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Syntheses and Different Chemical Behaviour of Precursors of Putative Dibasic Inhibitors of Human Mast Cell Tryptase

Gregor Radau*

Institute of Pharmacy, Ernst-Moritz-Arndt-University, Pharmaceutical/Medicinal Chemistry, D-17487 Greifswald, Germany

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Summary. Choosing the best conditions and pathways for the synthesis of peptidic compounds remains a challenge for the peptide chemist. Our efforts towards the syntheses of two precursors of potential tryptase inhibitors, building block **A** and **B**, led to the development of two different synthesis routes. Each of them is successful in the synthesis of only one of the two, structurally nearly identical target compounds.

Keywords. Peptide synthesis; Tryptase-inhibitor.

Introduction

Human mast cell tryptase (E.C. 3.4.21.59), a member of the trypsin-like serine proteases family and one of the major mast cell secretory proteases, is thought to play a central role in mast cell related pathologies. Elevated concentrations of tryptase were measured in allergic and inflammatory diseases, including asthma [1, 2], allergic conjunctivitis [3], allergic rhinitis [4], rheumatic arthritis [5], multiple sclerosis [6], interstitial cystitis [7], or psoriasis [8]. Tryptase is generated and stored as a catalytically active tetramer [9] and – as a result of mast cell activation – tryptase is released together with histamine, heparines, and other mast cell proteases. Inhibition of tryptase presents a notable measure for improving the treatment of the above mentioned disorders.

Results and Discussion

In the course of our continuing studies towards the synthesis of cyanopeptide-derived inhibitors of trypsin-like serine proteases [10–13], we have synthesised thrombin inhibitors (*e.g.* **RA-1002**, Fig. 1) using cyanopeptides like aeruginosin

* Corresponding author. E-mail: radau@pharmazie.uni-greifswald.de

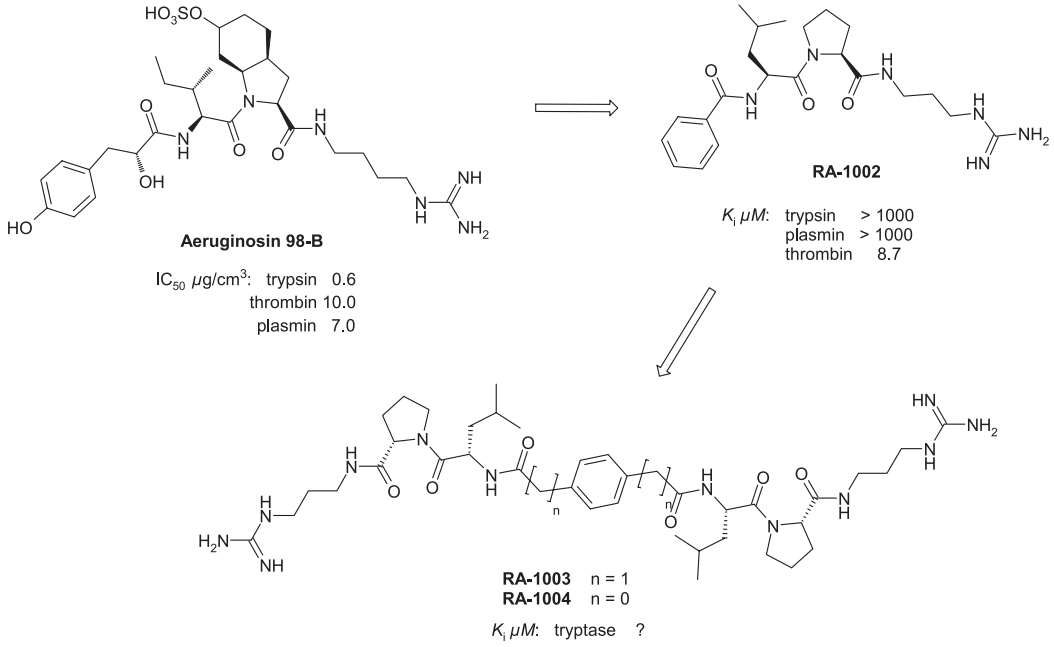
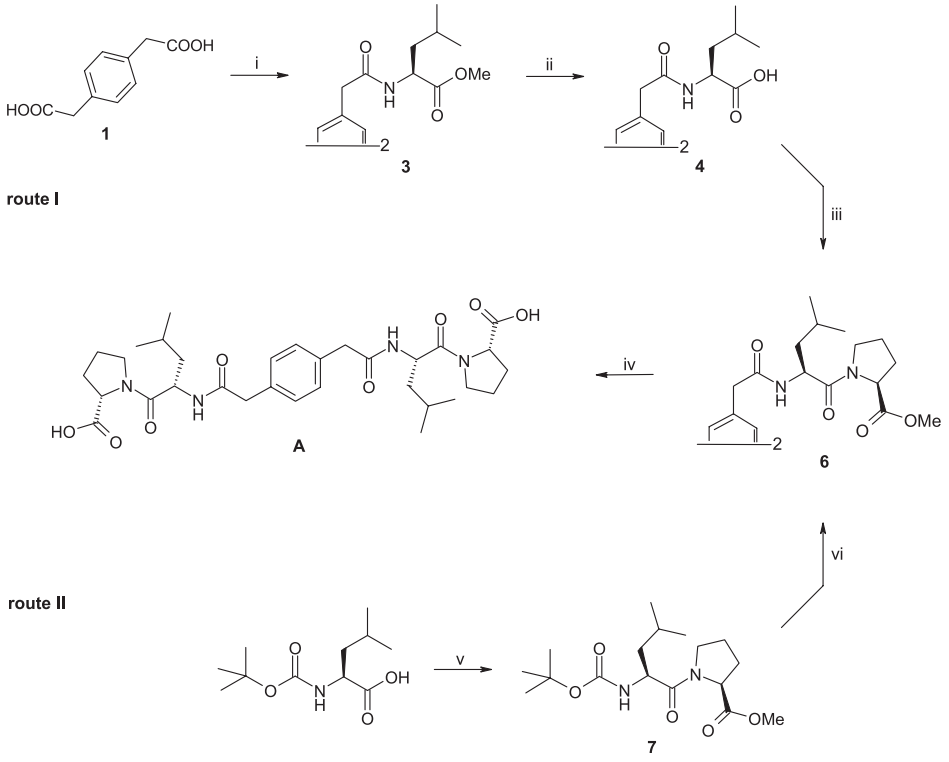


