

Synthesis and Evaluation of Enantiomeric Purity of Protected α -Amino and Peptide Aldehydes¹⁾

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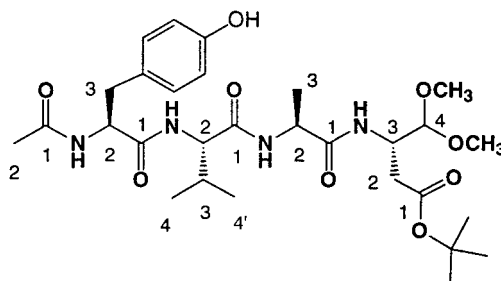
The synthesis of enantiomerically pure Ac-Tyr-Val-Ala-Asp(O'Bu)-H dimethyl acetal ((*S*)-**1**) is reported, a protected tetrapeptide C-terminal aldehyde belonging to a class of potent, reversible inhibitors of cysteine proteases (*e.g.*, interleukin-1 β -converting enzyme (ICE), also called caspase-1). The coupling of the precursors Ac-Tyr-Val-Ala-OH ((*S*)-**8**) and H-Asp(O'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-1 β -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'Bu)-H dimethyl acetal ((*S*)-**1**), with the main focus

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on the following: *i*) Preparation of the key building block (*S*)-**6** and determination of its enantiomeric purity by NMR spectroscopy (^1H - and ^{19}F -NMR of its MTPA-amides²) [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 ^1H -NMR spectroscopy, including full analytical characterization of (*S*)-**1**.



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

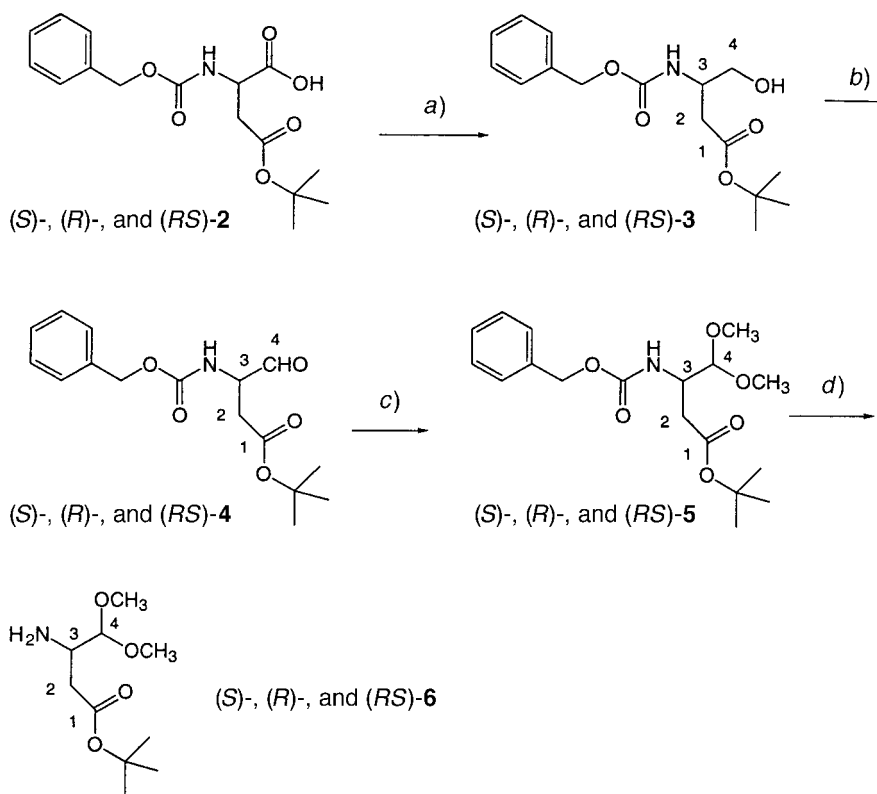
Results and Discussion. – When planning the synthesis of the target compound (*S*)-**1**, we realized that preparation of the key building block H-Asp(O^{*t*}Bu)-H dimethyl acetal ((*S*)-**6**) would be a critical step. In particular, no reliable information about conservation/loss of the chiral integrity 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 chloroformate (= 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-Methoxy-2-(trifluoromethyl)-2-phenylacetyl (= 3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl).

