46 (*s*, ^{t}Bu); 1.63 (br. *s*, NH_2); 2.23

(*dd*, ^2J = 16.1, ^3J = 8.8, 1 H–C(2)); 2.52 (*dd*, ^2J = 16.1, ^3J = 3.9, 1 H–C(2)); 3.22–3.31 (*m*, H–C(3)); 3.40, 3.42 (2*s*, (*Me*O)$_2$CH); 4.15 (*d*, ^3J = 5.8, H–C(4)). ^{13}C-NMR (DEPT; 75 MHz, $CDCl_3$): 27.9, 54.4, 54.8 (Me); 38.1 (CH_2); 49.5, 106.9 (CH); 80.0, 171.3 (C).

*(*R*)-3-Amino-4,4-dimethoxybutanoic Acid 1,1-Dimethylethyl Ester* ((*R*)-**6**; H-(*R*)-Asp(O^{t}Bu)-H dimethyl acetal). As described for (*S*)-**6**, from (*R*)-**2** (10.0 g, 30.9 mmol). Overall yield 52%. HPLC: purity >95%.

*(*RS*)-3-Amino-4,4-dimethoxybutanoic Acid 1,1-Dimethylethyl Ester* ((*RS*)-**6**; H-(*RS*)-Asp(O^{t}Bu)-H dimethyl acetal). As described for (*S*)-**6**. Overall yield 68%. Purity >95% by ^{1}H-NMR. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.46 (*s*, ^{t}Bu); 1.97 (br. *s*, NH_2); 2.25 (*dd*, ^2J = 16.2, ^3J = 8.8, 1 H–C(2)); 2.53 (*dd*, ^2J = 16.2, ^3J = 4.0, 1 H–C(2)); 3.27 (*ddd*, ^3J = 8.8, 5.8, 4.0, H–C(3)); 3.41, 3.43 (2*s*, (*Me*O)$_2$CH); 4.15 (*d*, ^3J = 5.8, H–C(4)). ■

*(3*S*)-4,4-Dimethoxy-3-{[(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl]amino}butanoic Acid 1,1-Dimethylethyl Ester* ((*S*)-**7**; (*S*)-MTPA-Asp(O^{t}Bu)-H dimethyl acetal). Under Ar at r.t., (–)-(2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride ((–)-(*R*)-MTPA-Cl) (140 mg, 0.48 mmol) was dissolved in dry pyridine (1.2 ml) and diluted with CCl_4 (1 ml). Subsequently, (*S*)-**6** (90 mg, 0.4 mmol) was added to the white suspension, the mixture stirred for 30 min at r.t., and then an excess of *N*,*N*-dimethylpropane-1,3-diamine (82 mg, 0.8 mmol) added. After 5 min, the soln. was diluted with Et_2O (50 ml) and extracted with 10% aq. citric acid (50 ml), 5% Na_2CO_3 (50 ml), and sat. NaCl soln. (2 × 20 ml). The org. phase was dried (Na_2SO_4) and evaporated: (*S*)-**7** (0.17 g, >90%; purity >90% by ^{1}H-NMR). Yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.39 (*s*, ^{t}Bu); 2.47 (*dd*, ^2J = 15.9, ^3J = 6.4, 1 H–C(2)); 2.53 (*dd*, ^2J = 15.9, ^3J = 5.4, 1 H–C(2)); 3.41, 3.43 (2*s*, (*Me*O)$_2$CH); 3.42–3.46 (*q*, ^5J(H,F) = 1.5 MeO (MTPA)); 4.40–4.53 (*m*, H–C(3), H–C(4)); 7.25 (*d*, ^3J = 10.1, NH); 7.30–7.60 (*m*, 5 arom. H). ^{19}F-NMR (282 MHz, $CDCl_3$): – 69.45 (*s*, CF_3); ee >98%.