Fig. 1. Development of putative tryptase inhibitors

98-B (isolated from *Microcystis aeruginosa* NIES-98) [14] as new lead structures. Compound **RA-1002**, our first selective acting inhibitor of thrombin, was the starting point for the development of putative tryptase inhibitors. The amino acid sequence of **RA-1002** was the base for molecular modeling studies on analogous compounds. Since these studies suggested that **RA-1003** and **RA-1004** should be able to inhibit tryptase in a bivalent manner, we chose the same sequence of amino acids by analogy to **RA-1002**. The connective bridges of the *L*-leucyl-*L*-proline dipeptides in **RA-1003** and **RA-1004** were xylyl and phenyl moieties.

Building block **A** – the precursor of **RA-1003** – was synthesised *via* two routes (routes I and II, Scheme 1). The *N*,*N*′-dicyclohexylcarbodiimide (*DCC*)-promoted reaction of 1,4-bis(carboxymethyl)benzene (**1**) with *L*-leucine methyl ester hydrochloride (**2**) represents the first step in the successive synthesis depicted in route I. After hydrolysis of **3** in a mixture of aqueous LiOH (1 *M*) and 1,2-dimethoxyethane (*DME*) the resulting acid **4** was condensed with *L*-proline methyl ester hydrochloride (**5**, *DIC*-catalysis) yielding dipeptide methyl ester **6**. The syntheses of dipeptide esters containing a C-terminal *L*-proline moiety and the following condensation with alcohols or amines using carbodiimides as coupling reagents strongly depend on the choice of appropriate reaction conditions [15]. In our hands, the combination of *N*,*N*′-diisopropylcarbodiimide (*DIC*)/dichloromethane was the most successful variation in both the synthesis of the dipeptide as well as the syntheses of the appropriate dipeptide esters or amides. The utilization of *DCC* at these steps was not satisfying, neither in dichloromethane nor in *DMF* (using *HOBt* as an additional activating reagent).

