HETEROCYCLES, Vol. 59, 2003, No. 1, pp. 189 - 198, Received, 17th June, 2002

HEXAFLUOROACETONE AS PROTECTION AND ACTIVATION REAGENT IN AMINO ACID AND PEPTIDE CHEMISTRY
REGIOSPECIFIC α-FUNCTIONALIZATION OF ASPARTIC ACID

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Abstract - A highly efficient method for regiospecific α-functionalization of aspartic acid is described. Key step is the synthesis of a *N*-protected and regioselectively α-carboxy-activated heterocyclic intermediate from aspartic acid and hexafluoroacetone. The new strategy offers *i.a.* a two step access to the sweetener Aspartame® and to libraries of aspartame analogues.

INTRODUCTION

Regioselective derivatization of multifunctional compounds like α- and/or ω-functionalization of ω-carboxy-α-amino acids requires sophisticated protection/activation concepts.[1,2] Therefore, syntheses of relatively simple target molecules like aspartic acid α-esters,[3] aspartic acid α-amides (isoasparagine),[4] e.g. of the sweetener Aspartame[5] are surprisingly laborious more-step procedures: The classical syntheses of Asp-α-OMe is a five-step,[6] and of Aspartame a seven step procedure.[7]

The state of the art synthesis of α-functionalized aspartic acid derivatives consists of a *N*-protection step, preferentially the introduction of an urethane protective group, and activation *via* an intramolecular anhydride.[8] The main drawback of this concept is the lack of regiospecifity of the aminolysis. Consequently, a separattion of regioisomers is neccessary. The final step involves *N*-deprotection.

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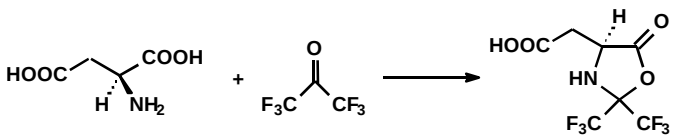
** Dedicated to Prof. Yuichi Kanaoka on the occasion of his 75th birthday.

Therefore, the development of new strategies, where activation of the α-carboxylic group and protection of the adjacent amino function can be accomplished in one step is of current interest. This challenge can be met by the „heterocyclic route“ *via* oxazolidinones,[9,10] *N*-thiocarboxy anhydrides[11], and Leuch´s anhydrides.[12] However, most oxazolidinones derived from aldehydes like formaldehyde,[13] require additonal *N*-protection and *N*-deprotection. We now report on an approach where separate *N*-protection and *N*-deprotection can be avoided.[14]

RESULTS AND DISCUSSION

α-Amino acids and hexafluoroacetone readily react in dimethyl sulfoxide at room temperature to give 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones in excellent yields.[15] With formation of a five-membered heterocyclic system, amino group protection and selective activation of the α-carboxy group are achieved in one step. This reaction tolerates additional functional groups in the side-chain, like ω-carboxy groups. On reaction of aspartic acid (**1**) with hexafluoroacetone (**2**) the five-membered lactone (**3**) is formed exclusively. No trace of a six-membered ring systems could by identified on monitoring the reaction by ^{19}F NMR spectroscopy.

