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# Proteolytically stable peptides by incorporation of α-Tfm amino acids

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Abstract: A series of model peptides containing α-trifluoromethyl-substituted amino acids in five different positions relative to the predominant cleavage site of the serine protease α-chymotrypsin was synthesized by solution methods to investigate the influence of α-Tfm substitution on the proteolytic stability of peptides. Proteolysis studies demonstrated absolute stability of peptides substituted in the $P_1$ position and still considerable proteolytic stability for peptides substituted at the $P_2$ and $P_2'$ positions compared with the corresponding unsubstituted model peptide. Comparison with peptides containing the fluorine-free disubstituted amino acid α-aminoisobutyric acid allowed to separate electronic from steric effects. Furthermore, the absolute configuration of the α-Tfm-substituted amino acid was found to exert considerable effects on the proteolytic stability, especially in $P_1'$ substituted peptides. Investigations of this phenomenon using empirical force field calculations revealed that in the (*S,R,S*)-diasteromer the steric constraints exhibited by the α-Tfm group can be outweighed by an advantageous interaction of the fluorine atoms with the serine side chain of the enzyme. In contrast, a favourable interaction between substrate and enzyme is impossible for the (*S,S,S*)-diastereomer. © 1997 European Peptide Society and John Wiley & Sons, Ltd.

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## Introduction

Some major disadvantages for the application of peptides as pharmaceuticals are their low bioavailability, their sensitivity to enzymatic degradation, and their low selectivity because of the considerable conformational flexibility leading to undesired interactions with different receptors [1–3]. Therefore, investigation of the biological properties and three-dimensional structure of peptides rich in the conformationally restricted $C^{\alpha,\alpha}$-disubstituted amino acids is of current interest. Certain $C^{\alpha,\alpha}$-dialkylated amino acids have been shown to impart well-defined and predictable conformations to the peptide backbone [4–7]. These amino acids, in particular α-methylalanine (α-aminoisobutyric acid; Aib), are present in naturally occurring peptide antibiotics [8], conferring a stable helical secondary structure on them and, thereby, ion-transporting properties. Furthermore, peptides containing these residues tend to slow down degradation processes dramatically [9].

Owing to the unique electronic properties of fluorine substituents, α-trifluoromethyl substituted amino acids (α-Tfm amino acids) form a special class of α,α-disubstituted amino acids and are, therefore, interesting building blocks for peptide synthesis [10]. A trifluoromethyl group in the α-position of an amino acid exerts considerable polarization effects on the neighbouring substituents. This structural alteration may influence the hydrolytic stability of peptides, resulting in retarded degradation by peptidases [11] and, consequently, in prolonged intrinsic activity. The often postulated quasi-isosterism

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between a methyl and a trifluoromethyl group is still controversial [12]. In several chemical reactions the trifluoromethyl group behaves as a much larger substituent than a butyl or phenyl group [13]. For that reason, the steric requirements of a trifluoromethyl group seem to be closer to those of an isopropyl group. Hence, severe conformational restrictions are imposed on a peptide chain by incorporation of α-Tfm amino acids [14]. Furthermore, owing to the high electron density, the trifluoromethyl group is capable of participating in hydrogen bonding as an electron pair donor. This property confers additional ways of interaction with enzyme or receptor subsites for trifluoromethyl-substituted peptides which cannot be found in the fluorine-free pendants.

We started to incorporate α-Tfm amino acids into peptides in order to investigate systematically the steric, electronic and hydrophobic effects of a trifluoromethyl group on the proteolytic stability. For this study, α-chymotrypsin was selected as a representative serine protease from human tissue. This pancreatic enzyme is one of the best investigated proteases concerning the catalytic mechanism, the substrate specificity and the three-dimensional structure [15]. Therefore, it represents an ideal model protease for the investigation of the proteolytic stability of peptides chemically modified by incorporation of non-natural amino acids.

## Materials and Methods

### General

Melting points (not corrected) were determined using a Tottoli apparatus (Büchi SMP-20). Elemental microanalyses were carried out with a Heraeus CHN-elemental analyser. $^{1}$H-NMR, $^{13}$C-NMR and $^{19}$F-NMR spectra were recorded with a Bruker AM 360 spectrometer at 360, 90 and 339 MHz, respectively. $^{19}$F-NMR spectra were obtained using a Bruker AC 250 (235 MHz) spectrometer. TMS was used as the reference standard for $^{1}$H- and $^{13}$C-spectra (internal), trifluoroacetic acid for $^{19}$F-NMR spectra (external). Owing to the low concentration of the model peptides, in some cases chemical shifts and coupling constants of the quaternary C-atoms could not be extracted. Mass spectra were recorded using thermospray ionization with a Hewlett Packard 5989 A instrument. The enzymatic hydrolysis reactions were monitored by RP-HPLC using a gradient system (eluent: 100% A to 65% B in 30 min; [A] = 95% $H_2O$/5% $CH_3CN$/0.1% TFA; [B] = 5% $H_2O$/95% $CH_3CN$/0.1% TFA) or isocratic systems containing acetonitrile/water/0.1% TFA mixtures in various volume ratios and a flow of 1.0–1.2 ml/min. Peptide and product ratios were detected at $\lambda = 254$ nm using a Vydac 218TP54 column (The Separation Group).

*N*,*N*′-diisopropylcarbodiimide (DIC), *N*-methylmorpholine (NMM), isobutylchloroformate, palladium on charcoal (Pd/C) were purchased from commercial suppliers and used without further purification. Solvents were dried using standard methods. Derivatives of the natural amino acids and DL-H-(α-Me)Phe-OH were obtained from Bachem (Switzerland) and Aldrich, respectively. Z-protection of DL-H-(α-Me)Phe-OH was achieved via the standard procedure. α-Chymotrypsin was purchased from Serva (Germany) and was used without further purification. α-Tfm amino acids and their protected derivatives were obtained as racemates via known procedures [16, 17]. Flash chromatography was performed using silica gel (0.032–0.064 μm) from Riedel de Haën with $CHCl_3$/methanol and ethyl acetate/hexanes as eluent, respectively.

### Peptide Syntheses

The syntheses of the model peptides were carried out using solution methods starting from the C-terminus. The activation of the Z-protected natural amino acids was achieved via mixed anhydrides while in the case of the Z-protected Aib and α-Tfm amino acids the activation via DIC proved to be the more favourable method [12, 18]. The Z-protecting group of all intermediates was cleaved by hydrogenolysis. The purification of all protected intermediates and of the final model peptides was carried out by flash chromatography and recrystallization. Generally, the diastereomer of the α-Tfm-substituted model peptides first eluated was denominated as "I" and the second eluated peptide as "II", respectively.

### Mixed Anhydride Method

Two mmol of NMM and 2.0 mmol diisopropylchloroformate were added to a stirred solution of 2.0 mmol Z-(α-Tfm)Xaa-OH in 20 ml of absolute ethyl acetate at −15°C. After 30 min, 2.0 mmol H-Yaa-R (R = $NH_2$; Zaa-$NH_2$) in 10 ml absolute ethyl acetate were added. The reaction mixture was stirred at −15°C for 1 h and then at room temperature for a further 12 h. The reaction mixture was extracted three times with $H_2O$, citric acid, $H_2O$, sat. $NaHCO_3$ and $H_2O$. The organic layer was dried ($MgSO_4$) and

evaporated *in vacuo*. Purification was achieved by flash chromatography.