Finally, hydrolysis of **6** (aqueous LiOH/*DME*) afforded building block **A** in high yields. Compound **1** was synthesised from α,α'-dichloro-*p*-xylene following

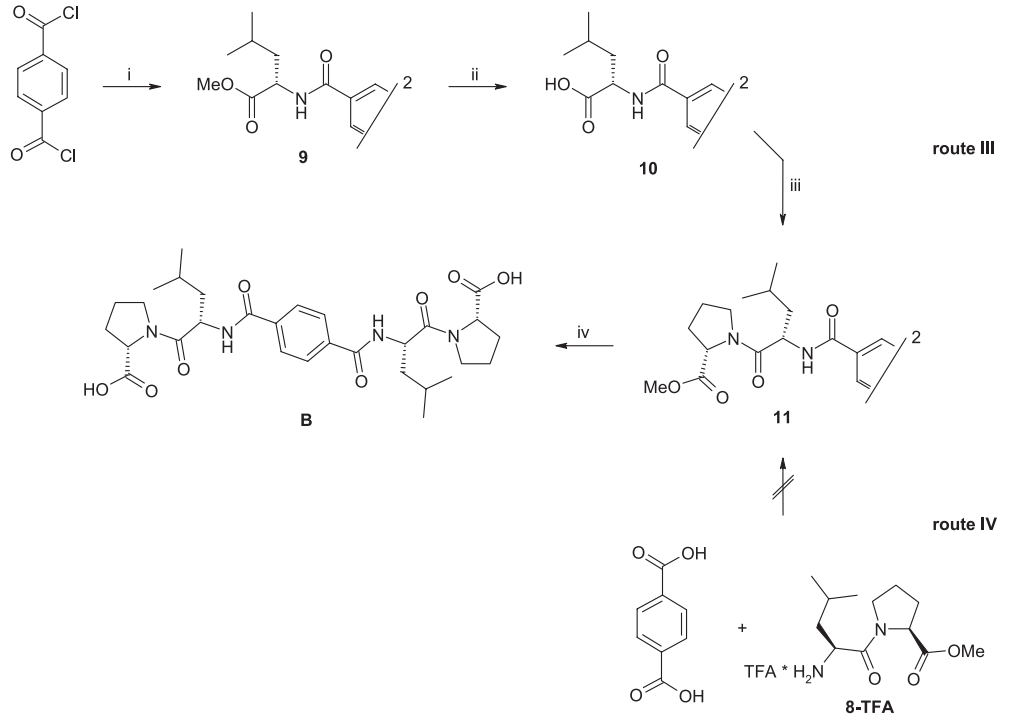


Scheme 1. i) *DCC*/*L-Leu*-O*Me*·HCl (**2**)/*DIPEA*/CH_2Cl_2, 0°C, 34%; ii) LiOH (1 *M*)/*DME*, rt, 74%; iii) *DIC*/*L-Pro*-O*Me*·HCl (**5**)/*DIPEA*/CH_2Cl_2, 0°C, 32%; iv) LiOH (1 *M*)/*DME*, rt, 82%; v) *DCC*/**5**/*DIPEA*/CH_2Cl_2, 0°C, 61%; vi) a) *TFA*/CH_2Cl_2, 0°C, b) **1**/*DIC*/*DIPEA*/CH_2Cl_2, 0°C, 64%

Kolbe's nitrile protocol. Acidic hydrolysis of the 1,4-bis(cyanomethyl)benzene furnished **1** in high yields. *L*-Leucine methyl ester hydrochloride (**2**) and *L*-proline methyl ester hydrochloride (**5**) were prepared according to the thionyl chloride method [16].

The synthesis of **6** *via* route II initially consists of the condensation of *N-Boc-L*-leucine with L-proline methyl ester hydrochloride (**5**). Cleavage of the *tert*-butyloxycarbonyl group by means of *TFA*/dichloromethane and subsequent transformation of the ammonium into the amino group with *DIPEA* generated the unprotected dipeptide methylester **8**, which was condensed with **1** to give **6** subsequent to *DIC*-activation of **1**.