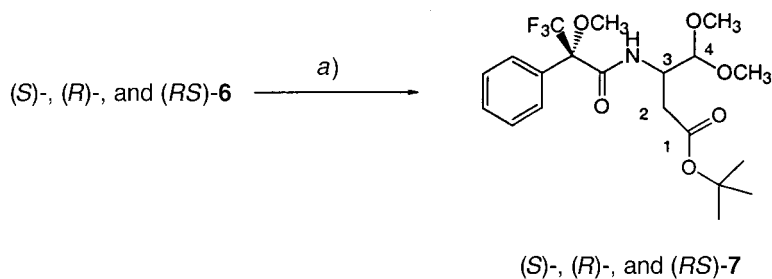
Scheme 1



a) Isobutyl chloroformate/*N*-methylmorpholine/THF, NaBH₄, -78°. b) (COCl)₂/DMSO/CH₂Cl₂, -45°, ⁱPr₂EtN. c) MeOH/TsOH/CH(OMe)₃, r.t. d) H₂, 10% Pd/C, MeOH, 48 h, r.t.

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

Scheme 2



a) (*R*)-MTPA-Cl, pyridine/CCl₄, 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 ^1H - 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 ^1H -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 ^1H -NMR Signals (CDCl_3) of (*S*)-MTPA-Asp(*O*^{*t*}Bu)-H Dimethyl Acetals

	$\delta(\text{F})$ [ppm] <i>s</i> of CF_3	$\delta(\text{H})$ [ppm] <i>s</i> of Asp (<i>O</i> ^{<i>t</i>} Bu)
(<i>S</i>)- 7	– 69.45	1.39
(<i>R</i>)- 7	– 69.35	1.45
(<i>RS</i>)- 7	– 69.35, – 69.45	1.45, 1.39

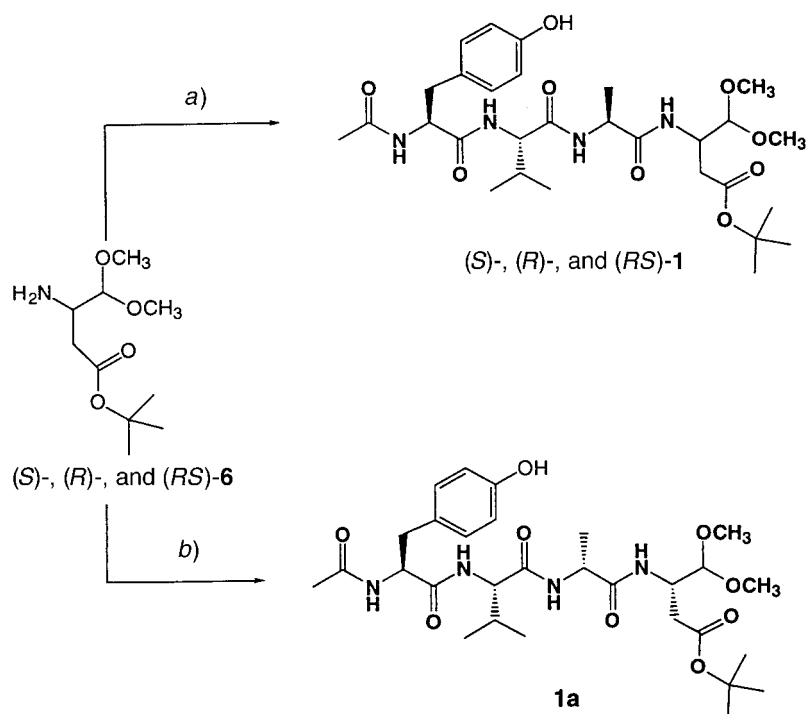
After having verified the quality of the key building block (*S*)-**6**, we concentrated on the final coupling to the target compound (*S*)-**1**. Thus, (*S*)-**8** and (*S*)-**6** were coupled in $\text{CH}_2\text{Cl}_2/\text{DMF}$ with $\text{EDCI}^3)/N$ -methylmorpholine and 1-hydroxybenzotriazole (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 ^1H -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*}Bu 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) $\text{EDCI} = 1$ -[3-(Dimethylamino)propyl]-3-ethylcarbodiimide.

