A New Method for the Synthesis of (*S*)-α-Methylisoserine and Its Incorporation into Peptides[1]

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(*S*)-(+)-Citramalic acid is converted into isocyanate **4** using hexafluoroacetone as the protecting agent. Isocyanate **4** represents a doubly activated species, which upon reaction with alcohols provides *N*-protected, carboxyl-activated α-methylisoserine derivatives, perfectly suited for peptide synthesis (**5** → **8**). Acylation of **4** with *N*-protected amino acids gives fully protected carboxyl-activated dipeptides. Ring opening with amino acid esters results in the formation of tripeptides (**9** → **10**).

Non-proteinogenic amino acids are of current interest for modification of native peptides and for the synthesis of peptide mimetics.[2] In this paper, we report on a new synthesis of the non-proteinogenic amino acid α-methylisoserine[3] and its incorporation into peptides using hexafluoroacetone as protecting and activating agent.

Hexafluoroacetone reacts with commercially available (*S*)-(+)-citramalic acid (**1**) furnishing compound **2** (Scheme 1). Protection of the α-hydroxy and activation of the α-carboxylic group is achieved simultaneously. The ω-carboxylic group remains unaffected and can be functionalized regioselectively.

On treatment of **2** with thionyl chloride the acid chloride **3** is formed in high yield. By this manipulation, the position of highest electrophilicity is transferred from the α- to the ω-carboxylic group. Heating of **3** with trimethylsilyl azide results in the formation of isocyanate **4** via Curtius rearrangement. Compound **4** represents a doubly activated α-methylisoserine derivative, in which the isocyanate function is the more reactive centre towards nucleophiles.

Addition of equimolar amounts of benzyl alcohol to isocyanate **4** results in the formation of the *Z*-protected, carboxyl-activated (*S*)-α-methylisoserine derivative **5** (Scheme 2). Transformation of **5** into *Z*-α-methylisoserine **6** is accomplished in one step with water/isopropanol (50 : 50, v/v) at room temperature. Hydrogenolytic deprotection of **6** can be achieved quantitatively to yield (*S*)-α-methylisoserine **7**.

Ring opening of **5** with phenylalanine *tert*-butyl ester affords dipeptide **8** with the α-methylisoserine moiety placed in the *N*-terminal position. Acylation of isocyanate **4** with Fmoc-Phe-OH leads to the fully protected, carboxyl-activated dipeptide derivative **9**, which can be transformed into tripeptides (e.g. **9** → **10**) on reaction with amino acid esters at room temperature.

All steps of the syntheses described above proceed in a stereoconservative manner (^{1}H NMR analysis).

Melting points were determined with a Totolli apparatus and are uncorrected. Optical rotations were measured at 589 nm (Na D line). ^{1}H NMR spectra were recorded at 300.075 MHz. Splitting multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), septet (sept), broad (br) and multipet (m). The chemical shifts are reported in ppm relative to TMS in $CDCl_3$, acetone-d_6, or DMSO-d_6; *J* values are given in Hertz (Hz). ^{13}C NMR spectroscopy was performed at 75.462 MHz. ^{19}F NMR spectra were recorded at 282.330 MHz with CF_3CO_2H as external standard. The IR spectra were recorded on a Specord (Carl-Zeiss-Jena) spectrometer as liquid films or KBr pellets. Mass spectra (EI) were obtained at 70 eV with a Masslab spectrometer. Elemental analyses were performed with a Heraeus RAPID analyzer. For flash chromatography, MERCK silica gel 60 (30–60 μm) was used with the solvent system given in the text. Organic solvents were dried and distilled prior to use. Proper precautions should be taken when using hexafluoroacetone (poison).

Reaction of Hexafluoroacetone with α-Functionalized Carboxylic Acids; General Procedure:

In a vessel equipped with a CO_2-condenser a solution of the α-functionalized carboxylic acid (200 mmol) in DMSO (100 mL) was reacted with hexafluoroacetone (69.8 g, 420 mmol). The progress of the reaction was monitored by ^{19}F NMR spectroscopy. The solution was then poured into water/CH_2Cl_2 (600 mL). The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layer was washed with water (2 × 100 mL) to completely remove DMSO and dried ($MgSO_4$). The solvent was evaporated in vacuo and the crude product was recrystallized from $CHCl_3$/hexanes.