Route II impresses with a noticeable higher over-all yield (39%) compared to route I (8%) concerning the synthesis up to intermediate **6**. On the other hand, in the case of phenylene derivative **B** the stepwise connection of each single building block is more successful (route III, Scheme 2) than the condensation of terephthalic acid with *L*-leucyl-*L*-proline methyl ester (**8**) which failed completely (route IV). Thus, commercially available terephthaloyl chloride was aminolised by *L*-leucine methyl ester hydrochloride (**2**) using an excess of *DIPEA*. The resulting methyl ester **9** was subjected to the analogous procedure that was utilized in route I. Hydrolysis of bisester **9** and condensation of the resulting acid **10** with two



Scheme 2. i) **2**/*DIPEA*/CH_2Cl_2, rt, 77%; ii) LiOH (1 *M*)/*DME*, rt, 95%; iii) *DIC*/**5**/*DIPEA*/CH_2Cl_2, 0°C, 58%; iv) LiOH (1 *M*)/*DME*, rt, 51%

equivalents of *L*-proline methyl ester hydrochloride (**5**) led to dipeptide derivative **11** in better yields compared to the synthesis of **6**. Building block **B** was obtained by hydrolysis of **11** – with an over-all yield of 22%.

In conclusion, we have examined the syntheses and the chemical properties of precursors of potential bivalent inhibitors of human mast cell tryptase. Interestingly, building blocks **A** and **B** display exact opposite behaviour during their syntheses bearing only one subtle distinction in their chemical structure, namely the benzylic methylene group. In the synthesis of building block **A** the synthetic pathway *via* the central dipeptide **7** (route II) is to be favoured, whereas this procedure fails in the preparation of building block **B** (route IV). The best way to synthesise **B** is the stepwise manner (route III), which was less successful in the synthesis of **A** (route I).

Choosing the best of several practicable pathways, the peptide chemist profits from his experience and synthetic talent, but it still stays nearly impossible to predict the most successful route. Thus, each route has to be checked concerning parameters like practicability, time-consumption, costs, and numbers of synthesis steps.

Experimental

General

Melting points are not corrected, Mikroheiztisch PHMK 80-2747 F. Küstner Nachf. KG Dresden. IR spectra (KBr): IR-Spektrometer Perkin-Elmer 1600 series FTIR. NMR spectra: Bruker DPX 300

(300 MHz), δ (ppm), solvents: $CDCl_3$, *DMSO*-d_6, internal standard: *TMS*. Elemental analysis: Perkin-Elmer Elemental Analyzer 2400 CHN, all compounds gave satisfactory elemental analyses. Chromatography: cc: Merck silica gel 60 (0.063–0.200 mm); tlc: Merck aluminium foils silica gel 60 F_{254}. Optical rotation: Polartronic D (Schmidt Haensch GmbH), determined with Na-D-line (589.3 nm) at 23°C. Abbreviations of amino acids follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature [17]. Other abbreviations: *Boc*: *tert*-butyloxycarbonyl, *DCC*: *N*,*N*′-dicyclohexylcarbodiimide, *DCU*: *N*,*N*′-dicyclohexylurea, *DIC*: *N*,*N*′-diisopropylcarbodiimide, *DIPEA*: diisopropylethylamine, *DME*: 1,2-dimethoxyethane, *Et*OAc: ethyl acetate, *PE*: petroleum ether, *TFA*: trifluoroacetic acid.

1,4-Bis(carboxymethyl)benzene (**1**)

α,α'-Dichloro-*p*-xylene (15.00 g, 85.7 mmol) and 10.50 g of powdered sodium cyanide (214 mmol) were stirred in 60 cm^3 of triethylene glycol at 90°C for 1 h. After cooling to room temperature, the mixture was poured into 150 cm^3 of H_2O and extracted with 3×75 cm^3 CH_2Cl_2. The combined CH_2Cl_2 layers were dried (Na_2SO_4), the solvent was evaporated, and the residue was recrystallised from *Et*OAc/*PE*. Yield 11.6 g (87%) of 1,4-bis(cyanomethyl)benzene, colourless crystals, mp 99°C (*Et*OAc/*PE*) (Ref. [18] 95–97°C); ^{1}H NMR ($CDCl_3$): $\delta = 7.36$ (4H, s, H_{arom}), 3.77 (4H, s, CH_2) ppm; IR: 3035, 2936, 2916, 2248, 1518, 1424, 1414, 1126, 1021, 931, 782, 751, 475 cm^{-1}.

1,4-Bis(cyanomethyl)benzene (5.50 g, 35.3 mmol) was stirred in a mixture of 35 cm^3 of acetic acid and 175 cm^3 of conc. HCl at 100°C for 2 h. After cooling to 0°C, the precipitate was collected, washed with H_2O, and recrystallised from H_2O. Yield 6.1 g (90%), colourless crystals, mp 252–253°C (*Et*OH/H_2O) (Ref. [19] 253–254°C). ^{1}H NMR (*DMSO*-d_6): $\delta = 12.31$ (2H, s, COOH), 7.19 (4H, s, H_{arom}), 3.52 (4H, s, CH_2) ppm; IR: 2800–3200, 2733, 2657, 1697, 1518, 1408, 1340, 1238, 1202, 1180, 899, 857, 784, 676, 515, 435 cm^{-1}.