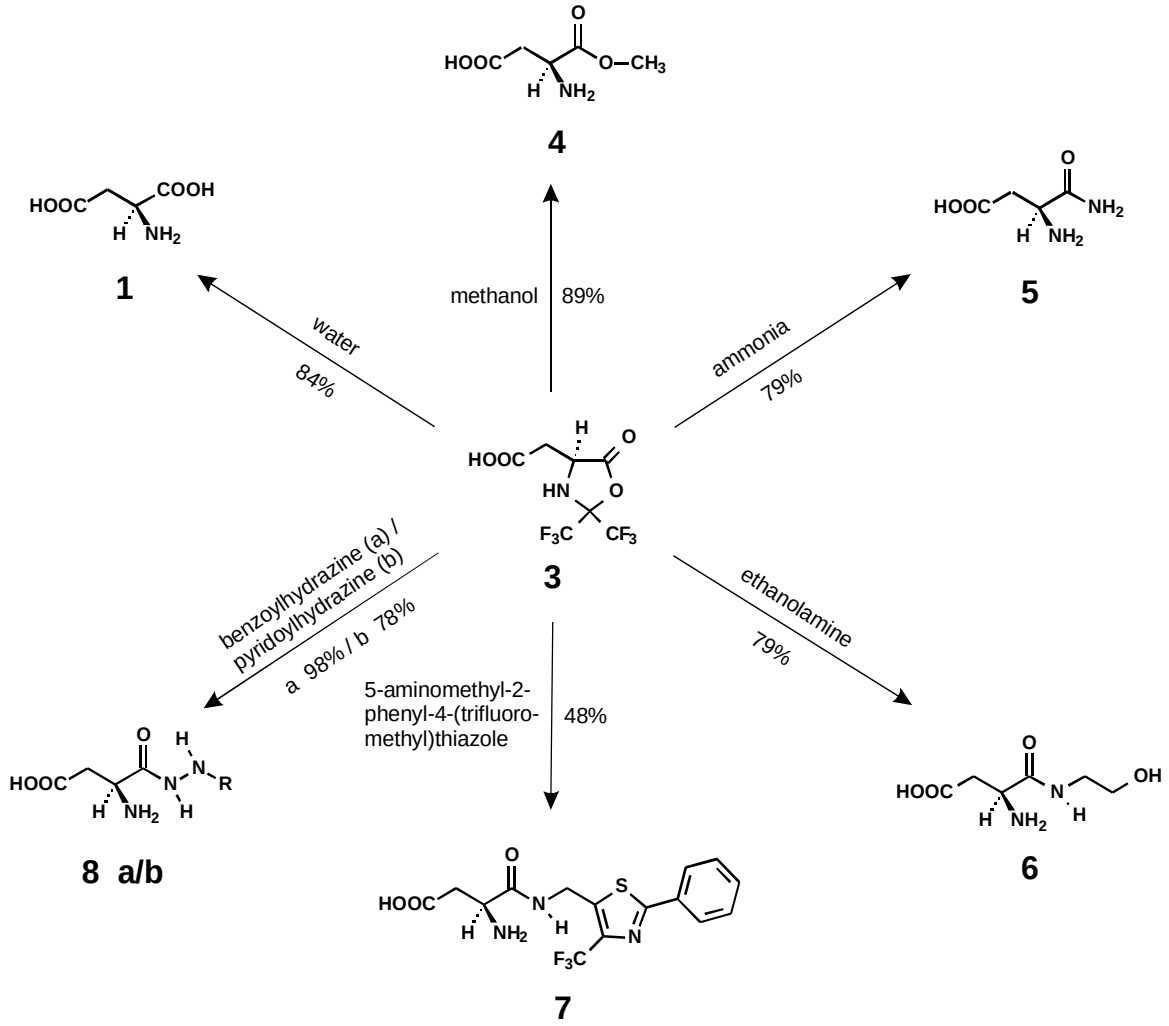


1 **2** **3**

Scheme 1

The cleavage of the lactone moiety of compound (**3**) by nucleophiles like water, alcohols, amines and hydrazines occurs regioselectively to give α-functionalized derivatives. Simultaneously, with the ring cleavage, deprotection of the α-amino group takes place. Therefore, the new general approach offers a two step route to α-functionalized aspartic acid derivates as well as to their homologues with enormous structural variability. Compounds (**4** - **8**) represent only a small selection of the synthetic potential of the new strategy.