### DIC Method

DIC, 2.2 mmol, and 2.0 mmol H-Yaa-R ($R = NH_2$; Zaa-$NH_2$) were added to a stirred solution of 2.0 mmol Z-(α-Tfm)Xaa-OH in 20 ml absolute dichloromethane. The reaction mixture was stirred at room temperature for 12 h. The precipitate was separated by filtration, the mother liquor was evaporated *in vacuo* and the remaining product was purified by flash chromatography.

### Hydrogenolysis

A small amount of Pd/C was added to a solution of 2.0 mmol Z-(α-Tfm)Xaa-R ($R = Yaa\text{-}NH_2$; Yaa-Zaa-$NH_2$) in 50 ml of methanol under a nitrogen atmosphere. The reaction mixture was stirred under a hydrogen atmosphere for 10–15 h. The catalyst was separated by filtration and the solution was evaporated. These unprotected amino acid and peptide derivatives were used for coupling reactions without further purification.

***2-(Trifluoromethyl)alaninamide DL-H(α-Tfm)Ala-NH₂.*** Thirty ml of ammonia were slowly added to a stirred solution of 2.84 mmol 4-methyl-4-trifluoromethyl-1,3-oxazolidin-2,5-one [19] in 15 ml absolute THF at − 40°C. After warming up to room temperature the solution was evaporated *in vacuo*, the remaining product was dissolved in water and carefully acidified with hydrochloric acid (1 M). The mixture was extracted three times with diethyl ether, the combined organic layer was dried ($MgSO_4$) and evaporated.

Yield, 0.2 g (45%); $^1$H-NMR ($CDCl_3$) $\delta$ 1.46 (s, 3H), 1.72 (s, br., 2H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 21.21, 171.9 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ −0.39 (s) p.p.m.

***N-Benzyloxycarbonyl-leucyl-2-(trifluoromethyl)-alaninamide Z-Leu-(α-Tfm)Ala-NH₂.*** Mixed anhydride method; yield, 82%.

*Diastereomeric mixture 1:1:* $^1$H-NMR ($CDCl_3$) $\delta$ 0.89–0.94 (m, 6H), 1.45–1.65 (m, 2H), 1.61–1.69 (m, 1H), 1.74/1.78 (s/s, 3H), 4.17/4.27 (m/m, 1H), 5.07/5.10 (s/s, 2H), 5.50/5.59 (m/m, 1H), 5.90/6.02 (m/m, 1H), 6.55–6.60 (m, 1H), 7.20 (s, br., 1H), 7.29–7.37 (m, 5H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 16.82/17.51, 21.62/21.83, 22.58/22.69, 24.55/24.62, 40.24/40.26, 54.12/54.45, 62.51 (q, $J = 27$ Hz), 67.29/67.44, 127.91/128.01, 128.27/128.35, 128.50/128.53, 135.67/135.83, 156.87, 167.82/167.98, 171.89/172.26 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 1.4/2.1 (s/s) p.p.m.

***N-Benzyloxycarbonyl-2-(trifluoromethyl)alanyl-alaninamide Z-(α-Tfm)Ala-Ala-NH₂.*** DIC method; yield, 83%; separation of the diastereomers by flash chromatography ($CHCl_3$/methanol = 20/1).

*Diastereomer I:* $^1$H-NMR ($CDCl_3$) 1.37 (d, $J = 7.9$ Hz, 3H), 1.76 (s, 3H), 4.48 (m, 1H), 5.10 (s, 2H), 5.50 (s, 1H), 5.91 (s, 1H), 6.70 (s, 1H), 6.76 (d, $J = 7.0$, 1H), 7.29–7.41 (m, 5H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 17.32, 17.58, 49.42, 63.01 (q, $J = 28$ Hz), 67.88, 124.18 (q, $J = 285$ Hz), 128.25, 128.61, 128.64, 135.20, 155.01, 164.95, 174.04 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 1.4 (s) p.p.m.

*Diastereomer II:* $^1$H-NMR ($CDCl_3$) 1.34 (d, $J = 7.0$ Hz, 3H), 1.76 (s, 3H), 4.44 (m, 1H), 5.09 (s, 2H), 5.72 (s, 1H), 6.39 (s, 1H), 6.82 (s, 1H), 7.16 (d, $J = 7.3$ Hz, 1H) 7.31–7.39 (m, 5H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 17.46, 18.09, 49.11, 62.42 (q, $J = 28$ Hz), 67.46, 124.24 (q, $J = 285$ Hz), 128.13, 128.41, 128.55, 135.52, 154.76, 165.84, 174.31 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 2.1 (s) p.p.m.

***N-Benzyloxycarbonyl-2-(trifluoromethyl)phenylalanyl-leucinamide Z-(α-Tfm)Phe-Leu-NH₂:*** Yield, 77%; M + H: 480.; Anal. calculated for $C_{24}H_{28}F_3N_3O_4$: C, 60.15; H, 5.84; N, 9.07; found: C, 60.17; H, 5.81; N, 9.07.

*Diastereomer I:* m.p., 134–136°C (ethyl acetate/hexanes); $^1$H-NMR ($CDCl_3$) $\delta$ 0.82–0.88 (m, 6H), 1.51–1.56 (m, 3H), 3.27 (d, $J = 14.6$ Hz, 1H), 3.99 (d, $J = 14.6$ Hz, 1H), 4.40 (m, 1H), 5.06 (d, $J = 12.6$ Hz, 1H), 5.18 (d, $J = 12.6$ Hz, 1H), 5.87 (s, 1H), 6.14 (s, 1H), 6.36 (m, 1H), 6.89 (d, $J = 7.6$ Hz, 1H), 7.04–7.18 (m, 5H), 7.34 (s, 5H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 21.75, 22.89, 24.49, 34.42, 40.79, 52.29, 65.90 (q, $J = 27$ Hz), 67.33, 124.85 (q, $J = 285$ Hz), 126.69, 127.61, 128.33, 128.41, 128.55, 128.60, 132.76, 135.85, 154.58, 164.54, 173.69 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 5.7 (s) p.p.m.

*Diastereomer II:* m.p., 138–140°C (ethyl acetate/hexanes); $^1$H-NMR ($CDCl_3$) $\delta$ 0.83–0.88 (m, 6H), 1.44–1.54 (m, 2H), 1.72 (m, 1H), 3.51 (d, $J = 14.8$ Hz, 1H), 3.78 (d, $J = 14.8$ Hz, 1H), 4.49 (m, 1H), 5.07 (d, $J = 12.1$ Hz, 1H), 5.16 (d, $J = 12.1$ Hz, 1H), 5.96–5.98 (m, 2H), 6.86–6.89 (m, 2H), 7.02–7.20 (m, 5H), 7.33–7.36 (m, 5H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 20.95, 22.96, 24.42, 34.42, 40.40, 52.47, 66.18 (q, $J = 26$ Hz), 67.72, 127.15 (q, $J = 287$ Hz), 127.29, 128.22, 128.48, 128.58, 128.89, 130.35,

133.31, 135.32, 155.18, 164.64, 174.42 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 5.8 (s) p.p.m.

***N*-Benzyloxycarbonyl-2-(trifluoromethyl)alanyl-phenylalanyl-leucinamide Z-(α-Tfm)Ala-Phe-Leu-NH$_2$.** Yield, 70%; m.p., 115–117°C ($CHCl_3$/hexanes); (M + H) 551. Anal. calculated for $C_{27}H_{33}F_3N_4O_5$: C, 58.92; H, 5.99; N, 10.17; found: C, 58.75; H, 5.75; N, 10.18.