1,4-Phenylenedi(acetyl-L-leucine methyl ester) (**3**, $C_{24}H_{36}N_2O_6$)

To an ice-cooled mixture of 3.00 g of **1** (15.5 mmol) in 200 cm^3 of CH_2Cl_2 a solution of 6.37 g of *DCC* (30.9 mmol) in 100 cm^3 of CH_2Cl_2 was added dropwise over a period of 90 min. After further 90 min of stirring, a mixture of 6.17 g of *L-Leu*-O*Me*·HCl (**2**, 34.0 mmol) and 4.83 g of *DIPEA* (37.4 mmol) in 100 cm^3 of CH_2Cl_2 was added at 0°C during 90 min. Stirring over night under warming up to room temperature was followed by evaporation of the solvent. The residue was purified by column chromatography (*Et*OAc:*PE* = 5:1). Yield 2.33 g (34%), colourless crystals, mp 147–148.5°C; ^{1}H NMR ($CDCl_3$): $\delta = 7.27$ (4H, s, H_{arom}), 5.80 (2H, d, 8.2 Hz, NH), 4.59–4.66 (2H, m, α-H), 3.70 (6H, s, OCH_3), 3.59 (4H, s, Ar-C$\underline{H}_2$), 1.43–1.62 (6H, m, C$\underline{H}(CH_3)_2 + \beta$-H), 0.90 (6H, d, $J = 6.3$ Hz, CH(C$\underline{H}_3)_2$), 0.89 (6H, d, $J = 6.3$ Hz, CH(C$\underline{H}_3)_2$) ppm; IR: 3261, 3069, 2956, 2870, 1745, 1641, 1553, 1439, 1353, 1276, 1248, 1203, 1165, 698, 541 cm^{-1}; $[\alpha]_D = -35.33$ deg cm^2 g^{-1} ($c = 1\%$, *Me*OH); $M = 448.56$.

1,4-Phenylenedi(acetyl-L-leucine) (**4**, $C_{22}H_{32}N_2O_6$)

Compound **3** (2.24 g, 4.98 mmol) was stirred in a mixture of 10 cm^3 of 1 *M* aqueous LiOH and 20 cm^3 of *DME* at room temperature for 2 h. The mixture was acidified ($pH = 4$) by addition of aqueous citric acid (10%) and extracted with 2×20 cm^3 of EtOAc. The combined organic layers were dried (Na_2SO_4) and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2:*Et*OAc:*PE*:*Me*OH = 10:10:10:1 → CH_2Cl_2:*Et*OAc:*Me*OH = 10:10:5). Yield 1.54 g (74%), colourless crystals, mp 184°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.48$ (2H, s, COOH), 8.31 (2H, d, $J = 8.0$ Hz, NH), 7.15 (4H, s, H_{arom}), 4.19–4.24 (2H, m, α-H), 3.42 (4H, d, $J = 14.1$ Hz, Ar-C$\underline{H}_2$), 1.48–1.75 (6H, m, C$\underline{H}(CH_3)_2 + \beta$-H), 0.88 (6H, d, $J = 6.4$ Hz, CH(C$\underline{H}_3)_2$), 0.81 (6H, d, $J = 6.4$ Hz, CH(C$\underline{H}_3)_2$) ppm;

IR: 3343, 2800–3500, 2857, 2929, 2870, 1712, 1625, 1549, 1512, 1328, 1291, 1270, 1244, 1209, 1165, 1128, 788, 658, 603, 551, 503 cm^{-1}. $[\alpha]_D = -28.5\ deg\ cm^2\ g^{-1}$ ($c = 2\%$, *Me*OH); $M = 420.51$.