Scheme 3



a) Ac-Tyr-Val-Ala-OH ((S)-**8**), HOBt, *N*-methylmorpholine, EDCl, CH₂Cl₂/DMF. b) Ac-Tyr-Val-(*R*)-Ala-OH ((*R*)-**8**), HOBt, *N*-methylmorpholine, EDCl, CH₂Cl₂/DMF; reaction carried out with (*S*)-**6** only.

Table 2. Relevant ¹H-NMR Signals ((D₆)DMSO) of the Diastereoisomeric Tetrapeptides

	δ(H) [ppm]	
	<i>d</i> of Me(3) (Ala)	<i>s</i> of ^t BuO (Asp)
(<i>S</i>)- 1	1.19	1.35
(<i>R</i>)- 1	1.17	1.36
(<i>RS</i>)- 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 ¹³C-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

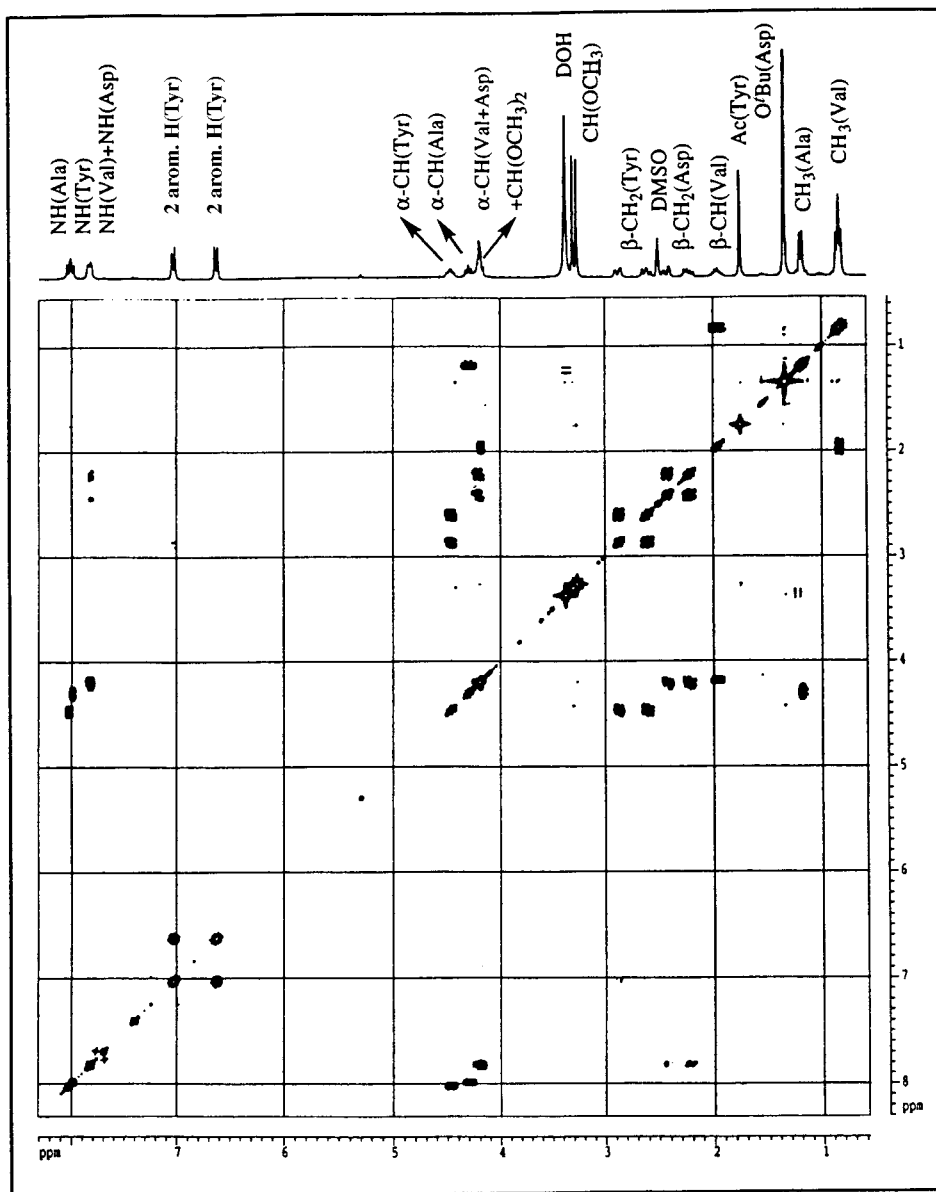


Figure. 2D-COSY(45) of Ac-Tyr-Val-Ala-Asp(O'Bu)-H dimethyl acetal ((S)-1) in (D_6)DMSO at 300 MHz

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.