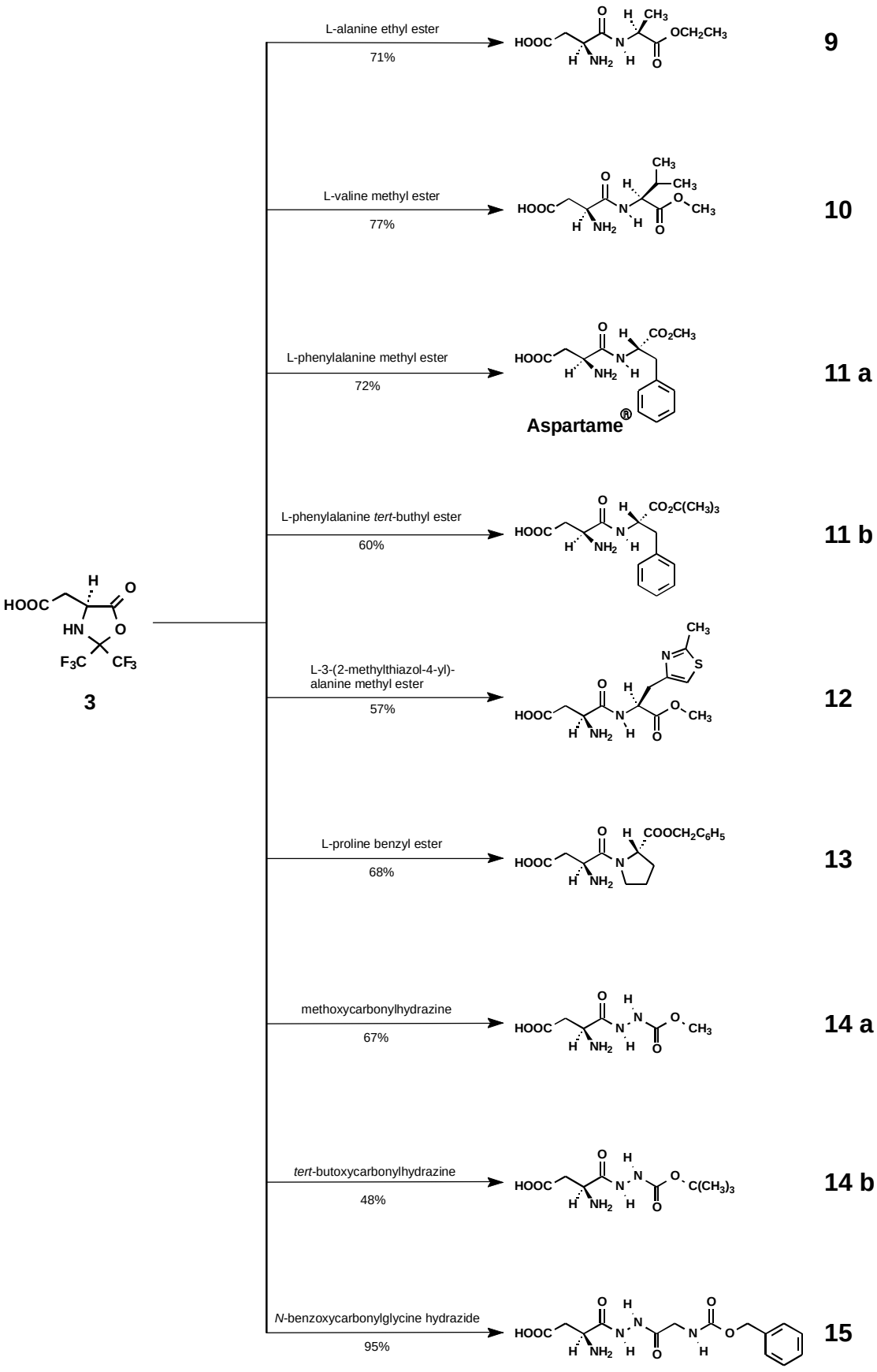
This route offers the shortest possible and the most efficient way to α-functionalized ω-carboxy α-amino acid derivatives known so far. Even enzymatic strategies[16] can not beat the minimum of two steps. The new strategy is applicable to the L- as well as to the D-series. Optimal yields are obtained, when solvent systems are used, where the products crystallize spontaneously. Furthermore, the new strategy can also be applied to the regioselective α-functionalization of α-hydroxy-[17] and α-mercapto-α,ω-dicarboxy acids.[18]



Scheme 2

Aminolysis of lactone (**3**) with amino acid esters results in the formation of dipeptide esters in acceptable yields. ^{19}F NMR spectral analysis indicates that the reactions are complete within 12 h at room temperature. From solvents like ether and ethyl acetate the dipeptide esters usually crystallize after a short induction period. The reason for the low solubility of the α-amides in these solvents is the formation of a betain structure by an internal protonation of the amino group by the β-carboxy moiety. In most cases the products are already analytically pure after filtration and careful trituration with ether. From NMR spectral data and optical rotation values of the crude products we conclude that epimerization is < 3%.

The new strategy offers an elegant two step route of the sweetener Aspartame in an over-all yield of 62%. Furthermore, the reaction sequence offers an efficient, preparative simple access to libraries of Aspartame analogues.[19] The new approach seems to be superior to recently published strategies. However, a major drawback of the new Aspartame synthesis is the high price of hexafluoroacetone and its toxicity. On reaction with alkoxycarbonylhydrazines, **3** can be transformed into dipeptide fragments of type Asp-AXaa-OR (**3** → **14**), and tripeptide fragments like Asp-Agly-ψ(CH_2NH)Xaa-OR (**3** → **15**) which represent valuable buildings blocks for the synthesis of peptide surrogates.[20]


