A New Convenient Synthesis of 2-Trifluoromethyl Substituted Aspartic Acid and its Isopeptides. Part 11 [1]

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The reaction of methyl 2-benzyloxycarbonylimino-3,3,3-trifluoropropionate with acetyl chloride–triethylamine yields methyl 2-benzyloxy-6-oxo-4,5-dihydro-4-trifluoromethyl-1,3-oxazine-4-carboxylate, a β-activated equivalent for 2-trifluoromethyl substituted aspartic acid. This reagent offers a versatile route to β-derivatized 2-trifluoromethyl substituted aspartic esters *via* nucleophilic ring cleavage; with amino acid esters isoaspartyl peptides are formed. The diastereoisomeric dipeptides have been separated by flash chromatography; for benzyl *N*-benzyloxycarbonyl-*R*-2-trifluoromethyl-β-aspartyl-(α-methylester)-*S*-prolinate an X-ray analysis is presented. Benzyl *N*-benzyloxycarbonyl-*S*-2-trifluoromethyl-β-aspartyl-(α-methylester)-*S*-prolinate exists as a mixture of *cis–trans* conformers in solution at room temperature.

Peptides with isoaspartyl substructures like compound **1** play an important role in biochemistry.[2,3] The linkage of aspartic acid to the next amino acid is formed with the β-carboxy group. Aging of peptides containing aspartic acid and mismatches during peptide synthesis in organisms lead to an accumulation of those isoaspartyl peptides *in vivo*. In eucaryotic cells, the mismatched peptides and proteins can be recognized and reisomerized by a repair mechanism. The free α-carboxy group of the isoaspartic moiety is methylated by the enzyme carboxy methylase. Loss of methanol leads to the intermediate formation of the succinimide **3** which is hydrolysed to give a mixture of aspartyl **4** and isoaspartyl peptides **1**, the latter re-entering the isomerization cycle (Fig. 1).

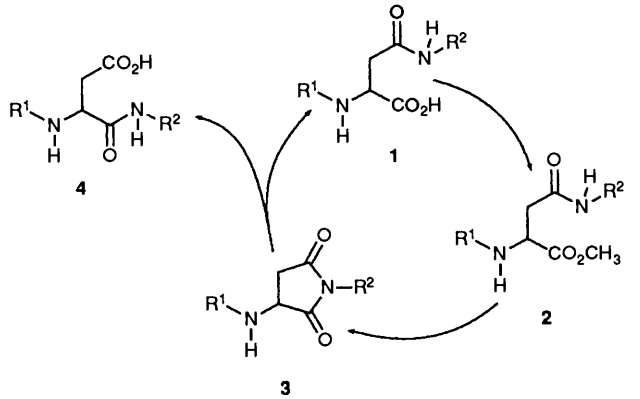
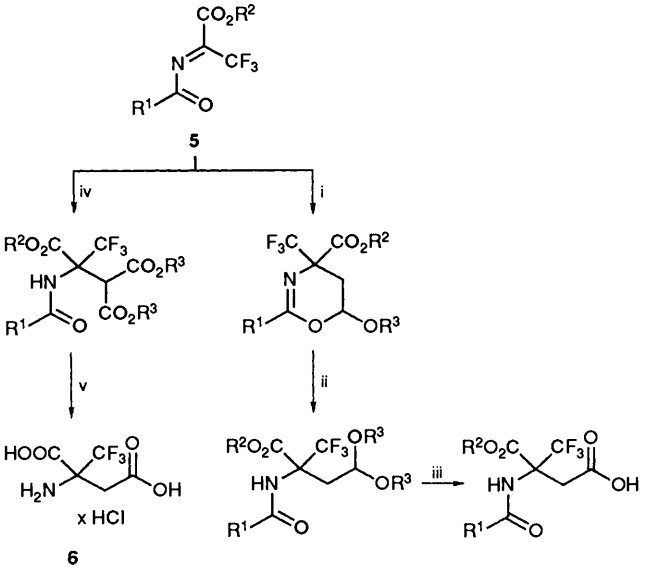


Fig. 1

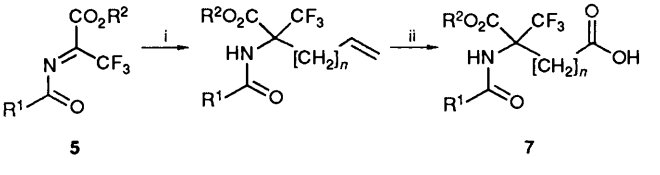
As correctly incorporated aspartyl residues are not methylated enzymatically, the net result of this reaction cycle is the conversion of isoaspartyl peptides to aspartyl peptides.[4] Mismatched peptides not being recognized by the enzyme are degraded partially and excreted; significant amounts of isoaspartyl peptides can be isolated from urine.

The introduction of a α-trifluoromethyl group into the backbone of aspartic acid would facilitate further investigations on the metabolism of isoaspartyl peptides *in vitro* by ^{19}F NMR spectroscopy. Furthermore, α-trifluoromethyl substituted amino acids are known to be potent inhibitors of pyridoxal phosphate-dependent enzymes, which catalyse transamination and decarboxylation processes.[5,6] The replacement of natural amino acids in peptides by non-natural amino acids is a widely used strategy for stabilization of the scissible peptide bond.



Scheme 1 *Reagents:* i, CH_2=$CHOR^3$; ii, R^3OH, H^+; iii, $KMnO_4$; iv, $H_2C(CO_2R^3)_2$; v, H_3O^+

There are only few reports in the literature on strategies for the synthesis of 2-trifluoromethyl substituted aspartic acid **6** [7–9] (Scheme 1). Most of them start from acyl imines **5** of 3,3,3-trifluoropyruvates.



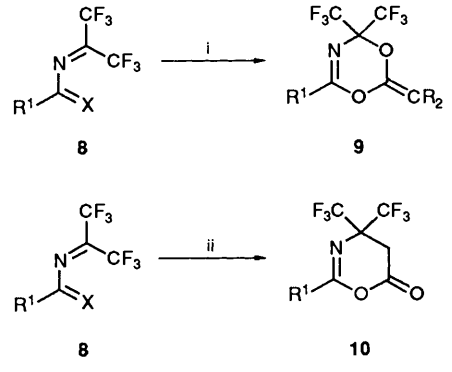
Scheme 2 *Reagents:* i, CH_2=$CH[CH_2]_nMgX$ (n = 1–4); ii, $KMnO_4$

Recently we presented a versatile route to *N*-protected 2-trifluoromethyl substituted ω-carboxylic α-amino acids **7** [10] (Scheme 2).

Results and Discussion

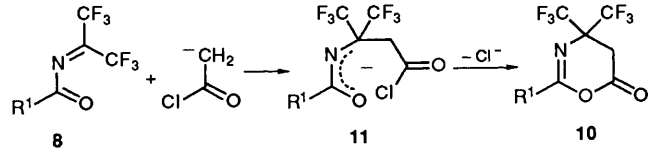
The acyl imines of hexafluoroacetone **8** or 3,3,3-trifluoropyruvates **5** can be considered as 1,4-dipolar species with nucleophilic character at position 1 and strongly electrophilic character at position 4.

During our investigation into the reaction behaviour of ketene and substituted ketene derivatives towards the acyl imines **8** of hexafluoroacetone (Scheme 3), originally reported by Gambaryan and Zeifman,[11] we concluded that, under the reaction conditions applied by these authors ('ketene generation' with acetyl chloride and base *in situ*), no free ketene should be involved.[12]



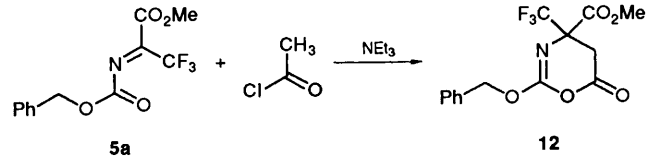
Scheme 3 *Reagents:* i, $R_2C{=}C{=}O$; ii, CH_3COCl, NEt_3

On reaction of the acyl imines with acetyl chloride–triethylamine, an anionic intermediate is formed by nucleophilic attack of the acetyl chloride anion (Scheme 4). It cyclizes to **10** by a 6-*exo-trig* process according to Baldwin's rules.[13] Stable substituted ketenes like diphenylketene or bis(trifluoromethyl)-ketene normally add to 4,4-bis(trifluoromethyl) substituted heterodienes like acyl imines **8**[12,14,15] to yield six-membered cycloadducts **9** across the ketene CO-bond.