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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): SiO₂ 60 (230–400 mesh, 0.040–0.063 mm) from *Bachem* and *Fluka*. TLC: precoated plates SiO₂-60 *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 SiMe₄ (¹H) or CFCl₃ (¹⁹F; 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'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 NaBH₄ (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/H₂O (100 ml), most of the solvent evaporated, the residue extracted with AcOEt (3 × 200 ml), the org. layer washed with 5% NaHCO₃ soln. (2 × 200 ml) and H₂O (100 ml), dried (Na₂SO₄), and evaporated, and the residue (9.5 g) purified by FC (SiO₂ (300 g), CH₂Cl₂/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 CCl₄): 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, CDCl₃): 1.40 (s, '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*, ²*J* = 12.2, PhCH₂); 5.82 (*d*, ³*J* = 8.6, NH); 7.35–7.23 (*m*, 5 arom. H). ¹³C-NMR (DEPT; 75 MHz, CDCl₃): 27.7 (Me); 37.0, 63.8, 66.5 (CH₂); 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'Bu)-H). Dry DMSO (3.95 g, 50.5 mmol) was dissolved under Ar at –45° in CH₂Cl₂ (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 CH₂Cl₂ (35 ml) was added dropwise at –45° and the white suspension stirred 30 min at this temp. Then ⁱPr₂EtN (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 CH₂Cl₂ (200 ml) and extracted with H₂O (50 ml), 1*N* NaHSO₄ (50 ml), and again with H₂O (3 × 50 ml). The org. phase was dried (Na₂SO₄) 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 CCl₄): 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, CDCl₃): 1.41 (s, 'Bu); 2.75 (*dd*, ²*J* = 17.1, ³*J* = 5.0, 1 H–C(2)); 2.89 (*dd*, ²*J* = 17.1, ³*J* = 5.0, 1 H–C(2)); 4.37 (*ddd*, ³*J* = 8.1, 5.0, 5.0, H–C(3)); 5.12 (s, PhCH₂); 6.09 (*d*, ³*J* = 8.1, NH); 7.39–7.26 (*m*, 5 arom. H); 9.60 (s, H–C(4)). ¹³C-NMR (DEPT; 75 MHz, CDCl₃): 27.8 (Me); 35.4, 67.0 (CH₂); 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'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 CH₂Cl₂ (200 ml), and the soln. extracted with 5% NaHCO₃ soln. (50 ml) and H₂O (50 ml). The extraction of the aq. layer with CH₂Cl₂ (200 ml) was repeated. The combined org. phase was dried (Na₂SO₄) and evaporated and the residue purified by FC (SiO₂ (300 g), AcOEt/hexane 1:4 with 0.5% Et₃N): (*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 CCl₄): 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, CDCl₃): 1.42 (s, 'Bu); 2.43 (*dd*, ²*J* = 15.6, ³*J* = 6.9, 1 H–C(2)); 2.53 (*dd*, ²*J* = 15.6, ³*J* = 5.7, 1 H–C(2)); 4.28–4.13 (*m*, H–C(3)); 3.39 (s, (MeO)₂CH); 4.33 (*d*, ³*J* = 4.0, (MeO)₂CH); 5.12, 5.06 (2*d*, ²*J* = 12.3, PhCH₂); 5.40 (*d*, ³*J* = 9.2, NH); 7.38–7.25 (*m*, 5 arom. H). ¹³C-NMR (DEPT; 75 MHz, CDCl₃): 27.8 (Me); 35.5, 66.5 (CH₂); 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'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 H₂. 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 CCl₄): 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, CDCl₃): 1.46 (s, 'Bu); 1.63 (br. s, NH₂); 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*, (MeO)₂CH); 4.15 (*d*, $^3J = 5.8$, H–C(4)). ¹³C-NMR (DEPT; 75 MHz, CDCl₃): 27.9, 54.4, 54.8 (Me); 38.1 (CH₂); 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^{*i*}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^{*i*}Bu)-H dimethyl acetal). As described for (*S*)-**6**. Overall yield 68%. Purity >95% by ¹H-NMR. ¹H-NMR (300 MHz, CDCl₃): 1.46 (*s*, ^{*t*}Bu); 1.97 (*br. s*, NH₂); 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*, (MeO)₂CH); 4.15 (*d*, $^3J = 5.8$, H–C(4)). ■