L-alanine ethyl ester
71%
9
L-valine methyl ester
77%
10
L-phenylalanine methyl ester
72%
11 a
Aspartame®
L-phenylalanine tert-buthyl ester
60%
11 b
3
L-3-(2-methylthiazol-4-yl)-
alanine methyl ester
57%
12
L-proline benzyl ester
68%
13
methoxycarbonylhydrazine
67%
14 a
tert-butoxycarbonylhydrazine
48%
14 b
N-benzoxycarbonylglycine hydrazide
95%
15

Scheme 3

Compound (**3**) derived from aspartic acid is a reactive species, reacting fastly and cleanly to give high yields of the corresponding α-amides. On monitoring aminolysis of the lactone derived from glutamic acid, surprisingly we found by-products *i.a. via* a fragmentation process starting with a base induced abstraction of the NH-hydrogen of the 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-one ring.[21]
On regioselective ω-functionalization ω-carboxy-α-amino acids and on regioselective α- and/or ω-functionalization of ω-carboxy-α-hydroxy and ω-carboxy-α-mercapto acids we report elsewhere.

EXPERIMENTAL

IR spectra were obtained on a Genesis ATI Mattson/Unicam FTIR spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz and 400 MHz. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS, δ = 0 ppm); *J* values are given in Hertz (Hz). ^{13}C NMR spectroscopy was performed at 75 MHz, 101 MHz and 151 MHz. ^{19}F NMR spectra were recorded at 282 MHz and 376 MHz with trifluoroacetic acid (TFA, δ = 0) as external standard. Optical rotations ($[\alpha]_D$) were measured using a Polatronic polarimeter (Schmidt & Haensch) in a 5 cm cell. Melting points were determined on a Boetius heating table. For C, H, N analyses a CHNO-Rapid-Elemental-Analyser (Hereaus) was used. For flash chromatography, silica gel (32-63 μm) was used with solvent systems given in the text. Organic solvents were dried and distilled prior to use.

[(4-L)-2,2-Bis(trifluoromethyl)-5-oxo-1,3-oxazolidin-4-yl]acetic acid (3). A solution of L-aspartic acid **(1)** (30.0 g, 225 mmol) in DMSO (100 mL) was stirred in an atmosphere of hexafluoroacetone (>2 equivalents) at rt. The apparatus was sealed with a dry-ice condenser. After completion of the reaction, the mixture was quenched with an ice/water mixture (500 mL) and the product was extracted with dichloromethane (3 x 150 mL). The combined organic layer was washed with ice-cold water (3 x 100 mL) and dried over $MgSO_4$, then the solvent was evaporated in vacuo. Yield 86% (54.5 g) **3**, mp 45 °C (from $CHCl_3$/hexanes). For data see lit.[22]

Hydrolysis of compound (3). 3 (2.81 g, 10 mmol) was heated in a H_2O/THF mixture (1:1, 20 mL) under reflux for 12 h. The suspension formed was evaporated to dryness *in vacuo*. The residue was carefully triturated with ether and recrystallized from water. Yield 84% (1.12 g) **L-aspartic acid** (**1**), mp 275 - 278 ^{o}C (decomp), lit.,[23] mp 270 ^{o}C (decomp), $[\alpha]_D$ = +7.0 ^{o} (c = 2, H_2O), lit.,[1] $[\alpha]_D$ = +6.7 ^{o} (c = 2, H_2O).

L-Aspartic acid α-methyl ester (**4**). A solution of **3** (2.81 g, 10 mmol) in dry methanol (20 mL) was heated under reflux. The ester (**4)** began to crystallize within minutes, after 30 min the reaction was complete. The sample was cooled down to 0 ^{o}C and the precipitate was filtered off. After careful

trituration with ice-cold ether and drying over P_2O_5 *in vacuo* compound (**4)** was analytically pure. Yield 89% (1.30 g), mp 183 – 186 °C (decomp), lit.,[24] mp 183 – 184 °C (decomp), $[\alpha]_D = +1.54^{o}$ (c =1.3, H_2O).

Aminolysis of 3. General method: A solution of **3** in dry ether (30 mL) was stirred with the corresponding amine (12 mmol) at rt. Within a few minutes the product began to crystallize. The reaction was complete within 24 h (^{19}F NMR spectral analysis). After cooling the reaction mixture to 0 ^{o}C the precipitate was filtered off, washed carefully with ice-cold ether and dried over P_2O_5 *in vacuo*.