Scheme 4

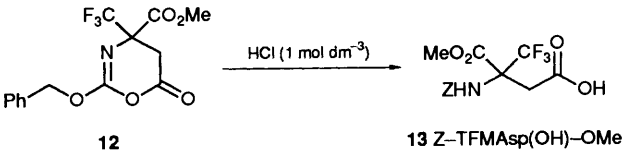
During the course of our research into the synthesis of trifluoromethyl substituted heterocycles and amino acids, we recognized that heterocycle **12**, formed by reaction of the 2-acylimino-3,3,3-trifluoropropionates **5a** with acetyl chloride in the presence of triethylamine, is a β-activated derivative of 2-trifluoromethylaspartic acid. Position 6 is highly activated towards nucleophilic attack due to its anhydride-like structure (Scheme 5).



Scheme 5

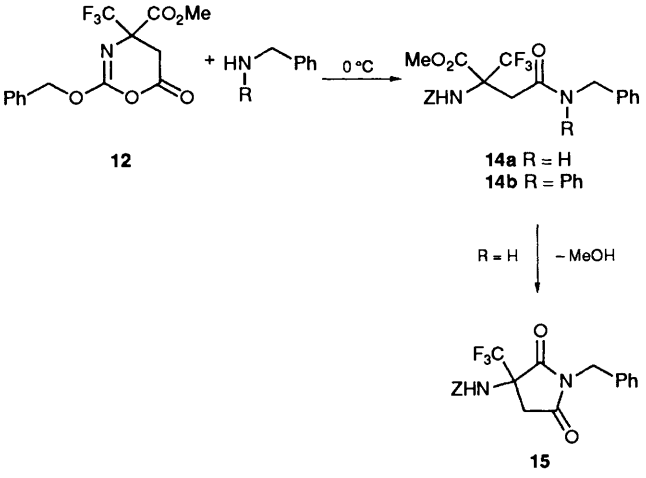
Ring cleavage by hydrochloric acid (1 mol dm^{-3}) at ambient temperature yields *N*-protected 2-trifluoromethylaspartyl α-methyl ester **13** (Scheme 6).

Benzylamine cleaves the six-membered ring **12** at room temperature within minutes. Under the reaction conditions applied product **14a** undergoes spontaneous cyclization to give



Scheme 6

succinimide **15** (Scheme 7) which is a trifluoromethyl substituted analogue of species **3** known from the biochemical studies mentioned above. Secondary amines like *N*-benzylaniline and **12** form derivatives of 2-trifluoromethylasparagine, a ring closure to give the succinimide now not being possible.



Scheme 7

With esters of α-amino acids the 2-trifluoromethyl-β-aspartyl dipeptides **16**, **18** and **19** are formed instantaneously at 0 °C (Scheme 8). Cyclization to succinimides can be prevented by bulky residues in the amino acid ester backbone. Minor amounts of succinimide **17** can be isolated with less sterically hindered amino acids like alaninates. They have been characterized by NMR and GC–MS.

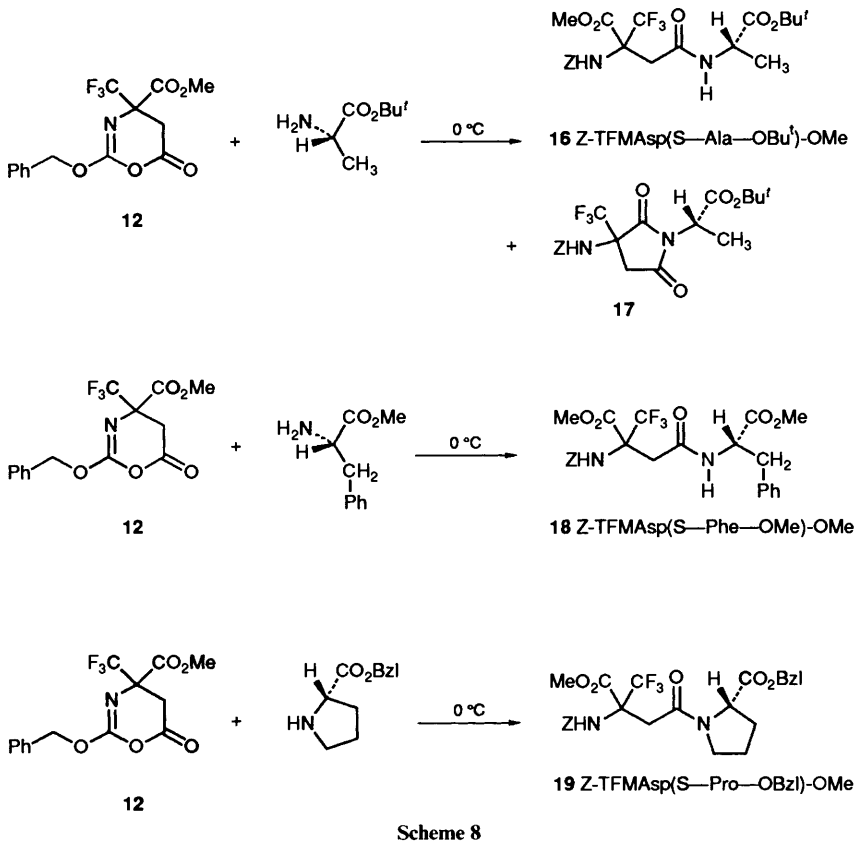
The benzyloxycarbonyl group can be cleaved by hydrogenation according to standard procedures (**18**→**20**) (Scheme 9).

The fully protected or, preferably, the *N*-deprotected diastereoisomeric dipeptides (*e.g.* **20**) are conveniently separated by flash chromatography. The strategy outlined offers a unique, preparatively simple access to dipeptides containing *N*-terminal 2-trifluoromethyl-β-aspartyl residues. Furthermore, the facile resolution of the diastereoisomers enables, after cleavage of the dipeptide, the preparation of enantiomerically pure 2-trifluoromethyl substituted aspartic acid. This fact is especially important as effective enantio- or diastereo-selective syntheses of 2-trifluoromethyl substituted amino acids are not reported in the literature. Synthetic routes to the optical isomers of 2-trifluoromethyl substituted amino acids generally rely on fluorination of an optically active precursor[17] or resolution by chemical[18] or biochemical[19] means.[20]

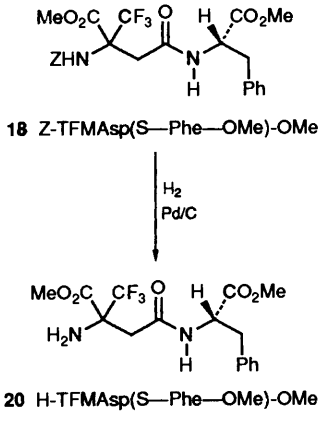
The structures of the products described are confirmed by standard analytical techniques. The most important diagnostic criterion for the identification of the succinimide of type **17** is loss of methanol; in the ^1H NMR spectra the signal corresponding to the methyl group of the ester function is no longer observed.

One diastereoisomer of the dipeptide Z-TFMAsp-(*S*-Pro-OBzl)-OMe* **19a** is obtained as a crystalline solid after

* TFM amino acids = trifluoromethyl amino acids; *e.g.* 2-TFMAsp = 2-trifluoromethyl aspartic acid.