*Diastereomeric mixture 1 : 1:* $^1$H-NMR ($CDCl_3$) $\delta$ 0.85 (d, $J$ = 6.7 Hz, 3H), 0.87 (d, $J$ = 6.4 Hz, 3H), 1.44–1.72 (m, 3H), 1.56/1.77 (s/s, 3H), 3.17–3.21 (m, 2H), 4.36–4.54 (m, 2H), 4.75/4.86 (d/d, $J$ = 12.2 Hz/12.2 Hz, 1H), 4.97/5.02 ($d_{AB}$/$d_{AB}$, $J$ = 12.2 Hz/12.2 Hz, 1H), 5.18 (s, 1H), 6.35 (s, 1H), 6.62 (s, 1H), 6.73–6.45 (m, 1H), 7.15 (m, 1H), 7.25–7.39 (m, 10H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 19.87, 20.73/20.88, 23.22/23.30, 24.55, 33.19, 39.56/39.77, 52.15, 55.47, 62.41 (q, $J$ = 25 Hz), 127.39/127.53, 127.92, 128.67, 128.72/128.77, 128.91, 129.09/129.99, 134.87, 135.84, 155.29, 167.45, 170.05/170.30, 175.18 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 1.5/3.5 (s/s) p.p.m.

***N*-Benzyloxycarbonyl-2-(trifluoromethyl)alanyl-alanyl-phenylalanyl-leucinamide Z-(α-Tfm)Ala-Ala-Phe-Leu-NH$_2$.** Yield, 70%; (M + H) 551. Anal. calculated for $C_{30}H_{38}F_3N_5O_6$*MeOH: C, 57.14; H, 6.44; N, 10.74; found: C, 56.74; H, 5.78; N, 11.04.

*Diastereomer I:* m.p., 124–126°C (methanol/$H_2O$); $^1$H-NMR ($CDCl_3$) $\delta$ 0.90 (d, $J$ = 6.1 Hz, 3H), 0.93 (d, $J$ = 6.1 Hz, 3H), 1.35 (d, $J$ = 7.3 Hz, 3H), 1.60–1.84 (m, 3H), 1.66 (s, 3H), 3.02 ($dd_{ABX}$, $J$ = 15.0 Hz, 11.6 Hz, 1H), 3.48 ($dd_{ABX}$, $J$ = 15.0 Hz, 3.7 Hz, 1H), 4.16 (m, 1H), 4.47 (m, 1H), 4.58 (m, 1H), 4.97 ($d_{AB}$, $J$ = 11.9 Hz, 1H), 5.01 ($d_{AB}$, $J$ = 11.9 Hz, 1H), 6.21 (s, 1H), 6.74 (s, br., 1H), 6.93 (s., br., 1H), 6.97 (m, 1H), 7.16 (m, 1H), 7.23–7.37 (m, 10H), 7.49 (d, $J$ = 6.4 Hz, 1H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 17.04, 17.48, 20.94, 23.21, 24.89, 36.03, 39.86, 52.08, 52.29, 55.61, 63.32 (q, $J$ = 28 Hz), 68.32, 126.72, 128.07, 128.39, 128.49, 128.79, 128.84, 134.54, 137.48, 155.81, 166.77, 171.49, 173.08, 175.30 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 1.7 (s) p.p.m.

*Diastereomer II:* m.p., 114–116°C (methanol/$H_2O$); $^1$H-NMR ($d_6$-DMSO) $\delta$ 0.82 (d, $J$ = 6.4 Hz, 3H), 0.86 (d, $J$ = 6.4 Hz, 3H), 1.41 (d, $J$ = 7.3 Hz, 3H), 1.44–1.62 (m, 3H), 1.47 (s, 3H), 2.83 (m, 1H), 2.99 ($dd_{ABX}$, $J$ = 13.7 Hz, 4.9 Hz, 1H), 4.18 (m, 1H), 4.45–4.48 (m, 2H), 4.99 ($d_{AB}$, $J$ = 12.4 Hz, 1H), 5.04 ($d_{AB}$, $J$ = 12.4 Hz, 1H), 6.94 (s, 1H), 7.05 (s, 1H), 7.17–7.35 (m, 10H), 7.45 (d, $J$ = 7.6 Hz, 1H), 7.88 (d, $J$ = 8.5 Hz, 1H), 8.26 (d, $J$ = 7.9 Hz, 1H), 8.56–8.63 (m, 1H) p.p.m.; $^{13}$C-NMR ($d_6$-DMSO) $\delta$ 14.36, 16.56, 21.65, 23.09, 24.23, 36.69, 41.01, 49.26, 51.11, 54.53, 61.94 (q, $J$ = 29 Hz), 62.99, 126.23, 126.49, 126.69, 128.10, 128.51, 129.16, 136.09, 137.85, 155.31, 168.01, 170.71, 172.71, 173.97 p.p.m.; $^{19}$F-NMR ($d_6$-DMSO) $\delta$ 1.0 (s) p.p.m.

***N*-Benzyloxycarbonyl-2-(methyl)phenylalanyl-leucinamide Z-(α-CH$_3$)Phe-Leu-NH$_2$.** Yield, 57%; (M + H) 425; m.p., 165–166°C (methanol/$H_2O$). Anal. calculated for $C_{24}$ $H_{31}N_3O_4$: C, 67.78; H, 7.29; N, 9.86; found: C, 67.29; H, 7.54; N, 9.67.

*Diastereomeric mixture 1 : 1:* $^1$H-NMR ($CDCl_3$) $\delta$ 0.89 (d, $J$ = 6.6 Hz, 3H), 0.90 (d, $J$ = 6.2 Hz, 3H), 1.34/1.54 (s/s, 3H), 1.40–1.52 (m, 2H), 1.71 (m, 1H), 3.01/3.21 ($d_{AB}$/$d_{AB}$, $J$ = 13.8 Hz/13.8 Hz, 1H), 3.20/3.36 ($d_{AB}$/$d_{AB}$, $J$ = 13.8 Hz/13.8 Hz, 1H), 4.45 (m, 1H), 4.95/5.11 (s/s, 1H), 5.05 ($d_{AB}$, $J$ = 12.4 Hz, 1H), 5.13 ($d_{AB}$, $J$ = 12.4 Hz, 1H), 6.25/6.27 (s, br./s, br., 1H), 6.98/7.00 (d/d, $J$ = 7.7 Hz/7.0 Hz, 1H), 7.07/7.08 (d, $J$ = 5.9 Hz/7.4 Hz, 1H), 7.18–7.41 (m, 10 H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 21.27/21.62, 23.14/23.25, 24.79/25.08, 40.00/40.27, 40.35/43.58, 51.78/51.86, 67.39/67.57, 127.01/127.48, 128.29/128.37, 128.41/128.48, 128.56/128.61, 128.70/128.75, 130.25/130.69, 134.94/134.96, 135.76/135.81, 155.69/155.90, 173.08/173.40, 174.43/174.61 p.p.m.