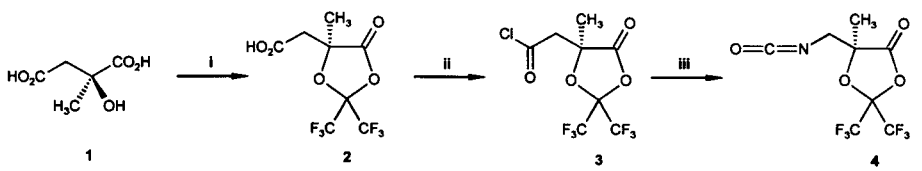
(5S)-2,2-Bis(trifluoromethyl)-5-methyl-4-oxo-1,3-dioxolan-5-ylacetic Acid (**2**):

Yield: 17.4 g (87%); mp 59°C; $[\alpha]_D^{25}$ −10.0 (*c* = 1.2, DMSO).

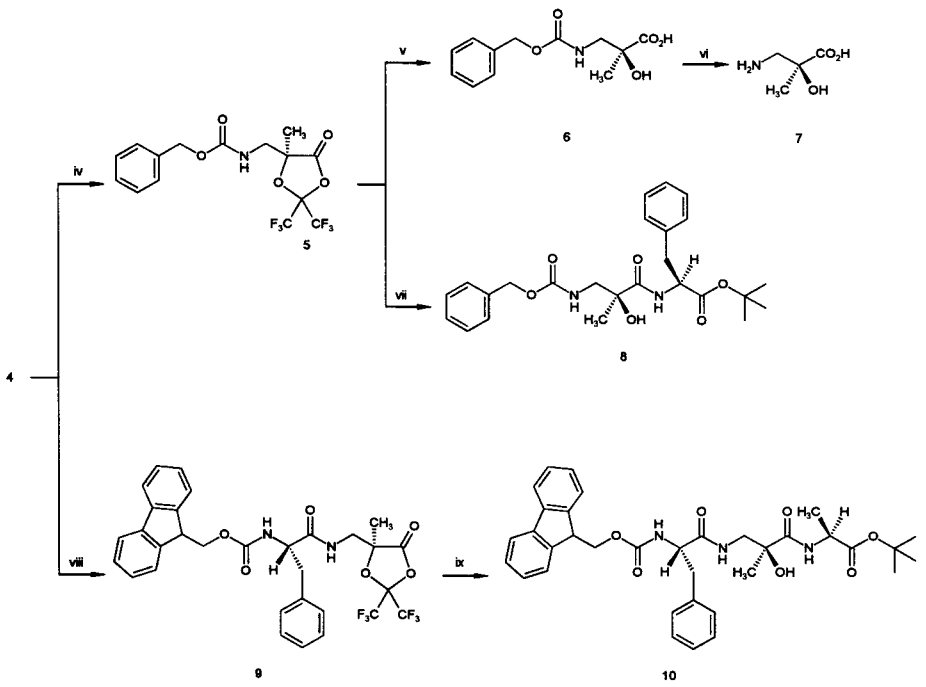
IR (KBr): ν = 3700–2400, 1840, 1710, 1450 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 1.78 (s, 3H, CH_3), 2.94 (s, 2H, CH_2), 11.76 (s, br, 1H, CO_2H).

^{13}C NMR (DMSO-d_6): δ = 22.8 (CH_3), 39.2 (CH_2), 79.4 (CCH_3), 97.3 [sept, *J* = 36 Hz, $C(CF_3)_2$], 118.9 (q, *J* = 288 Hz, CF_3), 119.1 (q, *J* = 289 Hz, CF_3), 168.9 (C=O, lactone), 173.0 (C=O, acid).



Scheme 1 i) $(CF_3)_2CO$/DMSO; ii) $SOCl_2$, reflux; iii) Me_3SiN_3, toluene, 80°C.



iv) BnOH›$CHCl_3$, reflux, v) H_2O/propan-2-ol (1 : 1), r.t.; vi) H_2/PdC/EtOH;
vii) Phe-OBu-*t*/Et_2O; viii) Fmoc-Phe-OH/toluene, 90 °C; ix) Ala-OBu-*t*/Et_2O.

Scheme 2

^{19}F NMR (DMSO-d_6): $\delta = -2.85$ (q, $J = 8$ Hz, CF_3), -2.63 (q, $J = 8$ Hz, CF_3).