Scheme 8



Scheme 9

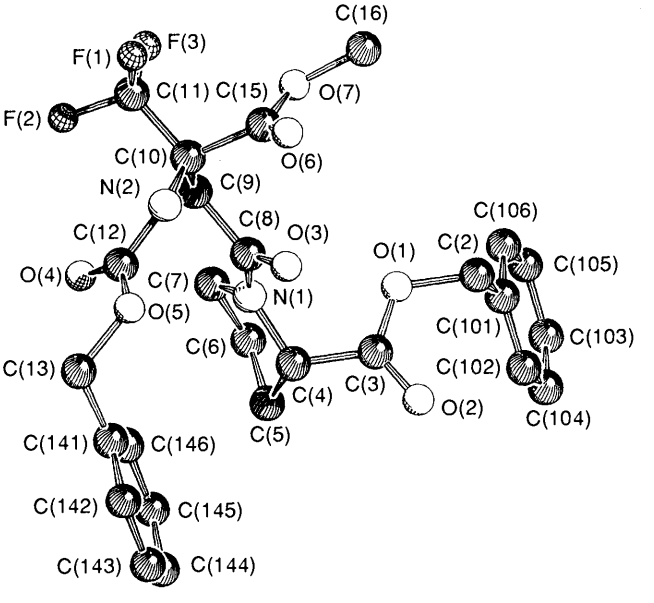


Fig. 2

chromatographic resolution. It was characterized by X-ray (Table 1) analysis (Fig. 2).*

The *S*-proline fragment provides a reference system for the absolute configuration at the chiral centre of the 2-trifluoromethylisoaspartyl moiety, which can therefore be identified as having the *R*-configuration. In the crystalline state, the *trans* configuration at the peptide bond is favoured. The second diastereoisomer **19b** of this dipeptide [Z-*S*-TFMAsp-(*S*-Pro-OBzl)-OMe] is a stable mixture of two conformers (**19ba**, **19bb**) in solution. Obviously, rotation around the peptide bond between the two amino acids is hindered, as is often observed in proline derivatives.[21,22] For peptides composed of α-amino acids, the *trans* conformers are generally expected to be the more stable ones. In the presence of proline, the energy difference between the *cis* and *trans* forms is thought to be quite low. In the case of the side chain of oxytocine (*S*-Bzl-Cys-Pro-Leu-Gly-NH_2), the *trans* form predominates in [$^2H_6$]DMSO over the *cis* form in an approximate ratio of 3:2.[21] The ratio of the conformers **19ba** and **19bb** as measured by ^{19}F NMR depends on the solvent polarity (in $CDCl_3$ 1.3:1; in [$^2H_4$]methanol 1.9:1). The structurally relevant NMR signals of the conformer mixtures have been assigned using COSY and C,H-correlation techniques.

The structural assignment is based on two-dimensional NOE measurements (Fig. 3). In one conformer (**19bb**), which is less populated, a spatial relation exists between the α-proton of

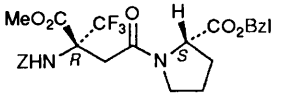
* Tables of bond lengths and bond angles, thermal parameters and hydrogen atom co-ordinates have been deposited with the Cambridge Crystallographic Data Centre (see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1992, Issue 1).

Table 1 Fractional atomic coordinates for **19a**

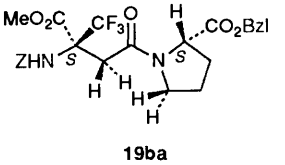
Atom	*X/a*	*Y/b*	*Z/c*
F(1)	0.263 82(7)	0.189 5(3)	0.136 88(6)
F(2)	0.304 48(7)	0.028 6(2)	0.061 13(5)
F(3)	0.332 69(8)	−0.095 3(2)	0.144 33(6)
N(1)	0.594 7(1)	0.156 3(3)	0.073 33(7)
N(2)	0.377 5(1)	0.420 5(3)	0.079 44(8)
O(1)	0.687 60(9)	0.223 9(3)	0.164 19(6)
O(2)	0.744 9(1)	0.505 4(3)	0.121 77(8)
O(3)	0.531 86(9)	0.428 0(3)	0.115 74(7)
O(4)	0.408 76(9)	0.288 6(3)	−0.009 76(6)
O(5)	0.394 4(1)	0.647 7(3)	0.007 47(6)
O(6)	0.386 2(1)	0.505 2(3)	0.190 50(6)
O(7)	0.425 50(9)	0.171 4(3)	0.213 45(6)
C(101)	0.801 1(1)	0.137 2(4)	0.218 06(9)
C(102)	0.867 0(2)	0.217 3(5)	0.193 9(1)
C(103)	0.931 9(2)	−0.107 6(6)	0.217 4(1)
C(104)	0.931 4(2)	0.094 4(7)	0.193 8(1)
C(105)	0.867 2(2)	−0.188 9(6)	0.242 0(1)
C(106)	0.802 1(2)	−0.067 3(5)	0.242 2(1)
C(2)	0.730 2(2)	0.267 3(6)	0.217 6(1)
C(3)	0.701 4(1)	0.356 1(4)	0.119 8(1)
C(4)	0.661 4(1)	0.290 8(4)	0.065 0(1)
C(5)	0.710 9(2)	0.149 4(5)	0.026 4(1)
C(6)	0.690 6(2)	−0.078 7(5)	0.043 9(1)
C(7)	0.605 7(1)	−0.068 7(4)	0.053 9(1)
C(8)	0.533 0(1)	0.240 9(4)	0.096 85(9)
C(9)	0.463 5(1)	0.099 4(4)	0.097 9(1)
C(10)	0.393 8(1)	0.231 9(4)	0.115 14(9)
C(11)	0.323 4(1)	0.088 0(4)	0.114 3(1)
C(12)	0.395 9(1)	0.435 4(4)	0.022 41(9)
C(13)	0.402 2(1)	0.695 1(5)	−0.054 2(1)
C(141)	0.482 2(1)	0.738 4(4)	−0.071 57(9)
C(142)	0.502 4(2)	0.938 6(5)	−0.093 3(1)
C(143)	0.574 6(2)	0.975 8(5)	−0.113 2(1)
C(144)	0.627 8(2)	0.816 3(5)	−0.110 9(1)
C(145)	0.609 3(2)	0.616 5(5)	−0.088 2(1)
C(146)	0.536 9(2)	0.578 1(5)	−0.068 6(1)
C(15)	0.401 8(1)	0.324 7(4)	0.177 21(9)
C(16)	0.431 4(2)	0.237 0(7)	0.274 0(1)

Table 2 Characteristic ^{13}C NMR shift values of compounds **19** (δ)

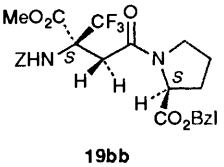
	19a	**19ba** (*trans*)	**19bb** (*cis*)
proline α-C	58.67	58.74	59.56
proline β-C	29.05	29.31	30.84
proline γ-C	24.53	24.48	22.64



19a



19ba



19bb

Scheme 10

proline (δ 4.64) and the more deshielded β-proton (δ 3.92) of TFMAsp, whereas in the other conformer (**19ba**), a relationship between one TFMAsp β-proton (δ 4.28) and the geminal δ-protons (δ 3.48/3.87) of the proline residue is found. Therefore, **19bb** has *cis* and **19ba** *trans* conformation (Scheme 10).

In addition, the carbon NMR shift values support this assignment. According to the literature, the proline γ-carbon atom should be more shielded in the *cis* isomer. Furthermore, the shift differences between the proline β- and γ-carbon atoms should be greater for the *cis* isomer (8–10 ppm) than for the *trans* isomer (5–6 ppm).[21] The observed data (Table 2) support the assignment.

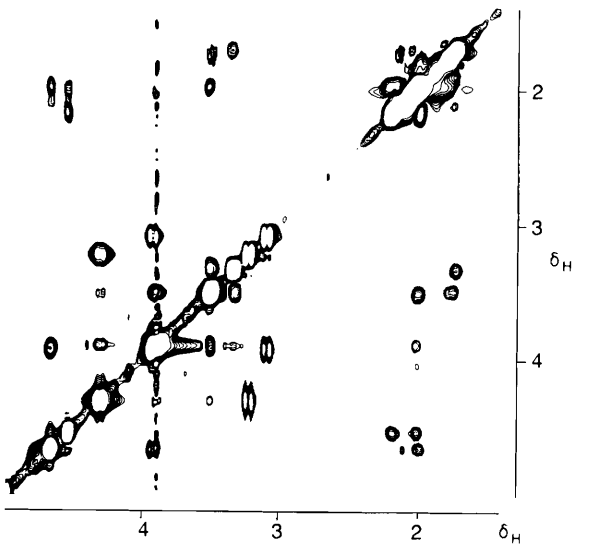


Fig. 3 NOESY spectrum of **19b**

The values in Table 2 clearly indicate that the diastereoisomer **19a** has a *transoid* conformation in $CDCl_3$ solution.

Experimental

For chromatography silica gel 60 (63–200 μm, Merck) and for flash chromatography silica gel 60 (30–63 μm, Riedel-de Haën) were used. Chloroform, dichloromethane and ethyl acetate were distilled over calcium chloride; diethyl ether and dioxane were predried over calcium chloride–potassium hydroxide and dried over sodium benzophenone ketyl under nitrogen.