*(3*R*)-4,4-Dimethoxy-3-{[(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl]amino}butanoic Acid 1,1-Dimethylethyl Ester* ((*R*)-**7**; (*S*)-MTPA-(*R*)-Asp(O^{t}Bu)-H dimethyl acetal). From (*R*)-**6**, as described for (*S*)-**7**. Yellow oil (0.17 g, >90%; purity >90% by ^{1}H-NMR/HPLC). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.45 (*s*, ^{t}Bu); 2.46 (*dd*, ^2J = 16.0, ^3J = 6.5, 1 H–C(2)); 2.51 (*dd*, ^2J = 15.9, ^3J = 5.4, 1 H–C(2)); 3.24, 3.28 (2*s*, (*Me*O)$_2$CH); 3.34 (*q*, ^5J(H,F) = 1.5, MeO(MTPA)); 4.31 (*d*, H–C(4)); 4.40 (*m*, H–C(3)); 7.23 (*d*, ^3J = 9.1, NH); 7.3–7.5 (*m*, 5 arom. H). ^{19}F-NMR (282 MHz, $CDCl_3$): – 69.35 (*s*, CF_3); ee >98%.

*(3*R*)- and (3*S*)-4,4-Dimethoxy-3-{[(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl]amino}butanoic Acid 1,1-Dimethylethyl Ester* ((*RS*)-**7**; (*S*)-MTPA-(*RS*)-Asp(O^{t}Bu)-H dimethyl acetal). From (*RS*)-**6**, as described for (*S*)-**7**. Yellow oil (0.17 g, >90%; purity >90% by ^{1}H-NMR). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.39 (*s*, ^{t}Bu ((*S*)-**7**)); 1.45 (*s*, ^{t}Bu ((*R*)-**7**)); 2.42–2.63 (*m*, 2 H–C(2)); 3.30, 3.35 (2*s*, (*Me*O)$_2$CH ((*R*)-**7**)); 3.41, 3.42 (2*s*, (*Me*O)$_2$CH ((*S*)-**7**)); 3.4–3.45 (*m*, MeO (MTPA)); 4.37–4.53 (*m*, H–C(3), H–C(4)); 7.15–7.25 (*m*, NH); 7.20–7.60 (*m*, 5 arom. H). ^{19}F-NMR (282 MHz, $CDCl_3$): – 69.45 (*s*, CF_3 ((*S*)-**7**)); – 69.35 (*s*, CF_3 ((*R*)-**7**)).