MS *m/z* (%) = 296 (M^+, 3.10), 250 (M − H_2O, CO^+, 2.1), 227 (M − CF_3^+, 6.6), 85 (M − HFA, −H, $-CO_2^+$, 72.4).

Anal. Calcd for $C_8H_6O_5F_6$ (296.1): C, 32.44%; H, 2.04%. Found C, 32.93%; H, 2.03%.

(5*S*)-2,2-Bis(trifluoromethyl)-5-methyl-4-oxo-1,3-dioxolan-5-ylacetyl Chloride (3):

Compound **2** (6.29 g, 163.9 mmol) and $SOCl_2$ (80 mL) were kept under reflux for 12 h. Excess of $SOCl_2$ was removed under reduced pressure and the remaining residue was distilled; yield: 42.48 g (82%); bp 81 °C/15 Torr; $[\alpha]_D^{20} -12.0$ (*c* = 1.0, $CHCl_3$).

IR (film): $\nu = 1850, 1810, 1235, 1130\ cm^{-1}$.

^1H NMR ($CDCl_3$): $\delta = 1.80$ (s, 3 H, CH_3), 3.41 (d, $J = 17$ Hz, 1 H, CH_2), 3.47 (d, $J = 17$ Hz, 1 H, CH_2).

^{13}C NMR ($CDCl_3$): $\delta = 22.2$ (CH_3), 52.6 (CH_2), 78.8 (CCH_3), 97.4 [sept, $J = 36$ Hz, $C(CF_3)_2$], 118.9 (q, $J = 288$ Hz, CF_3), 119.1 (q, $J = 289$ Hz, CF_3), 167.6, 168.0 (C=O, lactone, acid chloride).

^{19}F NMR ($CDCl_3$): $\delta -2.84$ (m, CF_3).

MS: *m/z* (%) = 296 (M − H_2O^+, 0.1), 279 (M − Cl^+, 5.0), 130 (M − HFA^+, 5.5), 69 (CF_3^+, 39).

Anal. Calcd for $C_8H_5O_4F_6Cl$ (314.6): C, 30.54; H, 1.60. Found C, 30.37; H, 1.66.

[(5*S*)-2,2-Bis(trifluoromethyl)-5-methyl-4-oxo-1,3-dioxolan-5-yl]-methyl Isocyanate (4):

To a solution of the acid chloride **3** (6.29 g, 20.0 mmol) in toluene (25 mL) was added dropwise a solution of Me_3SiN_3 (2.90 g, 25 mmol) in toluene (25 mL) and this was stirred at 80 °C for several hours until the evolution of N_2 ceased. After removal of the solvent, the residue was distilled; yield: 3.42 g (58%); bp 55 °C/0.1 Torr; $[\alpha]_D^{25} -16.5$ (*c* = 1.2, CH_2Cl_2).

IR (film): $\nu = 2150, 1840, 1775\ cm^{-1}$.

^1H NMR ($CDCl_3$): $\delta = 1.67$ (s, 3 H, CH_3), 3.66 (d, $J = 14$ Hz, 1 H, CH_2), 3.74 (d, $J = 14$ Hz, 1 H, CH_2).

^{13}C NMR ($CDCl_3$): $\delta = 20.4$ (CH_3), 48.4 (CH_2), 81.0 (CCH_3), 97.2 [sept, $J = 36$ Hz, $C(CF_3)_2$], 118.9 (q, $J = 288$ Hz, CF_3), 119.1 (q, $J = 288$ Hz, CF_3), 124.3 (N=C=O), 167.9 (C=O, lactone).

^{19}F NMR ($CDCl_3$): $\delta = -2.79$ (q, $J = 8$ Hz, CF_3), -2.49 (q, $J = 8$ Hz, CF_3).

MS: *m/z* (%) = 293 (M^+, 0.5), 237 (M − $C_2H_2NO^+$, 1.1), 69 (CF_3^+, 37).

Anal. Calcd for $C_8H_5NO_4F_6$ (293.1): C, 32.78; H, 1.72; N, 4.78. Found C, 33.24; H, 2.08; N, 5.80.

[(5*S*)-*N*-(Benzyloxycarbonyl)aminomethyl]-2,2-bis(trifluoromethyl)-5-methyl-1,3-dioxolan-4-one (5):

A mixture of **4** (2.63 g, 9.0 mmol) and benzyl alcohol (0.89 g, 8.5 mmol) in $CHCl_3$ (20 mL) was stirred under reflux for 40 h. Removal of the solvent and of the unreacted starting materials in vacuo afforded a white solid which was recrystallized from $CHCl_3$/hexanes; yield: 2.33 g (68%); mp 54 °C; $[\alpha]_D^{20} -6.0$ (*c* = 1.0, $CHCl_3$).