Melting points (not corrected) were determined using a Tottoli apparatus (Büchi SMP-20); elemental microanalyses were carried out with a Heraeus CHN-Elemental Analyzer. IR spectra were recorded using Perkin-Elmer 157 G or 257 spectrophotometers; ^1H, ^{13}C 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 JEOL FX 90 Q (84 MHz) and Bruker AC 250 (235 MHz) spectrometer. As reference standard TMS was used for ^1H and ^{13}C NMR spectra (internal) and trifluoroacetic acid for ^{19}F NMR spectra (external). All *J*-values are given in Hz. Mass spectra were recorded from electron ionization (EI, 70 eV) with a Varian MAT CH5 instrument. GC–MS analyses were carried out with a Carlo Erba 4160 gas chromatograph (column SE 30) and a Varian MAT M112S mass spectrometer. Optical rotation values were measured using a Perkin-Elmer 241 MC polarimeter. The X-ray analysis was performed on a Enraf-Nonius-CAD4-diffractometer using Mo-Kα radiation and a graphite monochromator.

Methyl 2-Benzyloxy-4,5-dihydro-4-trifluoromethy-6-oxo-1,3-oxazine-4-carboxylate **12**.—To a solution of acyl imine **5a**[23] (14.5 g, 50 mmol) and freshly distilled acetyl chloride (39.3 g, 500 mmol) in absolute diethyl ether (250 cm^3) at 0 °C, triethylamine (5.5 g, 55 mmol) was slowly added. The solution was stirred for 2 h at 0 °C, then hydrolysed with ice water and extracted with diethyl ether. The organic phases were combined, dried over $MgSO_4$, filtered, and evaporated to dryness. Filtration through silica gel (eluent, $CHCl_3$) yielded **12** (11.9 g, 72%) as a pale

yellow oil which partially crystallized on standing (Found: C, 50.70; H, 3.85; N, 4.35. $C_{14}H_{12}F_3NO_5$ requires C, 50.76; H, 3.65; N, 4.23%); ν_{max}(film)/cm^{-1} 1850 (CO) and 1760 (CH); δ_H(360 MHz, $CDCl_3$) 3.30 (1 H, d, J 17.4, 5-H), 3.40 (1 H, d, J 17.4, 5-H), 3.69 (3 H, s, CO_2Me), 5.19 (1 H, d, J 12.2. $C_6H_5CH_2O$), 5.28 (1 H, d, J 12.2, $C_6H_5CH_2O$) and 7.34 (5 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$ and DEPT-135) 45.32 (C-5), 53.84 (CO_2Me), 60.40 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 33.5, C-4), 69.05 ($C_6H_5CH_2O$), 122.70 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 281.7, CF_3), 147.43 (C-2), 159.98 (C-6), 164.44 (CO_2Me), 128.38, 128.77, 128.87 (CH_{arom}) and 134.51 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 5.2 (s); m/z 331 (M, 15%), 224 (2, M − $C_6H_5CH_2O$), 108 (46, $C_6H_5CH_2OH$), 107 (77, $C_6H_5CH_2O$) and 91 (100, $C_6H_5CH_2$).

Hydrolysis of Methyl 2-Benzyloxy-6-oxo-4-trifluoromethyl-4,5-dihydro-1,3-oxazin-4-carboxylate **12**.—Compound **12** (1.7 g, 5 mmol) was stirred at room temperature in a solution of hydrochloric acid (1 mol dm^{-3}; 5 cm^3) in dioxane (20 cm^3). The reaction progress was monitored by ^{19}F NMR. After 3 h, the reaction mixture was extracted with chloroform; the organic layer was dried over $MgSO_4$ and evaporated to dryness *in vacuo* to yield 1-methyl hydrogen *N*-benzyloxycarbonyl-2-trifluoromethylaspartate **13** (1.1 g, 62%) as an orange oil (Found: C, 48.25; H, 4.20; N, 3.95. $C_{14}H_{14}F_3NO_6$ requires C, 48.15; H, 4.04; N, 4.01%); ν_{max}(film)/cm^{-1} 3400 (OH), 3360–3260 (NH) and 1745 (CO); δ_H(360 MHz, $CDCl_3$) 3.32 (1 H, br d, J 17.3, H_β), 3.83 (3 H, s, CO_2Me), 4.19 (1 H, br d, J 17.3, H_β), 5.05 (1 H, d, J 12.3, $C_6H_5CH_2O$), 5.10 (1 H, s, J 12.3, $C_6H_5CH_2O$), 6.31 (1 H, br s, NH), 7.31 (5 H, m, Ar-H) and 10.07 (1 H, br, CO_2H); δ_C(90 MHz, $CDCl_3$) 33.09 (br, C_β), 54.52 (CO_2Me), 63.10 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.5, C_α), 67.39 (br, $C_6H_5CH_2O$), 123.35 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.0, CF_3), 154.30 (OCONH), 166.10 (CO_2Me), 173.37 (C_γ), 128.09, 128.39, 128.62 and 135.71 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 3.1 (s); m/z 349 (M, 4%), 331 (1 , M − H_2O), 224 (2, 331 − $C_6H_5CH_2O$), 108 (100, $C_6H_5CH_2OH$), 107 (49, $C_6H_5CH_2O$) and 91 (99, C_7H_7).

Reaction of **12** *with Benzylamine*.—A solution of **12** (2.6 g, 7.8 mmol) in absolute dichloromethane (10 cm^3) was treated with benzylamine (2 cm^3, 18.3 mmol) and stirred at room temperature. The progress of the slightly exothermic reaction was monitored by ^{19}F NMR. After 1 h, ice water (50 cm^3) was added. The reaction mixture was acidified (pH 6) with hydrochloric acid (1 mol dm^{-3}), extracted with dichloromethane (50 cm^3), and the organic layer was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The remaining yellow oil crystallized slowly after purification by column chromatography over silica gel (eluent, $CHCl_3$) to yield 1-benzyl-3-benzyloxycarbonylamino-3-trifluoromethylpyrrolidine-2,5-dione **15** (2.25 g, 71%), m.p. 96 °C (Found: C, 59.15; H, 4.50; N, 6.95. $C_{20}H_{17}F_3N_2O_4$ requires C, 59.11; H, 4.22; N, 6.89%); ν_{max}(KBr)/ cm^{-1} 3330 (NH), 1720 (CO) and 1705 (CO); δ_H(360 MHz, $CDCl_3$) 3.16 (1 H, d, J 18.6, 4-H), 3.31 (1 H, br, d, J 18.6, 4-H), 4.69 (2 H, br s, $C_6H_5CH_2N$), 5.06 (2 H, br s, $C_6H_5CH_2O$), 5.86 (1 H, s, NH) and 7.26–7.35 (10 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$, and DEPT-135) 36.27 (br, C-4), 43.27 ($C_6H_5CH_2N$), 62.15 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.9, C-3), 68.01 ($C_6H_5CH_2O$), 123.11 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 285.2, CF_3), 154.38 (OCONH), 169.71 (br, C-2), 171.56 (C-5), 128.11, 128.31, 128.35, 128.51, 128.64, 128.66 (CH_{arom}), 134.48, and 135.00 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 1.8 (s); m/z 406 (M, 1%), 315 (18, M − $C_6H_5CH_2$), 298 (7, M − $C_6H_5CH_2OH$), 255 (6, 298 − HNCO), 132 (15, C_6H_5CH=N–CO), 108 (22, $C_6H_5CH_2OH$) and 91 ($C_6H_5CH_2$).