***N*-Benzyloxycarbonyl-aminoisobutyryl-phenylalanyl-leucinamide Z-Aib-Phe-Leu-NH$_2$.** Yield, 74%; m.p., 168–170°C ($CHCl_3$/hexanes); (M + H): 497. Anal. calculated for $C_{27}H_{36}N_4O_5H_{32}$: C, 65.34; H, 7.25; N, 11.28; found: C, 65.38; H, 7.12; N, 11.22. $^1$H-NMR ($d_6$-DMSO) $\delta$ 0.76 (d, $J$ = 5.9 Hz, 3H), 0.80 (d, $J$ = 5.9 Hz, 3H), 1.11 (s, 3H), 1.16 (s, 3H), 1.47–1.49 (m, 3H), 2.93 (m, 1H), 3.08 (m, 1H), 4.10–4.12 (m, 1H), 4.33 (m, 1H), 4.86 (d, $J$ = 12.5 Hz, 1H), 4.99 (d, $J$ = 12.5 Hz, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.12–7.33 (m, 10H), 7.40–7.66 (m, 2H), 7.81 (d, $J$ = 7.3 Hz, 1H) p.p.m.; $^{13}$C-NMR ($d_6$-DMSO) $\delta$ 21.45, 23.24, 24.21, 24.67, 25.67, 36.05, 40.36, 51.31, 54.85, 65.72, 126.37, 127.90, 128.09, 128.57, 129.99, 136.70, 138.23, 155.69, 170.80, 174.23, 174.72 p.p.m.

***N*-Benzyloxycarbonyl-aminoisobutyryl-alanyl-phenylalanyl-leucinamid Z-Aib-Ala-Phe-Leu-NH$_2$.** Yield, 6%; m.p., 105–107°C ($CHCl_3$); (M + H): 568. Anal. calculated for $C_{30}H_{41}N_5O_6$*MeOH: C, 62.09; H, 8.61; N, 12.06; found: C, 61.93; H, 8.71; N, 12.05. $^1$H-NMR ($CDCl_3$) $\delta$ 0.87 (d, $J$ = 6.4 Hz, 3H), 0.89 (d, $J$ = 6.4 Hz, 3H), 1.29 (d, $J$ = 7.3 Hz, 3H), 1.43 (s, 3H),

1.47 (s, 3H), 1.73–1.80 (m, 3H), 3.02 ($dd_{ABX}$, $J = 15.0$ Hz, 11.4 Hz, 1H), 3.38 ($dd_{AB}$, $J = 15.0$ Hz, 11.4 Hz, 1H), 4.04 (m, 1H), 4.40–4.54 (m, 2H), 4.91 ($d_{AB}$, $J = 12.2$ Hz, 1H), 5.00 ($d_{AB}$, $J = 12.2$ Hz, 1H), 5.16 (s, 1H), 6.03 (s, 1H), 6.88 (s, 1H), 7.02 (s, br., 1H), 7.13–7.21 (m, 5H), 7.28–7.33 (m, 5H), 7.36 (d, $J = 8.2$ Hz, 1H), 7.61 (d, $J = 6.7$ Hz, 1H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 16.94, 20.86, 23.13, 23.72, 26.70, 24.79, 36.09, 39.81, 51.91, 55.68, 56.80, 67.27, 126.58, 127.83, 128.36, 128.39, 128.43, 128.62, 135.40, 137.56, 156.27, 171.94, 174.26, 175.64, 176.10 p.p.m.

***N-Benzyloxycarbonyl-phenylalanyl-2-(trifluoromethyl)alanyl-alaninamide Z-Phe-(α-Tfm)Ala-Ala-NH$_2$.*** Yield, 66%; (M + H), 509. Anal. calculated for $C_{24}H_{27}F_3N_4O_5$: C, 56.72; H, 5.31; N, 11.02; found: C, 56.55; H, 5.56; N, 11.06.

*(S,R,S)-Diastereomer:* m.p., 192–194°C ($CHCl_3$/methanol); $^1$H-NMR ($d_4$-methanol) 1.34 (d, $J = 8.6$ Hz, 3H), 1.66 (s, 3H), 2.92 ($dd_{ABX}$, $J = 14.0$ Hz, 8.9 Hz, 1H), 3.13 ($dd_{ABX}$, $J = 14.0$ Hz, 6.1 Hz, 1H), 4.30 (m, 1H), 4.41 (m, 1H), 5.08 (s, 2H), 7.24–7.38 (m, 10H) p.p.m.; $^{13}$C-NMR ($d_4$-methanol) $\delta$ 17.71, 19.10, 38.18, 50.70, 58.01, 63.58 (q, $J = 27$ Hz), 67.75, 125.80 (q, $J = 285$ Hz), 127.88, 128.67, 129.02, 129.47, 129.52, 130.37, 137.98, 138.07, 158.68, 167.88, 174.47, 177.25 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 0.9 (s) p.p.m.

*(S,S,S)-Diastereomer:* m.p., 195–197°C ($CHCl_3$/methanol); $^1$H-NMR ($d_4$-methanol $\delta$ 1.31 (d, $J = 7.0$ Hz, 3H), 1.49 (s, 3H), 2.92 ($dd_{ABX}$, $J = 13.8$ Hz, 8.4 Hz, 1H), 3.13 ($dd_{ABX}$, $J = 13.8$ Hz, 8.4 Hz, 1H), 4.21 (m, 1H), 4.38 (m, 1H), 5.05 (s, 2H), 7.21–7.29 (m, 10H) p.p.m.; $^{13}$C-NMR ($d_4$-methanol) $\delta$ 17.52, 18.05, 38.36, 50.94, 58.03, 63.86 (q, $J = 27$ Hz), 67.76, 125.68 (q, $J = 285$ Hz), 127.96, 128.67, 129.03, 129.49, 129.52, 130.52, 137.67, 138.12, 158.58, 167.75, 174.58, 177.08 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 4.9 (s) p.p.m.

***N-Benzyloxycarbonyl-phenylalanyl-leucyl-2-(trifluoromethyl)alaninamide Z-Phe-Leu-(α-Tfm)Ala-NH$_2$.*** Yield, 91%; (M + H), 551. Anal. calculated for $C_{27}H_{33}F_3N_4O_5$: C, 58.91; H, 5.99; N, 10.17; found: C, 59.60; H, 6.32; N, 10.01.

*Diastereomer I:* m.p., 95–97°C (methanol/$H_2O$); $^1$H-NMR ($CDCl_3$) $\delta$ 0.84–0.86 (m, 6H), 1.39–1.50 (m, 2H), 1.64 (m, 1H), 1.78 (s, 3H), 2.97 ($d_{ABX}$, $J = 13.8$ Hz, 6.8 Hz, 1H), 3.08 ($dd_{ABX}$, $J = 13.8$ Hz, 1H), 4.27 (m, 1H), 4.44 (m, 1H), 5.02 (s, 2H), 5.50 (m, 1H), 5.93 (s, 1H), 6.64 (m, 1H), 6.89 (s, 1H), 7.42 (s, 1H), 7.14–7.34 (m, 10H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 17.51, 21.79, 22.53, 24.50, 37.55, 39.40, 52.91, 56.27, 67.37, 62.83 (q, $J = 27$ Hz), 124.39 (q, $J = 285$ Hz), 126.54, 127.23, 128.08, 128.36, 128.54, 128.80, 129.13, 135.65, 156.32, 168.15, 171.19, 172.48 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 1.9 (s) p.p.m.