L-Aspartic acid α-amide (isoasparagine) (5). A solution of **3** (2.81 g, 10 mmol) in dry ether (30 mL) was stirred with the corresponding amine (12 mmol) for 24 h at rt. Within a few minutes the product began to crystallize. Yield 79% (1.03 g) **5**, mp 212 - 214 ^{o}C (water/ethanol) (decomp), lit.,[25] mp 204 -205 ^{o}C (water/methanol), $[\alpha]_D = +16.5^{o}$ (c = 2, H_2O), lit.,[4b] $[\alpha]_D = +17^{o}$ (c = 1, aq HCl).

L-Aspartic acid α-(β-hydroxyethylamide) (**6**). Ethanolamine (0.60 g, 10.0 mmol) was added to **3** (2.81 g, 10.0 mmol) in 40 mL of isopropanol. The reaction was complete within 24 h (^{19}F NMR spectral analysis). The precipitate was filtered off, washed carefully with ice-cold ether and purified by flash chromatography (eluent: methanol). Yield 79% (1.39 g) **6**, oil, $[\alpha]_D = -2^{o}$ (c = 1.0, CH_3OH). IR (film): ν = 3600 - 3200, 1712, 1414, 1217. ^{1}H NMR (D_2O, 200 MHz) δ 2.46 (2H, m), 3.05 (2H, m), 3.36 (2H, m), 3.90 (1H, dd, *J* = 7.5 Hz, *J* = 5.5 Hz). ^{13}C NMR (D_2O, 75 MHz) δ 41.4, 45.9, 55.2, 64.1, 173.9, 180.3. Anal. Calcd for $C_7H_{14}N_2O_4$ x 2 H_2O: C, 37.16; H, 8.02; N, 12.38, Found: C, 37.20; H, 8.18; N, 11.99.

L-Aspartic acid α-[(2-phenyl-4-trifluoromethylthiazol-4-yl)methyl]amide (**7**). **3** (1.50 g, 5.3 mmol) and 5-aminomethyl-2-phenyl-4-trifluoromethyl-1,3-thiazole react to give 48% (0.94 g) **7**, mp 170 ^{o}C, $[\alpha]_D = -7.0^{o}$ (c = 1.0, DMSO). IR (KBr): ν = 3700 - 2200, 1670, 1560 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.39 (1H, dd, *J* = 16.5 Hz, *J* = 8.5 Hz), 2.55 (1H, dd, *J* = 16.5 Hz, *J* = 5.0 Hz), 3.87 (1H, dd, *J* = 8.5 Hz, *J* = 5.0 Hz), 4.60 (2H, m), 6.28 (3H, br s), 7.57 (3H, m), 7.99 (2H, m), 9.34 (1H, br s). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 33.2, 37.7, 50.8, 121.0 (q, *J* = 267 Hz), 125.4, 126.3, 126.5 (q, *J* = 43.0 Hz), 129.3, 131.6, 149.7, 160.6, 171.1, 172.6. ^{19}F NMR (DMSO-d_6, 282 MHz) δ 17.0 (3F, s). Anal. Calcd for $C_7H_{14}N_3O_3F_3S$: C, 48.26; H, 3.78; N, 11.25. Found: C, 48.99; H, 4.09; N, 11.08.

L-Aspartic acid α-(***N*-benzoylhydrazide**) (**8a**). Benzoylhydrazine (2.52 g, 18.5 mmol) was added to a solution of **3** (5.20 g, 18.5 mmol) in ethyl acetate (50 mL) at rt with stirring. After 16 h the starting material **(3)** was consumed (^{19}F NMR spectral analysis). The precipitate was filtered off, carefully washed with cold ether and dried over P_2O_5 *in vacuo*. Yield 98% (4.54 g) **8a**, mp 211 - 212 ^{o}C (decomp); $[\alpha]_D =$

+28.5 ^{o} (c = 2.0, 1 N NaOH). IR (KBr): ν = 3350 - 2900, 1685, 1655, 1615 cm^{-1}. ^{1}H NMR (D_2O, NaOD, 300 MHz) δ 3.14 (1H, m), 3.37 (1H, m), 4.47 (1H, m), 8.17 - 8.20 (3H, m), 8.54 - 8.58 (2H, m). ^{13}C NMR (D_2O, NaOD, 75 MHz) δ 45.7, 54.9, 129.6, 131.1, 133.0, 138.6, 168.6, 173.0, 182.2. Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.72. Found: C, 52.55; H, 5.21; N, 16.60.