Ac-Tyr-Val-Ala-Asp(O^{t}Bu)-H Dimethyl Acetal ((*S*)-**1**). At 0–5°, 1-hydroxybenzotriazole monohydrate (1.53 g, 10 mmol), *N*-methylmorpholine (0.91 g, 9 mmol), (*S*)-**6** (1.1 g, 5 mmol), and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (1.16 g, 6.05 mmol) were added to a soln. of Ac-Tyr-Val-Ala-OH ((*S*)-**8**; 2.16 g, 5.5 mmol) in CH_2Cl_2/DMF 1:1 (25 ml). The reaction mixture was stirred for 1 h at 0–5° and additionally 18 h at r.t. After dilution with AcOEt (300 ml), the org. phase was extracted with 10% aq. citric acid (200 ml) and 5% $NaHCO_3$ soln. (200 ml), dried (Na_2SO_4), and evaporated and the residue purified by FC (SiO_2 (250 g), CH_2Cl_2/MeOH 20:1 with 0.5% Et_3N): (*S*)-**1** (2.6 g, 85%; purity >96% by ^{1}H-NMR). White crystals. M.p. 212–214° (dec.). $[\alpha]_D^{20} = -28.9$ ($c = 5.1 \cdot 10^{-3}$, MeOH). ee >98% (by comparison of the ^{1}H-NMR with that of the diastereoisomeric analogs (*R*)-**1** and **1a**). IR (1% in KBr): 3423*m* (N–H), 3092*w* (C–H, arom.), 2982*w* and 2936*w* (C–H), 2854*w* (C–O, acetal), 1743*m* (C=O, ester), 1642*s* (C=O, amide), 1554*m* and 1527*m* (amide II), 1458*w*, 1380*w* (Me), 1242*w* (C–O), 1169*m*, 1095*w*, 857*w*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 0.82 (*d*, ^3J = 6.8, Me(4) or Me(4′) (Val)); 0.85 (*d*, ^3J = 7.2, Me(4) or Me(4′) (Val)); 1.19 (*d*, ^3J = 7.1, Me(3) (Ala)); 1.35 (*s*, ^{t}Bu (Asp)); 1.76 (*s*, Ac); 1.87–2.04 (*m*, H–C(3) (Val)); 2.22 (*dd*, ^2J = 15.0, ^3J = 7.5, 1 H–C(2) (Asp)); 2.43 (*dd*, ^2J = 15.0, ^3J = 4.3, 1 H–C(2) (Asp)); 2.61 (*dd*, ^2J = 13.8, ^3J = 10.2, 1 H–C(3) (Tyr)); 2.87 (*dd*, ^2J = 13.8, ^3J = 3.8, 1 H–C(3) (Tyr)); 3.27, 3.30 (2*s*, each (*Me*O)$_2$CH); 4.13–4.25 (*m*, H–C(2) (Val), H–C(3) (Asp), H–C(4) (Asp)); 4.25–4.36 (*m*, H–C(2) (Ala)); 4.41–4.53 (*m*, H–C(2) (Tyr)); 6.63 (*d*, ^3J = 8.3, 2 arom. H (Tyr)); 7.03 (*d*, ^3J = 8.4, 2 arom. H (Tyr)); 7.74–7.90 (*m*, NH (Val), NH (Asp)); 7.93–8.11 (*m*, NH (Tyr), NH (Ala)); 9.17 (*s*, OH (Tyr)). ^{13}C-NMR (DEPT; 75 MHz, (D_6)DMSO): 18.0, 18.7, 19.9, 22.5, 27.7, 54.2, 55.8 (Me); 35.8, 36.5 (CH_2); 30.6, 47.8, 48.0, 54.3, 57.4, 104.8, 114.8, 130.1 (CH); 79.1, 128.1, 155.7, 169.2, 169.8, 170.2, 171.5, 171.6 (C). FAB-MS: 595.3 ([*M* + H]$^+$), 617.3 ([*M* + Na]$^+$).

*Ac-Tyr-Val-Ala-(*R*)-Asp(O^{t}Bu)-H Dimethyl Acetal* ((*R*)-**1**). As described for (*S*)-**1**, from (*R*)-**6** (1.39 g, 6.35 mmol): (*R*)-**1** (1.37 g, 35%; purity >95% by HPLC). M.p. 226–227°. White crystals. $[\alpha]_D^{20} = -13.7$ ($c = 5.1 \cdot 10^{-3}$, MeOH). ^{1}H-NMR (500 MHz, (D_6)DMSO): 0.83 (*d*, ^3J = 6.9, Me(4) or Me(4′) (Val)); 0.85 (*d*, ^3J = 6.7, Me(4) or Me(4′) (Val)); 1.17 (*d*, ^3J = 7.0, Me(3) (Ala)); 1.36 (*s*, ^{t}Bu (Asp)); 1.75 (*s*, Ac); 1.94–2.00 (*m*, H–C(3)

(Val)); 2.20 (*dd*, ^2J = 15.3, ^3J = 8.6, 1 H–C(2) (Asp)); 2.46 (*dd*, ^2J = 15.3, ^3J = 4.1, 1 H–C(2) (Asp)); 2.61 (*dd*, ^2J = 13.9, ^3J = 10.1, 1 H–C(3) (Tyr)); 2.87 (*dd*, ^2J = 14.1, ^3J = 3.9, 1 H–C(3) (Tyr)); 3.25, 3.31 (2*s*, (*Me*O)$_2$CH); 4.15–4.24 (*m*, H–C(2) (Val), H–C(3) (Asp), H–C(4) (Asp)); 4.25–4.33 (*m*, H–C(2) (Ala)); 4.42–4.50 (*m*, H–C(2) (Tyr)); 6.62 (*d*, ^3J = 8.6, 2 arom. H (Tyr)); 7.03 (*d*, ^3J = 8.6, 2 arom. H (Tyr)); 7.83 (*d*, ^3J = 8.9, NH (Val or Asp)); 7.87 (*d*, ^3J = 8.3, NH (Val or Asp)); 7.98 (*d*, ^3J = 7.4, NH (Ala or Tyr)); 8.5 (*d*, ^3J = 7.4, NH (Ala or Tyr)); 9.13 (*s*, OH (Tyr)).