Reaction of **12** *with* N-*Benzylaniline*.—A solution of **12** (1.7 g, 5 mmol) and *N*-benzylaniline (0.9 g, 5 mmol) in absolute dichloromethane (20 cm^3) was stirred at room temperature. The reaction progress was monitored by ^{19}F NMR. The solvent was removed *in vacuo*. The residue, a yellow oil, was purified by flash chromatography on silica gel (eluent, ethyl acetate–hexane, 1:10) yielding methyl N^β-benzyl-N^α-benzyloxycarbonyl-N^β-phenyl-2-trifluoromethylasparaginate **14b** (0.8 g, 31%) as a colourless oil (Found: C, 62.45; H, 4.85; N, 5.40. $C_{27}H_{25}F_3N_2O_5$ requires C, 63.03; H, 4.90; N, 5.44%); ν_{max}(film)/cm^{-1} 3390 (NH), 1750 (CO), 1730 (CO), 1650 (CO) and 1500 (N–CO); δ_H(360 MHz, $CDCl_3$) 3.07 (1 H, d, J 16.7, H_β), 3.91 (3 H, s, CO_2Me), 3.96 (1 H, d, J 16.7, H_β), 4.74 (1 H, d, J 14.4, $NCH_2C_6H_5$), 4.93 (1 H, d, J 14.4, $NCH_2C_6H_5$), 5.16 (2 H, s, $C_6H_5CH_2O$), 6.65 (1 H, br s, NH), 7.06 (2 H, m, Ar-H), 7.19 (2 H, m, Ar-H), 7.25 (3 H, m, Ar-H), 7.36 (4 H, m, Ar-H) and 7.42 (4 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$) 33.92 (C_β), 53.27 ($NCH_2C_6H_5$), 54.41 (CO_2Me), 63.81 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 28.6, C_α), 67.11 ($C_6H_5CH_2O$), 123.93 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.0, CF_3), 154.30 (OCONH), 167.12 (CO_2Me), 167.65 (C_γ), 127.70, 128.02, 128.39, 128.58, 128.59, 128.80, 128.81, 128.88, 130.00, 136.44, 137.26 and 141.30 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 3.4 (s); m/z 514 (M, 2%), 406 (30, M − $C_6H_5CH_2OH$), 347 (7, 406 − CO_2CH_3), 224 (13, 406 − $C_6H_5CH_2NC_6H_5$), 183 (13, $C_6H_5CH_2NHC_6H_5$), 108 (20, $C_6H_5CH_2OH$), 107 (17, $C_6H_5CH_2O$) and 91 (100, C_7H_7).

Synthesis of 2-Trifluoromethylisoaspartyl Peptides.—To a solution of **12** (2.5 g, 7.5 mmol) in absolute diethyl ether (100 cm^3) or absolute dichloromethane (100 cm^3) at 0 °C was slowly added a solution of the corresponding α-amino acid ester (10 mmol) in diethyl ether (10 cm^3) or dichloromethane (10 cm^3), respectively. The reaction mixture was stirred at 0 °C until the ^{19}F NMR spectrum of the solution indicated that the reaction had gone to completion. After evaporation of the solvent, polar impurities were removed by filtration through a 10 cm layer of silica gel (eluent, ethyl acetate–hexane, 2:1). The remaining colourless oil contained the two diastereoisomeric dipeptides and, in the case of alanine *tert*-butyl ester, minor amounts of non-polar impurities like the succinimide **17**. The products were purified by flash chromatography on silica gel (50 cm column; eluent, ethyl acetate–hexane, 1:5); resolution of the diastereoisomers was thereby achieved.

tert-*Butyl* N-*Benzyloxycarbonyl-2-trifluoromethyl-β-aspartyl-(α-methylester)-S-Alaninate* [*Z-TFMAsp*(S-*Ala-OBu*^{t})-*OMe*] **16**.—*tert*-Butyl alaninate (1.5 g, 10 mmol) was used to yield **16a** (0.8 g, 23%), **16b** (0.8 g, 23%) and the diastereoisomeric succinimides **17** (0.3 g, 8%) as colourless oils. Diastereoisomer **16a** (Found: C, 52.55; H, 5.70; N, 5.80. $C_{21}H_{27}F_3N_2O_7$ requires C, 52.94; H, 5.71; N, 5.88%); $[\alpha]_D^{25}$ −5.3 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3395 (NH), 1750 (CO), 1730 (CO), 1670 (CO) and 1510 (N–CO); δ_H(360 MHz, $CDCl_3$), 1.18 (3 H, d, J 7.0, Ala H_β), 1.45 (9 H, s, Ala CO_2Bu^t), 3.21 (1 H, d, J 15.3, TFMAsp H_β), 3.88 (1 H br, d, J 15.3, TFMAsp H_β), 3.90 (3 H, s, CO_2Me) 4.36 (1 H, dq, J 7.6, 7.0, Ala H_α), 5.07 (2 H, br s, $C_6H_5CH_2O$), 6.31 (1 H, br d, J 7.6, Ala NH), 6.45 (1 H, br s, TFMAsp NH) and 7.33 (5 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$) 18.25 (Ala C_β), 27.94 (Bu^t), 35.37 (br, TFMAsp C_β), 48.72 (Ala C_α), 54.28 (CO_2Me), 63.54 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.5, TFMAsp C_α), 67.10 ($C_6H_5CH_2O$), 82.18 (OBu^t), 123.63 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.2, CF_3), 154.22 (OCONH), 166.54 (TFMAsp CO_2Me/TFMAsp C_γ), 171.66 (Ala CO_2Bu^t), 128.04, 128.28, 128.57 and 135.84 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 2.8 (s); m/z 477 (M + 1, 0.1%), 476 (0.1, M), 420 (4, M − C_4H_8), 403 (2, M + 1 − C_4H_8 − H_2O), 375 (5, 403 − CO), 332 (13, M − $C_7H_{14}NO_2$), 268 (1, M + 1 − C_4H_8 − CO − H_2O − $C_6H_5CH_2O$), 267 (2, M − C_4H_8 − CO − H_2O − $C_6H_5CH_2O$), 108 (5, $C_6H_5CH_2OH$), 107 (6, $C_6H_5CH_2O$), 91 (100, C_7H_7), 57 (13, C_4H_9) and 44 (26, CO_2).

Diastereoisomer **16b** (Found: C, 52.95; H, 5.25; N, 5.90.

$C_{21}H_{27}F_3N_2O_7$ requires C, 52.94; H, 5.71; N, 5.88%); $[\alpha]_D^{25}$ −7.0 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3390 (NH), 1750 (CO), 1730 (CO), 1670 (CO) and 1510 (N–CO); δ_H(360 MHz, $CDCl_3$) 1.33 (3 H, d, *J* 7.0, Ala H_β), 1.44 (9 H, s, Ala CO_2Bu^t), 3.16 (1 H, d, *J* 14.1, TFMAsp H_β), 3.90 (1 H, br d, *J* 14.1, TFMAsp H_β), 3.91 (3 H, s, CO_2CH_3), 4.39 (1 H, dq, *J* 9.0, 7.0, Ala H_α), 5.08 (1 H, d, *J* 12.3, $C_6H_5CH_2O$), 5.17 (1 H, d, *J* 12.3, $C_6H_5CH_2O$), 6.39 (1 H, br s, TFMAsp NH), 6.48 (1 H, d, *J* 9.0, Ala NH) and 7.35 (5 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$) 18.38 (Ala C_β), 27.92 (Bu^t), 35.51 (TFMAsp C_β), 48.69 (Ala C_α), 54.26 (CO_2Me), 63.65 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.7, TFMAsp C_α), 67.30 ($C_6H_5CH_2O$), 82.01 (CO_2Bu^t), 123.63 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.4, CF_3), 154.41 (OCONH), 166.50 (TFMAsp CO_2Me), 166.80 (TFMAsp C_γ), 171.49 (Ala CO_2Bu^t), 128.09, 128.21, 128.51 and 135.84 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 2.9 (s); *m*/*z* 477 (M + 1, 0.1%) 476 (0.1, M), 420 (4, M − C_4H_8), 403 (2, M + 1 − C_4H_8 − H_2O), 375 (4, 403 − CO), 332 (12, M − $C_7H_{14}NO_2$), 268 (1, M + 1 − C_4H_8 − CO − H_2O − $C_6H_5CH_2O$), 267 (2, M − C_4H_8 − CO − H_2O − $C_6H_5CH_2O$), 108 (4, $C_6H_5CH_2OH$), 107 (7, $C_6H_5CH_2O$), 91 (100, C_7H_7), 57 (14, C_4H_9) and 44 (29, CO_2).