L-Aspartic acid α-(*N*-pyridoylhydrazide) (**8b**). Reaction of **3** (3.09 g, 11 mmol) and pyridoylhydrazine (1.43 g, 10.4 mmol) in ethyl acetate (50 mL) gave 78% (2.06 g) **8b**, mp 217 - 219 ^{o}C (decomp) after purification by flash chromatography (eluent: methanol), $[\alpha]_D$ = +26.0 ^{o} (c = 2.0, 1N NaOH). IR (KBr) ν 3500 - 2800, 1720, 1660, 1615 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.38 (1H, dd, *J* = 16.4 Hz, *J* = 9.0 Hz), 2.58 (1H, dd, *J* = 16.4 Hz, *J* = 4.6 Hz), 3.87 (1H, dd, *J* = 9.0 Hz, *J* = 4.6 Hz), 7.77 (2H, m), 8.74 (2H, m). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 38.0, 49.9, 121.3, 139.3, 150.4, 163.8, 171.0, 172.0. Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.51; H, 4.84; N, 22.00.

L-Aspartyl-L-alanine ethyl ester (**9**)**.** A solution of **3** (1.40 g, 5 mmol) in ether (20 mL) was stirred with L-alanine ethyl ester (0.64 g, 55 mmol) at rt until the starting material was consumed (^{19}F NMR spectral analysis). Then the precipitate was filtered off, carefully washed with cold ether and dried over P_2O_5 *in vacuo*. Yield 71% (0.82 g) **9**, mp 166 - 167 ^{o}C (from ethanol/water), $[\alpha]_D$ = -27.5 ^{o} (c = 2, H_2O). IR (KBr) ν 3310, 2990, 1735, 1665, 1630-1540 cm^{-1}. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.16 (3H, t, *J* = 7.0), 1.27 (3H, d, *J* = 7.2 Hz), 2.24 (1H, dd, *J* = 16.4 Hz, *J* = 9.8 Hz), 2.47 (1H, dd, *J* = 16.4 Hz, *J* = 4.0 Hz), 3.73 (1H, dd, *J* = 9.8 Hz, *J* = 4.0 Hz), 4.06 (2H, m), 4.25 (1H, m), 8.71 (1H, d, *J* = 5.2 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.0, 16.8, 37.5, 47.8, 50.4, 60.6, 170.0, 172.1, 173.1. Anal. Calcd for $C_9H_{16}N_2O_5$: C, 44.81; H, 7.10; N, 11.61. Found; C, 44.60; H, 6.83; N, 12.13.

L-Aspartyl-L-valine methyl ester (**10**)**.** To a solution of **3** (2.73 g, 9.7 mmol) in ethyl acetate (20 mL) a solution of L-valine methyl ester (1.27 g, 9.7 mmol) in ethyl acetate (20 mL) was added with stirring. Crystallization of the dipeptide ester started within a few minutes. After 24 h the precipitate was filtered off. The product was purified by RPC (eluent: methanol/water = 3:1) and dried in *vacuo*. Yield 77% (1.83 g) **10**, mp 105 - 107 ^{o}C, $[\alpha]_D$ = -12.0 ^{o} (c = 1, H_2O). IR (KBr): ν = 3309, 2966, 1744, 1670, 1555 cm^{-1}. ^{1}H NMR (CD_3OD, 200 MHz) δ 0.96 (3H, d, *J* = 6.8 Hz), 0.97 (3H, d, *J* = 6.9 Hz), 2.17 (1H, m), 2.53 (1H, dd, *J* = 17.0 Hz, *J* = 8.8 Hz), 2.75 (1H, dd, *J* = 17.0 Hz, *J* = 5.1 Hz), 3.71 (3H, s), 4.12 (1H, dd, *J* = 8.8 Hz, *J* = 5.1 Hz), 4.35 (1H, d, *J* = 5.7 Hz). ^{13}C NMR (CD_3OD, 75 MHz) δ 18.4, 19.5, 31.6, 39.2, 52.4, 52.6, 59.4, 171.7, 173.1, 176.8. Anal. Calcd for $C_{10}H_{18}N_2O_5$ x 1/2 H_2O: C, 47.05; H, 7.50; N, 10.97. Found: C, 47.16; H, 7.35; N, 10.99.