1,4-Phenylenedi(acetyl-L-leucyl-L-proline methyl ester) (**6**, $C_{34}H_{50}N_4O_8$)

Method A: Compound **4** (1.39 g, 3.31 mmol) was dissolved in 40 cm^3 of CH_2Cl_2 and cooled to 0°C. A solution of 0.84 g of *DIC* (6.62 mmol) in 10 cm^3 of CH_2Cl_2 was added dropwise over a period of 15 min. The mixture was stirred at rt for 45 min (solution A) while in a second vessel 1.04 g of *DIPEA* (8.01 mmol) in 10 cm^3 of CH_2Cl_2 was added to 1.21 g of *L*-proline methyl ester hydrochloride (**5**, 7.28 mmol) in 40 cm^3 of CH_2Cl_2 (solution B). The latter solution was added dropwise into solution A at 0°C (15 min) and this mixture was stirred for 16 h at rt and then it was evaporated *in vacuo*. The resulting residue was purified by column chromatography to yield 0.68 g (32%).

Method B: To an ice-cooled solution of 0.71 g of **1** (3.66 mmol) in 50 cm^3 of CH_2Cl_2 0.92 g of *DIC* (7.31 mmol) in 20 cm^3 of CH_2Cl_2 were added dropwise (15 min). The mixture was stirred for 45 min and the dipeptide solution (*L-Leu-L-Pro*-O*Me*) was added dropwise at 0°C (45 min). After stirring for 16 h the mixture was evaporated *in vacuo* and the residue was purified by column chromatography (*Et*O*Ac*:PE = 1:1 → CH_2Cl_2:*Et*O*Ac*:*PE*:*Me*OH = 10:10:10:1).

The dipeptide solution was obtained by treating 2.50 g of *Boc-L-Leu-L-Pro*-O*Me* (7.31 mmol) in a *TFA*/CH_2Cl_2-mixture (30/80 cm^3) at rt for 3.5 h. The mixture was evaporated *in vacuo* and the residue was dissolved in 20 cm^3 of methanol and the solvent was evaporated *in vacuo* again. To the remaining oil a solution of 1.04 g of *DIPEA* (8.04 mmol) in 50 cm^3 of CH_2Cl_2 was added and the mixture was stirred for 15 min at rt.

Yield 2.03 g (86%), colourless crystals, mp 106°C; ^{1}H NMR ($CDCl_3$): $\delta = 7.19$ (4H, s, H_{arom}), 6.46 (2H, d, $J = 8.7$ Hz, NH), 4.77–4.85 (2H, m, Leu-α-H), 4.46–4.51 (2H, m, Pro-α-H), 3.77–3.85 (2H, m, Pro-N-C$\underline{H}_2$), 3.70 (6H, s, OCH_3), 3.56–3.65 (2H, m, Pro-N-C$\underline{H}_2$), 3.50 (4H, s, Ar-C$\underline{H}_2$), 1.90–2.25 (8H, m, Pro-β-CH_2 + Pro-γ-CH_2) 1.58–1.70 (2H, sept, 6.5 Hz, C$\underline{H}(CH_3)_2$), 1.46–1.52 (4H, m, Leu-β-CH_2), 0.95 (6H, d, $J = 6.5$ Hz, CH(C$\underline{H}_3)_2$), 0.90 (6H, d, $J = 6.5$ Hz, CH(C$\underline{H}_3)_2$) ppm; IR: 3289, 3064, 2956, 2872, 1747, 1631, 1543, 1514, 1448, 1366, 1320, 1276, 1197, 1175, 1097, 1024, 997, 724, 682, 597, 561 cm^{-1}; $[\alpha]_D = -103.16\ deg\ cm^2\ g^{-1}$ ($c = 1.5\%$, *Me*OH); $M = 642.78$.

1,4-Phenylenedi(acetyl-L-leucyl-L-proline) (**A**, $C_{32}H_{46}N_4O_8$)