IR (KBr): $\nu = 3340, 1850, 1735, 1715, 1705, 1530\ cm^{-1}$.

^1H NMR ($CDCl_3$): $\delta = 1.62$ (s, 3 H, CH_3), 3.59 (dd, $J = 6, 15$ Hz, 1 H, CH_2), 3.73 (dd, $J = 7, 15$ Hz, 1 H, CH_2), 5.07 (s, br, 1 H, NH), 5.14 (s, 2 H, CH_2-Z), 7.36 (s, 5 H, aromatic-H).

^{13}C NMR ($CDCl_3$): $\delta = 20.5$ (CH_3), 46.4 (CH_2), 67.5 (CH_2-Z), 82.0 (CCH_3), 97.1 [sept, $J = 36$ Hz, $C(CF_3)_2$], 119.0 (q, $J = 288$ Hz, CF_3), 119.1 (q, $J = 289$ Hz, CF_3), 128.3, 128.4, 128.6, 135.9 (aromatic-C), 156.4 (C=O, carbamate), 168.8 (C=O, lactone).

^{19}F NMR ($CDCl_3$): $\delta = -2.82$ (q, $J = 8$ Hz, CF_3), -2.67 (q, $J = 8$ Hz, CF_3).

MS: *m/z* (%) = 401 (M^+, 1.6), 164 (M − $C_9H_{10}NO^+$, 4.1), 108 (M − $C_7H_8O^+$, 36.9), 91 (M − $C_7H_7^+$, 100).

Anal. Calcd for $C_{15}H_{13}NO_5F_6$ (401.3): C, 44.89; H, 3.27; N, 3.49. Found C, 44.48; H, 3.02; N, 4.05.

***N*-(Benzyloxycarbonyl)-α-methylisoserine (6):**

Compound **5** (1.20 g, 3.0 mmol) was stirred in propan-2-ol/water (10 mL, 1 : 1) for 5 d at r.t. The solvent was removed in vacuo and the residue was dried by lyophylization to give a white solid; yield: 0.46 g (60%); mp 103 °C; $[\alpha]_D^{25} +9.14$ (*c* = 1.0, DMSO).

IR (KBr): ν = 3420, 2930, 1710, 1525 cm^{-1}.

^1H NMR (DMSO-d_6): δ = 1.22 (s, 3H, CH_3), 3.19 (dd, J = 6, 13 Hz, 1H, CH_2), 3.31 (dd, J = 7, 13 Hz, 1H, CH_2), 5.02 (s, 2H, CH_2-Z), 7.03 (br t, 1H, NH), 7.35 (s, 5H, aromatic-H).

^{13}C NMR (DMSO-d_6): δ = 23.1 (CH_3), 48.8 (CH_2), 65.3 (CH_2-Z), 73.5 (CCH_3), 127.6, 127.7, 128.3, 137.1 (aromatic-C), 156.4 (C=O, carbamate), 176.1 (C=O, acid).

MS: m/z (%) = 253 (M^+, 0.2), 208 ($M-CO_2$, $-H^+$, 0.6), 91 ($C_7H_7^+$, 100).

Anal. Calcd for $C_{12}H_{15}NO_5$ (253.3): C, 56.91; H, 5.97; N, 5.53. Found C, 56.91; H, 6.05; N, 5.68.

α-Methylisoserine (7):

A mixture of Z-α-methylisoserine **6** (0.25 g, 1.0 mmol) and 10% Pd/C (200 mg) in EtOH (10 mL) was stirred under a H_2 atmosphere for 12 h. The mixture was filtered to remove the catalyst and the solvent was removed in vacuo. The remaining solid was dried by lyophylization; yield: 0.12 g (100%); mp 228 °C (dec.); $[\alpha]_D^{20}$ −11.3 (c = 1.0, water).

IR (KBr): ν = 3800–2300, 1700, 1615, 1560 cm^{-1}.

^1H NMR (D_2O): δ = 1.45 (s, 3H, CH_3), 3.15 (d, J = 13 Hz, 1H, CH_2), 3.32 (d, J = 13 Hz, 1H, CH_2).