*Ac-Tyr-Val-Ala-(*RS*)-Asp(O*^{t}*Bu)-H Dimethyl Acetal* ((*RS*)-**1**). As described for (*S*)-**1**, from (*RS*)-**6** (220 mg, 1.0 mmol) and (*S*)-**8** (433 mg, 1.1 mmol): (*RS*)-**1** (0.2 g, 40%; purity >95% by ^{1}H-NMR). White crystals. ^{1}H-NMR (300 MHz, (D$_6$)DMSO): 0.825 (*d*, ^3J = 5.1, Me(4) or Me(4′) (Val)); 0.85 (*d*, ^3J = 6.5, Me(4) or Me(4′) (Val)); 1.17, 1.19 (2*d*, ^3J = 6.7, 6.85, Me(3) (Ala, both diastereoisomers)); 1.348, 1.364 (2*s*, ^{t}Bu (Asp, both diastereoisomers)); 1.75 (*s*, Ac); 1.91–2.03 (*m*, H–C(3), (Val)); 2.14–2.33 (*m*, 1 H–C(2) (Asp)); 2.38–2.48 (*m*, 1 H–C(2) (Asp)); 2.61 (*dd*, ^2J = 13.7, ^3J = 10.0, 1 H–C(3) (Tyr)); 2.87 (*dd*, ^2J = 13.7, ^3J = 3.5, 1 H–C(3) (Tyr)); 3.27, 3.30 (2*s*, (*Me*O)$_2$CH ((*S*)-Asp diastereoisomer)); 3.25, 3.31 (2*s*, (MeO)$_2$CH ((*R*)-Asp diastereoisomer)); 4.07–4.33 (*m*, H–C(2) (Ala), H–C(2) (Val), H–C(3) (Asp), H–C(4) (Asp)); 4.41–4.53 (*m*, H–C(2) (Tyr)); 6.63 (*d*, ^3J = 8.3, 2 arom. H (Tyr)); 7.03 (*d*, ^3J = 7.4, 2 arom. H (Tyr)); 7.62–8.37 (*m*, NH (Val), NH (Asp), NH (Tyr), NH (Ala)); 9.11 (*s*, OH (Tyr)).

*Ac-Tyr-Val-(*R*)-Ala-Asp(O*^{t}*Bu)-H Dimethyl Acetal* (**1a**). As described for (*S*)-**1**, from (*S*)-**6** (110 mg, 0.5 mmol) and Ac-Tyr-Val-(*R*)-Ala-OH ((*R*)-**8**; 217 mg, 0.6 mmol): **1a** (0.25 g, 70%; purity >95% by ^{1}H-NMR). ee >98% (by comparison with the diastereoisomeric analogs). White crystals. ^{1}H-NMR (300 MHz, (D$_6$)DMSO): 0.83 (*d*, ^3J = 6.7, Me(4) (Val), Me(4′) (Val)); 1.15 (*d*, ^3J = 7.0, Me(3) (Ala)); 1.36 (*s*, ^{t}Bu (Asp)); 1.75 (*s*, Ac); 1.87–2.01 (*m*, H–C(3) (Val)); 2.22 (*dd*, ^2J = 15.2, ^3J = 8.5, 1 H–C(2) (Asp)); 2.43 (*dd*, ^2J = 15.5, ^3J = 4.0, 1 H–C(2) (Asp)); 2.61 (*dd*, ^2J = 13.9, ^3J = 10.1, 1 H–C(3) (Tyr)); 2.87 (*dd*, ^2J = 13.9, ^3J = 3.9, 1 H–C(3) (Tyr)); 3.26, 3.31 (2*s*, (*Me*O)$_2$CH); 4.10–4.37 (*m*, H–C(2) (Val), H–C(2) (Ala), H–C(3) (Asp), H–C(4) (Asp)); 4.40–4.52 (*m*, 1 H–C(2) (Tyr)); 6.63 (*d*, ^3J = 8.4, 2 arom. H (Tyr)); 7.02 (*d*, ^3J = 8.4, 2 arom. H (Tyr)); 7.80–8.15 (4*d*, ^3J = 7.7, 8.3, 8.4, 8.7, NH (Tyr), NH (Ala), NH (Val), NH (Asp)); 9.16 (*s*, OH (Tyr)).