*Diastereomer II:* m.p., 98–102°C (methanol/$H_2O$); $^1$H-NMR ($CDCl_3$) $\delta$ 0.82 (d, $J = 6.1$ Hz, 3H), 0.85 (d, $J = 6.1$ Hz, 3H), 1.42–1.49 (m, 2H), 1.61 (m, 1H), 1.73 (s, 3H), 2.98 ($dd_{ABX}$, $J = 14.0$ Hz, 8.2 Hz, 1H), 3.09 ($dd_{ABX}$, $J = 14.0$ Hz, 5.5 Hz, 1H), 4.19 (m, 1H), 4.42 (m, 1H), 4.97–5.07 (m, 2H), 5.37 (s, br., 1H), 6.09 (s, br., 1H), 6.67 (s, br., 1H), 6.83 (d, $J = 6.1$ Hz, 1H), 7.14–7.33 (m, 10 H), 7.49 (s, 1H) p.p.m.; $^{13}$C-NMR ($CDCl_3$) $\delta$ 17.89, 21.59, 22.71, 24.65, 37.57, 39.42, 53.27, 56.45, 62.53 (q, $J = 27$ Hz), 67.44, 127.32, 127.70 (q, $J = 286$ Hz), 128.17, 128.41, 128.55, 128.87, 129.08, 135.58, 135.69, 156.46, 171.23, 172.33, 172.51 p.p.m.; $^{19}$F-NMR ($CDCl_3$) $\delta$ 2.5 (s) p.p.m.

***N-Benzyloxycarbonyl-phenylalanyl-aminoisobutyryl-alaninamide Z-Phe-Aib-Ala-NH$_2$.*** Yield, 40%; (M + H), 455; m.p., 194–196°C (diethylether/methanol). Anal. calculated for $C_{24}H_{30}N_4O_5$: C, 63.46; H, 6.60; N, 12.32; found: C, 63.28; H, 6.79; N, 12.22.

$^1$H-NMR ($d_4$-methanol) $\delta$ 1.29–1.31 (m, 9H), 2.92 ($dd_{ABX}$, $J = 13.5$ Hz, 7.9 Hz, 1H), 3.02 ($dd_{ABX}$, $J = 13.5$ Hz, 7.9 Hz, 1H), 4.16–4.27 (m, 2H), 5.05 (s, 2H), 7.21–7.30 (m, 10H) p.p.m.; $^{13}$C-NMR ($d_4$-methanol) $\delta$ 17.78, 25.12, 25.81, 38.52, 57.96, 58.56, 67.81, 128.03, 128.78, 129.14, 129.60, 129.62, 130.63, 138.11, 138.30, 158.68, 174.37, 176.64, 178.10 p.p.m.

***N-Benzyloxycarbonyl-phenylalanyl-leucyl-aminoisobutyramid Z-Phe-Leu-Aib-NH$_2$.*** Yield, 56%; m.p., 120–122°C (ethyl acetate/hexanes); (M + H): 497. Anal. calculated for $C_{27}H_{36}N_4O_5$: C, 65.30, H, 7.25; N, 11.28; found: C, 65.39; H, 7.47; N, 11.30.

$^1$H-NMR ($d_4$-methanol) $\delta$ 0.93 (d, $J = 6.1$ Hz, 3H), 0.96 (d, $J = 6.1$ Hz, 3H), 1.48 (s, 3H), 1.52 (s, 3H), 1.60–1.62 (m, 3H), 2.88 ($dd_{ABX}$, $J = 14.0$ Hz, 4.9 Hz, 1H), 3.15 ($dd_{ABX}$, $J = 14.0$ Hz, 9.8 Hz, 1H), 4.28 (m, 1H), 4.45 (dd, $J = 9.6$ Hz, 4.9 Hz, 1H), 5.03–5.04 (m, 2H), 7.22–7.36 (m, 10H) p.p.m.; $^{13}$C-NMR ($d_4$-methanol) $\delta$ 22.38, 23.63, 25.05, 26.00, 26.63, 39.04, 41.42, 54.16, 57.88, 67.85, 127.97, 128.93, 129.19, 129.69, 129.70, 130.66, 138.35, 138.71, 158.58, 174.33, 174.94, 180.14 p.p.m.

***N*-Benzyloxycarbonyl-alanyl-phenylalanyl-leucinamide Z-Ala-Phe-Leu-$NH_2$.** Yield, 80%; m.p., 220–222°C (methanol/$H_2O$); (M + H), 483. Anal. calculated for $C_{26}H_{34}N_4O_5$: C, 64.75; H, 7.05; N, 11.61; found: C, 64.21; H, 7.04; N, 11.41.

$^1$H-NMR ($d_6$-DMSO) $\delta$ 0.83 (d, $J = 6.2$ Hz, 3H), 0.87 (d, $J = 6.6$ Hz, 3H), 1.12 (d, $J = 7.1$ Hz, 3H), 1.44–1.48 (m, 2H), 1.56 (m, 1H), 2.81 ($dd_{ABX}$, $J = 13.8$ Hz, 8.8 Hz, 1H), 3.00 ($dd_{ABX}$, $J = 13.8$ Hz, 4.8 Hz, 1H), 4.00 (m, 1H), 4.18–4.25 (m, 1H), 4.50 (m, 1H), 4.99 ($d_{AB}$, $J = 12.5$ Hz, 1H), 5.02 ($d_{AB}$, $J = 12.5$ Hz, 1H), 7.02 (s, 1H), 7.13 (s, 1H), 7.17–7.37 (m, 10H), 7.93 (d, $J = 8.4$ Hz, 1H), 8.00 (d, $J = 8.4$ Hz, 1H), 7.48 (d, $J = 7.5$ Hz, 1H) p.p.m.; $^{13}$C-NMR ($d_6$-DMSO) $\delta$ 17.92, 21.49, 22.99, 24.07, 30.63, 40.01, 50.23, 50.90, 53.74, 64.37, 126.15, 127.65, 127.74, 127.93, 128.29, 129.19, 136.86, 137.37, 155.63, 170.45, 172.34, 173.81 p.p.m.

***N*-Benzyloxycarbonyl-phenylalanyl-alanyl-alaninamide Z-Phe-Ala-Ala-$NH_2$.** Yield, 84%; m.p., 236–238°C (ethyl acetate/methanol); (M + H), 441. Anal. calculated for $C_{23}H_{28}N_4O_5$: C, 62.75; H, 6.36; N, 12.72; found: C, 62.58; H, 6.49; N, 12.71.

$^1$H-NMR ($d_6$-DMSO) $\delta$ 1.26 (d, $J = 7.0$ Hz, 3H), 1.29 (d, $J = 7.0$ Hz, 3H), 2.77 (m, 1H), 3.08 (m, 1H), 4.24 (m, 1H), 4.30–4.35 (m, 2H), 4.99 (s, 2H), 7.08 (s, 1H), 7.25 (s, 1H), 7.25–7.38 (m, 10H), 7.58 (d, $J = 7.4$ Hz, 1H), 7.91 (d, $J = 7.4$ Hz, 1H), 8.28 (d, $J = 8.5$ Hz, 1H) p.p.m.; $^{13}$C-NMR ($d_6$-DMSO) $\delta$ 18.31, 18.71, 37.60, 48.22, 51.25, 56.20, 65.51, 126.50, 127.69, 127.97, 128.33, 128.59, 129.51, 137.30, 138.44, 156.20, 171.89, 171.71, 174.34 p.p.m.

***N*-Benzyloxycarbonyl-phenylalanyl-leucyl-alaninamide Z-Phe-Leu-Ala-$NH_2$.** Yield, 97%; melting point 195–197°C (ethyl acetate/methanol); (M + H), 483. Anal. calculated for $C_{26}H_{34}N_4O_5$: C, 64.75; H, 7.05; N, 11.61; found: C, 64.31; H, 6.93; N, 11.33.