Compound **6** (2.42 g, 3.76 mmol) was stirred in a mixture of 10 cm^3 of 1 *M* aqueous LiOH and 20 cm^3 of *DME* at room temperature for 2 h. The mixture was acidified (*pH* = 4) by addition of aqueous citric acid (10%) and extracted with 2×20 cm^3 of *Et*O*Ac*. The combined organic layers were dried (Na_2SO_4) and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2:*Et*O*Ac*:*PE*:*Me*OH = 10:10:10:1 → CH_2Cl_2:*Et*O*Ac*:*Me*OH = 10:10:5). Yield 0.88 g (38%), colourless crystals, mp 190°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.41$ (2H, s br, COOH), 8.31 (2H, d, $J = 8.1$ Hz, NH), 7.12 (4H, s, H_{arom}), 4.49–4.56 (2H, m, Leu-α-H), 4.20–4.25 (2H, m, Pro-α-H), 3.60–3.71 (2H, m, Pro-N-C$\underline{H}_2$), 3.40–3.50 (2H, m, Pro-N-C$\underline{H}_2$), 3.39 (4H, m, Ar-C$\underline{H}_2$), 2.06–2.18 (2H, m, Pro-β-CH_2), 1.75–1.95 (6H, m, Pro-β-CH_2-γ-CH_2), 1.57–1.68 (2H, m, C$\underline{H}(CH_3)_2$), 1.39–1.49 (4H, m, Leu-β-CH_2), 0.89 (6H, d, $J = 6.6$ Hz, CH(C$\underline{H}_3)_2$), 0.84 (6H, d, $J = 6.6$ Hz, CH(C$\underline{H}_3)_2$) ppm; IR: 2850–3600, 3453, 2957, 1732, 1629, 1548, 1514, 1322, 1222, 1194, 668, 599, 562 cm^{-1}; $[\alpha]_D = -105.0\ deg\ cm^2\ g^{-1}$ ($c = 1\%$, *Me*OH); $M = 614.73$.

Terephthaloyldi(L-leucine methyl ester) (**9**, $C_{22}H_{32}N_2O_6$)

To a suspension of 5.08 g of terephthaloyl chloride (25.00 mmol) in 75 cm^3 of CH_2Cl_2 a solution of 9.08 g of *L*-leucine methyl ester hydrochloride (50.00 mmol) and 14.19 g of *DIPEA* (110.00 mmol) in 150 cm^3 of CH_2Cl_2 was added at rt (40 min). After stirring for 16 h the mixture was extracted with 100 cm^3 of 2 *N* HCl, the organic layer was dried (Na_2SO_4), and the solvent was evaporated *in vacuo*.

The resulting residue was recrystallised from *Et*OAc/*Et*OH. Yield 8.10 g (77%), colourless crystals, mp 234–235°C; ^{1}H NMR ($CDCl_3$): $\delta = 7.76$ (4H, s, H_{arom}), 7.19 (2H, d, $J = 8.3$ Hz, NH), 4.84–4.92 (2H, m, α-H), 3.80 (6H, s, OCH_3), 1.65–1.80 (6H, m, C<u>H</u>$(CH_3)_2 + \beta$-H), 0.97–1.00 (12H, 2d, CH(C<u>H</u>$_3)_2$) ppm; IR: 3325, 2955, 2870, 1745, 1634, 1549, 1502, 1340, 1278, 1210, 1162, 1017, 872, 846, 748, 730, 640 cm^{-1}; $[\alpha]_D = -18.66$ ($c = 2\%$, *Me*OH); $M = 420.51$.

Terephthaloyldi(L-leucine) (**10**, $C_{20}H_{28}N_2O_6$)

Compound **9** (1.57 g, 3.74 mmol) was stirred in a mixture of 10 cm^3 of 1 *M* aqueous LiOH and 20 cm^3 of *DME* at room temperature for 2 h. The mixture was acidified ($pH = 4$) by addition of aqueous citric acid (10%) and extracted with 2×20 cm^3 of *Et*OAc. The combined organic layers were dried (Na_2SO_4) and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2:*Et*OAc:*PE*:*Me*OH = 10:10:10:1 → CH_2Cl_2:*Et*OAc:*Me*OH = 10:10:5). Yield 1.38 g (95%), colourless crystals, mp 240–242°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.41$ (2H, s br, COOH), 8.72 (2H, d, $J = 7.9$ Hz, NH), 7.96 (4H, s, H_{arom}), 4.42–4.48 (2H, m, α-H), 1.59–1.82 (6H, m, C<u>H</u>$(CH_3)_2 + \beta$-H), 0.93 (6H, d, $J = 6.2$ Hz, CH(C<u>H</u>$_3)_2$), 0.89 (6H, d, $J = 6.2$ Hz, CH(C<u>H</u>$_3)_2$) ppm; IR: 3382, 2961, 2872, 2400–3500, 1728, 1632, 1542, 1500, 1270, 1234, 1170, 1017, 968, 870, 847, 748, 713, 660, 598 cm^{-1}; $[\alpha]_D = -5.87$ deg cm^2 g^{-1} ($c = 2\%$, *Me*OH); $M = 392.46$.