tert-*Butyl* 2-(3-*Benzyloxycarbonylamino*-3-*trifluoromethyl*-*pyrrolidin*-2,5-*dion*-1-*yl*)-*propionate* **17**, diastereoisomeric mixture, $C_{20}H_{23}F_3N_2O_6$; δ_H(360 MHz, $CDCl_3$) 1.42 (9 H, s, Bu^t), 1.50 (3 H, d, *J* 7.2, 3-H), 3.12/3.15 (1 H, d/d, *J* 17.1/17.1, pyrrolidindione 4-H), 3.48 (1 H, br, d, *J* 17.1, pyrrolidindione 4-H), 4.72/4.76 (1 H, q/q, 1 H, *J* 7.2/7.2, 2-H), 5.10 (2 H, br s, $C_6H_5CH_2O$), 5.99/6.00 (1 H, s/s, NH) and 7.34 (5 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$), 13.73/13.93 (C-3), 27.71 (Bu^t), 36.27/36.45 (pyrrolidindione C-4), 49.63/49.74 (C-2), 61.93/62.22 (q/q, $^2J[^{13}C^{19}F\{^1H\}]$ 30.0/30.1, pyrrolidindione C-3), 67.91/68.06 ($C_6H_5CH_2O$), 82.82/82.92 (OBu^t), 123.18 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 286.2, CF_3), 154.05/154.57 (OCONH), 167.23/167.35 (pyrrolidindione C-2), 168.91/169.10 (pyrrolidindione C-5), 170.96 (C-1), 128.37, 128.39, 128.61, 128.68, 128.70, 128.72, 135.12 and 135.32 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 0.7/0.9 (s); GC–MS: diastereoisomer 1: *m*/*z* 388 (M − C_4H_8, 4%), 370 (5, 388 − H_2O), 342 (2, 370 − CO), 253 (5, 388 − CO − $C_6H_5CH_2O$), 235 (3, 253 − H_2O), 209 (2, 253 − CO_2), 108 (14, $C_6H_5CH_2OH$), 107 (30, $C_6H_5CH_2O$), 91 (100, C_7H_7) and 57 (66, C_4H_9); diastereoisomer 2: *m*/*z* 388 (M − C_4H_8, 3%), 370 (3, 388 − H_2O), 342 (1, 370 − CO), 253 (3, 388 − CO − $C_6H_5CH_2O$), 235 (2, 253 − H_2O), 209 (2, 253 − CO_2), 108 (15, $C_6H_5CH_2OH$), 107 (30, $C_6H_5CH_2O$), 91 (100, C_7H_7) and 57 (72, C_4H_9).

Methyl N-*Benzyloxycarbonyl*-2-*trifluoromethyl*-β-*aspartyl*-(α-*methylester*)-S-*phenylalaninate* [*Z*-*TFMAsp*(S-*Phe*-*OMe*)-*OMe*] **18**.—Methyl S-phenylalaninate (1.9 g, 10 mmol) was used to yield the two diastereoisomeric dipeptides **18a** (1.2 g, 32%) and **18b** (1.2 g, 32%) as colourless oils. Diastereoisomer **18a** (Found: C, 56.25; H, 4.90; N, 4.95. $C_{24}H_{25}F_3N_2O_7$ requires C, 56.47; H, 4.94; N, 5.49%); $[\alpha]_D^{25}$ +6.9 (*c* 0.5 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3390 (NH), 1730 (CO), 1670 (CO) and 1500 (N–CO); δ_H(360 MHz, $CDCl_3$), 2.99 (1 H, dd, *J* 13.9, 6.6, Phe H_β), 3.10 (1 H, dd, *J* 13.9, 5.8, Phe H_β), 3.11 (1 H, d, *J* 15.2, TFMAsp H_β), 3.64 (3 H, s, CO_2Me), 3.86 (3 H, s, CO_2Me), 3.89 (1 H, br d, *J* 15.2, TFMAsp H_β), 4.74 (1 H, m, Phe H_α), 4.95 (1 H, d, *J* 12.1, $C_6H_5CH_2O$), 5.01 (1 H, d, *J* 12.1, $C_6H_5CH_2O$), 6.38 (1 H, s, TFMAsp NH), 6.51 (1 H, br d, *J* 8.5, Phe NH) and 7.07–7.34 (10 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$), 35.33 (TFMAsp C_β), 37.51 (Phe C_β), 52.27 (Phe CO_2Me), 53.36 (Phe C_α), 54.31 (TFMAsp CO_2Me), 63.62 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.6, TFMAsp C_α), 67.26 ($C_6H_5CH_2O$), 123.59 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.0, CF_3), 154.57 (OCONH), 166.38 (TFMAsp CO_2Me), 167.29 (TFMAsp C_γ), 171.32 (Phe CO_2Me), 127.20, 128.08, 128.22, 128.49, 128.67, 129.15, 135.72 and 135.80 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 2.9 (s); *m*/*z* 510 (M, 4%), 419 (2, M − C_7H_7), 348 [5, Z-TFMAsp(NH_2)-OMe], 241 (3, 348 − $C_6H_5CH_2O$), 162 (46, $C_6H_5CH{=}CHCO_2CH_3$) and 91 (100, C_7H_7).

Diastereoisomer **18b** (Found: C, 56.10; H, 4.80; N, 5.20. $C_{24}H_{25}F_3N_2O_7$ requires C, 56.47; H, 4.94; N, 5.49%); $[\alpha]_D^{25}$ + 19.9 (*c* 0.3 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3390 (NH), 1730 (CO), 1670 (CO) and 1500 (N–CO); δ_H(360 MHz, $CDCl_3$) 2.86 (1 H, dd, *J* 13.8, 6.0, Phe H_β), 3.02 (1 H, dd, *J* 13.8, 5.4, Phe H_β), 3.22 (1 H, d, *J* 15.3, TFMAsp H_β), 3.65 (3 H, s, CO_2Me), 3.88 (3 H, s, CO_2Me), 3.90 (1 H, br d, *J* 15.3, TFMAsp H_β), 4.78 (1 H, ddd, *J* 7.9, 6.0, 5.4, Phe H_α), 5.01 (1 H, d, *J* 12.3, $C_6H_5CH_2O$), 5.09 (1 H, d, *J* 12.3, $C_6H_5CH_2O$), 6.22 (1 H, br, *J* 7.9, Phe NH), 6.42 (1 H, s, TFMAsp NH), 7.08 (2 H, m, Ar-H) and 7.22–7.32 (8 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$) 35.06 (TFMAsp C_β), 37.92 (Phe C_β), 52.28 (Phe CO_2Me), 53.17 (Phe C_α), 54.33 (TFMAsp CO_2Me), 63.42 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.4, TFMAsp C_α), 67.07 ($C_6H_5CH_2O$), 123.62 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 287.9, CF_3), 154.23 (OCONH), 166.45 (TFMAsp CO_2Me), 166.80 (TFMAsp C_γ), 171.41 (Phe CO_2Me), 127.21, 127.90, 128.27, 128.57, 128.66, 129.25, 135.46 and 135.82 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 2.6 (s); *m*/*z* 510 (M, 5%), 419 (2, M − C_7H_7), 348 [6, Z-TFMAsp-(NH_2)-OMe], 241 (4, 348 − $C_6H_5CH_2O$), 162 (44, $C_6H_5CH{=}CHCO_2CH_3$) and 91 (100, C_7H_7).

Benzyl N-*Benzyloxycarbonyl*-R-2-*trifluoromethyl*-β-*aspartyl*-(α-*methylester*)-S-*prolinate* [*Z*-R-*TFMAsp*(S-*Pro*-*OBzl*)-*OMe*] **19**.—Benzyl *S*-prolinate (1.5 g, 10 mmol) was used to yield the two diastereoisomeric dipeptides **19a** (0.8 g, 20%) and **19b** (0.8 g, 20%). After resolution by flash chromatography, the diastereoisomer **19a** was recrystallized from benzene–hexane (m.p. 68 °C). Diastereoisomer **19a** (Found: C, 58.22; H, 4.98; N, 5.24. $C_{26}H_{27}F_3N_2O_7$ requires C, 58.21; H, 5.07; N, 5.22%); $[\alpha]_D^{25}$ −49.3 (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3400 (NH), 1750 (CO), 1640 (CO) and 1500 (N–CO); δ_H(360 MHz, $CDCl_3$) 1.90–2.05 (4 H, m, Pro HC_β/Pro H_γ), 3.22 (1 H, d, *J* 16.2, TFMAsp H_β), 3.58 (2 H, m, Pro H_δ), 3.81 (3 H, s, CO_2Me), 4.19 (1 H, br d, *J* 16.2, TFMAsp HC_β), 4.40 (1 H, dd, *J* 8.0, 3.0, Pro HC_α), 5.02 (1 H, d, *J* 12.4, $C_6H_5CH_2O$), 5.06 (1 H, d, *J* 12.4, $C_6H_5CH_2O$), 5.08 (1 H, d, *J* 12.4, $C_6H_5CH_2O$), 5.16 (1 H, d, *J* 12.4, $C_6H_5CH_2O$), 6.45 (1 H, br s, NH) and 7.25–7.35 (10 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$, and DEPT-135) 24.53 (Pro C_γ), 29.05 (Pro C_β), 33.06 (TFMAsp C_β), 46.97 (Pro C_δ), 54.20 (CO_2CH_3), 58.67 (Pro C_α), 63.19 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.0, TFMAsp C_α), 66.75 (2 × $C_6H_5CH_2O$), 123.75 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.0, CF_3), 154.17 (OCONH), 166.29 (TFMAsp CO_2Me), 166.65 (TFMAsp C_γ), 171.69 (Pro CO_2Bzl), 127.66, 127.92, 128.08, 128.22, 128.39, 128.46, 128.54, 135.73 and 136.18 (C_{arom}); δ_F(84 MHz, $CDCl_3$) 2.5 (s); *m*/*z* 536 (M, 5%), 428 (1, M − $C_6H_5CH_2OH$), 401 (7, M − $CO_2CH_2C_6H_5$), 332 (2, M − $C_{12}H_{14}NO_2$), 293 (7, 401 − $C_6H_5CH_2OH$), 204 (4, $C_{12}H_{14}NO_2$), 91 (83, C_7H_7) and 70 (100, C_4H_8N).