$^1$H-NMR ($d_6$-DMSO) $\delta$ 0.96 (d, $J = 6.6$ Hz, 3H), 1.00 (d, $J = 6.6$ Hz, 3H), 1.33 (d, $J = 7.2$ Hz, 3H), 1.57–1.61 (m, 2H), 1.73 (m, 1H), 2.84 (m, 1H), 3.12 (m, 1H), 4.30 (m, 1H), 4.38–4.42 (m, 2H), 5.01–5.08 (m, 2H), 7.33–7.43 (m, 11H), 7.12 (s, 1H), 7.61 (d, $J = 8.8$ Hz, 1H), 7.97 (d, $J = 7.4$ Hz, 1H), 8.23 (d, $J = 7.7$ Hz, 1H) p.p.m.; $^{13}$C-NMR ($d_6$-DMSO) $\delta$ 17.90, 21.08, 22.62, 23.61, 36.35, 40.21, 47.40, 50.63, 55.59, 64.69, 125.71, 126.90, 127.16, 127.50, 127.77, 128.70, 136.52, 137.62, 155.34, 170.87, 171.60, 173.48 p.p.m.

***N*-Benzyloxycarbonyl-phenylalanyl-leucyl-alanyl-alaninamide Z-Phe-Leu-Ala-Ala-$NH_2$.** Yield, 91%; m.p. 243–245°C (methanol/$H_2O$); (M + H), 554. Anal. calculated for $C_{29}H_{39}N_5O_6$: C, 62.95, H, 7.05; N, 12.65; found: C, 62.64; H, 6.87; N, 12.70.

$^1$H-NMR ($d_6$-DMSO) $\delta$ 0.79–0.86 (m, 6H), 1.14 (d, $J = 7.2$ Hz, 3H), 1.16 (d, $J = 7.2$ Hz, 3H), 1.40–1.47 (m, 2H), 1.58 (m, 1H), 2.68 (m, 1H), 2.95 (m, 1H), 3.96–4.29 (m, 4H), 4.85–4.89 (m, 2H), 6.95 (s, 1H), 7.18–7.24 (m, 11H), 7.42 (d, $J = 8.5$ Hz, 1H), 7.73 (d, $J = 7.2$ Hz, 1H), 7.99–8.06 (m, 2H) p.p.m.; $^{13}$C-NMR ($d_6$-DMSO) $\delta$ 18.00, 18.54, 21.78, 23.22, 24.27, 38.99, 40.39, 48.04, 48.34, 51.18, 56.18, 65.39, 126.36, 127.36, 127.80, 128.16, 128.42, 129.35, 137.17, 138.24, 155.97, 171.58, 171.63, 171.91, 174.13 p.p.m.

### α-Chymotrypsin Catalyzed Hydrolysis

Hydrolysis studies were performed with a total sample volume of 1 ml consisting of 300 μl DMSO, 500 μl buffer (0.2 M carbonate, pH 9.2), 100 μl substrate solution (0.04 mmol/ml DMSO) and 100 μl enzyme solution (20 mg α-chymotrypsin/ml $H_2O$). The buffer was slowly added to a stirred mixture of DMSO and substrate solution. The reaction was started by addition of the enzyme solution at 30°C. After 0, 2, 5, 10, 30, 60, 120, 240 min and 24 h, respectively, 50 μl were taken from the reaction mixture and added to 200 μl TFA solution (1% TFA in methanol/water 1/1 (v/v)) to stop enzymatic hydrolysis. These samples were stored at −18°C prior to analysis by HPLC.

### Molecular Modelling

The molecular modelling studies on the α-chymotrypsin–substrate complexes were performed employing the modelling software package QUANTA 4.1 [20] which is based on the CHARMm 23.1 force field.

## Results and Discussion

On the basis of the known sequence specificity for α-chymotrypsin [21] a series of peptides was designed and synthesized that allowed a fair comparison of α-Tfm substituted with the unsubstituted peptides (Figure 1). The model peptides synthesized by solution methods contained a well-defined cleavage site, namely an aromatic amino acid at $P_1$ position (P-nomenclature according to Schechter and Berger [22]) and an α-Tfm amino acid. Variation of the

cleavage site ↓

| $P_3$ | $P_2$ | $P_1$ | $P_1'$ | $P_2'$ | |
|---|---|---|---|---|---|
| | Z | **(α-Tfm)Phe** | Leu | $NH_2$ | |
| | Z | **(α-$CH_3$)Phe** | Leu | $NH_2$ | |
| Z | **(α-TfmAla)** | Phe | Leu | $NH_2$ | |
| Z | **Aib** | Phe | Leu | $NH_2$ | |
| Z | **(α-Tfm)Ala** | Ala | Phe | Leu | $NH_2$ |
| Z | **Aib** | Ala | Phe | Leu | $NH_2$ |
| | Z | Phe | **(α-Tfm)Ala** | Ala | $NH_2$ |
| | Z | Phe | **Aib** | Ala | $NH_2$ |
| | Z | Phe | Leu | **(α-Tfm)Ala** | $NH_2$ |
| | Z | Phe | Leu | **Aib** | $NH_2$ |

Figure 1 Sequences of the α-Tfm and α-methyl substituted model peptides; α-Tfm substituted peptides obtained as diastereomers where separated by flash chromatography.

position of the latter relative to the cleavage site (in the range $P_3$–$P'_2$) and qualitative determination of the hydrolysis rates directly revealed the influence of an α-Tfm amino acid substitution at a specific position of the peptide on its proteolytic stability.

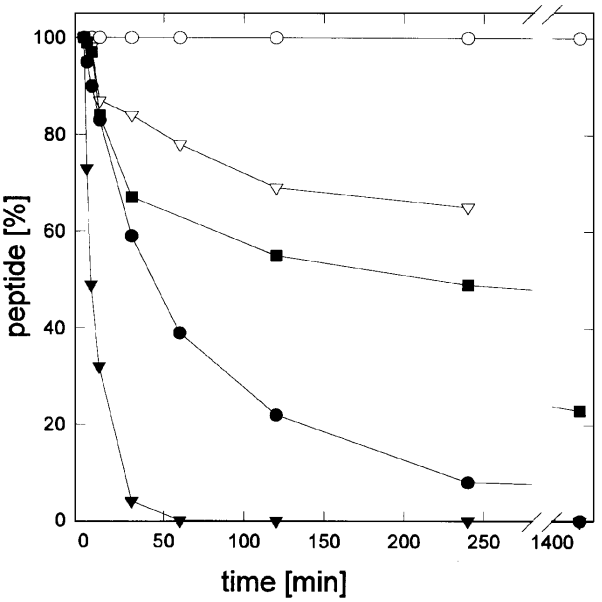


Figure 2 Optimization of the reaction conditions for the α-chymotrypsin catalysed proteolysis of the unsubstituted model peptide Z-Phe-Ala-Ala-$NH_2$ illustrated by the time-dependent decrease of the peptide concentration: ○ no enzyme, 25°C; ▽ 10 μl enzyme/40% DMSO, 30°C; ■ 100 μl enzyme/40% DMF, 35°C; ● 100 μl enzyme/40% DMF, 25°C; ▼ 100 μl enzyme/40% DMSO, 35°C.

Comparison with peptides containing the fluorine-free disubstituted amino acid Aib (α-aminoisobutyric acid) allowed to differentiate electronic from steric effects.