REFERENCES

[1] J. Jurczak, A. Golebiowski, *Chem. Rev.* **1989**, *68*, 14; M. Reetz, *Angew. Chem*. **1991**, *12*, 1559.
[2] K. Chapman, *Bioorg. Med. Chem. Lett*. **1992**, *6*, 613.
[3] T. D. Graybill, R. E. Dolle, C. T. Helaszek, R. E. Miller, M. A. Ator, *Int. J. Pept. Protein Res.* **1994**, *44*, 173.
[4] M. D. Mullican, D. J. Lauffer, R. J. Gillespie, S. S. Matharau, D. Kay, G. M. Porritt, L. Evans, J. M. C. Golec, M. A. Murcko, Y. P. Luong, S. A. Raybuck, D. J. Livingston, *Biorg. Med. Chem. Lett.* **1994**, *4*, 2359.
[5] A. Thornberry, *Nature (London)* **1992**, *356*, 768; F. G. Evan, *Cell (Cambridge, Mass.)* **1996**, *86*, 781; S. Nagata, *ibid*. **1997**, *88*, 355; N. A. Thornberry, Y. Lazebnik, *Science (Washington, D.C.)* **1998**, *281*, 1312.
[6] A. Ito, R. Takahashi, Y. Baba, *Chem. Pharm. Bull*. **1975**, *23*, 3081.
[7] J. March, 'Advanced Organic Chemistry', John Wiley & Sons, 4th Edition, New York – Chichester – Brisbane – Toronto – Singapore 1992, 890.
[8] T. Saino, T. Someno, H. Myazaki, S. Ishii, *Chem. Pharm. Bull*. **1982**, *30*, 2319.
[9] J. A. Dale, D. Dull, H. S. Mosher, *J. Org. Chem.* **1969**, *9*, 2543; J. A. Dale, H. A. Mosher, *J. Am. Chem. Soc*. **1973**, *95*, 512.
[10] G. Kokotos, *J. Org. Chem.* **1990**, *4*, 299.
[11] A. El Marini, M. L. Roumestant, Ph. Viallefont, D. Razafindramboa, M. Bonato, M. Follet, *Synthesis* **1992**, 1104.
[12] A. Manusco, S. Huang, D. Swern, *J. Org. Chem.* **1978**, *12*, 2480.
[13] ' Houben-Weyl, Methoden der organischen Chemie', Georg Thieme Verlag, Stuttgart, 1954, Vol. VII/1.
[14] J. Jones, 'The Chemical Synthesis of Peptides', Clarendon Press, Oxford, 1991; P. Bailey, 'An Introduction to Peptide Chemistry', Salle & Sauerländer, Aarau–Frankfurt–Salzburg, 1992; M. Bodanski, A. Bodansky, 'The Practice of Peptide Synthesis', Springer, Berlin, 1994, pp. 17, 28, and 199.
[15] N. L. Benoiton, *Biopolymers (Pept. Sci.)* **1996**, *40*, 245 and refs. cit. therein.

Received July 14, 1999