A single crystal was sealed in a glass capillary under argon.

Crystal data. $C_{26}H_{27}F_3N_2O_7$, *M* = 536.5. Orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 17.756(2), *b* = 6.171(1), *c* = 23.146(3) Å, *V* = 2536.16 Å^3, *Z* = 4, D_x = 1.405 g cm^{-3}. Colourless needles, μ(Mo-Kα) = 1.09 cm^{-1}.

Data collection and processing. CAD4 diffractometer, graphite-monochromated Mo-Kα radiation, λ = 0.710 69 Å, *T* = −55 °C, 4062 reflections measured, 3528 unique (R_{int} = 0.0203), giving 3284 with $F_0 \geq 2\sigma(F_0)$, no absorption correction.

Structure analysis and refinement. Direct methods (SHELXS-86[24]), anisotropic refinement with all non-hydrogen atoms; all hydrogen atoms found subsequently in difference Fourier maps and refined isotropically. The weighting scheme $w = 1.1673\ \sigma^{-2}(F_0)$ gave satisfactory agreement analyses; final *R* and R_w values are 0.036 and 0.027.

Benzyl N-*Benzyloxycarbonyl*-S-2-*trifluoromethyl*-β-*aspartyl*-(α-*methylesther*)-S-*prolinate* [*Z*-S-*TFMAsp*(*S*-*Pro*-*OBzl*)-*OMe*] **19b** (2 conformers; Found: C, 57.80; H, 4.80; N, 4.80.

$C_{26}H_{27}F_3N_2O_7$ requires C, 58.21; H, 5.07; N, 5.22%); $[\alpha]_D^{25}$ −49.2 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3390 (NH), 1750 (CO), 1730 (CO), 1650 (CO) and 1500 (N–CO); δ_H(conformer **19ba**, 360 MHz, $CDCl_3$) 1.96 (1 H, m, Pro H_γ), 2.00 (1 H, m, Pro H_γ), 2.00 (1 H, m, Pro H_β), 2.17 (1 H, m, Pro H_β), 3.20 (1 H, d, *J* 15.6, TFMAsp H_β), 3.48 (1 H, m, Pro H_δ), 3.87 (1 H, m, Pro H_δ), 3.87 (3 H, s, CO_2Me), 4.28 (1 H, br d, *J* 15.6, TFMAsp H_β), 4.51 (1 H, br dd, *J* 8.9, 3.4, Pro H_α), 5.04 (1 H, d, *J* 12.3, $C_6H_5CH_2O$), 5.06 (1 H, d, *J* 13.6, $C_6H_5CH_2O$), 5.10 (1 H, d, *J* 12.3, $C_6H_5CH_2O$), 5.13 (1 H, d, *J* 13.6, $C_6H_5CH_2O$), 6.40 (1 H, br s, NH) and 7.25–7.38 (10 H, m, Ar-H); δ_H(conformer **19bb**, 360 MHz, $CDCl_3$) 1.70 (1 H, m, Pro H_γ), 1.73 (1 H, m, Pro H_γ), 1.98 (1 H, m, Pro H_β), 2.10 (1 H, m, Pro H_β), 3.06 (1 H, d, *J* 15.6, TFMAsp H_β), 3.31 (1 H, m, Pro H_δ), 3.46 (1 H, m, Pro H_δ), 3.87 (3 H, s, CO_2Me), 3.92 (1 H, br d, *J* 15.6, TFMAsp H_β), 4.64 (1 H, br, d, *J* 7.0, Pro H_α), 4.97 (1 H, d, *J* 12.8, $C_6H_5CH_2O$), 5.08 (1 H, d, *J* 12.8, $C_6H_5CH_2O$), 5.15 (1 H, d, *J* 13.6, $C_6H_5CH_2O$), 5.21 (1 H, d, *J* 13.6, $C_6H_5CH_2O$), 6.41 (1 H, br s, NH) and 7.25–7.38 (10 H, m, Ar-H); δ_C(conformer **19ba**, 90 MHz, $CDCl_3$, and DEPT-135) 24.48 (Pro C_γ), 29.31 (Pro C_β), 33.38 (br, TFMAsp C_β), 47.16 (Pro C_δ), 54.22 (CO_2Me), 58.74 (Pro C_α), 63.41 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.0, TFMAsp C_α), 66.62, 66.71 (2 × $C_6H_5CH_2O$), 123.80 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 288.0, CF_3), 154.16 (OCONH), 166.66, 166.71 (TFMAsp CO_2Me/C_γ), 171.01 (Pro CO_2Bzl); δ_C(conformer **19bb**, 90 MHz, $CDCl_3$) 22.64 (Pro C_γ), 30.84 (Pro C_β), 33.52 (br, TFMAsp C_β), 46.37 (Pro C_δ), 54.30 (CO_2Me), 59.56 (Pro C_α), 63.43 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 29.1, TFMAsp C_α), 66.36, 67.12 (2 × $C_6H_5CH_2O$), 123.70 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 228.0, CF_3), 154.17 (OCONH), 166.33 (TFMAsp CO_2Me), 166.68 (TFMAsp-C_γ), 171.74 (Pro CO_2Bzl), 127.51, 127.88, 128.00, 128.06, 128.13, 128.24, 128.43, 128.44, 128.48, 128.53, 128.61, 128.73, 135.23, 135.87, 136.10 and 136.35 (C_{arom} of both conformers); δ_F(84 MHz, $CDCl_3$), 2.4 (s); 2.8 (s); ratio of integral values in $CDCl_3$ 1.9:1, in [$^2H_4$]methanol 1.3:1; *m/z* 536 (M, 2%), 428 (1, M − $C_6H_5CH_2OH$), 401 (4, M − $CO_2CH_2C_6H_5$), 332 (3, M − $C_{12}H_{14}NO_2$), 293 (6, 401 − $C_6H_5CH_2OH$), 204 (7, $C_{12}H_{14}NO_2$), 91 (100, C_7H_7) and 70 (92, C_4H_8N).

NOESY experiment: 32 scans (preceded by 2 dummy scans) were recorded at 25 °C into 2K data blocks for each of the 256 t_1 values with a mixing time of 600 ms, a relaxation delay of 2 s and spectral widths of 3597.12 Hz. Phase senstivity was achieved by the TPPI method. The longitudinal relaxation time T_1 was determined by an inversion-recovery experiment. The mixing time was randomized in the range ±3%. After zero-filling to 2K × 2K data were apodized with shifted square sine bell functions. After FT and phase correction, a baseline correction in both dimensions was applied.