The reaction conditions of the hydrolysis studies were optimized with respect to the organic solvent content, temperature and enzyme concentration (Figure 2) [23]. The diminished solubility of the α-Tfm and α-methyl-substituted peptides demanded a considerably higher percentage of organic solvent in the reaction mixture. Both DMF and DMSO were tested. In both cases 40% of the organic solvent was necessary for optimal solubilization of the substrates. However, DMSO allowed a higher enzyme stability than DMF, especially when long reaction times were necessary. The enzymatic activity at different temperatures was measured for the unsubstituted model peptide Z-Phe-Ala-Ala-$NH_2$. Furthermore, a substrate/enzyme ratio of 1 : 500 was necessary to achieve acceptable hydrolysis rates because of the high organic solvent content.

The unsubstituted model peptides chosen in this study were rapidly proteolysed. In contrast, all α-methyl- and α-Tfm-containing peptides showed considerable proteolytic stability depending on the position of the substituted amino acids. Peptides substituted at $P_1$-position were essentially unaffected by the protease used (Figure 3). Substitutions at $P_2$ resulted in significantly increased proteolytic stability towards enzymatic hydrolysis compared to the unsubstituted model peptide (Figure 4). Even for peptides substituted at $P_3$, slightly retarded degra-

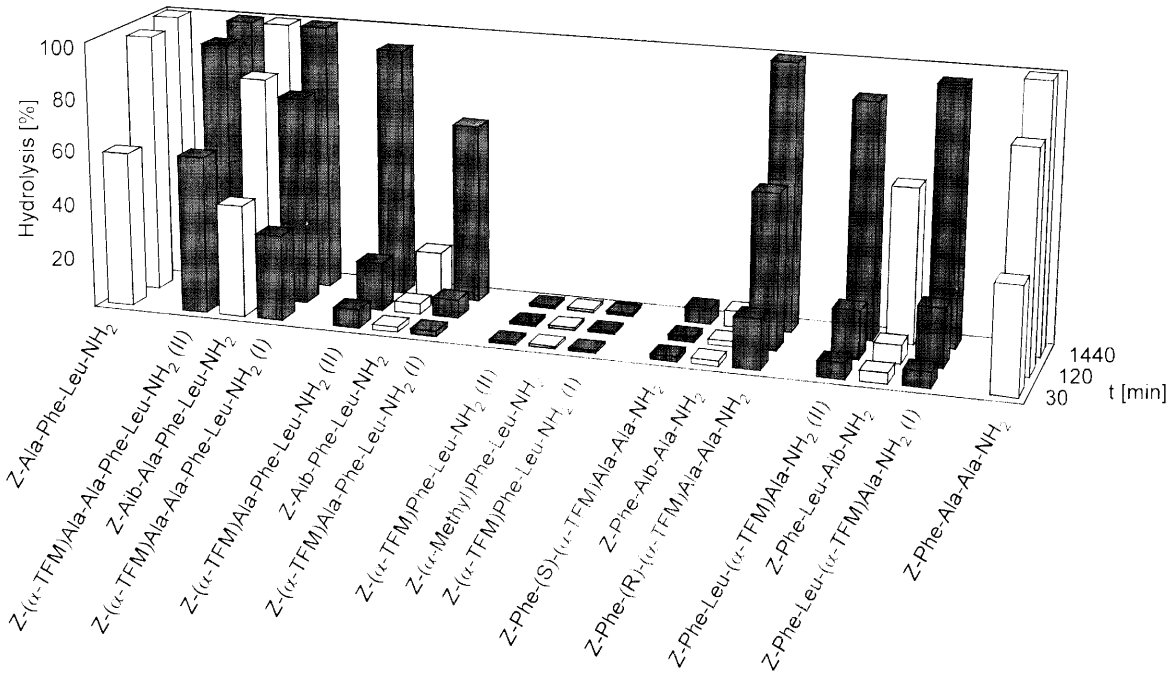


Figure 3 Summary of the hydrolysis study.

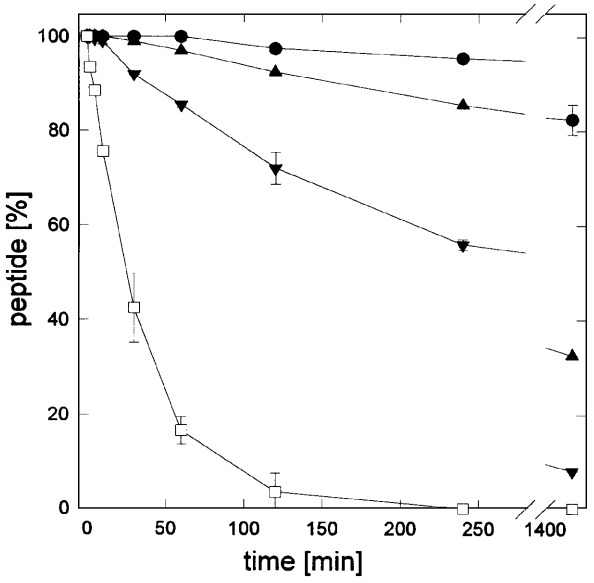


Figure 4 α-Chymotrypsin catalysed hydrolysis of model peptides substituted in $P_2$ by (α-Tfm)Ala or Aib: ▲ Z-(α-Tfm) Ala-Phe-Leu-$NH_2$(I); ▼ Z-(α-Tfm)Ala-Phe-Leu-$NH_2$ (II); ● Z-Aib-Phe-Leu, $NH_2$; □ Z-Ala-Phe-Leu-$NH_2$.

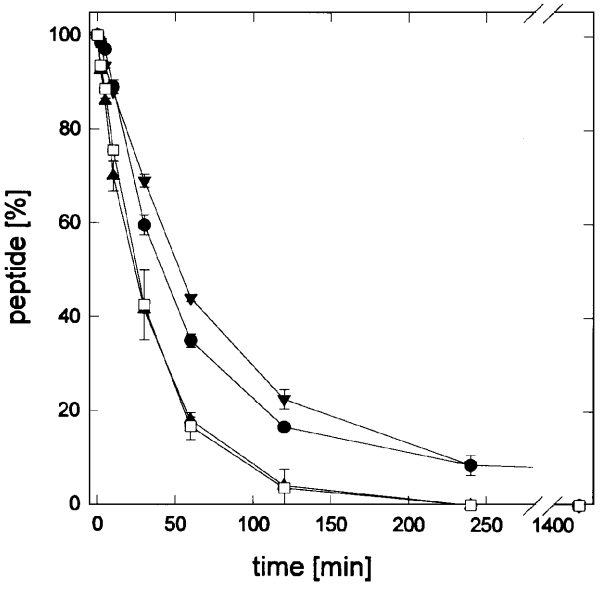


Figure 5 α-Chymotrypsin catalysed hydrolysis of model peptides substituted in $P_3$ by (α-Tfm)Ala or Aib: ▲ Z-(α-Tfm)Ala-Ala-Phe-Leu-$NH_2$(I); ▼ Z-(α-Tfm)Ala-Ala-Phe-Leu-$NH_2$ (II), ● Z-Aib-Ala-Phe-Leu-$NH_2$ □ Z-Ala-Phe-Leu-$NH_2$.