^{13}C NMR (D_2O): δ = 23.6 (CH_3), 46.7 (CH_2), 72.9 (CCH_3), 179.4 (C=O, acid).

MS: m/z (%) = 119 (M^+, 7.6), 74 ($M-CO_2$, $-H^+$, 100).

Anal. Calcd for $C_4H_9NO_3$ (119.1): C, 40.33; H, 7.62; N, 11.76. Found C, 40.22; H, 8.14; N, 11.76.

[*N*-(Benzyloxycarbonyl)-α-methylisoseryl]phenylalanine *tert*-Butyl Ester [H-MeIse(Z)-Phe-OBu-*t*] (8):

A mixture of **5** (1.61 g, 4.0 mmol) and phenylalanine *tert*-butyl ester (0.91 g, 4.1 mmol) in Et_2O (20 mL) was stirred for 3 d at r.t. The solvent was evaporated in vacuo and the residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried ($MgSO_4$) and evaporated. The resulting solid was purified by flash chromatography (eluent: EtOAc/hexanes, 1 : 1) to give a white solid; yield: 1.10 g (60%); mp 82 °C; $[\alpha]_D^{20}$ −21.0 (c = 1.0, $CHCl_3$).

IR (KBr): ν = 3600–3120, 2990, 1730, 1700, 1640 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.33 (s, 3H, CH_3), 1.41 [s, 9H, $C(CH_3)_3$], 2.99 (dd, J = 7, 14 Hz, 1H, CH_2-Phe), 3.07 (dd, J = 6, 14 Hz, 1H, CH_2-Phe), 3.40 (m, 2H, CH_2-MeIse), 4.64 (s, br, 1H, OH), 4.69 (m, 1H, CH-Phe), 5.09 (d, J = 12 Hz, 1H, CH_2-Z), 5.13–5.17 (m, 2H, CH_2-Z, NH), 7.13–7.46 (m, 11H, aromatic-H, NH).

^{13}C NMR ($CDCl_3$): δ = 24.1 (CH_3-MeIse), 28.0 [$C(CH_3)_3$], 38.4 (CH_2-Phe), 49.3 (CH_2-MeIse), 53.5 (CH-Phe), 67.5 (CH_2-Z), 77.3 (C-CH_3), 82.3 [$OC(CH_3)_3$], 127.0, 128.2, 128.4, 128.6, 129.4, 136.0, 136.3 (aromatic-C), 159.0 (C=O, carbamate), 170.2, 174.5 (C=O, amide, ester).

MS: m/z (%) = 456 (M^+, 0.07), 164 ($C_9H_{10}NO_2^+$, 17.2), 91 ($C_7H_7^+$, 69).

Anal. Calcd for $C_{25}H_{32}N_2O_6$ (456.5): C, 65.77; H, 7.06; N, 6.14. Found C, 65.45; H, 7.24; N, 6.52.

(5*S*)-2,2-Bis(trifluoromethyl)-*N*-[(9-fluorenylmethoxycarbonyl)phenylalanylaminomethyl]-5-methyl-1,3-dioxolan-4-one (9):

Isocyanate **4** (1.17 g, 4.0 mmol) and Fmoc-Phe-OH (1.36 g, 3.5 mmol) were stirred in toluene (40 mL) at 100 °C for 20 h. The mixture was concentrated in vacuo and the excess of isocyanate removed by distillation. The remaining residue was purified by flash chromatography (eluent: EtOAc) to give a white solid which was recrystallized from CH_2Cl_2/hexanes; yield: 1.39 g (55%); mp 111 °C; $[\alpha]_D^{25}$ −6.3 (c = 1.1, CH_2Cl_2).

IR (KBr): ν = 3655–3120, 1845, 1670, 1545 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.44 (s, 3H, CH_3-MeIse), 3.01–3.13 (m, 2H, CH_2-Phe), 3.63 (m, 2H, CH_2-MeIse), 4.20 (m, 1H, CH-Fmoc), 4.38–4.49 (m, 3H, CH-Phe, CH_2-Fmoc), 5.20 (s, br, 1H, NH), 6.04 (s, br, 1H, NH), 7.18–7.35 (m, 7H, aromatic-H), 7.39–7.44 (m, 2H, aromatic-H), 7.52–7.55 (m, 2H, aromatic-H), 7.76–7.79 (m, 2H, aromatic-H).