Terephthaloyldi(L-leucyl-L-proline methyl ester) (**11**, $C_{32}H_{46}N_4O_8$)

Compound **10** (3.44 g, 8.79 mmol) was dissolved in 130 cm^3 of CH_2Cl_2 and cooled to 0°C. A solution of 2.22 g of *DIC* (17.58 mmol) in 30 cm^3 of CH_2Cl_2 was added dropwise over a period of 30 min. The mixture was stirred at rt for 1 h (solution A) while in a second vessel 2.74 g of *DIPEA* (21.27 mmol) in 30 cm^3 of CH_2Cl_2 were added to 3.20 g of *L*-proline methyl ester hydrochloride (**5**, 19.34 mmol) in 100 cm^3 of CH_2Cl_2 (solution B). The latter solution was added dropwise into solution A at 0°C (45 min) and this mixture was stirred for 16 h at rt and then the solvent was evaporated *in vacuo*. The resulting residue was purified by column chromatography (*Et*OAc:*PE* = 1:1 → *Et*OAc:*PE* = 2:1). Yield 3.13 g (58%), colourless crystals, mp 195–203°C; ^{1}H NMR ($CDCl_3$): $\delta = 7.69$–7.84 (4H, m, H_{arom}), 7.03 (2H, d, NH), 5.01–5.20 (2H, m, Leu-α-H), 4.41–4.56 (2H, m, Pro-α-H), 3.85–4.00 (2H, m, Pro-N-C<u>H</u>$_2$), 3.69 (6H, s, OCH_3), 3.55–3.66 (2H, m, Pro-N-C<u>H</u>$_2$), 1.90–2.25 (8H, m, Pro-β-CH_2 + Pro-γ-CH_2), 1.40–1.85 (6H, m, Leu-β-CH_2 + C<u>H</u>$(CH_3)_2$), 0.89–1.07 (12H, m, CH(C<u>H</u>$_3)_2$) ppm; IR: 3454 (NH), 2956, 2872, 1745 (C=O), 1632 (C=O), 1544, 1498, 1439, 1290, 1366, 1343, 1198, 1174, 872, 729, 606 cm^{-1}; $[\alpha]_D = -59.41$ deg cm^2 g^{-1} ($c = 2\%$, *Me*OH); $M = 614.73$.

Terephthaloyldi(L-leucyl-L-proline) (**B**, $C_{30}H_{42}N_4O_8$)

Compound **11** (2.08 g, 3.39 mmol) was stirred in a mixture of 10 cm^3 of 1 *M* aqueous LiOH and 20 cm^3 of *DME* at room temperature for 2 h. The mixture was acidified ($pH = 4$) by addition of aqueous citric acid (10%) and extracted with 2×20 cm^3 of *Et*OAc. The combined organic layers were dried (Na_2SO_4) and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2:*Et*OAc:*PE*:*Me*OH = 10:10:10:1 → CH_2Cl_2:*Et*OAc:*Me*OH = 10:10:5). Yield 1.01 g (51%), colourless crystals, mp 247°C (dec); ^{1}H NMR (*DMSO*-d_6): $\delta = 12.48$ (2H, s br, COOH), 8.71 (2H, d, $J = 7.8$ Hz, NH), 7.97 (4H, s, H_{arom}), 4.72–4.81 (2H, m, Leu-α-H), 4.18–4.30 (2H, m, Pro-α-H), 3.73–3.85 (2H, m, Pro-N-C<u>H</u>$_2$), 3.56–3.62 (2H, m, Pro-N-C<u>H</u>$_2$), 2.10–2.20 (2H, m, Pro-β-CH_2), 1.65–2.00 (10H, m, Pro-β-CH_2-γ-CH_2 + Leu-β-CH_2), 1.45–1.53 (2H, m, C<u>H</u>$(CH_3)_2$), 0.90 (6H, d, $J = 6.6$ Hz, CH(C<u>H</u>$_3)_2$), 0.86 (6H, d, $J = 6.6$ Hz, CH(C<u>H</u>$_3)_2$) ppm; IR: 3445, 2958, 1729, 1633, 1541, 1497, 1451, 1317, 1224, 1193, 870, 668, 600 cm^{-1}; $[\alpha]_D = -42.16$ deg cm^2 g^{-1} ($c = 2\%$, *Me*OH); $M = 586.67$.

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