L-Aspartyl-L-phenylalanine methyl ester (**Aspartame®**) (**11a**). **3** (5.62 g, 20 mmol) and L-phenylalanine methyl ester (4.25 g, 24 mmol) were reacted in ether (30 mL) to give 72% (4.24 g) **11a**, mp 250 °C, lit.,[11] mp 243 °C, $[\alpha]_D$ = +27.5 ° (c = 1.0, acetic acid). IR (KBr): ν 3320, 1735, 1665 cm^{-1}. ^1H NMR (D_2O, 200 MHz) δ 2.66 (1H, dd, *J* = 17.5 Hz, *J* = 8.0 Hz), 2.77 (1H, dd, *J* = 17.5 Hz, *J* = 5.5 Hz), 3.06 (1H, dd, *J* = 14.0 Hz, *J* = 9.0 Hz), 3.24 (1H, dd, *J* = 14.0 Hz, *J* = 6.0 Hz), 3.73 (3H, s), 4.17 (1H, dd, *J* = 8.0 Hz, *J* = 5.5 Hz), 4.73 (1H, m), 7.26 - 7.41 (5H, m). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 36.6, 37.2, 50.7, 51.9, 53.6, 126.6, 128.1, 129.1, 136.9, 171.4, 171.6, 172.4. Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 55.44; H, 6.26; N, 9.24. Found: C, 55.59; H, 6.24; N, 9.20.

L-Aspartyl-L-phenylalanine *tert*-butyl ester (**11b**). **3** (5.62 g, 20 mmol) and L-phenylalanine *tert*-butyl ester (6.70 g, 30 mmol) were reacted in ether (80 mL) to give 60% (4.04 g) **11b**, mp 138 °C, $[\alpha]_D$ = -7.5 ° (c = 2.0, DMSO). IR (KBr): ν 3700-3100, 1735, 1670, 1585, 1540 cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz) δ 1.33 (9H, s), 2.23 (1H, dd, *J* = 16.5 Hz, *J* = 9.5 Hz), 2.49 (1H, dd, *J* = 16.5 Hz, *J* = 4.0 Hz), 2.90 (1H, dd, *J* = 14.0 Hz, *J* = 8.5 Hz), 2.98 (1H, dd, *J* = 14.0 Hz, *J* = 6.0 Hz), 3.68 (1H, dd, *J* = 9.5 Hz, *J* = 4.0 Hz), 4.36 (1H, m), 7.15 - 7.30 (5H, m), 8.66 (1H, m). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 27.5, 36.8, 37.7, 50.5, 54.2, 80.9, 126.5, 128.2, 129.2, 137.0, 170.1, 171.0, 172.5. Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.18; N, 8.33. Found: C, 59.15; H, 7.18; N, 7.77.

L-Aspartyl-L-3-(2-methylthiazol-4-yl)alanine methyl ester (**12**). **3** (1.41 g, 5 mmol) and L-3-(2-methylthiazol-4-yl)alanine methyl ester (1.30 g, 6.5 mmol) were reacted in ether (30 mL) to give 57% (0.90 g) **12**, mp 151 °C (from methanol), $[\alpha]_D$ = -11.0 ° (c = 1.0, H_2O). IR (KBr) ν 3660 - 2800, 1730, 1660 cm^{-1}. ^1H NMR (D_2O, 200 MHz) δ 2.66 (3H, s), 2.67 (1H, dd, *J* = 17.5 Hz, *J* = 8.0 Hz), 2.77 (1H, dd, *J* = 17.5 Hz, *J* = 5.5 Hz), 3.25 (2H, m), 3.74 (3H, s), 4.22 (1H, dd, *J* = 8.0 Hz, *J* = 5.5 Hz), 4.78 (1H, m), 7.11 (1H; s). ^{13}C NMR (D_2O, 75 MHz) δ 20.4, 34.3, 39.5, 53.1, 55.7, 55.8, 119.3, 152.1, 171.5, 172.0, 175.3, 178.3. Anal. Calcd for $C_{12}H_{17}N_3O_5S$ x H_2O: C, 43.20; H, 5.70; N, 12.60. Found: C, 43.78; H, 5.98; N, 12.47.