^{13}C NMR ($CDCl_3$): δ = 20.5 (CH_3-MeIse), 38.2 (CH_2-Phe), 44.3 (CH_2-MeIse), 47.1 (CH-Fmoc), 56.4 (CH-Phe), 67.3 (CH_2-Fmoc), 81.4 (CCH_3-MeIse), 97.0 [sept., J = 36 Hz, $C(CF_3)_2$], 118.9 (q, J = 287 Hz, CF_3), 119.0 (q, J = 288 Hz, CF_3), 120.0, 125.1, 127.1, 127.8, 128.6, 128.7, 129.4, 136.4, 141.3, 143.7 (aromatic-C), 156.1 (C=O, carbamate), 168.6 (C=O, lactone), 171.6 (C=O, amide).

^{13}F NMR ($CDCl_3$: δ = −2.83–2.72 (m, $2CF_3$).

MS: m/z (%) = 636 (M^+, 11.7), 179 ($C_{14}H_{11}^+$, 65), 69 (CF_3^+, 29.7).

Anal. Calcd for $C_{31}H_{26}N_2O_6F_6$ (636.6): C, 58.49; H, 4.12; N, 4.40. Found C, 58.73; H, 4.39; N, 4.60.

{[*N*-(9-Fluorenylmethoxycarbonyl)phenylalanyl]-α-methylisoseryl} alanine *tert*-Butyl Ester [H-MeIse(Fmoc-Phe)-Ala-OBu-*t*] (10):

A mixture of compound **9** (0.45 g, 0.7 mmol) and alanine *tert*-butyl ester (0.12 g, 0.8 mmol) was stirred in Et_2O (10 mL) for 2 d. The white precipitate was filtered and washed thoroughly with Et_2O to give a white powder; yield: 0.27 g (71%); mp 140 °C; $[\alpha]_D^{20}$ −18.0 (c = 1.0, $CHCl_3$).

IR (KBr): ν = 3600–3160, 2980, 1730, 1690, 1650 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.33–1.36 (m, 6H, CH_3-MeIse, CH_3-Ala), 1.47 [s, 9H, $C(CH_3)_3$], 3.06 (m, 2H, CH_2-Phe), 3.37 (dd, J = 5.5, 14 Hz, 1H, CH_2-MeIse), 3.57 (m, 1H, CH_2-MeIse), 4.16 (m, 1H, CH-Fmoc), 4.30–4.44 (m, 3H, CH-Ala, CH_2-Fmoc), 4.70 (m, 1H, CH-Phe), 5.28 (d, J = 6.5 Hz, 1H, NH), 7.18–7.78 (m, 15H, aromatic-H, 2NH).

^{13}C NMR ($CDCl_3$): δ = 18.2 (CH_3-Ala), 24.0 (CH_3-MeIse), 28.0 [$C(CH_3)_3$], 38.5 (CH_2-Phe), 47.1, 48.8 (CH-Ala, CH-Fmoc), 48.4 (CH_2-MeIse), 56.2 (CH-Phe), 67.3 (CH_2-Fmoc), 76.5 (CCH_3-MeIse), 82.0 [$OC(CH_3)_3$], 120.0, 125.1, 127.1, 127.8, 128.6, 128.7, 129.4, 136.4, 141.3, 143.7 (aromatic-C), 156.1 (C=O, carbamate), 171.9, 174.1, 174.7 (C=O, 2 × amide, ester).

MS: m/z (%) = 615 (M^+, 1.10), 179 ($C_{14}H_{11}^+$, 100).

Anal. Calcd for $C_{35}H_{41}N_3O_7$ (615.7): C, 68.27; H, 6.71; N, 6.83. Found C, 67.81; H, 6.86; N, 6.78.

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(1) Part of this work was presented at the 4 Congress on Amino Acids, Peptides and Analogues, Vienna, Austria, Aug. 1995.

(2) See for example:
Tetrahedron Symposia-in-print 50, Peptide Secondary Structure Mimetics, *Tetrahedron* **1993**, *49*, 3433ff.
Giannis, A.; Kolter, Th. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244.
Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699.

(3) For alternative syntheses of α-methylisoserine see:
Melikoff, *Liebigs Ann. Chem.* **1886**, *234*, 217.
Kay, F.W. *Liebigs Ann. Chem.* **1908**, *362*, 327.
Cativiela, C.; Diaz-de-Villegas, M.D.; Gálvez, J.A. *Tetrahedron* **1996**, *52*, 687.