Hydrogenolytic Cleavage of the N-*Protecting Group. Methyl 2-Trifluoromethyl-β-aspartyl-(α-methylester)-S-phenylalaninate* [H-*TFMAsp*(S-*Phe-OMe*)-*OMe*] **20**.—A solution of the diastereoisomeric mixture of the dipeptide Z-TFMAsp(L-Phe-OMe)-OMe **18** (2.0 g, 4 mmol) in absolute methanol (20 cm^3) was treated with hydrogen in presence of palladium on charcoal (50 mg, 10% Pd) until hydrogen was no longer consumed. The catalyst was filtered off, the solvent was evaporated *in vacuo*, and the two diastereoisomers **20a** (0.7 g, 48%) and **20b** (0.7 g, 48%) were resolved by flash chromatography on silica gel (50 cm column, eluent ethyl acetate–hexane, 1:2); diastereoisomer **20a** (Found: C, 50.70; H, 5.00; N, 7.45. $C_{16}H_{19}F_3N_2O_5$ requires C, 51.07; H, 5.09; N, 7.44%); $[\alpha]_D^{25}$ +38.9 (*c* 1.2 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3380 (NH), 3340 (NH), 1745 (CO), 1670 (CO) and 1540 (N–CO); δ_H(360 MHz, $CDCl_3$) 2.33 (2 H, br s, NH_2), 2.59 (1 H, d, *J* 15.7, TFMAsp H_β), 3.01 (1 H, dd, *J* 13.9, 6.7, Phe H_β), 3.08 (1 H, br, d, *J* 15.7, TFMAsp H_β), 3.11 (1 H, dd, *J* 13.9, 5.6, Phe H_β), 3.71 (3 H, s, CO_2Me), 3.73 (3 H, s, CO_2Me), 4.83 (1 H, ddd, *J* 7.8, 6.7, 5.6, Phe H_α), 7.00 (1 H, br d, *J* 7.8, Phe NH), 7.10 (2 H, m, Ar-H) and 7.24–7.32 (3 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$) 37.84 (Phe C_β), 37.86 (TFMAsp C_β), 52.41 (Phe, CO_2Me), 53.31 (Phe C_α), 53.66 (TFMAsp CO_2Me), 62.94 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 27.5, TFMAsp C_α), 124.22 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 285.4, CF_3), 168.00 (TFMAsp C_γ), 168.94 (br, TFMAsp CO_2Me), 171.89 (Phe CO_2Me), 127.23, 128.69, 129.22 and 135.77 (C_{arom}); δ_F(84 MHz, $CDCl_3$) −0.74 (s); *m/z* 376 (M, 11%), 317 (19, M − CO_2CH_3), 285 (5, M − C_7H_7), 180 (28, $C_5H_3F_3O_3$), 170 [17, $CF_3C(CO_2CH_3)NH_2CH_2$], 162 (100, $C_6H_5CH{=}CHCO_2CH_3$), 156 [18, $CF_3C(CO_2CH_3){=}NH_2$], 91 (15, C_7H_7) and 88 (36, $C_4H_8O_2$).

Diastereoisomer **20b** (Found: C, 50.80; H, 5.10; N, 7.15. $C_{16}H_{19}F_3N_2O_5$ requires C, 51.07; H, 5.09; N, 7.44%); $[\alpha]_D^{25}$ + 121.1 (*c* 1.7 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3370 (NH), 3340 (NH), 1740 (CO), 1665 (CO) and 1535 (N–CO); δ_H(360 MHz, $CDCl_3$) 2.38 (2 H, br, s, NH_2), 2.59 (1 H, d, *J* 15.7, TFMAsp H_β), 3.03 (1 H, dd, *J* 14.0, 6.5, Phe H_β), 3.07 (1 H, br d, *J* 15.7, TFMAsp H_β), 3.13 (1 H, dd, *J* 14.0, 5.6, Phe H_β), 3.71 (3 H, s, CO_2Me), 3.80 (3 H, s, CO_2Me), 4.81 (1 H, ddd, *J* 7.5, 6.5, 5.6, Phe H_α), 6.97 (1 H, br d, *J* 7.5, Phe NH), 7.11 (2 H, m, Ar-H) and 7.26–7.30 (3 H, m, Ar-H); δ_C(90 MHz, $CDCl_3$) 37.65 (Phe C_β), 37.81 (TFMAsp C_β), 52.42 (Phe, CO_2Me), 53.30 (Phe C_α), 53.73 (TFMAsp CO_2Me), 62.95 (q, $^2J[^{13}C^{19}F\{^1H\}]$ 27.5, TFMAsp C_α), 124.18 (q, $^1J[^{13}C^{19}F\{^1H\}]$ 285.4, CF_3), 167.90 (TFMAsp C_γ), 168.78 (br, TFMAsp CO_2Me), 171.68 (Phe CO_2Me), 127.23, 128.66, 129.20 and 135.74 (C_{arom}); δ_F(84 MHz, $CDCl_3$) −0.76 (s); *m/z* 376 (M, 11%), 317 (16, M − CO_2CH_3), 285 (7, M − C_7H_7), 180 (23, $C_5H_3F_3O_3$), 170 [17, $CF_3C(CO_2CH_3)NH_2CH_2$], 162 (100, $C_6H_5CH{=}CHCO_2CH_3$), 156 [18, $CF_3C(CO_2CH_3){=}NH_2$], 91 (15, C_7H_7) and 88 (39, $C_4H_8O_2$).

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References

1 Synthesis of 2-trifluoromethyl α-amino acids, Part 11. – Part 10. K. Burger, K. Gaa and K. Mütze, *Chem.-Ztg.*, in the press.
2 S. Santikarn, D. H. Williams, R. J. Smith, S. J. Hammond, D. P. Botes, A. A. Tuinman, P. L. Wessels, C. C. Viljoen and H. Kruger, *J. Chem. Soc., Chem. Commun.*, 1983, 652.
3 E. D. Murray, jr. and S. Clarke, *J. Biol. Chem.*, 1984, **259**, 10722.
4 (*a*) P. N. McFadden and S. Clarke, *J. Biol. Chem.*, 1986, **261**, 11503; (*b*) P. N. McFadden and S. Clarke, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 2595; (*c*) R. C. Stephenson and S. Clarke, *J. Biol. Chem.*, 1989, **264**, 6164; (*d*) B. A. Johnson and D. W. Asward, *Biochem.*, 1990, **29**, 4373.
5 V. P. Kuk'har, Yu. L. Yagupol'skii and V. A. Soloshonok, *Usp. Khim.*, 1990, **59**, 149; *Russ. Chem. Rev.*, 1990, **59**, 89.
6 C. Walsh, *Tetrahedron*, 1972, **38**, 871.
7 S. N. Osipov, A. F. Kolomiets and A. V. Fokin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1989, 746; *Bull. Acad. Sci. USSR, Chem. Sect.*, 1989, 673.
8 S. N. Osipov, N. D. Chkanikov, Yu. V. Shklyaev, A. F. Kolomiets and A. V. Fokin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1989, 2131; *Bull. Acad. Sci. USSR, Chem. Sect.*, 1989, 1962.
9 K. Burger, K. Gaa and K. Geith, *J. Fluorine Chem.*, 1988, **41**, 429.
10 K. Burger and K. Gaa, *Chem.-Ztg.*, 1990, **114**, 101.
11 Yu. V. Zeifman, N. P. Gambaryan, L. A. Simonyan, R. B. Minasyan and I. L. Knunyants, *Zh. Obsch. Khim.*, 1967, **37**, 2476; *J. Gen. Chem. USSR*, 1967, **37**, 2355.
12 K. Burger, E. Huber, N. Sewald and H. Partscht, *Chem.-Ztg.*, 1986, **110**, 83.
13 J. E. Baldwin, *J. Chem. Soc., Chem. Commun.*, 1976, 734.
14 N. P. Gambaryan and Yu. V. Zeifman, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1969, **9**, 2059.
15 K. Burger and R. Ottlinger, *J. Fluorine Chem.*, 1978, **11**, 29.
16 E. Wünsch, in *Houben-Weyl*, ed. E. Müller, Thieme. Stuttgart, 1974, vol. XV 1/2, and literature cited therein.

17 J. Kollonitsch, S. Marburg and L. Perkins, *J. Org. Chem.*, 1976, **41**, 3107.
18 G. Gal, J. M. Chemerda, D. Reinbold and R. Purick, *J. Org. Chem.*, 1977, **42**, 142.
19 J. W. Keller and B. J. Hamilton, *Tetrahedron Lett.*, 1986, **27**, 1249.
20 For a review see: J. T. Welch and S. Eswarakrishnan, in *Fluorine in Bioorganic Chemistry*, Wiley, New York, 1991.
21 V. R. Hruby, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, ed. B. Weinstein, Marcel Dekker, New York, 1974, vol. 3, p. 53.
22 R. DesLauriers, R. Walter and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, 1972, **48**, 854.
23 K. Burger, E. Höß, K. Gaa, N. Sewald and Ch. Schierlinger, *Z. Naturforsch.*, 1991, **46b**, 361.
24 G. M. Sheldrick, SHELXS-86, Program for Crystal Structure Determinations, Goettingen 1986.

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