L-Aspartyl-L-proline benzyl ester (**13**). **3** (2.81 g, 10 mmol) and L-proline benzyl ester (2.90 g, 14 mmol) were reacted in ether (30 mL) to give 68% (2.24 g) **13**, mp 124 °C (methanol/water), $[\alpha]_D$ = -76.0 ° (c = 1.0, DMSO). IR (KBr): ν 3600 - 3300, 2980, 1740, 1640 cm^{-1}. ^1H NMR (D_2O, 200 MHz) δ 1.95 - 2.04 (3H, m), 2.30 (1H, m), 2.46 (1H, dd, *J* = 17.5 Hz, *J* = 10.5 Hz), 2.72 (1H, dd, *J* = 17.5 Hz, *J* = 3.0 Hz), 3.60 (1H, m), 3.70 (1H, m), 4.49 (1H, dd, *J* = 10.5 Hz, *J* = 3.0 Hz), 4.56 (1H, m), 5.17 (1H, d, *J* = 12.0 Hz), 5.23 (1H, d, *J* = 12.0 Hz), 7.45 (5H, m). ^{13}C NMR (D_2O, 75 MHz) δ 27.3, 31.2, 37.8, 50.1,

52.5, 62.4, 70.5, 131.1, 131.5, 131.6, 137.8, 170.4, 175.8, 178.0. Anal. Calcd for $C_{16}H_{20}N_2O_5$: C, 59.98; H, 6.29; N, 8.74. Found: C, 59.70; H, 6.31; N, 8.96.

L-Aspartyl-azaglycine methyl ester (**14a**)**. 3** (1.62 g, 5.8 mmol) and methoxycarbonylhydrazine (0.51 g, 5.8 mmol) were reacted in ethyl acetate (20 mL) to give 67% (0.80 g) **14a**, mp 145 ^{o}C (decomp) after purification by RPLC (eluent: water), $[\alpha]_D$ = -55.0 ^{o} (c = 1.0, H_2O). IR (KBr) ν 3400 - 2900, 1730, 1700, 1605 cm^{-1}. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.34 (1H, dd, *J* = 16.4 Hz, *J* = 9.0 Hz), 2.51 (1H, m), 3.59 (3H, s), 3.83 (1H, dd, *J* = 9.0 Hz, *J* = 4.4 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 37.7, 49.6, 52.0, 156.5, 170.6, 172.6. Anal. Calcd for $C_6H_{11}N_3O_5$: C, 35.13; H, 5.40; N, 20.48. Found: C, 34.80; H, 5.61; N, 20.61.

L-Aspartyl-azaglycine *tert*-butyl ester (**14b**)**. 3** (3.08 g, 10.9 mmol) and BOC-hydrazine (1.44 g, 10.9 mmol) were reacted in ether (50 mL) to give 48% (1.30 g) **14b**, mp 126-128 ^{o}C (after trituration with ether). IR (KBr) ν 3500 - 2800, 1760-1550 cm^{-1}. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.46 (9H, s), 2.59 (1H, m, *J* = 16.8 Hz, *J* = 9.7 Hz), 2.75 (1H, m, *J* = 16.8 Hz, *J* = 4.4 Hz), 4.08 (1H, dd, *J* = 9.7 Hz, *J* = 4.4 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 28.0, 37.7, 49.6, 79.4, 155.1, 170.6, 172.4. Anal. Calcd for $C_9H_{17}N_3O_5$: C, 43.72; H, 6.93; N, 16.99. Found: C, 44.04; H, 6.75; N, 17.08.

L-Aspartyl-azaglycyl-retro(CH_2NH)glycine benzyl ester (**15**)**. 3** (1.93 g, 6.8 mmol) and *N*-benzoxy-carbonylglycine hydrazide (1.53 g, 6.8 mmol) were reacted in ether (40 mL). The precipitate was carefully washed with cold ether and dried in *vacuo*. Yield 95% (2.20 g) **15**, mp 166 - 168 ^{o}C (after trituration with ether), $[\alpha]_D$ = -6.5 ^{o} (c = 2.0, DMSO). IR (KBr) ν 3210, 3034, 1695, 1618, 1538 cm^{-1}. ^{1}H NMR (CD_3OD, 300 MHz) δ 2.68 (1H, dd, *J* = 17.2 Hz, *J* = 8.9 Hz), 2.81 (1H, dd, *J* = 17.2 Hz, *J* = 4.8 Hz), 3.91 (2H, s), 4.21 (1H, dd, *J* = 8.9 Hz, *J* = 4.8 Hz), 5.10 (2H, s), 7.30 - 7.36 (5H, m). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 38.0, 42.0, 49.8, 65.5, 127.7, 128.4, 137.0, 156.5, 167.9, 169.9, 172.3. Anal. Calcd for $C_{14}H_{18}N_4O_6$: C, 49.70; H, 5.36; N, 15.56. Found: C, 49.25; H, 5.45; N, 15.40.

ACKNOWLEDGEMENTS

The authors are grateful to the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support.

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