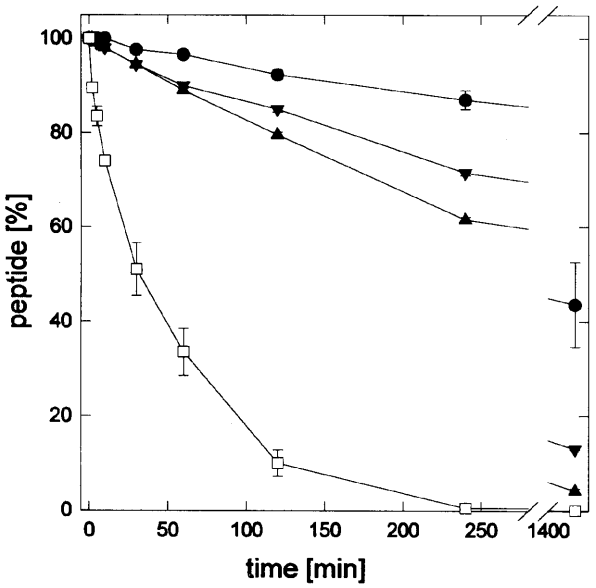


Figure 6 α-Chymotrypsin catalysed hydrolysis of model peptides substituted in $P'_2$ by (α-Tfm)Ala or Aib: ▲ Z-Phe-Leu-(α-Tfm)Ala-$NH_2$ (I); ▼ Z-Phe-Leu-(α-Tfm)Ala-$NH_2$ (II); ● Z-Phe-Leu-Aib-$NH_2$; □ Z-Phe-Leu-Ala-$NH_2$.

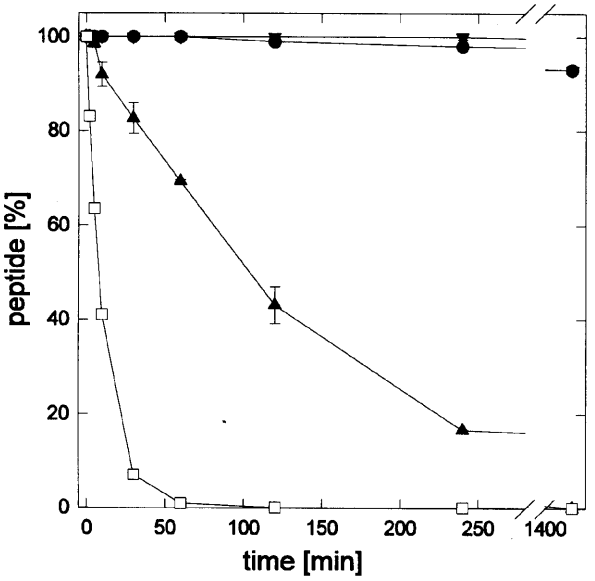


Figure 7 α-Chymotrypsin catalysed hydrolysis of model peptides substituted in $P'_1$ by (α-Tfm)Ala or Aib: ▲ Z-Phe-(α-Tfm)Ala-Ala-$NH_2$ (I); ▼ Z-Phe-(α-Tfm)Ala-Ala-$NH_2$ (II); ● Z-Phe-Aib-Ala-$NH_2$ □ Z-Phe-Ala-Ala-$NH_2$.

dation was observed (Figure 5). Substitutions at the $P'_2$ position provided nearly the same effect on the proteolytic behaviour of peptides as substitutions at $P_2$ (Figure 6).

A significant difference between α-methyl- and α-Tfm-substituted peptides was noted in the sense that the former generally showed a stronger retardation to proteolysis at all positions examined (Figure 3). The comparison of the proteolysis data of all α-Tfm-substituted peptides indicates that not only the position of the substitution but also the absolute configuration of the α-Tfm amino acid significantly influences the proteolytic stability of peptides. The strongest influence of the configuration was detected for the diastereomeric model peptides containing an α-Tfm amino acid in the $P'_1$ position (Figure 7). The absolute configuration of the diastereomers of Z-Phe-(α-Tfm)Ala-Ala-$NH_2$ was determined by X-ray diffraction (manuscript in preparation). While the (*S*,*R*,*S*)-diastereomer was rapidly hydrolysed, the (*S*,*S*,*S*)-diastereomer showed an extraordinarily high proteolytic stability similar to that of the Aib-substituted peptide. This surprising effect cannot be explained by the steric constraints of the Tfm group, since the corresponding Aib-substituted peptide with the even smaller methyl groups also exhibits a high stability to proteolysis. Moreover, the hydrolysis rate of the (*S*,*R*,*S*)-configured Tfm-substituted diastereomer compares to that of the alanine-containing model peptide. The different proteolytic stabilities of the diastereomers seem to result from a specific interaction between the substrate and the enzyme induced by the high electron density of the Tfm group.

In order to obtain information on the binding between the substrates Z-Phe-Aib-Ala-$NH_2$, (*S*,*R*,*S*)-Z-Phe-(α-Tfm)Ala-Ala-$NH_2$, (*S*,*S*,*S*)-Z-Phe-(α-Tfm)Ala-Ala-$NH_2$), Z-Phe-Ala-Ala-$NH_2$ and α-chymotrypsin the corresponding complexes were examined by molecular modelling. The structures of the complexes were built on the basis of a X-ray study on an α-chymotrypsin/phenyl boronic acid complex taken from the Brookhaven Protein Data Bank [24]. The identical backbone of the four model peptides was fitted into the active site of α-chymotrypsin considering both the correspondence to the phenyl boronic acid in some parts and the mechanistic requirements of the α-chymotrypsin catalysis, so that the phenylalanine residue occupied the hydrophobic pocket and the peptide bond to be cleaved was close to the Ser 195. Based on this structure, the actual starting arrangements for all complexes were generated by the corresponding variations of the substituents of the $P'_1$ amino acid and subsequently optimized.

The catalytic triad of α-chymotrypsin consists of Ser 195, His 57 and Asp 102. In the first step of the α-chymotrypsin-catalysed hydrolysis, a nucleophilic

attack takes place by the Ser 195 oxygen on the carbonyl function of the substrate. A tetrahedral transition state stabilized by two additional hydrogen bonds between substrate and enzyme is formed. The reaction is accompanied by a proton transfer from the Ser 195 oxygen to the nitrogen of His 57 [25].

In Figure 8 a section of the energy-minimized complex between α-chymotrypsin and the (*S*,*R*,*S*)-diastereomer is shown. In this case the steric constraints exhibited by the α-Tfm group are obviously outweighed by an advantageous interaction of the fluorine atoms with the serine side chain of the enzyme. The fluorine substituents can presumably act as a proton acceptor in hydrogen bonding enhancing the nucleophilicity of the serine oxygen and, thus, facilitating the nucleophilic attack on the carbonyl group of the substrate. In contrast, such a favourable interaction between substrate and enzyme is impossible for the (*S*,*S*,*S*)-diastereomer. As shown in Figure 9 the Tfm group in this diastereomer points towards the opposite direction now although a backbone conformation rather comparable to that of the (*S*,*R*,*S*)-diastereomer can be realized.

## Conclusions

Considerable polarization effects on the neighbouring substituents and conformational restrictions on the peptide chain are exerted on incorporation of an α-Tfm group into peptides. These structural alterations result in an increased proteolytic stability of peptides depending on the relative position of the α-Tfm group to the predominant cleavage site defined by the substrate specificity of the protease and the absolute configuration of the α-Tfm amino acid. Furthermore, owing to the high electron density, the trifluoromethyl-substituted peptides are capable of interacting with enzyme or receptor subsites in a manner which is impossible for the fluorine-free pendants.

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