(*3S*)-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^{*i*}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 CCl₄ (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 Et₂O (50 ml) and extracted with 10% aq. citric acid (50 ml), 5% Na₂CO₃ (50 ml), and sat. NaCl soln. (2 × 20 ml). The org. phase was dried (Na₂SO₄) and evaporated: (*S*)-**7** (0.17 g, >90%; purity >90% by ¹H-NMR). Yellow oil. ¹H-NMR (300 MHz, CDCl₃): 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*, (MeO)₂CH); 3.42–3.46 (*q*, $^5J(\text{H,F}) = 1.5$ 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). ¹⁹F-NMR (282 MHz, CDCl₃): –69.45 (*s*, CF₃); ee >98%.

(*3R*)-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^{*i*}Bu)-H dimethyl acetal). From (*R*)-**6**, as described for (*S*)-**7**. Yellow oil (0.17 g, >90%; purity >90% by ¹H-NMR/HPLC). ¹H-NMR (500 MHz, CDCl₃): 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*, (MeO)₂CH); 3.34 (*q*, $^5J(\text{H,F}) = 1.5$, 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). ¹⁹F-NMR (282 MHz, CDCl₃): –69.35 (*s*, CF₃); ee >98%.

(*3R*)- and (*3S*)-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^{*i*}Bu)-H dimethyl acetal). From (*RS*)-**6**, as described for (*S*)-**7**. Yellow oil (0.17 g, >90%; purity >90% by ¹H-NMR). ¹H-NMR (300 MHz, CDCl₃): 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*, (MeO)₂CH ((*R*)-**7**)); 3.41, 3.42 (2*s*, (MeO)₂CH ((*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). ¹⁹F-NMR (282 MHz, CDCl₃): –69.45 (*s*, CF₃ ((*S*)-**7**)); –69.35 (*s*, CF₃ ((*R*)-**7**)).

Ac-Tyr-Val-Ala-Asp(O^{*i*}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 CH₂Cl₂/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% NaHCO₃ soln. (200 ml), dried (Na₂SO₄), and evaporated and the residue purified by FC (SiO₂ (250 g), CH₂Cl₂/MeOH 20:1 with 0.5% Et₃N): (*S*)-**1** (2.6 g, 85%; purity >96% by ¹H-NMR). White crystals. M.p. 212–214° (dec.). [α]_D²⁰ = –28.9 (*c* = 5.1 · 10^{–3}, MeOH). ee >98% (by comparison of the ¹H-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*. ¹H-NMR (300 MHz, (D₆)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 (MeO)₂CH); 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)). ¹³C-NMR (DEPT; 75 MHz, (D₆)DMSO): 18.0, 18.7, 19.9, 22.5, 27.7, 54.2, 55.8 (Me); 35.8, 36.5 (CH₂); 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^{*i*}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. [α]_D²⁰ = –13.7 (*c* = 5.1 · 10^{–3}, MeOH). ¹H-NMR (500 MHz, (D₆)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 (2s, (MeO)₂CH); 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^tBu)-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 ¹H-NMR). White crystals. ¹H-NMR (300 MHz, (D₆)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 (2s, ^tBu (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 (2s, (MeO)₂CH ((S)-Asp diastereoisomer)); 3.25, 3.31 (2s, (MeO)₂CH ((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^tBu)-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 ¹H-NMR). ee >98% (by comparison with the diastereoisomeric analogs). White crystals. ¹H-NMR (300 MHz, (D₆)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, ^tBu (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 (2s, (MeO)₂CH); 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)).

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